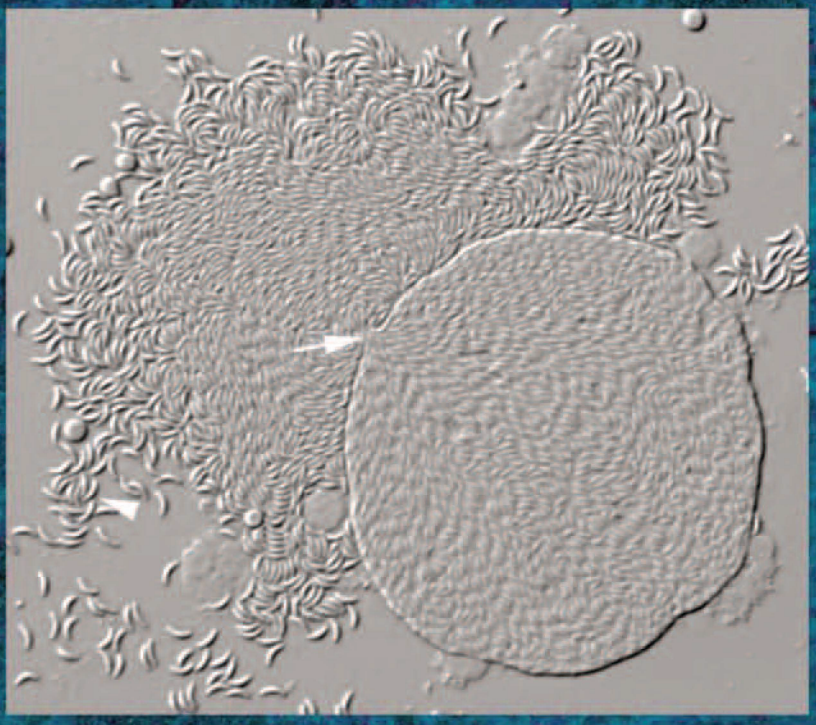
TOXOPLASMOSIS

of ANIMALS and HUMANS

Second Edition



J. P. Dubey

CRC Press
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USDA/ARS
Beltsville, Maryland, U.S.A.

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number: 978-1-4200-9236-3 (Hardback)

Library of Congress Cataloging-in-Publication Data

Dubey, J. P.
Toxoplasmosis of animals and humans / Jitender Prakask Dubey. -- 2nd ed.
p. ; cm.
Rev. ed. of: Toxoplasmosis of animals and man / authors, J.P. Dubey, C.P. Beattie. c1988.
Includes bibliographical references and index.
ISBN 978-1-4200-9236-3 (hardcover : alk. paper)
1. Toxoplasmosis in animals. 2. Toxoplasmosis. I. Dubey, J. P. Toxoplasmosis of animals and man. II. Title.
[DNLM: 1. Toxoplasmosis. 2. Toxoplasmosis, Animal. WC 725 D714t 2010]

SF809.T6D83 2010
636.089'6936--dc22 2009027561

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Dedication

I would like to dedicate this book to my uncle, Mr. G. L. Dubey. Without his kindness, generosity, and encouragement I would have been ploughing land in a remote village in India.[352a]

Contents

Preface to the Second Edition

In the two decades since the first edition of this book was published in 1988, there has been an explosion of knowledge concerning the parasite *Toxoplasma gondii* and toxoplasmosis. One reason for this is because *T. gondii* has been and continues to be used extensively as a model for the cell biology of apicomplexan parasites. Although several books have been published on toxoplasmosis, the present book provides unique information on all known host types for this parasite including humans and bats, and is compiled by a single author.

I view Chapter 1 as the strength of this edition as it focuses in detail on the biology of the parasite. Many scientists use *T. gondii* to investigate problems in cell biology and genetics, and in support I have provided information on methods of cultivation and safety procedures. Chapter 2, which deals with toxoplasmosis in humans, was originally written by my former professor and coauthor of the first edition, C. P. Beattie, who passed away in 1987 before the first edition was published. I have revised the content but kept the original format, and added worldwide prevalence of toxoplasmosis in pregnant women. Each of the remaining chapters deals with toxoplasmosis in one of the known host types of *T. gondii*, including a chapter on marine mammals that are dying from toxoplasmosis. In these chapters, I have extensively reviewed literature from 1988 to 2008, provided factual data, and have avoided speculation.

During the last 45 years I have had the privilege of working with virtually all hosts of *T. gondii*, including all livestock species, wildlife, and zoo animals. I feel blessed to have collaborated with hundreds of scientists. In the interest of brevity and economy, references cited in the first edition, although mentioned, are not listed in the literature cited section in the present edition. Because the literature on toxoplasmosis is vast (over 20,000 references), I have extensively borrowed tables from literature (mostly from my papers) to reduce citations. In the present edition, there are approximately 2,000 citations.

I would like to acknowledge those who made this book possible; I feel guilty that I cannot possibly list all of them. Oliver Kwok, Anna Thorn, Leandra Ferreira, Rajenderan Chelliah, Linda Okai, Yara Al-Kappany, Edwards Frazão-Teixeirac, colleagues and staff in my laboratory who've helped with proofreading and preparations of tables. To Fournet Valsin I am grateful for helping me with illustrations. I am also grateful to Drs. Sonia Almeria, Daniel Azenberg, Eva Bartová, Władystaw Cabaj Vern Carruthers, Marie Dardé, Bill Everson, David Ferguson, Jack Frenkel, Ray Gamble, Ruth Gilbert, Mike Grigg, Dolores Hill, Ke Hu, Solange Gennari, Jeffery Jones, David Lindsay, Joan Lunney, Rima McLeod, P. Marty, Manami Nishi, Eskild Petersen, Gereon Schares, David Sibley, Borris Striepen, Chunlei Su, Philippe Thulliez, Eric Villegas, Louis Weiss, and others who have contributed to this book in more than one way. I appreciate the love and support of both my sons, Ravi and Raj who had to make sacrifices during their growing up because I was either working in the laboratory or writing at home. Finally I am indebted to my wife, Niti Dubey, without whose support and love I could not have succeeded in completing this book, and life in general.

J. P. Dubey

The Author

J. P. Dubey, M.V.Sc. Ph.D., was born in India. He received his veterinary degree in 1960, and Masters in Veterinary Parasitology in 1963, from India. He obtained a Ph.D. in Medical Microbiology in 1966 from the University of Sheffield, England. He obtained postdoctoral training with Dr. J. K. Frenkel, Department of Pathology and Oncology, University of Kansas Medical Center, Kansas City, from 1968 to 1973. From 1973 to 1978, he was Associate Professor of Veterinary Parasitology, Department of Pathobiology, Ohio State University, Columbus. He was Professor of Veterinary Parasitology, Department of Veterinary Science, Montana State University, Bozeman, from 1978 to 1982. He is presently a Senior Scientist, Animal Parasitic Diseases Laboratory, Animal Natural and Resources Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland.

Dr. Dubey has spent over 45 years researching *Toxoplasma gondii* and related cyst-forming coccidian parasites of man and animals. He has published over 1200 research papers in international journals, half of which are on toxoplasmosis. He was also instrumental in discovering the life cycle of *Toxoplasma gondii* in the cat. In 1985 he was chosen to be the first recipient of the Distinguished Veterinary Parasitologist Award by the American Association of Veterinary Parasitologists. Dr. Dubey is recipient of the 1995 WAAVP Pfizer award for outstanding contributions to research in veterinary parasitology. He also received the 2005 Eminent Parasitologists Award from the American Society of Parasitologists. The Thomas/Institute for Scientific Information identified him as one of the world's most cited authors in plant and animal sciences for the last decade. Dr. Dubey was the recipient of the U.S. Presidential Meritorious Award in 2003. Recently, he was selected for the newly created Senior Scientific Research Service (SSRS), and is one of the 11 scientists and executives within the USDA's Agricultural Research Service; selection for this position is by invitation only, and is approved by the Secretary of Agriculture. He is the only veterinarian and parasitologist in this grade.

Abbreviations

CFT: complement fixation test
CNS: central nervous system
DMSO: dimethylsulfoxide
DT: Sabin-Feldman dye test
ELISA: enzyme linked immunoabsorbent assay
ER: Endoplasmic reticulum
H and E: hematoxylin and eosin
IFA: indirect fluorescent antibody
IgAGA: immunoabsorbent agglutination assay
IgG: immunoglobin G
IgM: immunoglobin M
IgM-ISAGA: Immunoglobin M immunoabsorbent agglutination assay
IHA: indirect hemagglutination
IMC: inner membrane complex
hr: hour
i.m.: intramuscular
i.p.: intraperitoneal
i.v.: intravenous
LAT: latex agglutination test
MAT: modified agglutination test
min: minutes
mo: month
PAS: Periodic acid Schiff
p.i.: post inoculation
PV: parasitophorous vacuole
p.v.m.: parasitophorous vacuolar membrane
s.c.: subcutaneous
T. gondii: *Toxoplasma gondii*
TCM: tissue culture medium
TMN: tubular membranous network
wk: week
yr: year

CHAPTER 1

General Biology

1.1 INTRODUCTION AND HISTORY

Toxoplasma gondii is one of the most well studied parasites because of its medical and veterinary importance. It is used extensively as a model for cell biology of apicomplexan organisms. Two books were recently published that dealt with cell biology and molecular biology of this parasite.[13,1350] Some of the advantages of using *T. gondii* for cell biology are that it is large enough to be easily seen under the light microscope, it can be grown in virtually any warm-blooded cell line, it can be maintained indefinitely in mice and cell culture, and there is only one species that infects all hosts. Additionally, this parasite is readily amenable to genetic manipulation, with refined protocols for classic and reverse genetics, high transfection efficiency, and expression of epitope tags.[14,1350]

It has been 100 years since the discovery of the parasite in 1908 and the event was celebrated with publication of historical accounts of the parasite and its biology.[413,420,500,619,779,1335] Historic landmarks are summarized in Table 1.1.

Nicolle and Manceaux[977] found a protozoan in tissues of a hamster-like rodent, the gundi, *Ctenodactylus gundi,* which was being used for leishmaniasis research in the laboratory of Charles Nicolle at the Pasteur Institute in Tunis. Nicolle initially believed the parasite to be a piroplasm,[13] then *Leishmania,* but soon realized that he had discovered a new organism and named it *Toxoplasma gondii* based on the morphology (mod. L. *toxo* = arc or bow, *plasma* = life) and the host.[978] Thus, its complete designation is *Toxoplasma gondii*.[977,978] In retrospect, the correct name for the parasite should have been *Toxoplasma gundii*; Nicolle and Manceaux[977] had incorrectly identified the host as *Ctenodactylus gondi*.[413] Splendore[1226] discovered the same parasite in a rabbit in Brazil, also erroneously identifying it as *Leishmania*, but he did not name it.

1.2 STRUCTURE AND LIFE CYCLE

T. gondii is a coccidian parasite with cats as the definitive host, and warm-blooded animals as intermediate hosts. It belongs to

Phylum: Apicomplexa; Levine, 1970
Class: Sporozoasida; Leukart, 1879
Subclass: Coccidiasina; Leukart, 1879
Order: Eimeriorina; Leger, 1911
Family: Toxoplasmatidae, Biocca, 1956
Genus: Toxoplasma Nicolle and Manceaux, 1909

There is only one species of *Toxoplasma, T. gondii*.

Table 1.1 Summary of Landmarks in the History of *Toxoplasma gondii*[a]

Finding	Reference[b]
Etiologic Agent	
Protozoa found in the rodent *Ctenodactylus gundi* in Tunisia	Nicolle and Manceaux (1908)
Protozoa found in a rabbit in Brazil	Splendore (1908)
Name *Toxoplasma gondii* proposed (taxon = bow, plasma = image)	Nicolle and Manceaux (1909)
First viable *T. gondii* isolate obtained from an animal	Sabin and Olitsky (1937)
First isolate of *T. gondii* from human	Wolf et al. (1939)
Human and animal *T. gondii* proven identical	Sabin (1941)
Pathogenesis of toxoplasmosis, including hydrocephalus	Frenkel and Friedlander (1951); Frenkel (1953,1956)
Parasite Morphology and Life Cycle	
Tachyzoite (Trophozoite, Feeding Form, Proliferative Form, Endodyozoite)	
Term tachyzoite proposed (tachy-fast, zoite-life)	Frenkel (1973)
Endodyogeny described	Goldman et al. (1958)
Ultrastructure described	Gustafson et al. (1954); Sheffield and Melton (1968)
Tissue cyst, bradyzoite, cystozoite	
Cyst recognized	Levaditi et al. (1928)
Cyst described cytologically	Frenkel and Friedlander (1951); Frenkel (1956)
Ultrastructure described	Wanko et al. (1962); Ferguson and Hutchison (1987)
Term bradyzoite proposed (bradys = slow, zoon = animal)	Frenkel (1973)
Term tissue cyst proposed	Dubey and Beattie (1988)
Bradyzoite resistance to digestive enzymes recognized	Jacobs et al. (1960a)
Development of tissue cysts and bradyzoites described	Dubey and Frenkel (1976)
Complete biology of bradyzoites and tissue cysts reviewed	Dubey et al. (1998)
Feline Entroepithelial Stages	
Coccidian phases described	Frenkel et al. (1970); Hutchison et al. (1970); Sheffield and Melton (1970); Dubey and Frenkel (1972)
Oocyst morphology described	Dubey et al. (1970b)
Five asexual *T. gondii* types (A–E) described	Dubey and Frenkel (1972)
Ultrastructure of coccidian stages described	Sheffield (1970); Piekarski et al. (1971); Ferguson et al. (1974, 1975, 1979a,b); Christie et al. (1978); Speer, Clark, and Dubey (1998); Speer and Dubey (2005)
Transmission	
Congenital	
Transmission demonstrated in human	Wolf et al. (1939)
Repeated transmission found in house mouse	Beverley (1959)
Congenital transmission found in a large wild animal species, white-tailed deer	Dubey et al. (2008)

Table 1.1 Summary of Landmarks in the History of *Toxoplasma gondii*[a] (Continued)

Finding	Reference[b]
Carnivorism, Transmission by Meat of Intermediate Hosts	
Suggested carnivorous transmission	Weinman and Chandler (1954)
Transmission by meat found in humans	Desmonts et al. (1965)
Fecal-Oral	
Transmission by a resistant fecal form of *T. gondii* demonstrated	Hutchison (1965)
Coccidian phase recognized	Hutchison et al. (1970, 1971); Frenkel et al. (1970); Dubey et al. (1970a,b); Sheffield and Melton (1970); Overdulve (1970)
Definitive and intermediate hosts defined, including shedding of oocysts only by felids	Frenkel et al. (1970); Miller et al. (1972); Jewell et al. (1972)
First oocyst-inhaled/ingested human toxoplasmosis outbreak described	Teutsch et al. (1979)
Genetics and Different Genetic *T. gondii* Strains	
Recombinants and genetic crosses produced	Pfefferkorn and Pfefferkorn (1980)
Isoenzyme differences used to distinguish *T. gondii* strains	Dardé et al. (1987); Tibayrene et al. (1991)
Restriction fragment length polymorphism used to group *T. gondii* strains into 3 types (I,II,III)	Sibley et al. (1992); Howe and Sibley (1995)
National, continental, intercontinental, and pandemic *T. gondii* strains distinguished	Lehmann et al. (2006)
T. gondii genome anointed	Khan et al. (2005)
Immunity and Protection	
T. gondii neutralizing antibody recognized	Sabin and Ruchman (1942)
Antibodies found to kill extracellular but not intracellular *T. gondii*	Sabin and Feldman (1948)
Protection transferred by immune lymphoid cells but not by antibodies	Frenkel (1967)
Interferon gamma found to be main cytokine for protection	Suzuki et al. (1988)
Role of CD4+ and CD8+ cells in protection defined	Gazzinelli et al. (1991)
Toxoplasmosis in Humans	
Congenital	
First proven case of congenital toxoplasmosis described	Wolf et al. (1939)
Typical tetrad clinical signs described (hydrocephalus or microcephalus, chorioretinitis, intracerebral calcification)	Sabin (1942)
Acquired	
First case in a child	Sabin (1941)
Fatal toxoplasmosis in adults found	Pinkerton and Weinman (1940)
Lymphadenopathy recognized as the most frequent symptom	Siim (1956); Beverley and Beattie (1958)
Susceptibility to toxoplasmosis in AIDS patient recognized	Luft et al. (1983)

(*continued*)

Table 1.1 Summary of Landmarks in the History of *Toxoplasma gondii*[a] (Continued)

Finding	Reference[b]
Chronic Infection	
Cysts found in autopsy slides, indicating chronic asymptomatic infection	Plout (1946); Kean and Grocott (1947)
Toxoplasmosis in Other Animals	
Toxoplasmosis found in a domestic animal, dog	Mello (1910)
Immunosuppressive canine distemper virus influenced clinical toxoplasmosis outcome in dogs	Campbell et al. (1955)
Epidemic toxoplasmosis abortions in sheep recognized	Hartley and Marshall (1957)
Toxoplasmosis in animals reviewed critically	Dubey and Beattie (1988)
Toxoplasmosis found a common infection in a marine mammal species, sea otter	Cole et al. (2000)
Diagnosis	
Novel Sabin-Feldman dye test described	Sabin and Feldman (1948)
Toxoplasma skin test as a survey tool	Frenkel (1948)
Tests developed to detect IgM antibodies in cord blood	Remington et al. (1968); Desmonts et al. (1981)
Simple direct agglutination test developed (DAT, MAT)	Desmonts and Remington (1980); Dubey and Desmonts (1987)
First validation of a serologic test using isolation of the parasite as standard	Dubey et al. (1995a); Dubey (1997)
PCR test developed to detect *T. gondii* DNA using B1 gene	Burg et al. (1989)
Treatment	
Sulfonamides found effective against *T. gondii*	Sabin and Warren (1942)
Pyrimethamine found synergistic with sulfonamides against dividing tachyzoites	Eyles and Coleman (1953)
Folic acid and yeast improves activity of sulfadiazine and pyrimethamine	Frenkel and Hitchings (1957)
Spiramycin found to have anti-toxoplasmic activity	Garin and Eyles (1958)
Clindamycin found to be anti-toxoplasmic	McMaster et al. (1973); Araujo and Remington (1974)
Prevention and Control	
Prophylactic treatment, and screening of pregnant women initiated in Austria and France to reduce congenital toxoplasmosis	Thalhammer (1973, 1978); Desmonts and Couvreur (1974a,b)
Hygienic measures advocated to prevent human exposure to oocysts	Frenkel and Dubey (1972)
Thermal curves to kill *T. gondii* in meat by cooking, freezing, and irradiation constructed	Dubey et al. (1986); Dubey et al. (1990); Kotula et al. (1991)
Animal production practices developed to reduce *T. gondii* infection in farm animals	Dubey et al. (1995b); Weigel et al. (1995)
Low prevalence of *T. gondii* in pigs correlated with reduction of seroprevalence of *T. gondii* in humans	Dubey et al. (2005); Jones et al. (2007)
Vaccination	
A vaccine to reduce fetal losses in sheep commercialized	Wilkins and O'Connel (1983); Buxton and Innes (1995)
Ts-4 vaccine for intermediate host	Waldeland and Frenkel (1983)
T-263 vaccine to prevent oocyst shedding by cats	Frenkel et al. (1991)

[a] From Dubey.[420]
[b] For references see Dubey.[420]

Coccidia are among the most important parasites of animals, and they were the first protozoa discovered. The oocyst is the key stage of all coccidians, and their classification was based on the structure of the oocyst.[279,329] Oocysts with four sporocysts, each with two sporozoites (total eight sporozoites), are classified as *Eimeria*. Oocysts containing two sporocysts, each with four sporozoites, historically were classified as *Isospora*. Coccidiosis due to *Eimeria* species is one of the most economically important diseases of poultry, cattle, sheep, goats, and many other herbivores; it is difficult to raise livestock coccidia-free.

Before the discovery of the life cycle of *T. gondii,* coccidians were considered to be host-specific with a simple one-host life cycle. Infection was confined to the intestines and usually to enterocytes. With few exceptions, eimerians still follow this life cycle. The host becomes infected by ingesting sporulated oocysts of *Eimeria*. After excystation, the sporozoites penetrate intestinal epithelial cells and multiply asexually before forming male and female gamonts. Oocysts are produced after fertilization, and are passed in feces in an unsporulated stage. Sporulation occurs outside the host. Unlike *Eimeria,* the life cycle of which has been known for many years, little was known of the complete life cycle of most *Isospora* species until 1970, when the life cycle of *T. gondii* was discovered. Until then, *Isospora* species were considered parasites of carnivores (dogs, cats) and birds and were not thought to be host specific.[437] In 1970, *T. gondii*, a parasite previously known to parasitize extraintestinal tissues of virtually all warm-blooded hosts, was found to be an intestinal coccidium of cats and to have an isosporan-like oocyst (Table 1.1). This finding was a major breakthrough in medical and veterinary sciences and eventually led to the recognition of several new taxa of economically important *Toxoplasma*-like parasites (e.g., *Neospora, Sarcocystis*) and discovery of their life cycles.

Historically, *T. gondii* originated probably as a coccidian parasite of cats with a fecal-oral cycle.[432] With domestication, it adapted transmission by several modes, including transmission by fecal-oral cycle, by carnivorism, and transplacentally.[535] There are three infectious stages of *T. gondii*: the tachyzoites, the bradyzoites, and oocysts. These stages are linked in a complex life cycle (Figure 1.1).

1.2.1 Tachyzoite

Tachyzoite (tachos = speed in Greek) is the rapidly multiplying stage. The tachyzoite is crescent-shaped, approximately 2 × 6 μm (Figure 1.2A), with a pointed anterior (conoidal) end and a rounded posterior end. In histological sections, tachyzoites are often round with a central nucleus (Figure 1.2D). Ultrastructurally, it has a complex structure with several organelles. For details of the ultrastructure of organelles and their function of *T. gondii* stages see the following references: 49, 112, 363, 438, 447, 492, 499, 500, 521, 600, 684, 698, 750, 815, 889, 913, 945, 946, 953, 1027, 1164, 1180, 1220, 1238, 1302, and 1340. The tachyzoite consists of various organelles and inclusion bodies including a pellicle (outer covering), cytoskeleton (inner membrane complex, subpellicular microtubules, apical rings, polar rings, a conoid), secretory (rhoptries, micronemes, dense granules) micropore, a mitochondrion, endoplasmic reticulum, a Golgi complex, ribosomes, rough and smooth endoplasmic reticula (ER), micropore, nucleus, amylopectin granules, Golgi apparatus, and an apicoplast (Figures 1.3–1.6). The pellicle consists of three membranes, a plasmalemma, and two closely applied membranes that form an inner membrane complex (IMC). The IMC is formed from a patchwork of flattened vesicles. The inner membrane is discontinuous at the anterior tip above the polar rings, at the micropore, and at the basal complex posterior pore at the extreme posterior tip of the zoite. There are two apical and two polar rings (outer and inner). The apical rings are located at the anterior tip of the parasite and consist of electron-dense material. The outer ring encircles the top of the resting conoid. The outer polar ring is an electron-dense thickening of the inner membrane complex at the anterior end of the tachyzoite. The inner ring anchors the subpellicular microtubules. The conoid (0.3 × 0.4 μm) is a truncated hollow cone and consists of

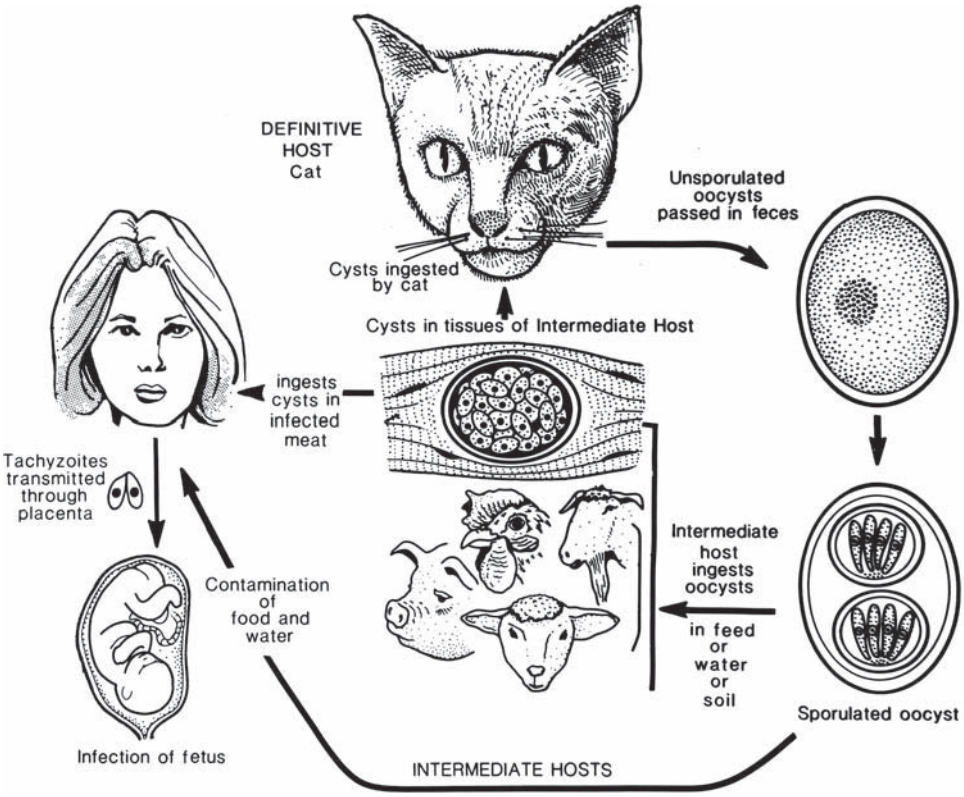

Figure 1.1 Life cycle of *Toxoplasma gondii*.

tubulin structures wound like compressed springs (Figure 1.4). Twenty-two subpellicular microtubules originate from the inner polar ring and run longitudinally almost the entire length of the cell, just beneath the inner membrane complex. They are evenly spaced, and their distal ends are not capped. In addition, there are two 400-nm-long intraconoidal, tightly bound microtubules. The subpellicular microtubules are like a rib cage and are arranged in a gentle counterclockwise spiral. Individual microtubules have prominent transverse striations. Between the anterior tip and the nucleus there are up to ten club-shaped organelles called rhoptries (Figures 1.3–1.6). A rhoptry consists of an anterior narrow electron-dense neck up to 2.5 μm long that extends into the interior of the conoid, and a sac-like, often labyrinthine posterior end (up to 0.25×1 μm). Micronemes are electron-dense rod-like structures that occur at the anterior end of the parasite (Figures 1.3–1.6). Dense granules are electron dense and scattered throughout the tachyzoite but mostly in the posterior end. The nucleus is usually situated toward the central area of the cell. It consists of a nuclear envelope with pores, clumps of chromatin, and a centrally located nucleolus. The mitrochondrium is branched and has bulbous cristae, thought to be an endosymbiont. Considerable biological curiosity has been focused on a non-photosynthetic organelle called the plastid (Figure 1.2C). It is a membrane bound, alga-derived obligatory endosymbiont structure (Figure 1.3). Although tachyzoites can move by gliding, flexing, undulating, and rotating, they have no visible means of locomotion such as cilia, flagella, or pseudopodia (Figure 1.5). Instead, their motility is powered by the actin-myosin motor complex anchored to the inner membrane complex. The intracellular components and the extracellular mileu has been called the gliodosome.[1215a,1230a] *T. gondii* is nonmotile at room temperature.

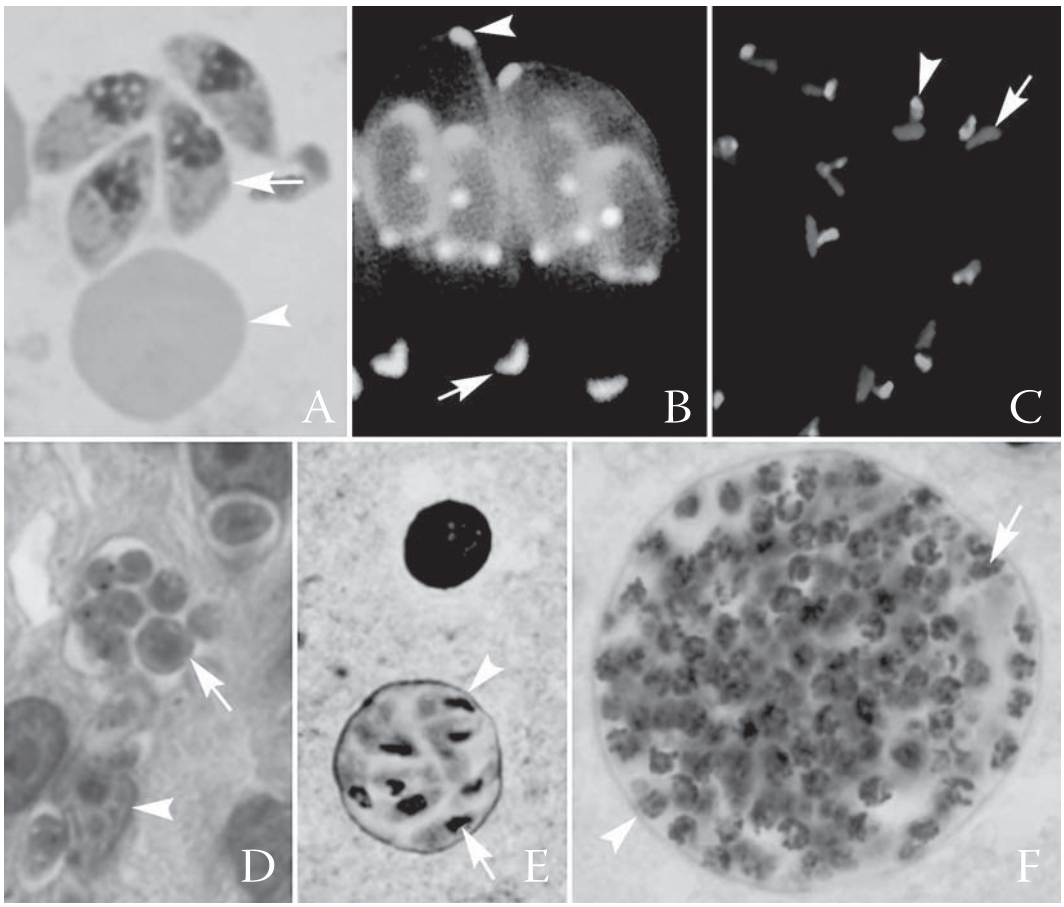

Figure 1.2 (Please see color insert following page 46.) Tachyzoites (A–D) and tissue cysts (E,F) of *T. gondii*. **A.** Tachyzoites (arrow) are crescent shaped and as long as the red blood cell (arrowhead), impression smear, mouse lung. Giemsa. **B.** Fluorescent image of two dividing tachyzoites, expressing YFP-±-tubulin (cyan) and RFP-TgMORN1 (yellow). The apical ends (arrowhead) of the parasites are oriented toward the top of the image. The two dome-like structures in each parasite are daughter cortical cytoskeletons highlighted by YFP-±-tubulin (cyan) and RFP-TgMORN1 (yellow). Arrow points to nonoconidal end. Courtesy of Drs. K. Hu and John Murray. **C**. Group of tachyzoites showing Golgi (Grasp-mRFP; red, arrow) and apicoplast (anti-acyl carrier protein with Alexa 488; green, arrowhead) with DAPI staining. Courtesy of Dr. Boris Striepen. **D.** Group of tachyzoites (arrow) in a parasitophorous vacuole in the lamina propria of small intestine of cat. Dividing tachyzoites (arrow) are bigger in size and plump. Arrowhead points to a host cell nucleus. Histologic section. H and E. **E.** Small tissue cyst. Note thin silver-positive cyst wall (arrowhead) and bradyzoites with terminal nuclei (arrow). Impression smear, mouse brain. Wilder's silver stain plus Giemsa. **F.** Tissue cyst with a thin PAS-negative (or faintly stained) cyst wall (arrowhead) and intensely PAS-positive bradyzoites (arrow). Histologic section of brain. PAS reaction counter stained with hematoxylin.

Functions of the conoid, rhoptries, micropores, and micronemes are not fully known but are probably associated with host cell penetration and creating an intracellular environment suitable for parasite growth and development, and exit from the host cell. The conoid can rotate, tilt, extend, and retract as the parasite probes the host cell plasmalemma immediately before penetration. Rhoptries have a secretory function associated with host cell penetration, secreting their contents through the plasmalemma just above the conoid to the exterior. The micropore is a cytosome-like structure formed by the invagination of the outer membrane. The mechanical events involved in zoite attachment and penetration include (1) gliding of the zoite; (2) probing of the host cell with the zoite's conoidal tip; (3) indenting the host cell

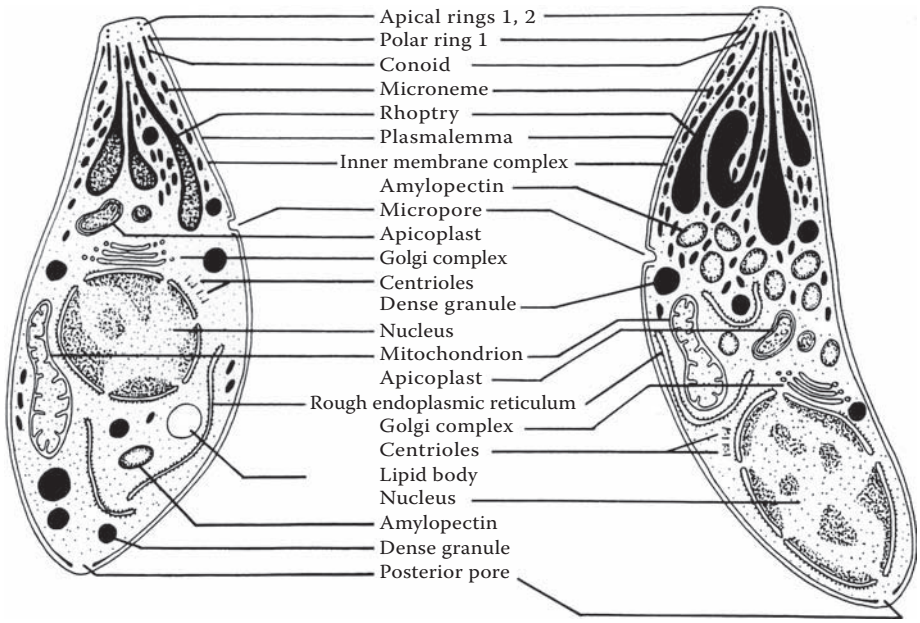

Figure 1.3 Schematic drawings of a tachyzoite and a bradyzoite of *T. gondii*. The drawings are composites of electron micrographs. From Dubey et al.[363]

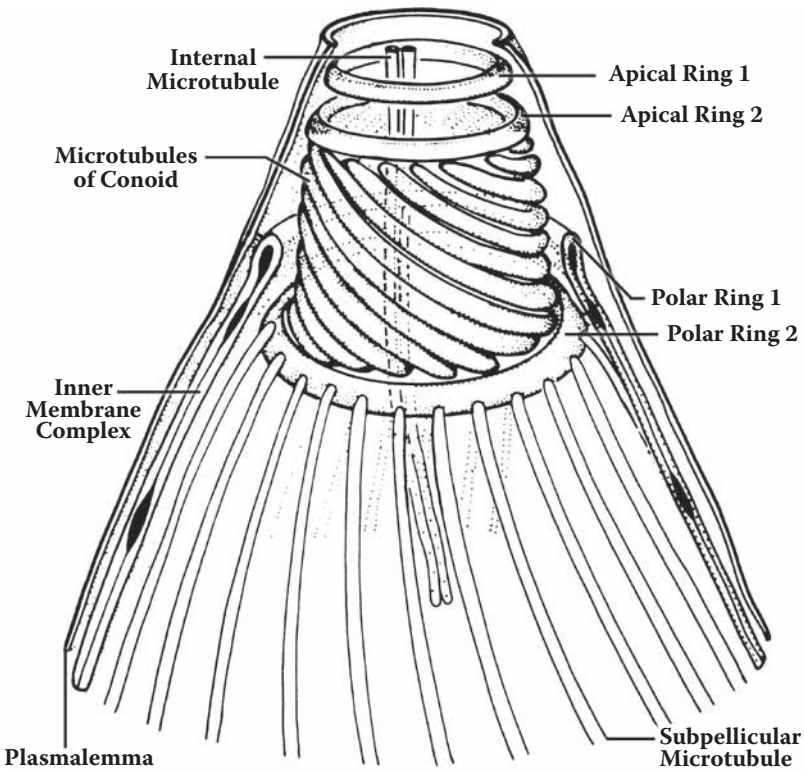

Figure 1.4 Schematic representation of apical complex of *T. gondii*. From Dubey et al.[363]

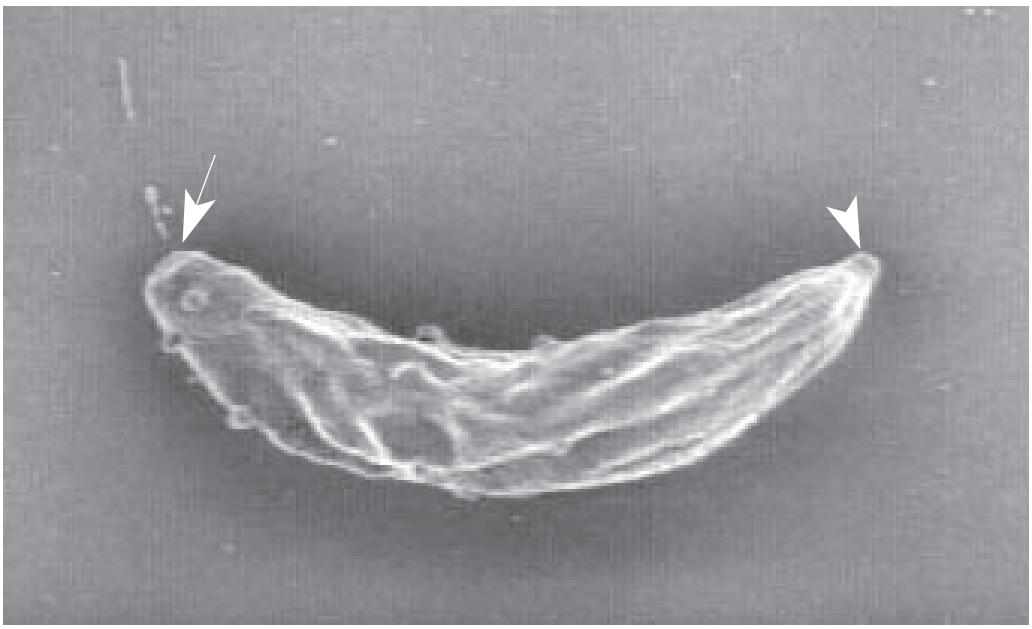

Figure 1.5 Field scanning electron microscopic surface view of a tachyzoite in gliding motion, leaving a slime trail (arrow) at the non conoidal end. The conoid (arrowhead) is a nipple-like structure that can protrude and retract. Courtesy of Dr. Vern Carruthers.

plasmalemma; (4) forming a moving junction (Figure 1.7) that moves posteriorly along the zoite as it penetrates into the host cell; and (5) partially exocytosing micronemes, rhoptries, and dense granules. *T. gondii* can penetrate a variety of cell types from a wide range of hosts, indicating that the biochemical receptors involved in attachment and penetration are probably common to most animal cells. *T. gondii* can penetrate the host plasmalemma in 26 seconds. The invasion involves two Ca^{2+}-dependent events of protrusion of the conoid and secretion of microneme and rhoptry contents forming a moving junction. The tachyzoite is temporarily deformed as it penetrates and squeezes through the host plasmalemma. The parasite is quickly surrounded by a membrane that is evidently derived from the host cell plasmalemma minus host proteins. This membrane is destined to become the parasitophorous vacuole (PV) membrane (PVM); and a number of parasite proteins associate with it including rhoptry proteins, ROP2, -3, -4, and -7. ROP2 is located on the host cell cytoplasmic side of the PVM suggesting a role in host/parasite biochemical communication. Within a few minutes after penetration, tachyzoites modify the newly formed PV and the PVM with parasite proteins and a tubular membranous network (TMN) forms within the PV. The TMNs are antigenically and structurally different from PVM but have connections to it. The PVM acquires pore structures that freely allow charged molecules up to 1,200 kDals to diffuse bidirectionally between the PV and host cell cytoplasm. Dense granule associated proteins (GRA) are secreted into the PV. Collectively, these modifications establish a parasite-friendly environment within the host cell cytoplasm conducive to parasite replication. The PVM is not a homogenous structure of uniform thickness. The volume of PV may increase many fold within 24 hours of its formation.

One interesting and elusive phenomenon is association of host mitochondria and ER to the PV. Within minutes of tachyzoite invasion into the host cell, host mitochondria and ER are tightly associated with PVM and the extent of this juxtaposed association does not change with enlargement of the PV. The *T. gondii* vacuole does not fuse with host lysosomes.

Most of the available information concerning the interaction of *T. gondii* zoites with host cells has been derived from *in vitro* cultivation with tachyzoites. Limited information is available on initial

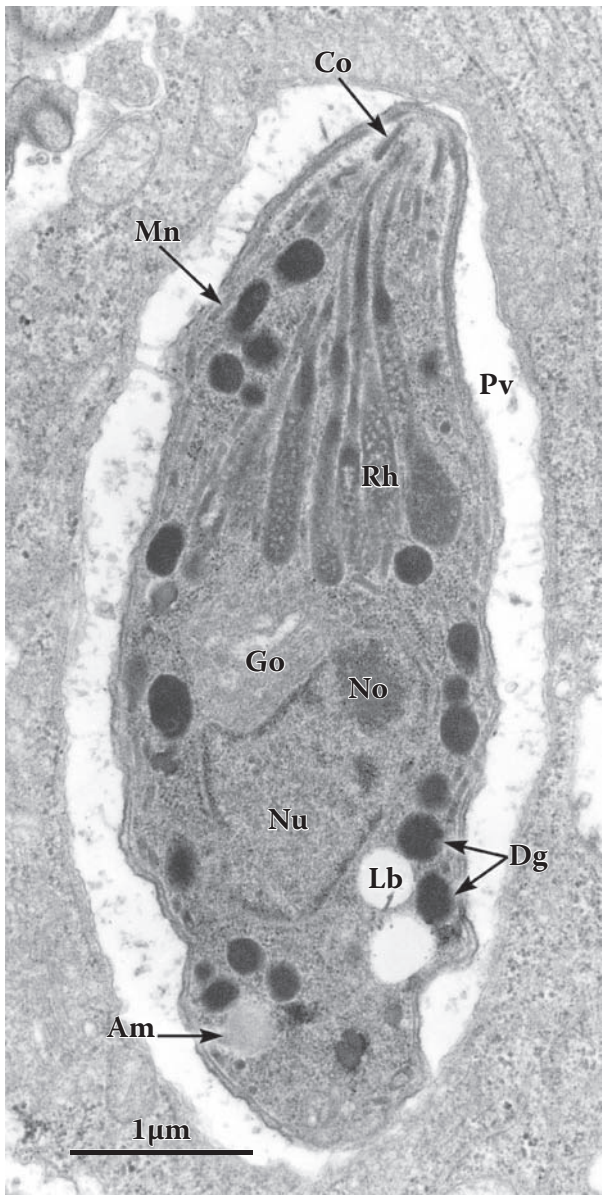

Figure 1.6 TEM of a *T. gondii* tachyzoite in a mouse peritoneal exudate cell. Am, amylopectin granule; Co, conoid; Dg, electron-dense granule; Go, Golgi complex; Mn, microneme; No, nucleolus; Nu, nucleus; Pv, parasitophorous vacuole; Rh, rhoptry. Lb, lipid. From Dubey et al.[363]

host cell interaction with bradyzoites and sporozoites.[841,1145] In one study, host ER, but not mitochondria, surrounded the initial phases of PV formation,[614] and some bradyzoites divided into tachyzoites whereas others gave rise to bradyzoites directly, apparently without conversion to tachyzoites.[1349] In the only report on the interaction of sporozoites and cultured cells, an extra vacuole was formed around invading sporozoites.[1219]

There is limited information on *in vivo* zoite–host cell interactions. In mice fed oocysts, sporozoites excysted and passed through enterocytes and goblet cells of the mouse ileal epithelium and entered the lamina propria where they infected all cells of the host, except red blood cells, and underwent

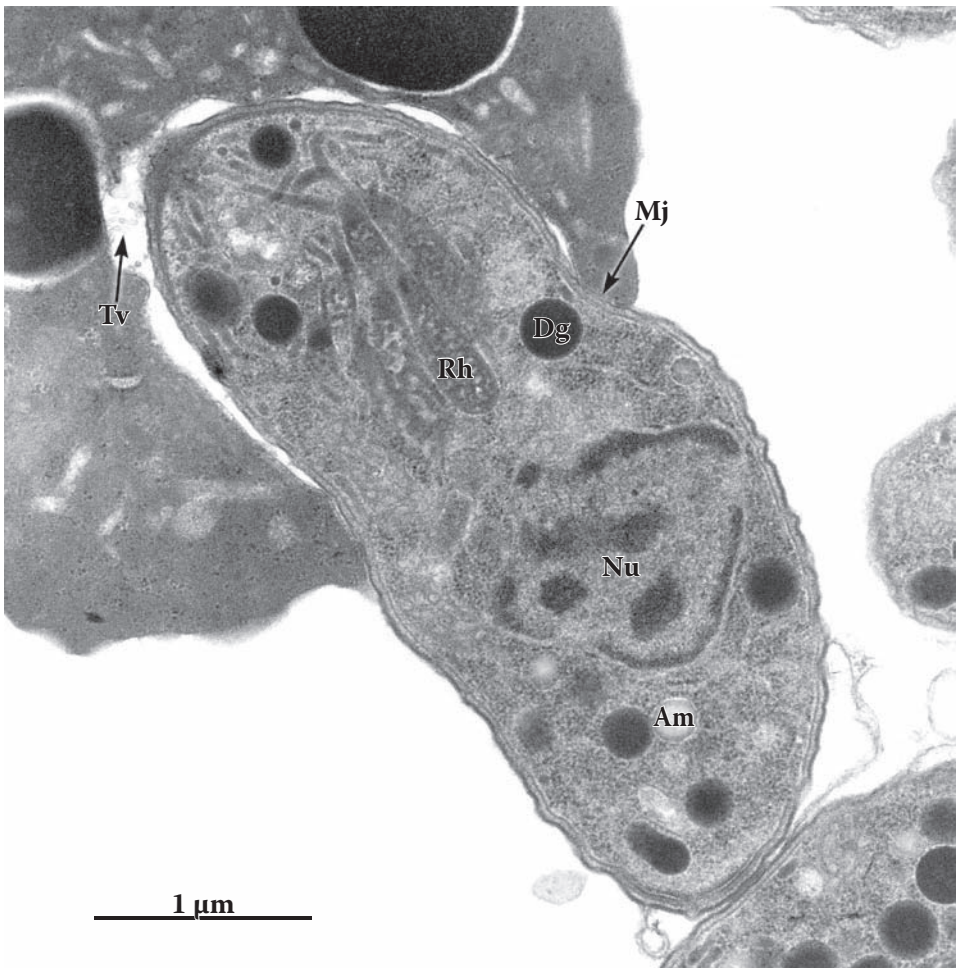

Figure 1.7 TEM of a tachyzoite of VEG strain of *T. gondii* penetrating a neutrophil in mouse peritoneum; note the moving junction (Mj) at the site of penetration into the neutrophil and the extraordinary early development of the tubulovesicular membranes (Tv) in the space between the neutrophil and the tip of the tachyzoite. Am, amylopectin granule; Dg, electron-dense granule; Rh, rhoptry. From Dubey et al.[363]

endodyogeny to form tachyzoites. Even though the sporozoites were just passing through ileal enterocytes, they still formed PVs that contained exocytosed dense granule material and well-developed TMNs (Figures 1.8 and 1.9). Exocytosed material and TMNs associated with the parasitophorous vacuoles (PVs) of sporozoites in transit across the intestinal epithelium indicate that parasite replication does not always follow the formation of a parasite-modified PV. After entering cells in the lamina propria, sporozoites presumably formed a second PV and multiplied by endodyogeny.[359]

Tachyzoites multiply asexually within the host cell by repeated endodyogeny (Figure 1.10), a specialized form of reproduction in which two progeny form within the parent parasite (Figure 1.2B), consuming it. Nishi et al.[982] detailed the chronological development of endodyogeny. In endodyogeny, the Golgi complex and the apicoplast divide, first becoming two at the anterior end of the nucleus, along with the formation of two centrosomes from the nucleus. Next, the conoid, the anterior portions of the inner membrane complexes, and the subpellicular microtubules of the progeny cells appear as two dome-shaped structures anteriorly. The parasite nucleus becomes horseshoe-shaped and the ends of the nucleus move into the dome-shaped anterior ends of the developing progeny.

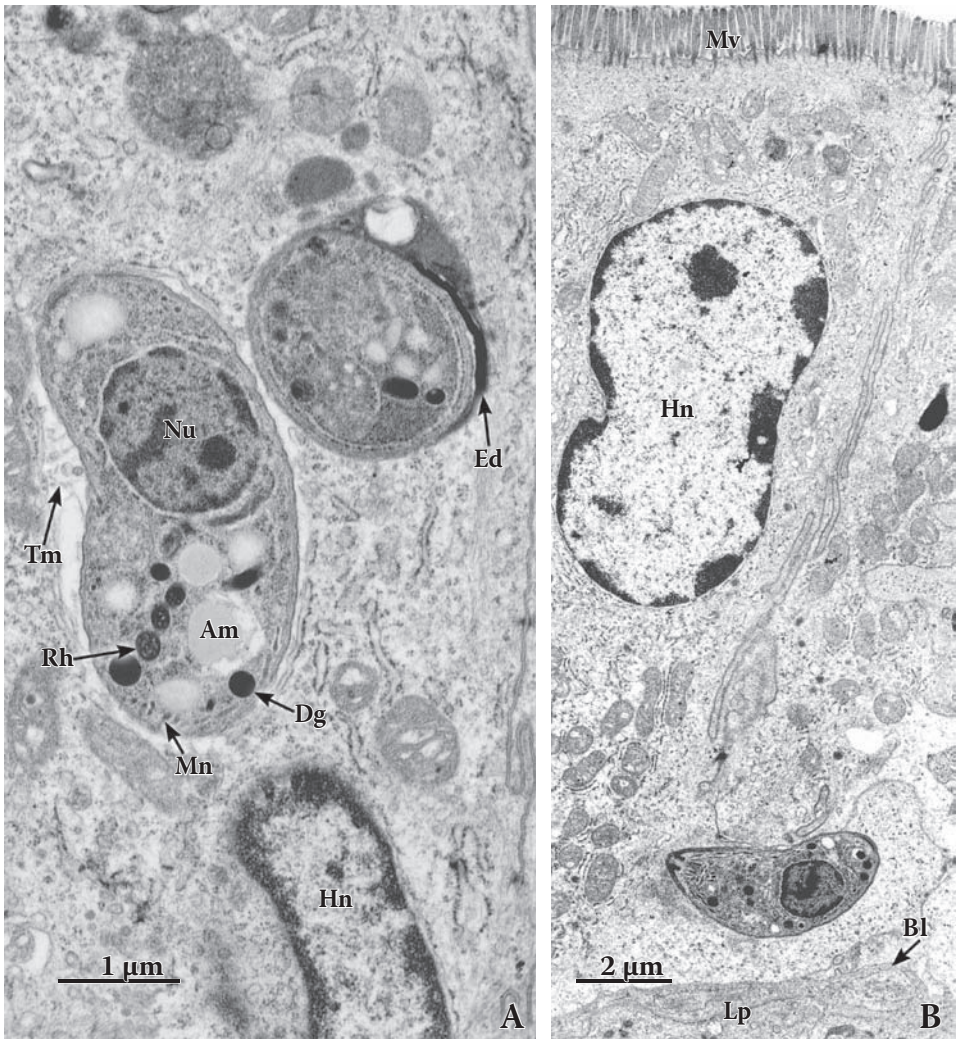

Figure 1.8 TEM of sporozoites of the VEG strain of *T. gondii* in mouse ileum at 2 hr after feeding of oocysts. **A.** Two intracellular sporozoites located above the host nucleus (Hn) in an ileal enterocyte; note the electron-dense material (Ed) in the parasitophorous vacuole (pv) surrounding the sporozoite cut in cross-section and the tubulovesicular membranous network (Tm) in the other sporozoite. Am, amylopctin; Dg, dense granule; Mn, microneme; Nu, nucleus of sporozoite; Rh, rhoptry. **B.** Sporozoite located at the base of an ileal enterocyte. Bl, basal lamina of the intestinal epithelium; Hn, host cell nucleus; Mv, microvilli of enterocyte; Lp, lamina propria. From Speer and Dubey.[1221]

The inner membrane complex and subpellicular microtubules continue to extend posteriorly and surround one half of the nucleus, which eventually pinches into two. Partitioning of the endoplasmic reticulum, and the mitochondrium continues. Rhoptries and micronemes are synthesized *de novo* in the progeny. The progeny continue to grow until they reach the surface of the parent. The inner membrane complex of the parent disappears, and its outer membrane becomes the plasmalemma of the progeny cells. Tachyzoites continue to divide by endodyogeny. *In vivo*, most groups of tachyzoites are arranged randomly due to asynchronous cycles of endodyogeny (Figure 1.11). However, occasionally rosettes are formed due to synchronous division. In rapidly dividing tissue culture–adapted strains, *T. gondii* within a vacuole may divide synchronously, but this is not the

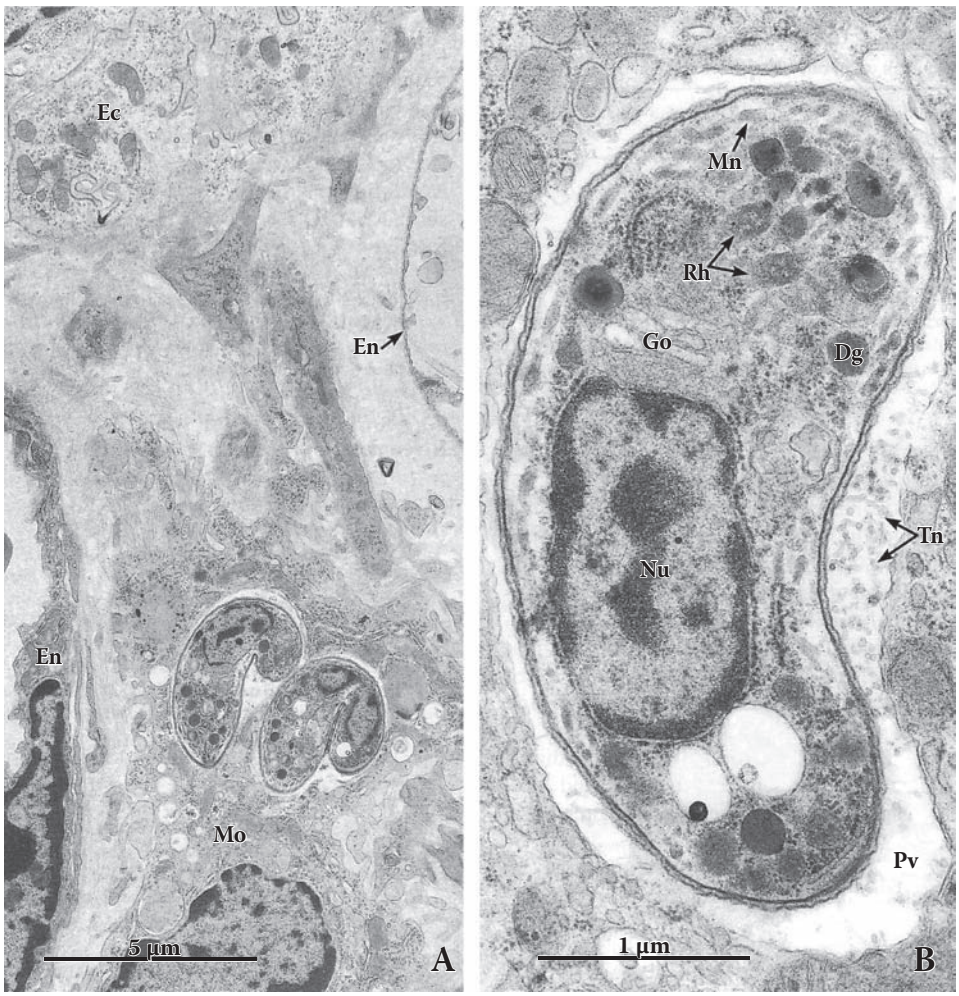

Figure 1.9 TEM of sporozoites in ileal lamina propria of a mouse at 6 hr p.i of sporulated oocysts of *T. gondii* irradiated at 0.5 kGy. **A.** Two sporozoites in Pv within a macrophage (Mo). Ec, enterocyte; En, endothelial cell of blood vessel. **B.** Sporozoite within a macrophage. Dg, dense granule; Go, Golgi complex; Mn, microneme; Nu, nucleus; Pv, parasitophorous vacuole; Rh rhoptry; Tn, tubulovesicular membrane network. From Dubey et al.[364]

norm. Rarely, tachyzoites of certain strains may divide by binary fission. The host cell ruptures when it can no longer support growth of tachyzoites. Exit from the host cell is mediated by a protein (TgPLP1) secreted by the parasite.[751]

The rate of invasion and growth vary depending on the strain of *T. gondii* and the host cells. After entry of tachyzoites into a host cell, there is a variable period of lag phase before the parasite divides, and this lag phase is partly parasite dependent. Mouse virulent strains of *T. gondii* grow faster in cell culture than "avirulent" strains, and some strains of *T. gondii* form more rosettes than others.

1.2.2 Bradyzoites and Tissue Cysts

The bradyzoite is the encysted stage of the parasite in tissues. Bradyzoites are also called cystozoites. To avoid confusion between the terms cyst (also called pseudocyst) in tissues versus oocyst in feces, Dubey and Beattie[302] proposed the term tissue cyst for the encysted stage of the parasite. Tissue

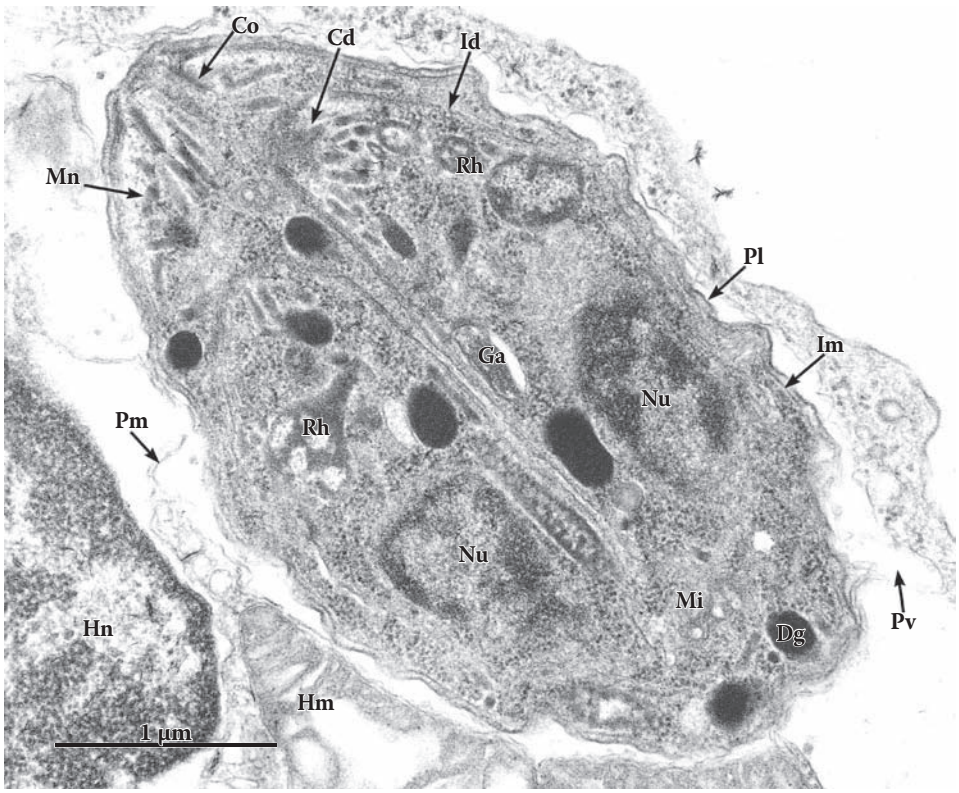

Figure 1.10 TEM of *in vivo T. gondii* tachyzoites undergoing endodyogeny. Cd, conoid of developing tachyzoite; Co, conoid of mother tachyzoite; Dg, electron-dense granule; Ga, Golgi adjunct; Hm, host cell mitochondrion; Hn, host cell nucleus; Id, inner membrane complex of developing tachyzoite; Im, inner membrane complex of mother tachyzoite; Mi, microchdrion; Mn, microneme; Nu, nuclei of daughter tachyzoites; Pl, plasmalemma of mother tachyzoite; Pm, parasitophorous vacuolar membrane; Pv, parasitophorous vacuole; Rh, rhoptries of daughter tachyzoites. From Dubey et al.[363]

cysts (Fig. 1.2E) grow and remain intracellular as the bradyzoites divide by endodyogeny. Tissue cysts vary in size; young tissue cysts may be as small as 5 µm in diameter and contain only two bradyzoites, while older ones may contain thousands of organisms (Figures 1.2E,F, 1.12–1.15). Occurrence of odd numbers of bradyzoites in young tissue cysts indicates asynchronous division. In histological sections, tissue cysts in the brain are often spheroidal and rarely reach a diameter of 70 µm, whereas intramuscular cysts are elongated and may be 100 µm long. Although tissue cysts may develop in visceral organs, including the lungs, liver, and kidneys, they are more prevalent in the neural and muscular tissues, including the brain, eyes, and skeletal and cardiac muscles.

The tissue cyst wall is elastic, argyrophilic, and <0.5 µm thick (Figure 1.2E,F), and it encloses hundreds of crescent-shaped bradyzoites, 5–8.5 × 1–3 µm in size. The tissue cyst wall is only faintly periodic acid-Schiff (PAS) positive compared to the enclosed bradyzoites (Figure 1.2F). The tissue cyst develops within the PV in the host cell cytoplasm (Figure 1.13B). The PV is rarely visible in older tissue cysts. The thickness and the character of the cyst wall may vary depending on the age of the parasite and the host cell parasitized (Figures 1.13–1.15). The tissue cyst wall is uneven in thickness and appears rough. The outer limiting membrane, probably derived both from the host and the parasite, is lined with granular material (hereafter termed granular layer, GL), which also fills the space between the bradyzoites. In fixed material, the GL consists of granules or particulate material with few vesicles. In older tissue cysts, the GL has deep invaginations of the outer limiting membrane

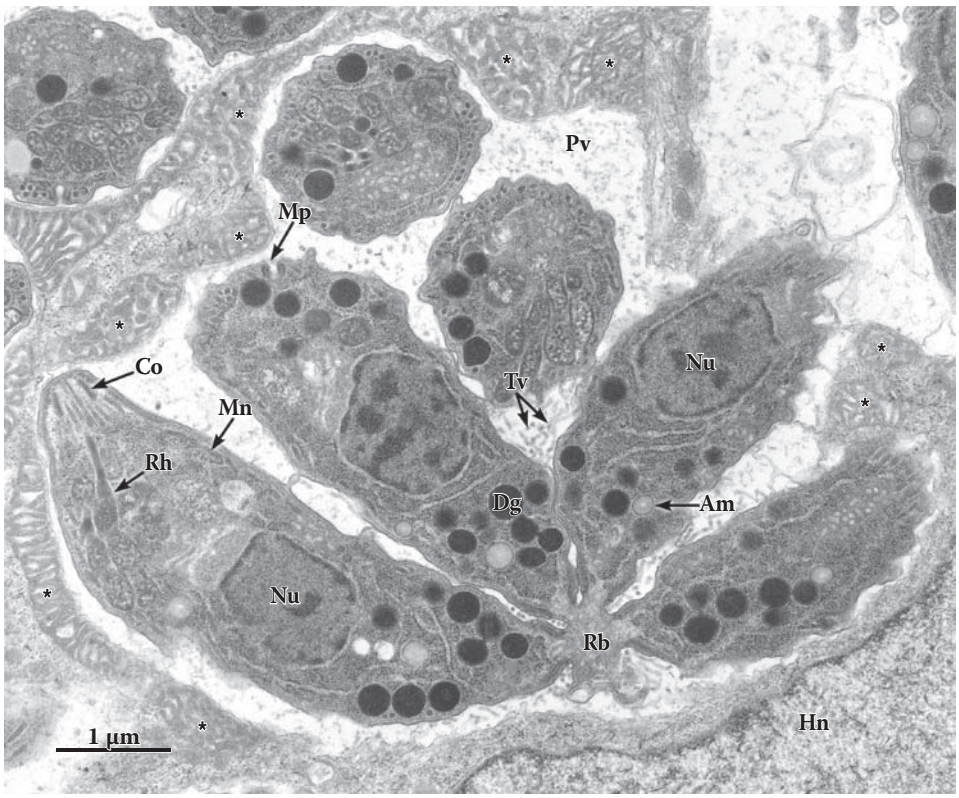

Figure 1.11 TEM of four *T. gondii* tachyzoites in final stages of endodyogeny that are still attached by their posterior ends to a common residual body (Rb); note that several host cell mitochondria (*) are situated in close proximity to the parasitophorous vacuole (Pv), which contains extensively developed tubulovesicular membranes (Tv). Am, amylopectin granule; Co, conoid; Dg, electron-dense granule; Hn, host cell nucleus; Mn, microneme; Mp, micropore; Nu, nucleus; Rh, rhoptry. From Dubey et al.[363]

with interconnecting branches or channels extending up to half the width of the GL. The bradyzoites are justaxposed against the GL (Figure 1.15). Some bradyzoites degenerate, especially in older tissue cysts. In younger tissue cysts, the bradyzoites are often plump and contain relatively fewer organelles than in older tissue cysts (Figure 1.13B). Factors regulating the formation of tissue cysts *in vivo* are not fully understood. The PV in tissue cysts lacks typical TMN found in tachyzoites (Figure 1.13B). The matrix between bradyzoites has vesicles, lipid-like bodies, and tubular structures. The tissue cyst may or may not be surrounded by host mitochondria, depending on the host cell parasitized or unknown factors. Mitochondria were not surrounded in the tissue cyst in Figure 1.13B, but many were present in the tissue cyst in Figure 1.15B; both tissue cysts were in mouse brain.

Bradyzoites differ structurally only slightly from tachyzoites (Table 1.2). They have a nucleus situated toward the posterior end, whereas the nucleus in tachyzoites is more centrally located. The contents of rhoptries in bradyzoites are usually electron dense, whereas those in tachyzoites are labyrinthine. However, the contents of rhoptries in bradyzoites vary with the age of the tissue cyst. Bradyzoites in younger tissue cysts may have labyrinthine rhoptries, whereas those in older tissue cysts are electron dense. Also, most bradyzoites have one to three rhoptries that are looped back on themselves (Figure 1.15A). Bradyzoites contain several amylopectin granules that stain red with PAS reagent; such material is either in discrete particles or is absent in tachyzoites. Bradyzoites are more slender than are tachyzoites. There are more micronemes in bradyzoites, reaching posterior to the nucleus (Figure 1.15B). Even in the youngest tissue cyst containing only two bradyzoites, the nucleus can be terminally located

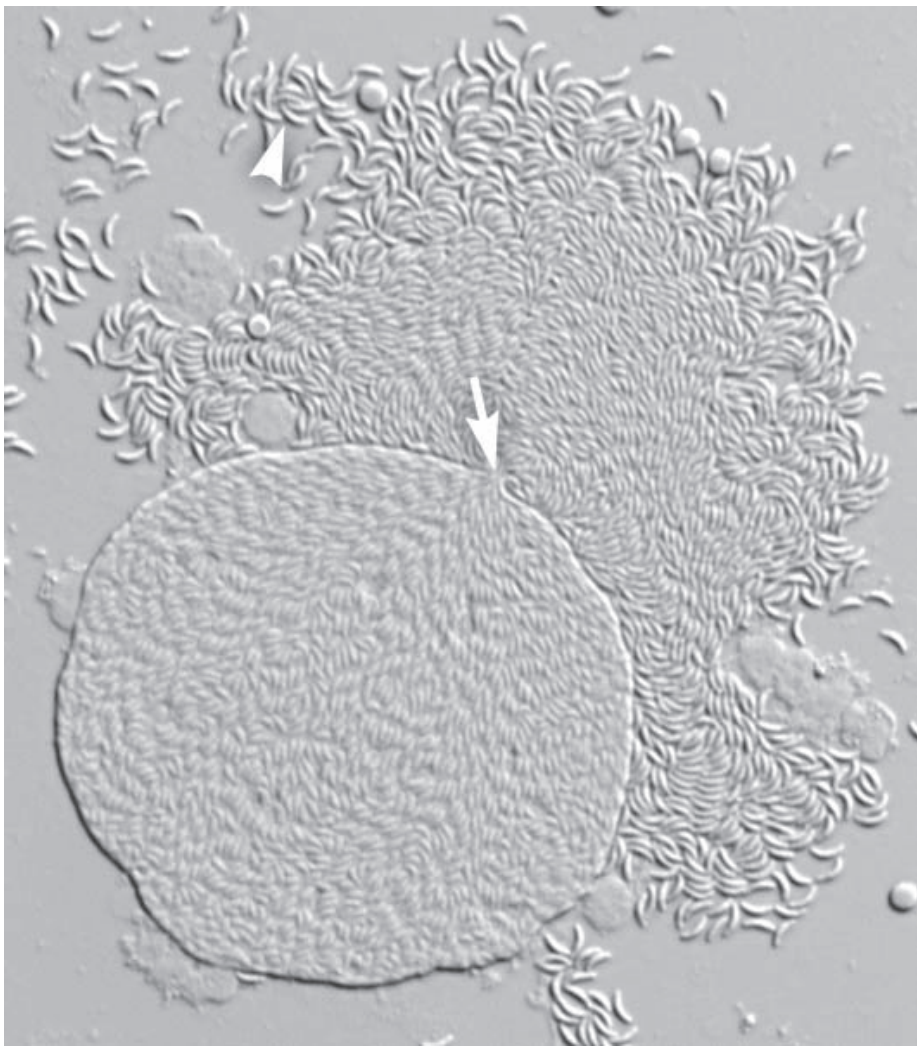

Figure 1.12 A tissue cyst mechanically ruptured to release hundreds of bradyzoites (arrowhead) through a hole in cyst wall (arrowhead). Courtesy of Mr. Fournet Valsin and Dr. Dolores Hill.

(Figure 1.13A). Tissue cysts are an integral part of the life cycle of *T. gondii* and are formed as early as 3 days post inoculation (p.i.).[277] In my opinion, there are no cystless strains of *T. gondii* in nature.

Tissue cyst distribution is in part controlled by the host.[356,357] Distribution of tissue cysts in different organs of animals fed the GT-1 strain of *T. gondii* is shown in Table 1.3. Muscular tissues were parasitized more than the brain. Similar observations were made with naturally infected animals (Table 1.4). However, in mice, the brain tissue has more tissue cysts than other organs.

Although more tissue cysts are found per gram of tissue in mice than other hosts, the fate of tissue cysts is difficult to study in mice because mice are never completely immune to *T. gondii*, and new tissue cysts are formed even in chronic infections. Factors affecting tissue cyst rupture are largely unknown. *T. gondii* tissue cyst rupture has been rarely documented.[352,498] Tachyzoites may persist in chronic infection. The mechanism of formation of new generations of tissue cysts in chronically infected mice is unknown. Clusters of tissue cysts are found in mice infected with certain strains of *T. gondii*, and sometimes in clinically normal mice. Whether bradyzoites

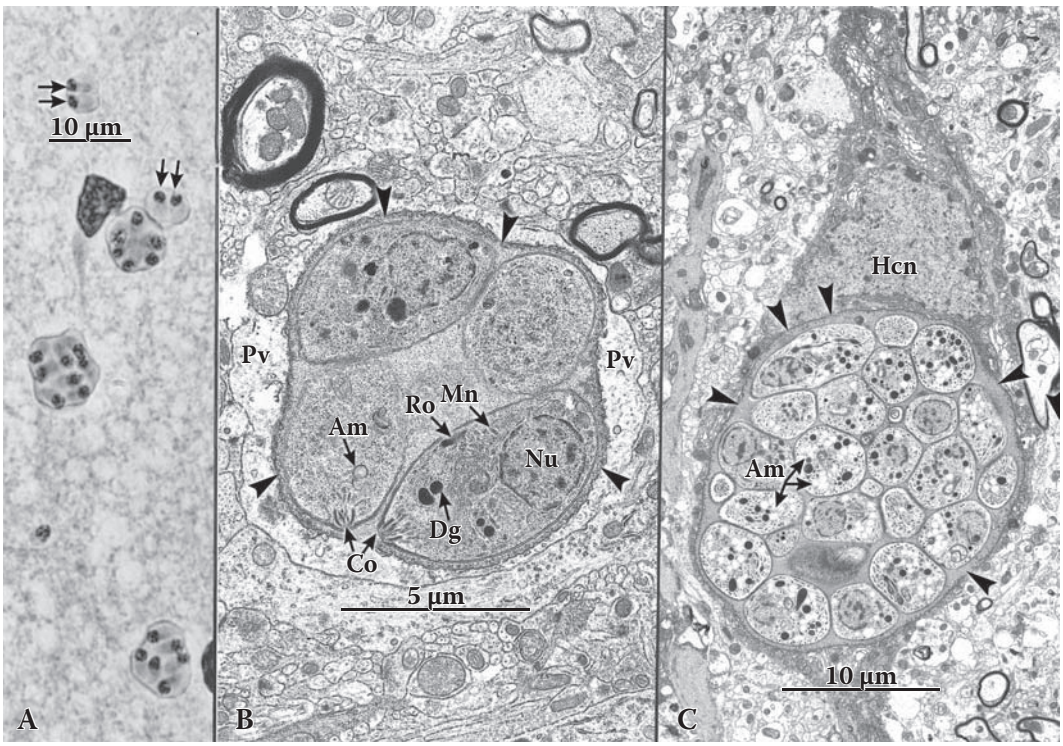

Figure 1.13 *T. gondii* tissue cysts in mouse brain. **A.** Impression smear. Five small tissue cysts with silver positive cyst wall. Silver stain and Giemsa. **B.** TEM of a tissue cyst with only four bradyzoites. This is the youngest tissue cyst that I have examined by TEM. Note well-defined cyst wall clearly within a parasitophorous vacuole (Pv). The enclosed organisms have only a few organelles (micronemes: Mn, dense granules: Dg, and a conoid: Co), but the nucleus (Nu) is terminal. **C.** TEM of a small intracellular cyst with well-developed cyst wall. Note cyst wall (arrowheads) and amylopectin (Am) in bradyzoites. The host cell nucleus (Hcn) is closely applied to the cyst wall. The Pv is no longer visible.

leak out from intact tissue cysts is uncertain but unlikely. The rupture of tissue cysts and subsequent multiplication of tachyzoites can lead to fulminating toxoplasmosis even in chronically infected mice.

Little information is available concerning the mechanisms of relapse. Intact tissue cysts probably do not cause any harm and persist for the life of the host. When and how tissue cysts rupture is largely unknown. Treatment with corticosteroids, anti-lymphocyte serum, and anti-interferon antibody are known to induce immunosuppression and relapse due to toxoplasmosis. The animal model, route of inoculation, stage of *T. gondii* used for primary infection, and criteria used for evaluation of relapses are all important considerations. For example, hamsters are more corticosteroid sensitive than mice or rats, and oral administration of corticosteroids is less effective than parenterally administered corticosteroids.

1.2.3 Life Cycle in the Definitive Host, the Cat

Cats, not only domestic (*Felis domesticus*), but nearly all species of felids (Table 1.5), can shed *T. gondii* oocysts.

Cats shed oocysts after ingesting any of the three infectious stages of *T. gondii*, i.e., tachyzoites, bradyzoites, and oocysts (Figure 1.16). Prepatent periods (time to the shedding of

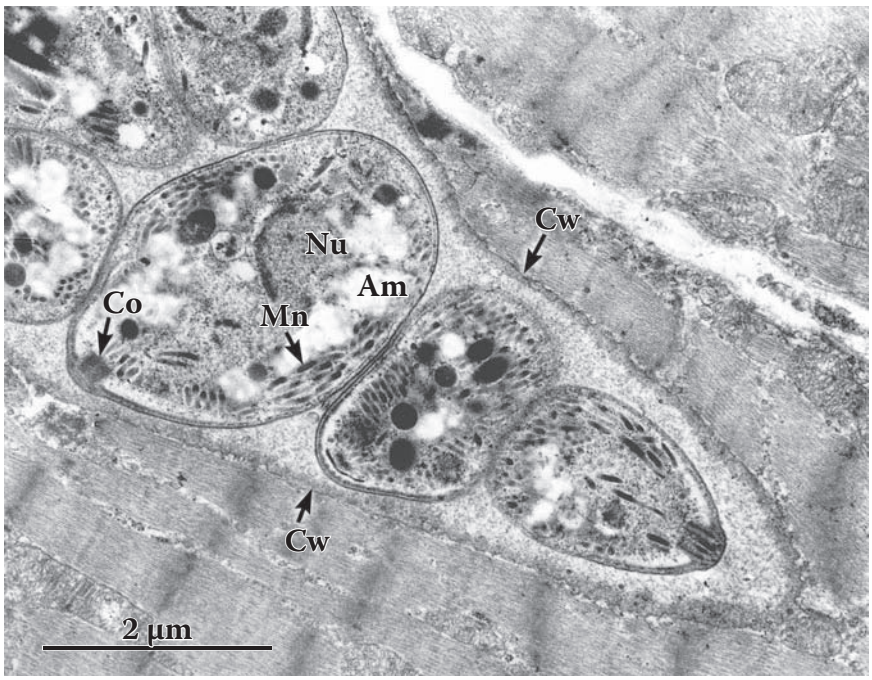

Figure 1.14 TEM of a *T. gondii* tissue cyst in the heart of a naturally infected possum from Australia. The cyst wall (Cw) is butted against the cytoplasm of the cardiac myocyte. Note 1 plump bradyzoite with an anterior conoid (Co), subterminal nucleus (Nu), amylopectin (Am), and numerous micronemes (Mn).

oocysts after initial infection) and frequency of oocyst shedding vary according to the stage of *T. gondii* ingested. Prepatent periods are 3 to 10 days after ingesting tissue cysts, and more than 18 days after ingesting oocysts, irrespective of the dose.[377] The prepatent period after ingesting tachyzoites may vary.[397] Fewer than 50% of cats shed oocysts after ingesting tachyzoites or oocysts, whereas nearly all cats shed oocysts after ingesting tissue cysts.[273,277, 349,377,385,397] *T. gondii* is adapted to be transmitted biologically by carnivorism in cats and transmission by the oocysts is more efficient in non-feline hosts; bradyzoites are more infective to cats and oocysts are more infective to mice.[349,352,535] To illustrate this point, bradyzoites were released from tissue cysts, diluted 10-fold, and inoculated orally into cats, and orally and subcutaneously (s.c.) into mice. The same procedure was followed with suspensions of sporulated oocysts. Cats could be infected orally with as few as one bradyzoite, but not the mice (Table 1.6). Cats fed few bradyzoites shed millions of oocysts (Table 1.7). The reverse was true with oocyst infection; oocysts were more infective orally to mice than cats (Table 1.8).

1.2.3.1 Bradyzoite-Induced Cycle in the Cat

Details of only the bradyzoite-induced cycle are known in cats. For detailed review and references to the following sections see references 273, 363, 499, and 1223. After the ingestion of tissue cysts by cats, the tissue cyst wall is dissolved by proteolytic enzymes in the stomach and small intestine. The released bradyzoites penetrate the epithelial cells of the small intestine and initiate the development of numerous generations of *T. gondii*. Five morphologically distinct types of *T. gondii* develop in intestinal epithelial cells before gametogony begins. These stages are designated types A to E instead of generations because there are several generations within each *T. gondii* type. These asexual stages in the feline intestine are structurally distinct from tachyzoites that also develop in the lamina

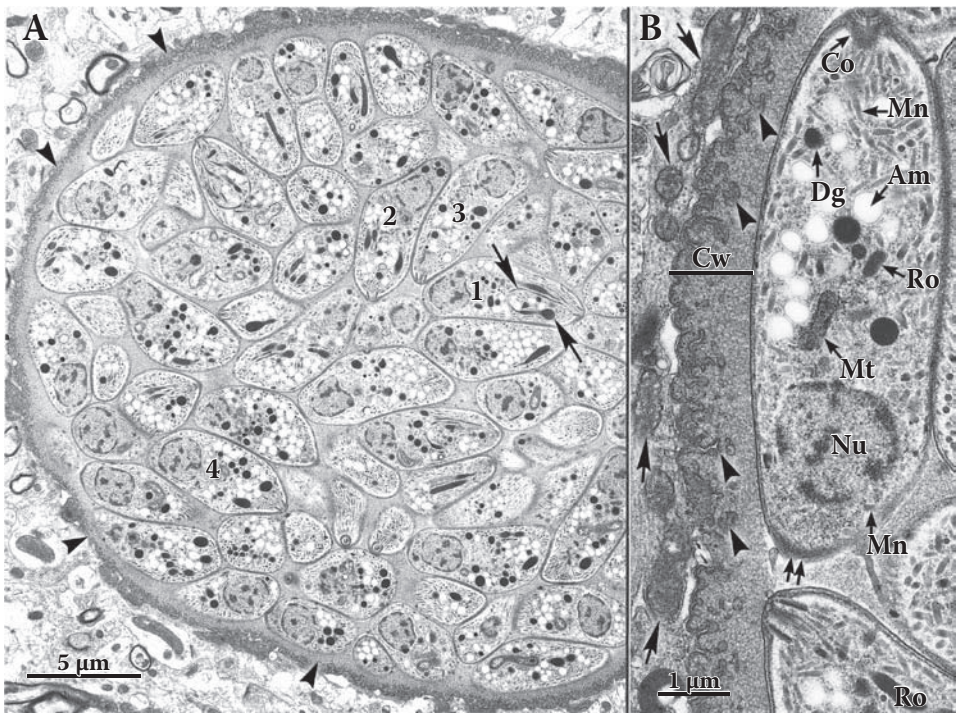

Figure 1.15 TEM of a *T. gondii* tissue cyst in mouse brain, 6 mo p.i. **A.** Note numerous bradyzoites are enclosed in cyst wall (arrowheads). Several bradyzoites (marked 1–4) have looped rhoptries; 1 rhoptry is almost as long as the bradyzoite and the blunt end of the rhoptry is pointed towards conoid (arrows). **B.** Longitudinal section of a bradyzoite with an anterior conoid (Co), few rhoptries (Ro), numeous haphazardly arranged micronemes (Mn), a mitochondrium (Mt); the posterior end is thickened (double arrows). The cyst wall membrane is invaginated and the branches extend half way into the interior of the cyst wall (arrowheads). Host mitochondria (arrows) surround the cyst wall.

propria. The entroepithelial stages (types A–E, gamonts) are formed in the intestinal epithelium and the development of types B–E schizonts in enterocytes has been confirmed ultrastructurally (Table 1.9). Occasionally, type B and C schizonts develop within enterocytes that are displaced beneath the epithelium into the lamina propria. I would like to emphasize that the asexual types A–C are time-dependent stages, few in number, and difficult to find; they are not artifacts, abnormal stages, or tachyzoites as some have suggested.[499] Tachyzoites occur exclusively within the lamina propria. Schizonts and gamonts develop exclusively in enterocytes (Figure 1.17). Structural differences among *T. gondii* types are summarized in Table 1.9. The PV surrounding type B and

Table 1.2 Relative Numbers of Organelles and Inclusion Bodies in Sporozoites, Tachyzoites, and Bradyzoites of the VEG Strain of *T. gondii* as Determined by TEM[a]

	Mean No. (Range)				
Stage	**Rhoptries**	**Micronemes**	**Dense Granules**	**Amylopectin**	**Lipid**
Sporozoite	5.9 (2–11)	55 (40–78)	9.4 (5–15)	7.8 (3–13)	1.25 (1–3)
Tachyzoite	6.7 (2–11)	25 (19–38)	9.1 (5–17)	2.4 (1–6)	0.6 (0–2)
Bradyzoite	5.5 (2–8)	75.5 (36–112)	2.7 (1–5)	21.8 (7–38)	0

[a] From Dubey et al.[363]

Table 1.3 Persistence of *T. gondii* in Tissues of Animals Fed Oocysts the GT–1 strain[a]

Species	Day(s) p.i.	Liver	Kidneys	Brain	Skeletal Muscles	Heart	Diaphragm	Reference[d]
Sheep	97–173	6/8[b]	5/8	4/8	6/8	7/8	6/8	Dubey (1984)
Goats	335–441	6/6	3/6	5/6	6/6	6/6	6/6	Dubey (1982)
Horses	33–476	0/13	1/13	2/13	2/13	3/13	ND[c]	Dubey (1985)
Cattle	256, 267	2/2	1/2	0/2	1/2	0/2	0/2	Dubey (1983)
Pigs	38–171	2/8	2/8	8/8	5/8	8/8	4/8	Dubey et al. (1984, 1986); Dubey (1988)
Elk	73	1/2	0/2	2/2	1/2	1/2	2/2	Dubey et al. (1980)
Bison	28	1/1	0/1	0/1	0/1	0/1	ND	Dubey (1983)
Coyotes	49–84	ND	1/4	3/4	4/4	2/4	2/4	Dubey (1982)
Foxes	36	2/2	ND	2/2	2/2	2/2	ND	Dubey et al. (1983)
Dogs	166, 167	1/4	0/4	0/4	4/4	3/4	ND	Dubey (1985)

[a] From Dubey et al.[363]
[b] Number of infected animals/number fed *T. gondii*. Pepsin digests of tissues (50 g) were bioassayed in mice.
[c] ND, not done.
[d] For references, see Dubey et al.[363]

C schizonts consists of a single membrane, whereas those surrounding types D and E schizonts are comprised of 2–4 electron-dense membranes (Figures 1.18–1.20). Types C, D, and E multiply by schizogony. In schizogony, the nucleus divides two or more times without cytoplasmic division. Whether the daughter organism (merozoite) formation begins after four or more nuclei have been formed is uncertain. Before or simultaneous with the last nuclear division, merozoite formation is initiated near the center of the schizont. The merozoites eventually move towards the periphery of the schizont and the schizont plasmalemma invaginates around each merozoite forming the plasmalemma of the merozoite. The merozoites separate from the schizont at their posterior ends; sometimes leaving a residual body. *T. gondii* schizonts and merozoites are not infective to mice.[273,1264]

The sexual cycle starts two days after ingestion of tissue cysts by the cat. The origin of gamonts has not been determined, but the merozoites released from schizont types D and E probably initiate gamete formation. Gamonts occur throughout the small intestine but most commonly in the ileum, 3 to 15 days after inoculation. They occur above the nucleus of the host epithelial cell near the tips of the villi of the small intestine (Figure 1.17B,C). Female (macro) gamonts are subspherical and each contains a single centrally located nucleus and several PAS-positive granules (Figure 1.21). Ultrastructurally, the mature female gamete contains several micropores, rough and smooth endoplasmic reticulum, numerous mitochondria, double-membraned vesicles, and wall-forming bodies

Table 1.4 Prevalence (%) of Viable *T. gondii* in Brain and Muscles of Naturally Infected Asymptomatic Animals

Host	Number Bioassayed	Brain	Muscle[a]	Reference
Sheep	31	29	58 (D)	717
Chickens	136	49.2	89.5 (H)	431
Goats	10	30	100 (S)	283
Dogs	43	46.5	81.3 (H)	406, 410, 414
Cats	60	20	56.6 (S)	286, 293, 394,401,408

[a] D = diaphragm, H = heart, S = skeletal muscle.

Table 1.5 Wild Felids as Definitive Host for *T. gondii*[a]

Definitive Host	Oocyst Shedding		Reference
	Experimental	Natural	
African wild cat (*Felis lybica*)	Yes[b]	No	Polomoshnov, 1979
Amur leopard cat (*Felis euptilurus*)	No	Yes[b]	Lukešová and Literák, 1998
Asian leopard (*Felis bengalensis*)	Yes	No	Janitschke and Warner, 1972
	Yes[b]	No	Miller et al., 1972
Bobcat (*Lynx rufus*)	No	Yes[b]	Marchiondo et al., 1976
	Yes[b]	No	Miller et al., 1972
Cheetah *(Acinonyx jubatus)*	No	Yes[b]	Marchiondo et al., 1976
	Yes[b]	No	Polomoshnov, 1979
Cougar *(Felis concolor)*	No	Yes[b]	Marchiondo et al., 1976
	Yes[b]	No	Miller et al., 1972
Cougar *(Felis concolor vancouverensis*)	No	Yes[b]	Aramini et al., 1998
Geoffroy's cat (*Oncifelis geoffroyi*)	No	Yes[c]	Pizzi et al., 1978
	No	Yes[b]	Lukešová and Literák, 1998
Iriomote cat (*Felis iriomotensis*)	No	Yes[b]	Akuzawa et al., 1987
Jaguarundi (*Felis yagouaroundi*)	Yes[b]	No	Jewell et al., 1972
Lion (*Panthera leo*)	No	Yes[d]	Ocholi et al., 1989
	Yes[b]	No	Polomoshnov, 1979
Mountain lion (*Felis concolar*)	No	Yes[b]	Marchiondo et al., 1976
Ocelot (*Felis pardalis*)	Yes[b]	No	Jewell et al., 1972
	No	Yes	Patton et al., 1986
Pallas cat (*Felis manul*)	No	Yes[b]	Basso et al., 2005
	No	Yes[d]	Dubey et al., 1988
	Yes[b]	No	Polomoshnov, 1979
Pampas cat (*Oncifelis colocolo*)	No	Yes[c]	Pizzi et al., 1978
Siberian tiger (*Panthera tigris altaica*)	No	Yes[b]	Dorny and Fransen, 1989
Wild cat (*Felis silvestris*)	No	Yes[b]	Lukešová and Literák, 1997

[a] From Dubey.[436] For references, see this paper.
[b] Confirmed by bioassay in mice.
[c] Confirmed by bioassay in pigs.
[d] Immunohistochemical post-mortem examination.

(WFB) (Figures 1.21 and 1.22). The double-membraned bodies are located near the nucleus and are probably derived from it. The WFB are of two types: I and II. Type I forming bodies are about 0.35 μm in diameter, electron-dense, and appear before type II WFB. The WFB II are moderately electron-dense, fewer in number than WFB, and are 1.2 μm in diameter. In addition, there are electron dense bodies, recently identified as veil forming bodies (VEB), slightly smaller in size than WFB type 1.[499]

Mature male gamonts (microgamonts) are ovoid to ellipsoidal in shape (Figure 1.23). During microgametogenesis, the nucleus of the microgamont divides to produce 21–30.[273,499] Ultrastructurally, after several nuclear divisions, nuclei migrate to the periphery of the gamont, and microgametes bud from the surface, leaving behind a large residual body containing various organelles and inclusion bodies including electron-lucent residual nuclei, amylopectin granules, Golgi complexes, endoplasmic reticula, multiple membrane-bound vacuoles, mitochondria, and numerous ribosomes (Figure 1.23). Microgametes are biflagellate and contain a perforatorium, 2 flagella (up to 10 μm long) that arise from 2 basal bodies immediately behind the perforatorium, a single mitochondrion, an electron-dense nucleus, and an inner membrane complex associated with approximately 12 microtubules. The inner membrane complex and subpellicular

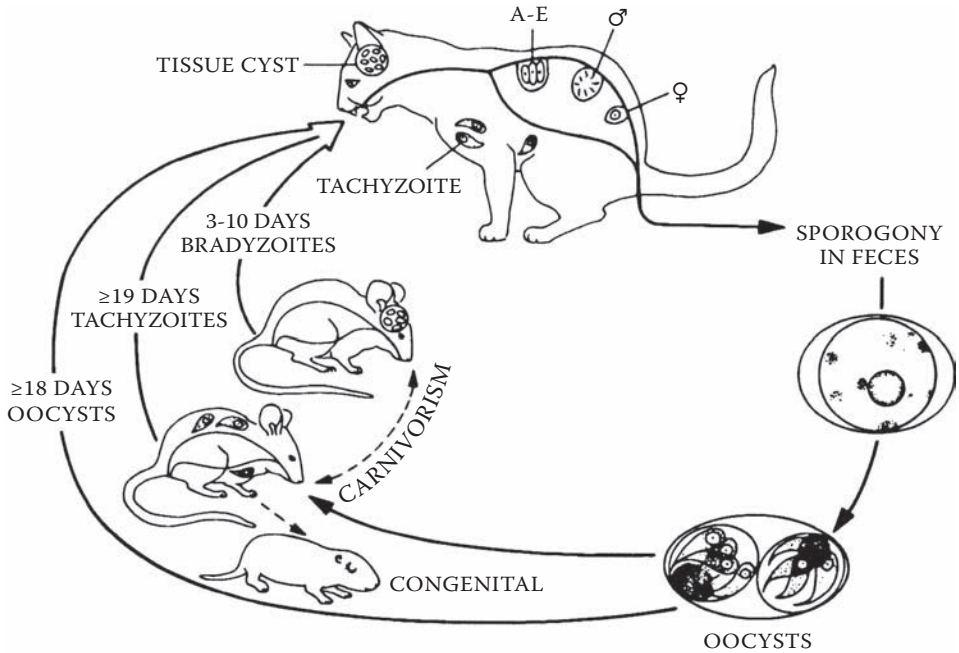

Figure 1.16 *T. gondii* life cycle in the cat. The prepatent is long (18 days or higher) after feeding tachyzoites or oocysts compared with short (3–10 days) after feeding bradyzoites.

microtubules occur in the region immediately above the mitochondrion but are absent in the region near the microgamete nucleus. The PV surrounding the microgamonts is filled with a clear, electron-lucent material.

Microgametes use their flagella to swim to, penetrate, and fertilize mature macrogametes to form zygotes. After fertilization, an oocyst wall is formed around the parasite. Infected epithelial cells rupture and discharge oocysts into the intestinal lumen. Five layers of the oocyst wall are formed at the surface of the zygote. No extensive cytoplasmic changes occur in the zygote while layers 1, 2, and 3 are formed, but WFB type Is disappear with the formation of layer 4, and type II WFB disappear with layer 5.

By light microscopy, unsporulated oocysts are subspherical to spherical and are 10×12 μm in diameter (Figures 1.17E, 1.24); the oocyst wall consists of two colorless layers. Polar granules are absent and the sporont almost fills the oocyst. Sporulation occurs outside the cat within 1 to 5 days

Table 1.6 A Comparison of Infectivity of *T. gondii* Bradyzoites to Cats (Oocyst Shedding) and Mice[a]

	Cats[b]		Mice[c]			
No of Bradyzoites	**Oral**	**%**	**Oral**	**%**	**s.c.**	**%**
1,000	8/13	61.5	12/24	50	24/24	100
100	11/16	68.7	4/24	16.6	17/17	100
10	5/18	27.7	3/24	12.5	8/24	33.3
1	3/18	16.6	0/24	0	5/24	20.8

[a] Data were pooled from two studies (Dubey[377] VEG strain – type III, and Dubey[398] TgCkAr23 strain – type I).
[b] Number of cats shed *T. gondii* oocysts/number of cats fed bradyzoites.
[c] Number of mice infected with *T. gondii*/number of mice inoculated.

Table 1.7 Numbers of Oocyst Shed by Cats Fed 1 or 10 or *Toxoplasma gondii* Bradyzoites[a]

Day Post Feeding	Cat 503 (1[b])	Cat 519 (10)[b]	Cat 501 (10)[b]
6	6,250,000	0	<100,000
7	26,750,000	27,000,000	21,000,000
8	55,000,000	22,000,000	<100,000
9	8,750,000	1,000,000	<100,000
10	6,250,000	500,000	<100,000
11	No feces	2,000,000	0
12	<100,000	<100,000	0
13	<100,000	0	0
14	0	0	0

[a] From Dubey.[377]
[b] Number of bradyzoites fed.

depending upon aeration and temperature. Sporulated oocysts are subspherical to ellipsoidal and are 11 × 13 μm in diameter. Each sporulated oocyst contains two ellipsoidal sporocysts without Stieda bodies. Sporocysts measure 6 × 8 μm. A sporocyst residuum is present; there is no oocyst residuum. Each sporocyst contains four sporozoites (Figure 1.24D).

Oocyst ultrastructure during sporulation was described by Ferguson et al.[495–497] The cytoplasm of an unsporulated oocyst (zygote) has a large nucleus with an amorphous nucleoplasm and a distinct nucleolus. The zygote is limited by a unit membrane with few micropores. The nucleus divides twice giving rise to four nuclei that are situated at the periphery of the zygote; at this stage, a second limiting membrane is formed. After cytoplasm divides, two spherical sporoblasts are formed, each with two nuclei.

As sporulation continues, the sporoblasts elongate and sporocysts are formed. The two outer membranes of the sporoblasts become the outer layer of the sporocyst wall, and the plasmalemma of the cytoplasmic mass becomes the inner layer. Ultimately, as the sporocyst develops, four curved plates form the inner-most layer of the sporocyst. The plates are joined by four lip-like sutures with a depression on the surface of the sporocyst wall at the junction point. Sporozoite formation begins when two dense plaques (analagen) appear at both ends of the sporocyst. Each nucleus divides into two and is incorporated into elongating sporozoite analagen. Thus, four sporozoites are formed by endodyogeny

Table 1.8 Infectivity of *T. gondii* Oocysts to Cats and Mice[a]

	Cats	Mice	
No. of Oocysts	Oral	Oral	s.c.
10,000	3/4[b] (0)[c]	5/5[d]	5/5[d]
1,000	3/4 (1)	5/5	5/5
100	1/4 (0)	5/5	5/5
10	0/4 (0)	4/5	5/5
1	Not done	2/5	2/5
<1	Not done	1/5	1/5

[a] From Dubey.[398]
[b] Number of cats infected/number of cats fed oocysts.
[c] Number of cats shed oocysts.
[d] Number of mice infected/five mice inoculated. All infected mice died of toxoplasmosis.

Table 1.9 Ultrastructural Differentiation of Tachyzoites and Types B–E Schizonts and Merozoites of *Toxoplasma gondii* in the Small Intestine of Cats Fed Bradyzoites[a]

	Characteristic Parasite or Schizont Type				
	Tachyzoites	**B**	**C**	**D**	**E**
Location	Lamina propria	Epithelium, lamina propria	Epithelium, lamina propria	Epithelium	Epitheliuim
Division	Endodyogeny, asynchronous	Endodyogeny, asynchronous	Endopolygeny, synchronous	Endopolygeny, synchronous	Endopolygeny, synchronous
Parasitophorous vacuole					
TMN	Present	Present	Absent	Absent	Absent
PVM	Thin	Thin	Thin	Thick	Thick
Granular material	Absent	Absent	Present	Absent	Absent
Residual body	Absent	Absent	Present	Present	Present
Schizont size, number measured		26 × 17 µm, 10	16 × 10 µm, 8	7.3 × 5.6 µm, 6	12 × 8.8 µm, 10
Number of merozoites per schizont		2–48	24–36	12–18	50–80
Tachyzoites or merozoites					
Size, number measured	4.7 × 2.1 µm, 10	5.8 × 2.9 µm, 10	6.1 × 1.5µm, 8	5.8 × 1.3 µm, 10	4.5 × 1.1 µm, 10
Amylopectin	Few	Few	Several	Few	Few
Rhoptries	Labyrinthine	Labyrinthine	Electron-dense	Electron-dense	Electron-dense
Granular vacuole	Absent	Absent	Absent	Present	Absent
Granular body	Absent	Absent	Absent	Absent	Present

[a] From Speer and Dubey.[1223]

at the periphery in each sporocyst. A prominent residual body is left after sporozoites are formed. The residual body is enclosed in a single-unit membrane. Ultrastructurally, the wall of sporulated oocysts consists of three layers: an outer electron-dense layer, an electron-lucent middle layer, and a moderately electron-dense inner layer.[1220] The middle layer consists of remnants of two membranes that evidently were laid down between the inner and outer layers during oocyst wall formation. Treatment of oocysts with 1.3% sodium hypochlorite (Clorox) removes the outer layer. The oocyst wall contains a single micropyle, which is relatively small and located randomly in the oocyst wall.[1220] The micropyle is a 350–nm-diameter indentation that consists of three layers that are continuous with the three layers in the oocyst wall. However, the outer layer of the micropyle is thin and moderately electron-dense and the inner layer is electron-dense, disk-shaped, and slightly thicker than the inner layer of the oocyst wall. Although the function of the micropyle is not known, it might represent a permeable site in the oocyst wall that is susceptible to the actions of CO_2 and various enzymes that allow entry of bile salts and trypsin that stimulate excystation of sporozoites from sporocysts.

The sporocyst wall consists of two distinct layers with a thin electron-dense outer layer and thicker, moderately electron-dense inner layer. The inner layer consists of four curved plates (Figure 1.25). At sites of apposition between two plates, there are two apposing liplike thickenings and an interposed strip in the inner layer. During excystation in the presence of bile salts and trypsin, the sporocyst ruptures suddenly, evidently at the sites of apposition between plates, releasing the sporozoites. As the plates separate, they curl inward to form conical coils. The sporocyst residuum consists of amylopectin granules and lipid bodies.

Ultrastructurally, the sporozoite is similar to the tachyzoite except that there is an abundance of micronemes, rhoptries, and amylopectin granules in the former. Sporozoites are 2 × 6–8 µm with a subterminal nucleus (Figure 1.25). There is no crystalloid body nor are there any refractile bodies in

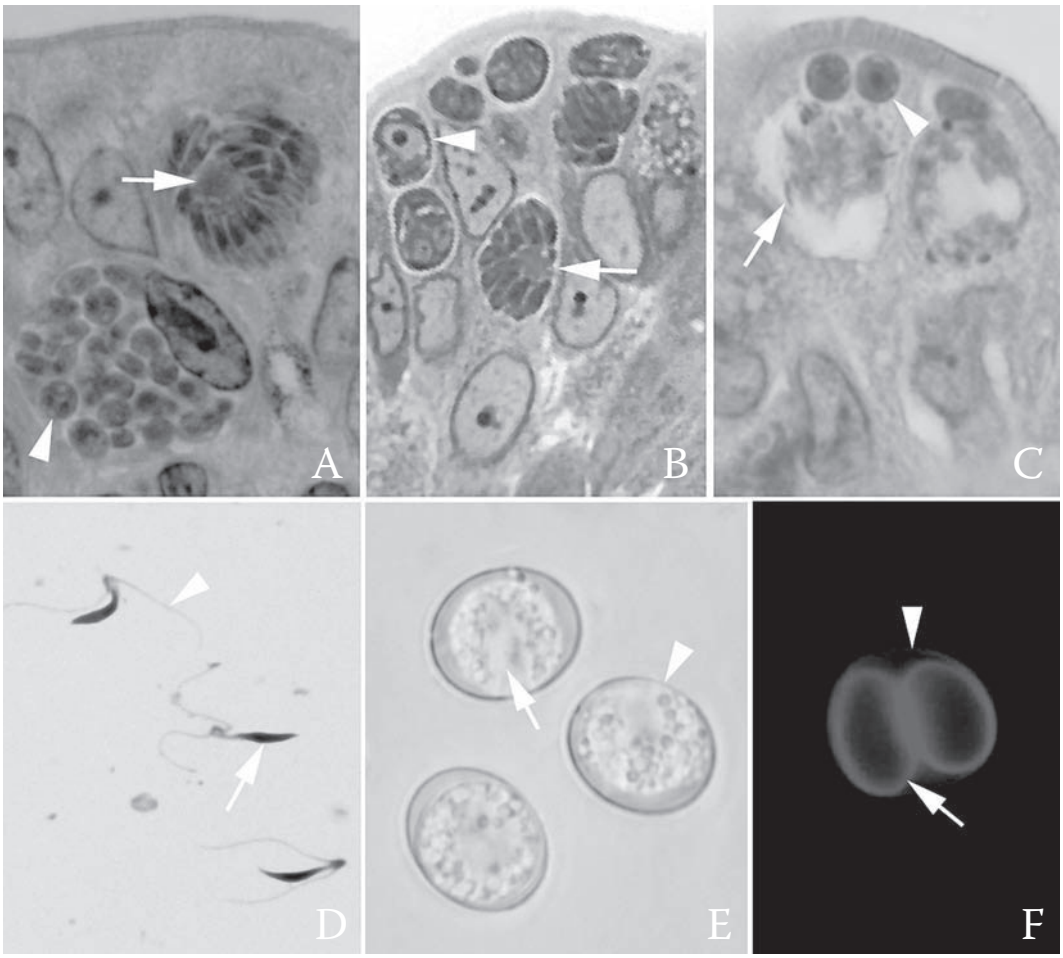

Figure 1.17 (Please see color insert following page 46). Enteroepithelial stages of *T. gondii.* **A.** Type C (arrow) schizont with a residual body and a type B schizont with a hypertrophied host cell nucleus (arrowhead) in sections of small intestine of cat fed tissue cysts 52 hr p.i. **B**. A mature schizont (arrow) and developing stages (arrowhead) in section of the epithelium of small intestine. 7 days p.i. All stages are located above the nuclei of enterocytes. Toluidine Blue. Courtesy of Dr. David Ferguson. **C.** Two male gamonts with peripherally located microgametes (arrow) and uninucleate organisms (arrowhead) in section of the epithelium of small intestine. 5 days p.i. Hand E stain.**D.** Three biflagellated microgametes. Note long slender flagella (arrowhead) and the small microgamete nucleus (arrow). Impression smear of small intestine, 5 days p.i. Giemsa stain **E.** Three unsporulated oocysts in cat feces. Note thin oocyst wall (arrowhead) and an unsporulated mass called sporont (arrow). Unstained. **F.** An autofluorescing sporulated oocyst, ultraviolet. Note thin oocyst wall (arrowhead) and two sporocysts (arrow). Courtesy of Dr. Bill Everson.

T. gondii sporozoites that are present in other coccidian parasites. *T. gondii* oocysts are autofloures-cent (Figure 1.17F).[483,836] *T. gondii* oocysts are negatively charged.[535,1253]

1.2.3.2 Oocyst-Induced Cycle in the Cat

As stated earlier, cats shed oocysts with a minimum prepatent period of 18 days after feeding on sporulated oocysts, irrespective of the dose,[349,398,548] and not all cats that become infected shed oocysts. Enteroepithelial stages preceding the formation of gamonts have not been found. It has been hypothesized that sporozoites first convert to tachyzoites, and tachyzoites convert to

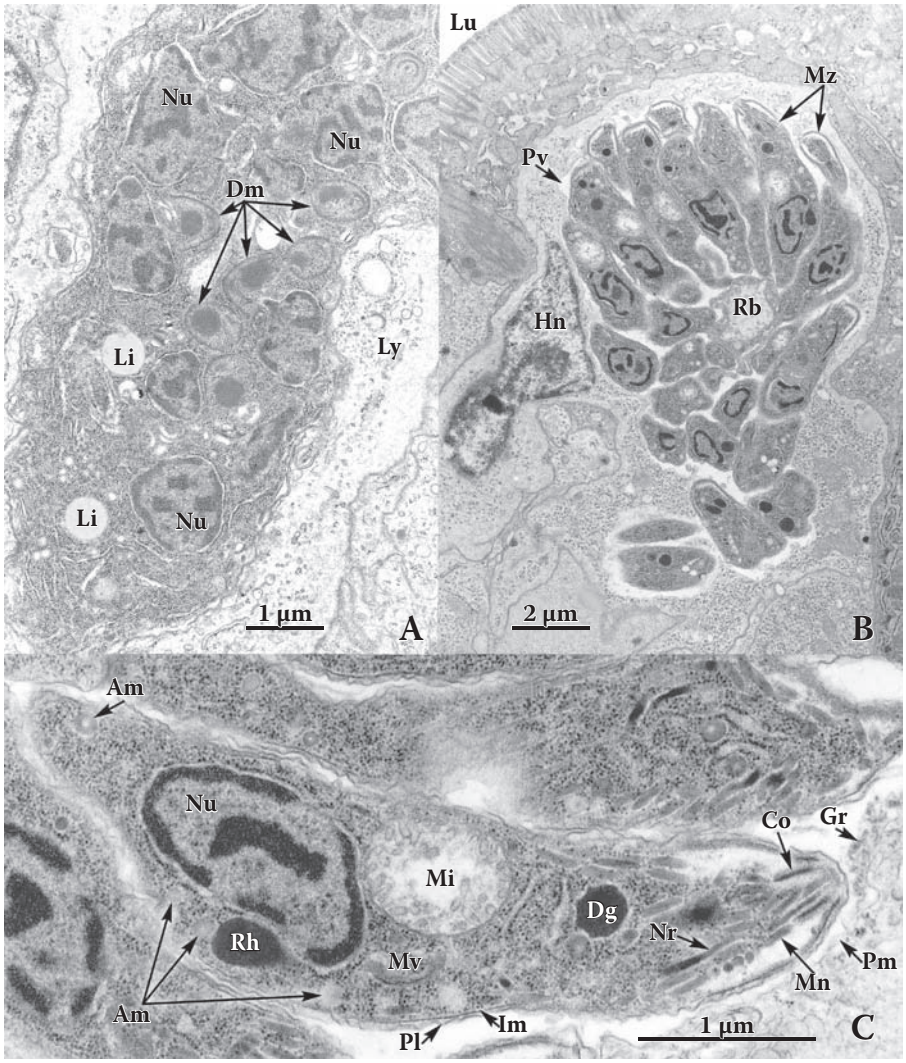

Figure 1.18 TEM of type C schizonts (A, B) and merozoites (C) in intraepithelial lymphocytes (Ly); 46 hr p.i. **A.** Intermediate schizont in an early stage of endopolygeny; note nuclei (Nu), developing merozoites (Dm), lipid bodies (Li) and parasitophorous vacuolar membrane closely associated with the schizont plasmalemma. **B.** Nearly mature type C schizont with merozoites (Mz) budding from a centrally located residual body (Rb); note narrow parasitophorous vacuole (Pv); Hn, host cell nucleus; Lu, lumen of intestinal tract. **C.** Higher magnification of type C merozoite in Fig. 1.18B showing scattered amylopectin granules (Am), electron-dense rhoptry (Rh), large mitochondrion (Mi), conoid (Co), dense granule (Dg), granules (Gr) in the parasitophorous vacuole, inner membrane complex (Im), micronemes (Mn), multiple-membrane bound vacuole (Mv), neck of rhoptry (Nr), plasmalemma (Pl) of merozoite and parasitophorous vacuolar membrane (Pm). From Speer and Dubey.[1223]

bradyzoites, and when tissue cysts rupture, a few bradyzoites return to the intestinal epithelium to initiate the enteroepithelial cycle; this event is unpredictable.

1.2.3.3 Tachyzoite-Induced Cycle in the Cat

Cats can shed oocysts after feeding on tachyzoites with a prepatent period of 18 days or more, similar to the oocyst-induced cycle. However, in some cats fed tachyzoites, the prepatent period

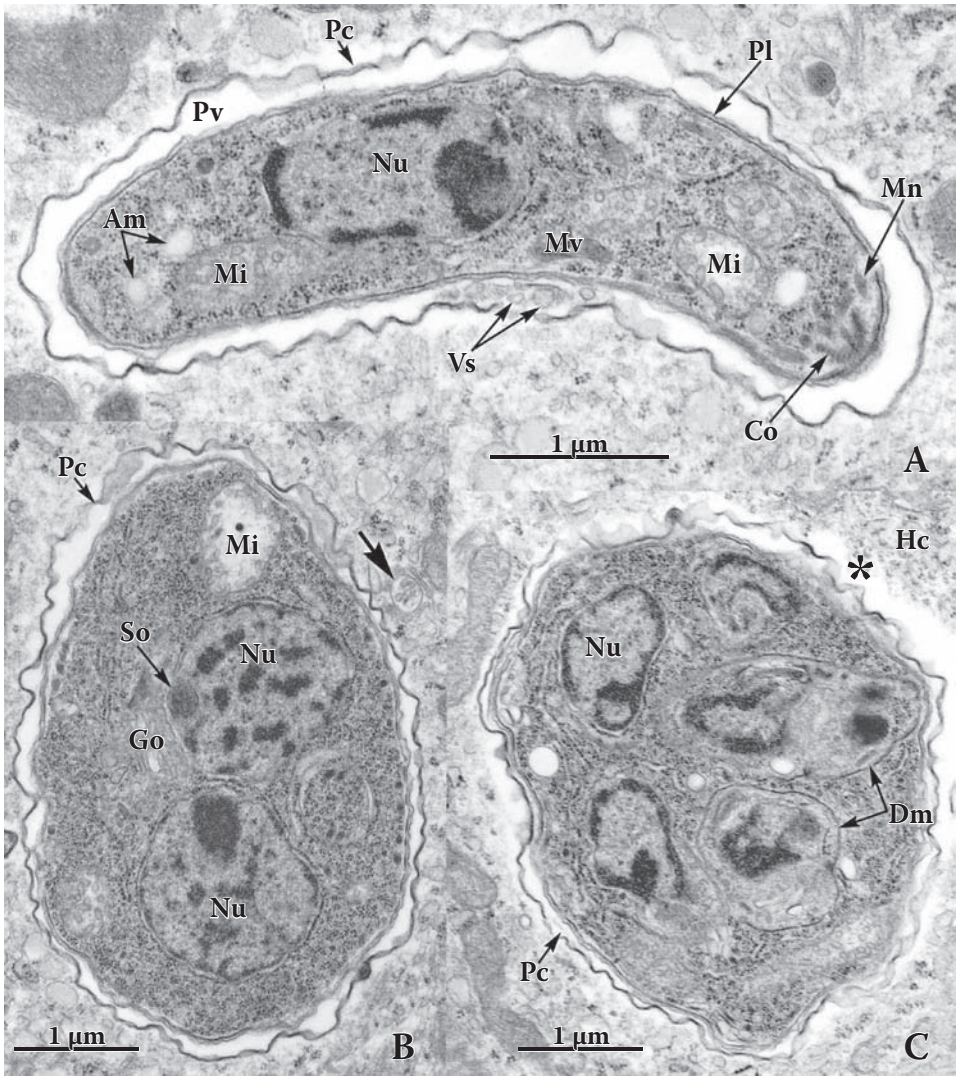

Figure 1.19 TEM of developmental stages of type D schizonts; 46 hr p.i. **A.** Type C merozoite in early stage of transformation to a type D schizont; note merozoite contains several amylopectin granules (Am) and several prominent mitochondria (Mi), and electron-dense, undulating parasitophorous vacuolar membrane complex (Pc) consisting of 2–4 fused membranes. Co, conoid; Mn, microneme; Mv, multiple-membrane bound vacuole; Nu, nucleus of merozoite; Pl, plasmalemma of merozoite; Vs, vesicles in parasitophorous vacuole (Pv). **B.** Intermediate type D schizont showing two nuclei (Nu). Go, Golgi complex; Mi, mitochondrion; Pc, parasitophorous vacuolar membrane complex; Sc, spindle polar cone; arrow points to host cell vacuole containing several small vesicles. **C.** Schizont showing merozoites developing (Dm) by endopolygeny; note space (*) between host cell cytoplasm (Hc) and the parasitophorous vacuolar membrane complex (Pc). From Speer and Dubey.[1223]

was 11–17 days,[397] which probably related to the ingestion of *T. gondii* transitional stages between tachyzoites and bradyzoites. The transition of bradyzoite to tachyzoite and tachyzoite to bradyzoite is not an all-or-none phenomenon, and the transitional stage has not been morphologically and biologically characterized. For example, tachyzoites lack PAS-positive granules that are numerous in bradyzoites, and their synthesis and accumulation is gradual.[277] Although tachyzoites are not as

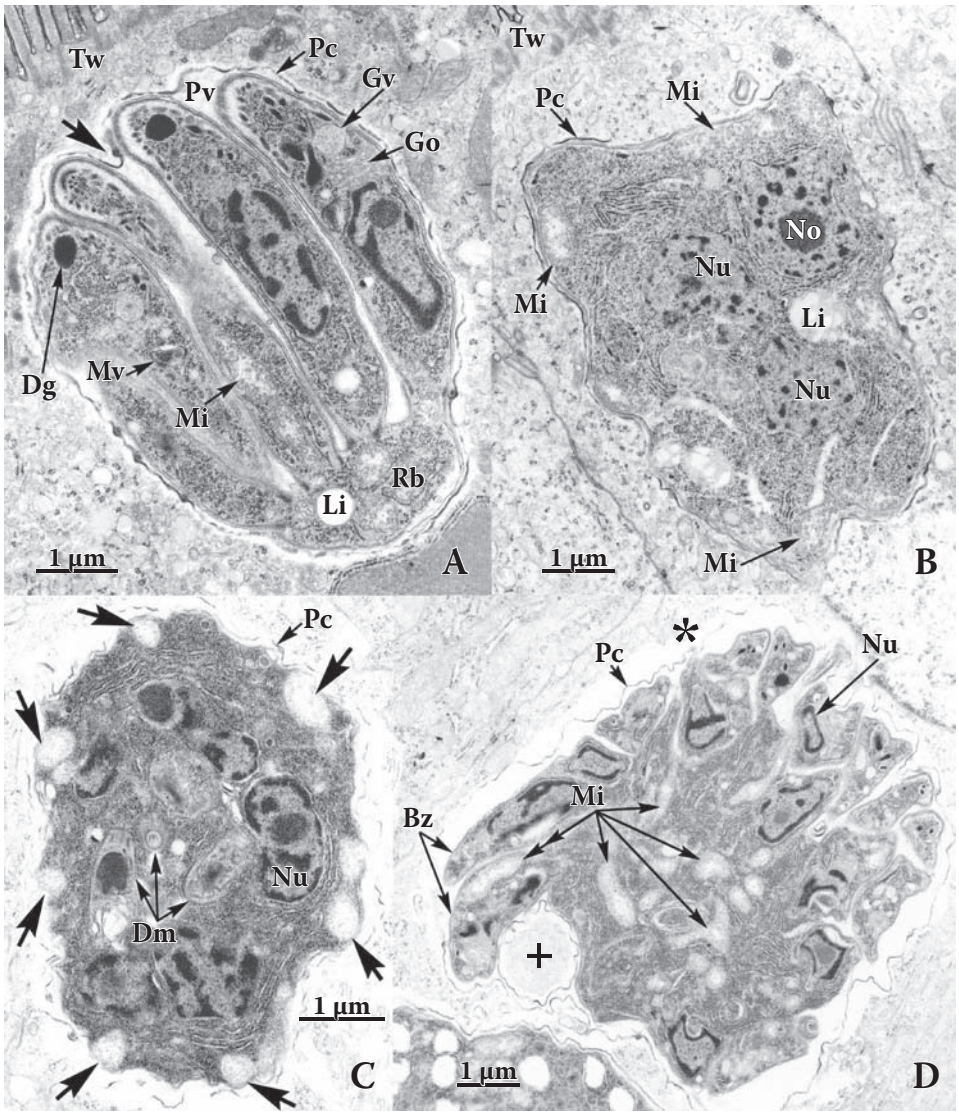

Figure 1.20 TEM of type D (A) and E (B-D) schizonts in enterocytes. **(A)** Type D schizont with merozoites budding from a residual body (Rb). Merozoites show a prominent vacuole (Gv) containing granular material immediately above maturation face of Golgi complex, electron-dense rhoptries (Rh) and large mitochondria (Mi); the parasitophorous vacuolar membrane complex (Pc) is indented between two merozoites (arrow); Dg, dense granule; Li, lipid body; Mv, multiple-membrane bound vacuole; Pv, parasitophorous vacuole; Tw, terminal web of enterocyte; 46 h p.i. **(B)** Intermediate type E schizont showing angular shape, numerous mitochondria (Mi) immediately beneath the surface of the schizont, and several lipid bodies (Li); schizont is surrounded by a characteristic parasitophorous vacuolar membrane complex (Pc); No, nucleolus; Nu, nucleus; Tw, terminal web of enterocyte; 6 days p.i. **(C)** Type E schizont in early stage of merozoite development (Dm) by endopolygeny; note characteristic angular shape of schizont and mitochondria (arrows) located immediately beneath the pellicle of the parasite; Nu, nucleus; Pc, parasitophorous vacuolar membrane complex; 6 days p.i. **(D)** Type E schizont with budding merozoites (Bz); note numerous mitochondria (Mi) one of which (tandem arrows) has been incorporated into a budding merozoite, the large vacuole (+) containing fine granular material within the parasitophorous vacuole, and the space between the host cell cytoplasm and the parasitophorous vacuolar membrane complex (Pc); a portion of a macrogamont (Ma) is visible at the lower left; Nu, nucleus of budding merozoite; 4 days p.i. From Speer and Dubey.[1223]

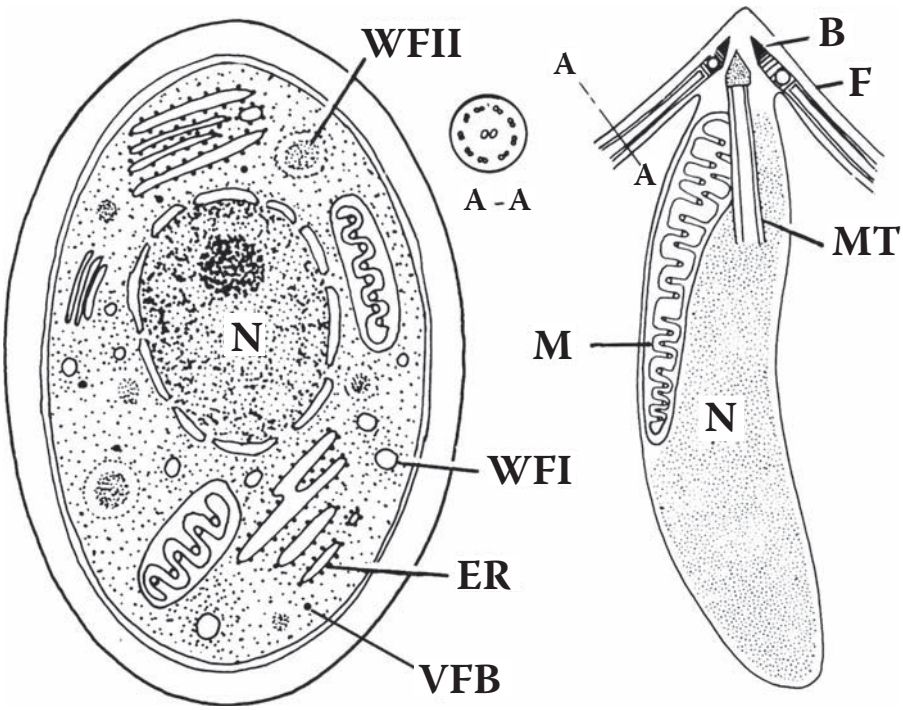

Figure 1.21 Schematic drawings of a macrogamete (left) and a microgamete (right). A–A, cross section of flagellum. ER, endoplasmic reticulum; F, flagellum; MT, microtubules; M, mitochondrion; N, nucleus; WFI, wall-forming bodies of the first type; WFII, wall-forming bodies of the second type; VFB, veil forming bodies.

resistant to acid as bradyzoites, it is likely that some tachyzoites might penetrate pharyngeal-buccal mucosa when tachyzoites are poured into the mouth of the cat.[385,397] Seventeen of 31 cats administered tachyzoites by a stomach tube also became infected, indicating that the tachyzoites survived acid-pepsin digestion in the stomach.[365,397] From a practical point of view, bradyzoites are likely to be present in most infected animals, even during acute infection, because bradyzoites were found in mice infected for only 3 days.[277] These results indicate that humans can become infected if they accidentally ingest *T. gondii* tachyzoites in the laboratory.

1.2.4 Life Cycle in the Intermediate Hosts, Including Humans and Cats

The life cycle of oocyst-transmitted infection has been studied in mice.[359,1221] After ingestion of sporulated oocysts, sporozoites excyst, penetrate enterocytes and goblet cells of the intestinal epithelium, and are carried to the lamina propria via an unknown mechanism (Figures 1.8 and 1.9). Sporozoites were found in the PV in enterocytes and goblet cells as early as 2 hr p.i. post-feeding (Figure 1.8). Some sporozoites can be found circulating in peripheral blood as early as 4 hr p.i. after ingestion. However, most remain in the lamina propria where they multiply in a variety of cells including the vascular endothelium, fibroblasts, mononuclear cells, and segmented leukocytes; but not in erythrocytes. It is likely that sporozoites break out of the PV before reaching the lamina propria. At 12 hr p.i., sporozoites had divided into two tachyzoites. By 6 days p.i., bradyzoites and tissue cysts had formed, as demonstrated by IHC staining with bradyzoite-specific antibodies and by bioassays in cats. Tissue cysts persisted in many organs of mice even during chronic infection.

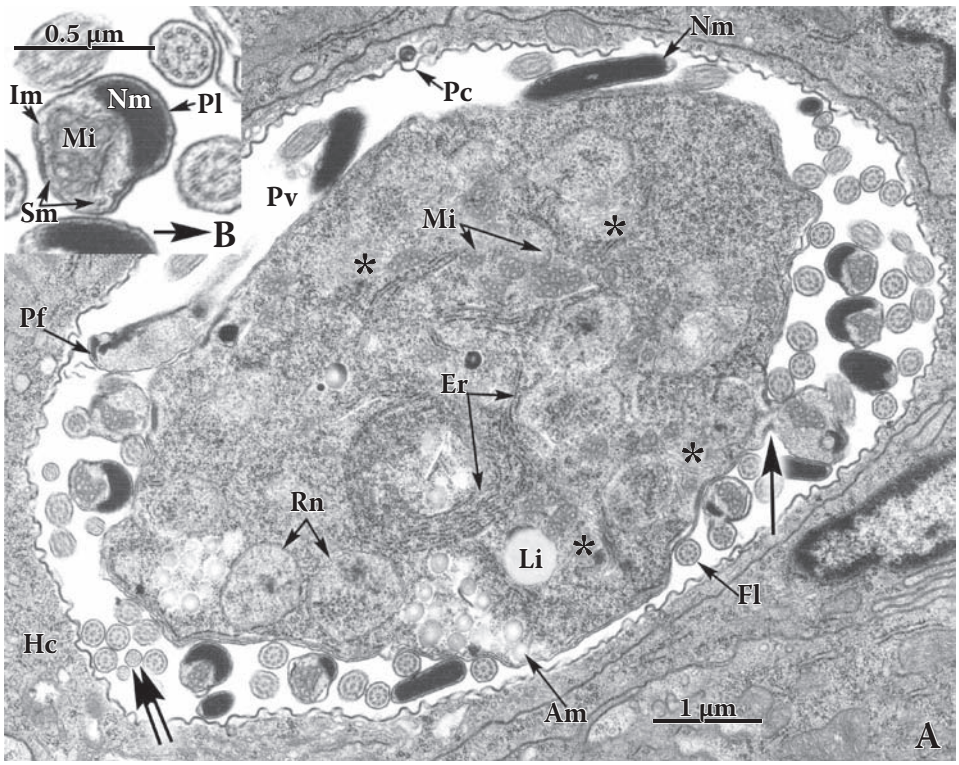

Figure 1.22 TEM of a mature microgamont, 6 days p.i. Microgamont with microgametes budding at the surface (arrow); microgamete flagellum (Fl) contains typical 9 + 2 microtubules except at the flagellar tips (double arrow) where the microtubules are arranged singularly; residual body contains numerous amylopectin granules (Am), Golgi complexes (*), mitochondria (Mi), residual nuclei (Rn), endoplasmic reticulum (Er), and lipid bodies (Li). Hc, host cell cytoplasm; Nm, electron-dense nucleus of microgamete; Pc, parasitophorous vacuolar membrane complex; Pf, perforatorium of microgamete; Pv, parasitophorous vacuole. Inset a (arrow) is a higher magnification of cross section of microgamete showing inner membrane complex (Im) and subpellicular microtubules (Sm) in region near mitochondrion (Mi). Nm, nucleus of microgamete; Pl, plasmalemma of microgamete. From Speer and Dubey.[1223]

The bradyzoite-induced cycle in the intermediate host is similar to that of the oocyst-induced cycle but bradyzoites are less infective to mice than sporozoites. After feeding bradyzoites to mice, bradyzoites were found in enterocytes and in the lamina propria at 1 hr p.i. By 2 days p.i., tachyzoites were formed, and tissue cysts were formed by 6 days p.i. Tissue cysts persisted in several organs.

1.3 MOLECULAR BIOLOGY

A full review of this subject is beyond the scope of this book. To help molecular biologists and cell biologists not familiar with *T. gondii* biology, I have summarized information concerning the origin and maintenance of the *T. gondii* strains commonly used for molecular studies (Tables 1.10 and 1.11). Additionally, in the section on techniques, I have given procedures to obtain different stages of the parasite and precautions necessary while handling this parasite.

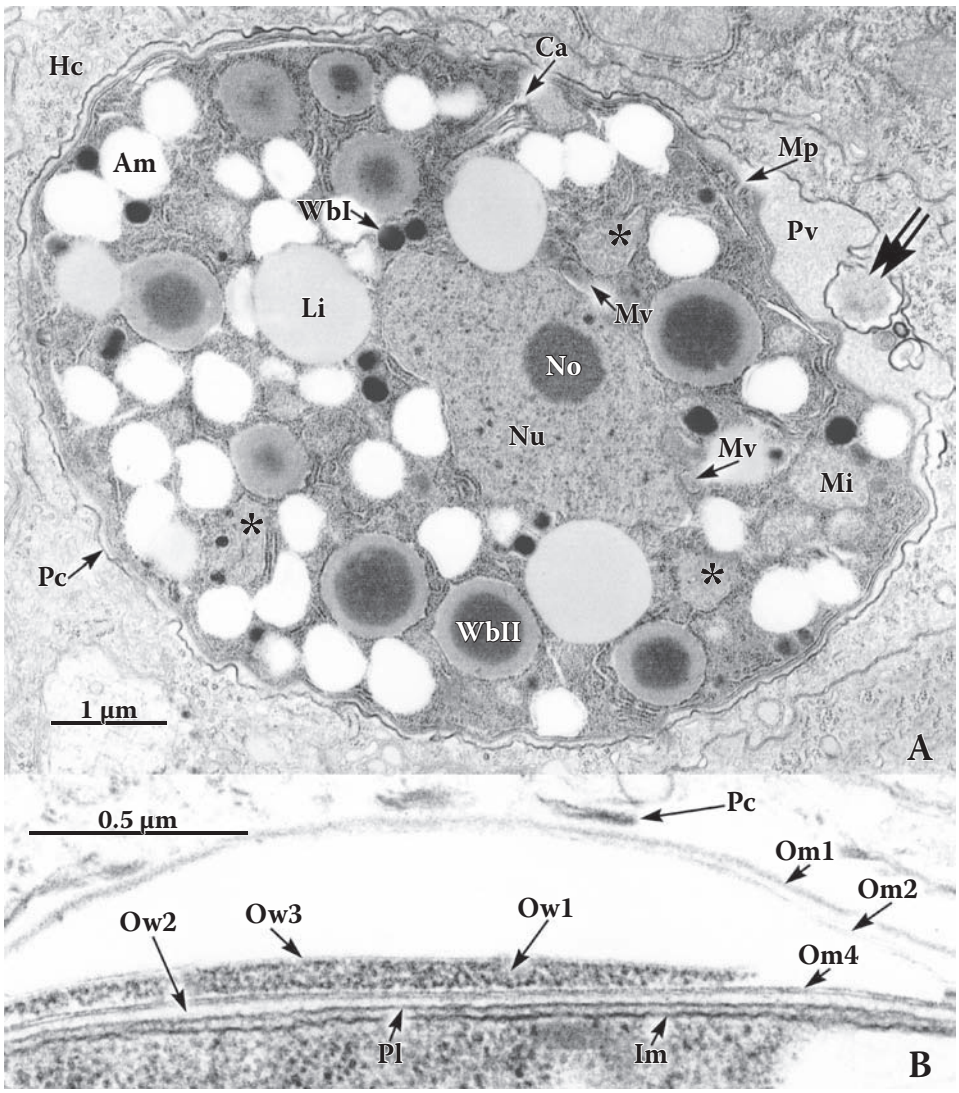

Figure 1.23 TEM of a mature macrogamont (A) and an early stage of oocyst wall formation (B). **A.** Mature macrogamont showing characteristic canaliculi (Ca), wall-forming bodies I and II (WbI, WbII), lipid bodies (Li), amylopectin granules (Am) and centrally located nucleus (Nu) with prominent nucleolus (No); pellicle (Pe) consists of plasmalemma and inner membrane complex; note large vacuole (double arrows) with granular material within the parasitophorous vacuole (Pv). *, Golgi complex; Hc, host cell cytoplasm; Mi, mitochondrion; Mp, micropore; Mv, multiple membrane bound vacuole; Pc, parasitophorous vacuolar membrane complex; 4 days p.i. **B.** High magnification of surface of zygote with developing oocyst wall; portions of the parasitophorous vacuolar membrane complex (Pc) have disappeared; the outer layer of the oocyst wall (Ow1) is forming between membranes 3 (Om3) and 4 (Om4) and the inner layer (Ow2) is beginning to form between the plasmalemma (Pl) of the zygote and membrane 4 (Om4); Im, inner membrane complex of pellicle; 6 days p.i. From Speer and Dubey.[1223]

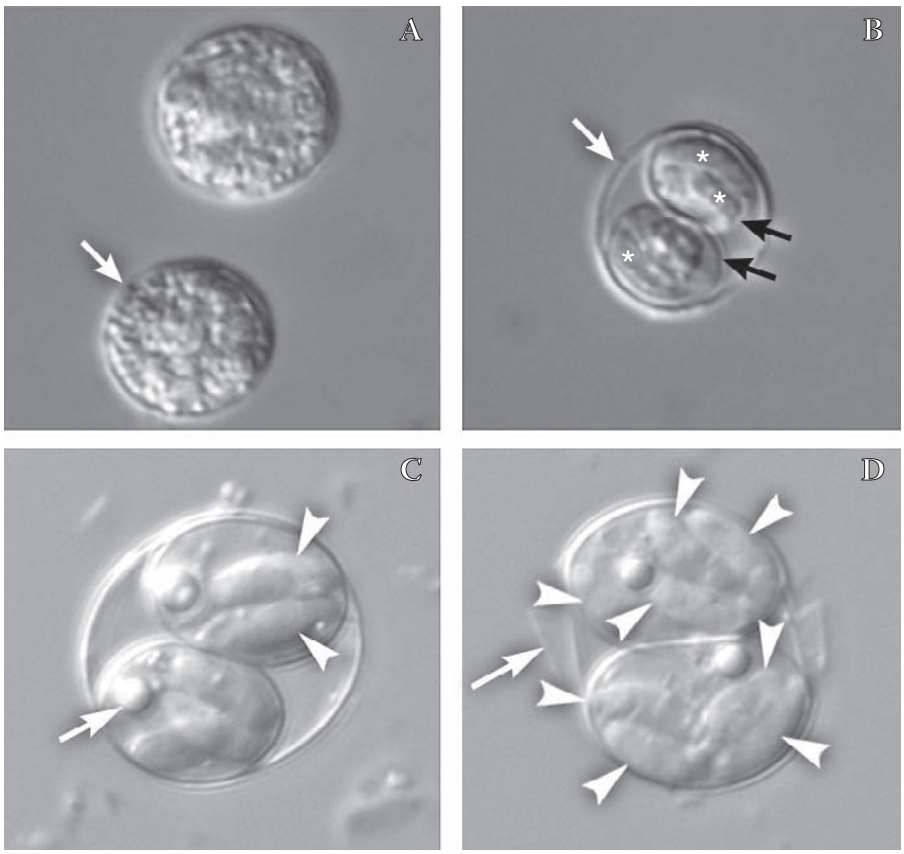

Figure 1.24 *T. gondii* oocysts. **A.** Unsporulated oocysts. **B.** Sporulating oocyst. Note the oocyst wall (white arrows) and sporocyst walls (black arrows), and developing sporozoites (asterisks). **C.** Fully sporulated oocyst with sporozoites (arrowheads) and a residual body (arrow). **D.** Sporulated oocyst flattened with pressure to show four sporozoites in each of the two sporocysts. The outer oocyst wall (arrow) has partially ruptured (arrow). Nomarski phase contrast. A and B courtesy of Dr. Eric Villegas, and C and D courtesy of Mr. Fournet Valsin and Dr. Dolores Hill .The scale bar is missing but the oocysts are approximately 12 µm long.

The *T. gondii* nucleus is haploid except during the sexual division in the intestine of the cat.[1054] Sporozoites are the results of meiosis and seem to follow classical Mendelian laws. The total haploid genome contains 14 chromosomes and 7793 genes, with a total genome size of 63,495,144 base pairs.[765] It is an unusual parasite because of its broad host range and with only one species in the genus. Prior to the development of genetic markers, *T. gondii* isolates were grouped by their virulence to outbred mice. During the 1980s and 1990s methods were developed to recognize genetic differences among *T. gondii* isolates from humans and animals.[218,222,681,1055,1179,1285] Based on restriction fragment length polymorphism (RFLP), Howe and Sibley[681] classified *T. gondii* into three genetic types (I, II, III) and linked mouse virulence to genetic type. They proposed that type I isolates were 100% lethal to mice, irrespective of the dose, and that types II and III generally were avirulent for mice.[682] Lehmann et al.[818] made the first in-depth study of genetic variability among more than 275 *T. gondii* isolates obtained worldwide from one host (free-range chicken) and in one laboratory[384] and found geographic differences, with some isolates confined to Brazil whereas others were worldwide in distribution. Phenotypically, *T. gondii* isolates from asymptomatic chickens from Brazil were mouse virulent.[384] Recent studies have indicated a

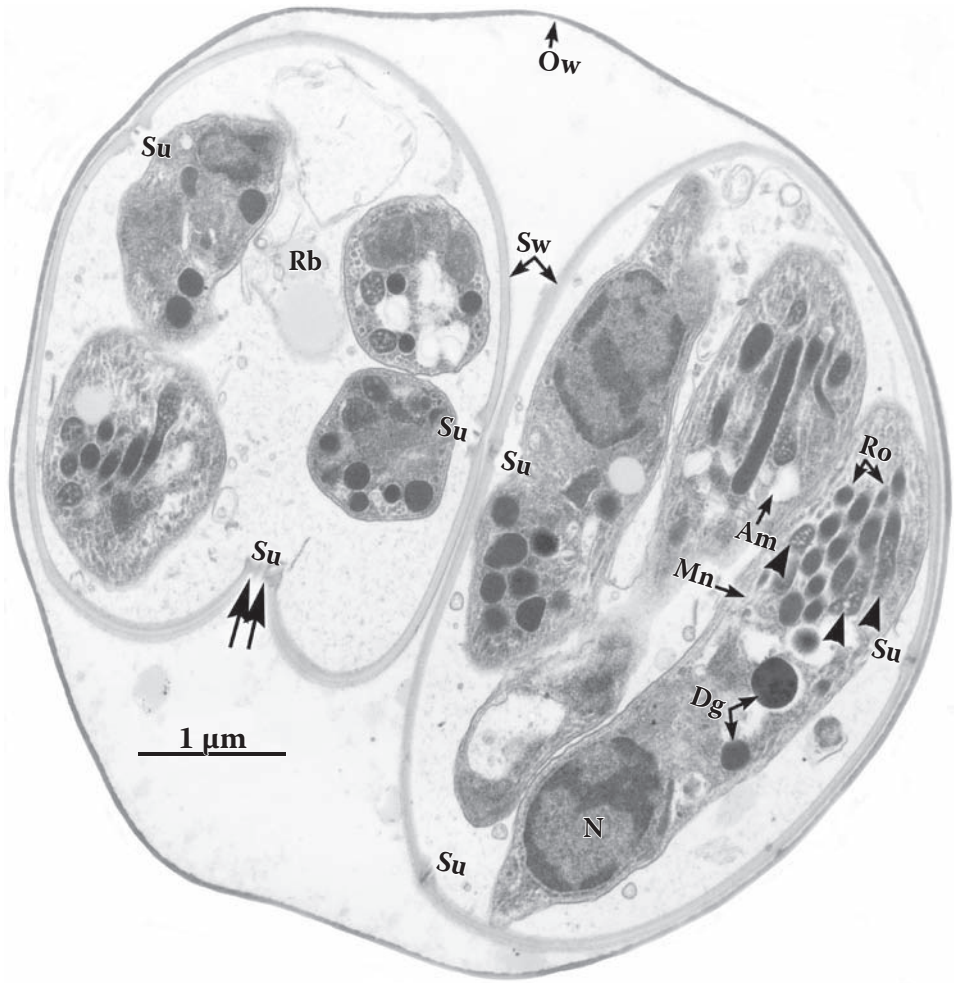

Figure 1.25 TEM of a *T. gondii* sporulated oocyst. Note the thin oocyst wall (Ow) enclosing two sporocysts. Note sporocyst walls (Sw) with platelike sutures (Su). The sporozoites are as long as the sporocyst, one of which is cut longitudinally. The sporozoites contain rhoptries (Ro); their contents vary from labyrinthine to electron dense (arrowheads), terminal nucleus (N), micronemes (Mn), and amylopectin (Am). From Dubey et al.[363]

higher genetic diversity among *T. gondii* isolates in livestock and wildlife in the Americas.[432] Population structure of *T. gondii* and genetic variablity among isolates are now under extensive investigation.[257,258,584,764,1180a,1254,1310]

1.4 TRANSMISSION

1.4.1 Transmission by Oocysts

1.4.1.1 Cats Are Everywhere

Oocysts are essential in the life cycle of *T. gondii* and as stated earlier, both domestic cats (*F. domesticus)* and other felids may shed oocysts. Cats are everywhere, except the frozen arctic.

Table 1.10 Commonly Used Strains of *T. gondii* for Research

T. gondii Strain (Year Isolated)	Source	ATCC No[a] or BRC TOXO N[b]	Summary Genetic Type	
			Type (See Table 11)	Ref. for Genetic Data
RH (1937)	Human, brain, US Sabin[1127]	50838	I	16, 408, 1042
Beverley (BEV) (1956)	Rabbit, brain, UK Beverley[96]	50854	II	
Me–49 (1965)	Sheep, muscle, US Lunde and Jacobs[871]	50840	II	
PTG	(clone from Me–49)	50841	II	408, 1042
VEG (1989)	Human, blood, US Parmley et al.[1026]; Dubey et al.[353]	50861	III	1042
GT1 (1978)	Goat, muscle, US Dubey[282]	50853	I	1042, 408
TgPgUs15 (P89) (1991)	Pig, heart, US Dubey et al.[340], Velmurugan et al.[1311]	50879	Atypical	1042, 1311
TgCgCa1 (Cougar) (1998)	Cougar, oocysts, Canada Aramini et al.[39] Dubey et al.[429]		Atypical	408, 1042
TgCatBr5 (2003)	Cat, tissues, Brazil Dubey et al.[394]		Atypical	408, 1042
C (CTG, CEP) (1976)	Cat, oocysts, US Pfefferkorn et al.[1053]	50842	III	17, 408, 1042
PRU (Prugniaud) (1964)	Human, fetal tissues, congenital, France Dardé et al.[219]	H–001	II	16, *
CASTELLS (1993)	Sheep, placenta, Uruguay Dardé[220]		Atypical	1241a, 17
MAS (1991)	Human, fetal tissues, congenital, France Dardé et al.[219]	50870	Atypical	17, 408, 1042
NED (1989)	Human, placenta, congenital, France Dardé et al.[219]	H–002	III	17*
RUB (1992)	Human–adult, broncho alveolar fluid, immunocompetent, French Guiana Dardé et al.[221]	H–003	Atypical	17*
IPP–2002–URB (2002)	Human–newborn, peripheral blood, congenital, France Ajzenberg et al.[17]	H–004	Atypical	17*
GUY–2002–KOE (2002)	Human–adult, peripheral blood, immunocompetent French Guiana Ajzenberg et al.[17]	H–005	Atypical	17*
GUY–2003–MEL (2003)	Human–adult, peripheral blood, immunocompetent, French Guiana Ajzenberg et al.[17]	H–006	Atypical	17*
RMS–2003–DJO (2003)	Human–adult, cerebral biopsy, AIDS, Benin Ajzenberg et al.[18]	H–007	Atypical	17*

* See Table 1.11.
[a] Deposited by Dr. David Sibley.
[b] See http://www.toxobrc.com, courtesy of Drs. Ajzenberg and Dardé.

Table 1.11 Genetic Characterization of Commonly Used *T. gondii* Strains Shown in Table 1.10

	Genetic Markers																
***T. gondii* Strains**	**SAG1**	**(5'+3') SAG2***	**SAG2†**	**SAG3**	**BTUB**	**GRA6**	**c22–8**	**c29–2**	**L358**	**PK1**	**Apico**	**TUB2**	**W35**	**TgM–A**	**B18**	**B17**	**M33**
RH	I	I	I	I	I	I	I	I	I	I	I	1	1	1	1.3	1	1.2
GT1	I	I	I	I	I	I	I	I	I	I	I	1	1	1	1.3	1	1.2
PTG	II or III[a]	II	II	II	II	II	II	II	II	II	II	2.3	2.3	2	2	2.3	1.2
Me–49	II or III	II	II	II	II	II	II	II	II	II	II	2.3	2	2	2	2.3	1.2
Beverley (BEV)	II or III	II	II	II	II	II	II	II	II	II	I	2.3	2	2	2	2.3	1.2
PRU (Prugniaud)	II	II	II	II	II	II	II	II	II	II	I	2.3	2.3	2	2	2.3	1.2
TgCgCa1 (COUGAR)	I	II	II	III	II	II	II	u–1	I	u–2	I	2.3	2.3	3	2	2.3	1.2
C (CTG,CEP)	II or III	III	III	III	III	III	III	III	III	III	III	2.3	3	3	1.3	2.3	3
VEG	II or III	III	III	III	III	III	III	III	III	III	III	2.3	2.3	3	1.3	2.3	3
NED	III	III	III	III	III	III	III	III	III	III	III	2.3	3	3	1.3	2.3	3
MAS	u–1	I	II	III	III	III	u–1	I	I	III	I	1	2	3	4	10	1.2
TgCatBr5	I	III	III	III	III	III	I	I	I	u–1	I	1	2.3	3	1.3	10	3
TgPgUs15 (P89)	I	III	III	III	III	III	II	III	III	III	III	1	2.3	3	1.3	6	3
CASTELLS	u–1	I	II	III	III	III	III	I	I	III	I	4	2	2	2	8	1.2
RUB	I	I	II	I	III	III	III	III	I	III	III	2.3	3	3	7	9	4
IPP–2002–URB	u–1	I	II	III	III	III	u–1	I	I	III	I	4	2	2	1.3	7	1.2
GUY–2002–KOE	I	I	II	I	III	III	III	I	III	III	I	2.3	4	4	5	4	3
GUY–2003–MEL	I	II	I	I	III	III	I	III	I	III	III	2.3	2	4	2	5	5
RMS–2003–DJO	I	I	I	III	I	II	u-1	I	I	I	I	1	1	3	1.3	1	3
RMS–2001–MAU	u–1	II	II	II	II	II	II	II	I	II	I	2.3	2	5	1.3	2.3	1.2

Courtesy of Drs. Daniel Ajzenberg and Chunlei Su. Microsatellite data are from laboratory of Daniel Ajzenberg and Marie Dardé and PCR –RFLP data are from the laboratory of Chunlei Su.
Drs. Daniel Ajzenberg and Chunlei Su made this table.

The feline population closely parallels the human population, especially in the Western world. For example, approximately one-third of households in the United States own a cat, and this number is steadily increasing. There are approximately 78 million domestic cats and 73 million feral cats. It is probable that every farm in the United States has cats. In a study of pig farms in Illinois, 366 cats were trapped on 43 farms, a mean of 8.5 cats per farm, with a mean of 6 seropositive cats on each farm.[1347] *T. gondii* oocysts were detected in cat feces, feed, soil, or water samples on six farms,[344,1347] and soil samples around human dwellings.[546] Thus there is strong potential for *T. gondii* transmission in rural settings.[31]

1.4.1.2 Shedding of Oocysts by Naturally Infected Domestic Cats

Experimentally, most cats seroconverted during the second and third week after they were fed tissue cysts and usually after they had shed oocysts.[273,304,342] Thus, it is a reasonable assumption that most seropositive cats have already shed oocysts. For epidemiological studies, seroprevalence data are more meaningful than determining the prevalence of *T. gondii* oocysts in feces. Up to 100% of cats in worldwide surveys (see Chapter 3) were found to have *T. gondii* antibodies, indicating that seropositive cats have already shed oocysts and caused widespread environmental contamination.

At any given time, approximately 1% of cats are expected to shed oocysts, based on the observation that most cats only shed oocysts for about 1 week in their life, and this is supported by fecal surveys (Table 1.12).

Most reports in Table 1.12 were based on microscopic examination of feces and would have missed low numbers of oocysts. The threshold of detection of *T. gondii* by microscopic examination of feces is low (approximately 1,000 oocysts per gram). Bioassay in mice can detect even a few viable *T. gondii* oocysts. However, only a few studies bioassayed all fecal samples in mice, irrespective of microscopic examination.[344,546] Although polymerase chain reaction (PCR) can detect low numbers of oocysts (see Section 1.11.5), it does not reveal the viability of oocysts. Salant et al.[1133] detected *T. gondii* DNA in feces of 11% of 122 cats; oocysts were not detected microscopically in these samples. Dabritz et al.[211] had the opposite experience; they found *T. gondii* oocysts in feces of three cats but did not detect *T. gondii* DNA in any of the samples. Schares et al.[1153] performed an extensive investigation to identify *T. gondii* DNA in a large number of samples, but the identity was not confirmed by bioassays. Schares et al.[1153] found *T. gondii*-like oocysts in feces of 48 of 24,106 cats from Germany and other European countries; of these 26 were identified as *T. gondii* and 22 as *Hammondia hammondi* based on PCR; bioassays were not performed. Up to 13 million *T. gondii* oocysts were present per gram of cat feces.[1153] This study also demonstrates the importance of proper identification of *T. gondii* oocysts in cat feces because half of the cats were shedding *H. hammondi* oocysts, which have no zoonotic significance.

Both young (<6 mo) and older (>6 mo) cats have been found shedding oocysts in nature (Table 1.13). Even suckling kittens[330] and cats as old as 13 years were found shedding oocysts.[1153]

Little information is available concerning the immune status and shedding of oocysts. In limited studies, both seropositive and seronegative cats were found shedding oocysts, but most data were based on the dye test (DT) (Table 1.13). Experimentally, the DT is less sensitive than the modified agglutination test (MAT) for the detection of onset of IgG antibodies in cats.[304] Occasionally cats that shed oocysts remained seronegative by the DT for a long time but acquired protective immunity as evidenced by challenge studies.[273]

The number of oocysts shed by naturally infected domestic cats is largely unknown. There are only a few studies on quantitative estimation of oocysts in cat feces (Table 1.14). Whether all oocysts were counted in feces by Schares et al.[1153] is uncertain because bioassays were not made. However, data reported by us[39,293] were based on bioassays in mice.

Table 1.12 Prevalence of *T. gondi*-Like Oocysts in Feces of Cats (1988–2008)[a]

Country	No. of Cats	Grams of Feces Tested	Method			No. Pos (%)	Reference
			Micro	Bioassay	PCR		
Argentina	50	NS	Yes	Yes	No	**1 (2.0)[d]**	Venturini et al., 1992, 1997a
Austria	1368	NS	Yes	No	No	27 (2)	Edelhofer and Aspöck, 1996
Belgium	30	NS	Yes	No	No	0	Vanparijs et al., 1991
Brazil	237	1	Yes	Yes	Yes	3 (1.2)	Pena et al., 2006
	327	NS	Yes	No	No	2 (0.6)	625
Colombia	18	NS	Yes	No	No	12 (66.6)	Londoña et al., 1998
	143	2–10	Yes	Yes	No	0	Dubey et al., 2006
Czech Republic	390	NS	Yes	Yes	No	0	Svobodová et al., 1998
	620	NS	Yes	No	No	8 (1.2)	1257
France	322	NS	Yes	No	No	0	Afonso et al., 2006
Germany	264	NS	Yes	No	No	0	Knaus and Fehler, 1989
	70[b]	NS	Yes	No	No	(17.1)	Beelitz et al., 1992
	24106	NS	Yes	No	Yes	26 (0.11)	Schares et al., 2008
	2473	NS	Yes	No	No	22 (0.9)	Barutzki and Schaper, 2003
	2472	NS	Yes	No	No	26 (1.0)	Epe et al., 1993
	441	NS	Yes	No	No	3 (0.7)	Epe et al., 2004
India	9	NS	Yes	Yes	No	**1 (11)**	Shastri and Ratnaparkhi, 1992
Iran	50	NS	Yes	No	No	0	Hooshyar et al., 2007
	100	1	Yes	No	No	0	Sharif et al., 2009
Israel	122	3	Yes	No	Yes	11(9)[e]	Salant et al., 2007
Japan	335	NS	Yes	Yes	No	**1 (0.3)**	Oikawa et al., 1990
Mexico	200	NS	Yes	Yes	No	**14 (7.0)**	de Aluja and Aguilar,1977
	200	NS	Yes	No	No	0	Guevara Collazo et al., 1990
New Zealand	63	NS	Yes	No	No	0	Langham and Charleston, 1990
Nigeria	52	NS	Yes	No	No	0	Umeche, 1990
Panama	383	NS	NS	Yes	No	**2 (0.5)**	Frenkel et al., 1995
People's Republic of China	26	10	Yes	Yes	No	0	Dubey et al., 2007
Singapore	722	1–2	Yes	No	No	0	Chong et al., 1993
Spain	382	3	Yes	No	No	0	Miró et al., 2004
	592	NS	Yes	No	No	0	Montoya et al., 2008
Taiwan	96	NS	Yes	No	No	0	Lin et al., 1990
Turkey	72	NS	Yes	No	No	0	Karatepe et al., 2008
United States	274[c]	NS	No	Yes	No	**5 (1.8)**	Dubey et al., 1995
	206	NS	Yes	No	No	0	Hill et al., 2000
	450	NS	Yes	No	No	3 (0.7)	Rembiesa and Richardson, 2003
	326	NS	Yes	No	No[f]	3 (0.9)	Dabritz et al., 2007b
	263	NS	Yes	No	No	3 (1.1)	Victor Spain et al., 2001
	34	NS	yes	yes	No	0	de Camps et al., 2008

[a] Modified from Jones and Dubey.[739] For references, see this paper.
[b] Litters of kittens from farms.
[c] Cats from pig farms.
[d] **Confirmed by bioassay in mice**.
[e] Oocysts not found by microscopic examination.
[f] Negative by PCR.

Table 1.13 Select Reports of Oocysts Shedding (Proven by Bioassays in Mice) and Age and Antibody Status of Naturally Infected Cats

Country	No. Exam.	No. Pos.	*T. gondii* Antibodies			Age		Ref
			Test	Neg.	Pos.	<6 mo	>6 mo	
Brazil	237	4	MAT	4	0	2	2	1041
Costa Rica	237	55[a]	DT	35	20	24	31	1121
Japan	446	4	DT	1	3	4	0	713
United States	1604	12	DT	5	7	6	6	1331
United States	1000	7	DT	4	3	Unknown		280

[a] Oocysts were detected microscopically in only 7 of the 55 cats.

1.4.1.3 Repeat Shedding of Oocysts

Whether naturally infected cats shed oocysts more than once in their life is unknown. Apart from challenge, at present there are no reliable and practical criteria to judge the immune status of the cat with respect to shedding of oocysts. The presence of antibodies to *T. gondii* cannot be relied upon to judge the immune status. Immunity to shedding of *T. gondii* oocysts in experimentally infected cats may be different to that in naturally infected cats based on their antibody status at the time of challenge.[341] Eleven of 18 adult feral cats with naturally acquired *T. gondii* infections, as judged by seropositivity in dye test, shed *T. gondii* oocysts after being fed tissue cysts.[271,1060]

It is generally believed that cats that have once shed *T. gondii* oocysts become immune to re-shedding of oocysts based on studies in experimentally infected cats.[228] In one study, five cats were immune to *T. gondii* when challenged 39 days after primary infection, but four of nine cats had lost that immunity by 77 mo p.i.[341] Three of the four cats that re-excreted oocysts after the 6-year challenge had been challenged earlier at 39 days.[341]

The number of oocysts shed during the secondary infection is usually lower than the primary infection.[276] As stated earlier, several factors can affect the shedding of oocysts by cats both during primary infection and during challenge. These factors include age of the cat, strain of *T. gondii,* nutritional status of the cat, and the number of tissue cysts fed during primary and challenge infections. None of these factors have been evaluated in depth to make final conclusions. Ruiz and Frenkel[1121] found re-shedding of *T. gondii* oocysts in one of seven cats that were fed calorie-deficient diets.

Infection of cats chronically infected with *T. gondii* with the feline coccidium *Isospora felis* induces re-excretion of *T. gondii* oocysts (Table 1.15) in the absence of any clinical signs.[171,278] The *I. felis*-induced relapse is specific because the other species of feline *Isospora, Isospora rivolta,* does not induce relapse of *T. gondii* infection in cats, and the effect can be blocked by immunization of cats with *I. felis* before *T. gondii* infection.[278,281] The interaction between *T. gondii* and *I. felis* in the gut of the cat remains unknown.[1006,1007] How often this re-shedding after *I. felis* occurs in nature is not known, but the point is that it can happen.

Table 1.14 *Toxoplasma gondii* Oocysts per Gram (Opg) of Feces in Naturally Infected Cats

Cat Type	No. of Cats	OPG or Total	Ref
Felis catus	2	7.6×10^6, 1.2×10^6	713
	1	1×10^5	293
	26	Up to 13×10^6	1153
	1	10^6	997
Felis concolor	2	12.5×10^6, 2.4×10^5	39

Table 1.15 Shedding of *Toxoplasma gondii* Oocysts by Cats after Challenge with *I. felis*[a]

	No. of Oocysts Shed	
Cat No.	Primary Infection	After Challenge with *I. felis*
3	$10^{8.19}$	$10^{7.22}$
4	$10^{7.38}$	$10^{6.57}$
5	$10^{7.27}$	$10^{7.17}$
6	$10^{7.50}$	$10^{6.24}$
7	$10^{7.86}$	$10^{6.69}$
8	$10^{8.13}$	$10^{6.34}$
9	$10^{7.14}$	10^{4}
10	$10^{8.13}$	None
11	$10^{7.43}$	$10^{6.17}$
12	$10^{7.15}$	$10^{5.21}$

[a] Modified from Dubey.[278]

Whether other concomitant infections can induce re-shedding of *T. gondii* oocysts is also not known. In particular, co-infection with feline immunodefficiency virus (FIV) did not affect the repeat shedding of oocysts.[802] FIV is a retrovirus related to human immunodeficiency virus (HIV), and is known to cause immunosuppression in cats (see Chapter 3).

Immunosuppression induced by large doses of corticosteroids can also induce oocyst shedding from *T. gondii*-infected cats, dependent on the dosage of corticosteroids used and the immune status of cats.[276,799] Three of six naturally infected cats in the United States shed *T. gondii* oocysts 3–11 days after administration of 80 mg/kg body weight of methyl prednisolone acetate.[276] Samad et al.[1137] reported shedding of *T. gondii*-like oocysts 9–13 days after administration of corticosteroids in three of six cats in Bangladesh. The dose of corticosteroid is important as low doses (5–20 mg of methyl prednisolone acetate) did not induce re-shedding of oocysts.[799]

1.4.1.4 Shedding of Oocysts by Naturally Infected Wild Felids

In addition to domestic cats, wild felids can shed oocysts. The number of bobcats in the United States is thought to be in the millions, and one study estimated thousands of cougars.[186] Prevalence of *T. gondii* infection in wild felids can be high (see Chapter 3). Young and weak white-tailed deer and small mammals are common prey for bobcats and 60% of white-tailed deer in Pennsylvania, were seropositive for *T. gondii* (see Chapter 16). In addition to live prey, eviscerated tissues (gut piles) from hunted deer and black bears would be a source of infection for wild cats. Thus, a sylvatic cycle of *T. gondii* in rural areas is feasible and appears to be efficient.

Shedding of oocysts by naturally infected feral felids needs a comment. As indicated in Table 1.5, oocysts were detected in rectal contents (sample A) of a wild trapped cougar and in a fecal pile (sample B) in the vicinity of a municipal water reservoir.[39] It is noteworthy that 12.5 million *T. gondii* oocysts were present in sample A and 250,000 in sample B[39]; the data were based on bioassays in mice. Genotyping data on the *T. gondii* isolates from these cougars now suggest that both fecal samples might have been from the same cougar.[429]

Lukešová and Literák[870] found *T. gondii*-like oocysts in feces of 8% of 175 *Felis silvestris* and 4% of *Felis euptilurus* in zoos in the Czech Republic; some of these oocysts were proven to be *T. gondii* by bioassays in mice.

Dorny and Fransen[267] reported 200,000 per gram of feces in a Siberian tiger in a zoo. The animal had diarrhea and had a MAT titer of 1:128. The animal died and was found to have diaphragmatic

hernia and pneumonia. *T. gondii* was not demonstrable by bioassays in mice and by histopathology. Ocholi et al.[996] also found numerous *T. gondii*-like oocysts in two cage-mate lions that died of acute toxoplasmosis (see Chapter 3). Oocysts from the tiger and the lions were not bioassayed and thus a definitive diagnosis could not be made.

1.4.1.5 Dispersal of Oocysts in the Environment

It has been said that the cat is a clean animal that licks its fur clean and buries its feces. Up to a point this is true, but it would be more correct to say "hides its feces," and kittens do not even do this. It is not uncommon to see uncovered cat feces on street pavements, parks, hay, animal feed, and of course in sandboxes. Moreover, oocysts can be brought to the surface by flies, cockroaches, earthworms, and by climatic conditions such as rain and snow. Oocysts may accumulate in defecation sites in parks and other public areas frequented by cats.[10]

The fate of cat feces disposed of in the toilet or in domestic trash destined for landfills is unknown. It is anticipated that the heat generated and lack of oxygen will kill some or all oocysts, depending on the conditions. It is likely that oocysts are carried into our homes on shoes contaminated with oocysts from street pavements.

Freshwater runoff has been suggested as a risk factor for *T. gondii* infection in California sea otters.[186,923] Based on an estimate of 10 million oocysts shed by each cat and a 36% defecation rate outdoors, a burden of 36 oocysts/m^2 over the region was calculated by Dabritz et al.[209] These authors considered this level of land contamination as likely to be high enough for oocysts to reach marine waters.[209] Sea otters eat approximately 25% of their body weight in invertebrate prey each day.[186] Cold-blooded animals, including fish, are not hosts for *T. gondii*.[1011] Before the discovery of the oocyst stage of *T. gondii,* numerous experiments were done to infect various invertebrates including arthropods with *T. gondii* tachyzoites, and although the parasite survived for various amounts of time, there was no evidence that it multiplied in cold-blooded animals.[302] With respect to oocysts, it is not known if the sporozoite excysts after ingestion by cold-blooded animals. Molluscs, however, can act as transport hosts for *T. gondii* oocysts.[45,848,851,926] In addition, sea otters might ingest oocysts directly from marine water. How much marine water is cycled through the gut of sea otters in a day is unknown, but it is likely to be a large quantity. *T. gondii* oocysts can survive in sea water at room temperature for up to 18 mo.[856]

Whether oocysts can be aerosolized in nature is uncertain. In October 1977, an outbreak of acute toxoplasmosis occurred in patrons of a riding stable in Atlanta, Georgia.[1274] An epidemiological investigation suggested that the patrons acquired *T. gondii* from oocysts aerosolized during the riding activity, although attempts to isolate oocysts from 29 samples of soil, sand, and sawdust from different parts of the stable were unsuccessful.[285] Attempts were made to isolate *T. gondii* from animals trapped in and around the stable. Viable *T. gondii* were isolated from tissues of 2 of 4 kittens, 3 of 3 adult cats, and 4 of 4 mice trapped in the stable in November, 1977 (sample 1) but not from 12 mice, 3 rats and 4 cotton rats trapped in the stable in December, 1977 (sample 2). All four mice and the five cats from whose tissues viable *T. gondii* were isolated had no detectable antibodies to *T. gondii* at a 1:2 serum dilution, suggesting recent infection. Bulldozing of the arena between collection of samples 1 and 2 might have contributed to differences in results obtained with these two samples.

1.4.1.6 Environmental Resistance of Oocysts

Little is known of the sporulation or survival rate of oocysts openly exposed to the sun and other environmental conditions. Oocysts survived outdoors in Texas (6–36°C) in native cat feces, uncovered, for 46 days, for 334 days when covered,[1381] and outdoors in soil buried at the depth of

Table 1.16 Effect of Environmental Influences on *T. gondii* Oocysts[a]

Temperature (°C) Indoors	Medium	Duration of Treatment	Killed	Ref
–21	Water	28 days	No	539
4	2% H_2SO_4	578 days	No	279
4	Water	1620 days	No	366
10	Water	200+ days	No	366
15	Water	200+ days	No	366
20–22	Water	548 days	No	697
30	Water	107 days	No	366
35	Water	32 days	No	366
40	Water	28 days	Yes	366
40	Water	24 hr	No	366
45	Water	3 day	No	366
50	Water	60 min	No	271
52	Water	5 min	No	366
55	Water	1 min	No	366
		2 min	Yes	366
60		1 min	Yes	366
Drying at relative humidity	19%	11 days	Yes	537
	0%	2 days	No	537
Texas 6.5–37.5	Native feces	334–410 days	No	1381
Kansas 20–35	Native feces	548 days	No	541
Costa Rica 15–30	Native feces	56–357 days	No	541

[a] Modified from Dubey.[403]

3–9 cm in Kansas for 18 mo.[541] Drying under low humidity and high temperatures were deleterious to oocysts (Table 1.16). Unsporulated oocysts are more thermal susceptible than sporulated oocysts;[849,850] unsporulated oocysts exposed to 37°C for 24 hr were killed[272] whereas sporulated oocysts were not. *T. gondii* oocysts are highly resistant to disinfectants (Table 1.17) but are killed by temperatures above 60°C (Table 1.17). Ultraviolet rays also have a deleterious effect on oocysts, depending on the dose (Table 1.16), but as of yet there is no practical way of killing oocysts in large water reservoirs.

1.4.1.7 Detection of Oocysts in Environmental Samples

Detection of *T. gondii* oocysts in environmental samples (soil, water feed) is technically difficult (for review see Dumètre and Dardé;[440] Jones and Dubey;[739] Borchardt et al.[113]). Considerable progress has been made in detecting oocyst or DNA of the genus *Cryptosporidium*. Traditionally, oocysts were concentrated from large volumes of water by filtration or centrifugation, isolation from concentrated particulates by immunomagnetic separation (IMS) or other methods, and detection by immunofluorescence microscopy, infection of cultured cells, biochemistry, bioassays in mice or cell culture, molecular techniques, or combinations of these. For *T. gondii* oocysts there are no commercially available IMS techniques or immunofluorescent reagents. Although DNA from *T. gondii* oocysts can be detected from feces of cats (see Section 1.11.5) there is no rapid, accurate, sensitive PCR method for the detection of oocysts, but progress is being made.[113]

Attempts to detect *T. gondii* in water have been largely unsuccessful.[170,1355] Two large waterborne outbreaks of toxoplasmosis in humans were epidemiologically linked to oocyst contamination of

Table 1.17 Effect of Disinfectants on *T. gondii* Oocysts*

Reagent	Concentration (%)	Duration of Treatment	Killed	Ref
Formalin	10	48 hr	No	714
Sulfuric acid + dichromate	63/7	30 min	No	271
	63/7	24 hr	Yes	
Ethanol + acetic acid	95/5	1 hr	No	271
	95/5	24 hr	Yes	
Ammonium hydroxide	5.0	10 min	No	271
	5.0	30 min	Yes	271
Sodium hyporchlorite (Purex)	6.0	24 hr	No	271
Sodium lauryl sulfate	0.1	24 hr	No	271
Cetyl trimethyl ammonium	0.1	24 hr	No	271
Tween 80	0.1	24 hr	No	271
Ammonia[a], liquid	5.5	1 hr	No	537
	5.5	3 hr	Yes	537
Tincture of iodine	2.0	10 min	No	537
	2.0	3 hr	Yes	537
	7.0	10 min	Yes	537
Aldesol[b]	33	24 hr	No	789
Tincture of hibisept[c]		24 hr	No	789
Izosan-G[d]	.02	24 hr	No	789
Lomasept	1	1 hr	No	714
		3 hr	Yes	714
Neo Kurehasol	5	24 hr	No	714
Paracetic acid	5	48 hr	Yes	714
Chlorination of water	100 mg/L	24 hr	No	1329
Ozone treatment of water				
	6 mg/L	12 min	No	1329
	9.4 mg/L	20 min	No	442
Ultraviolet irradiation	>500 mJ/cm^2		Yes/No	1330
	40 mJ/cm^2		Yes/No	442
High pressure processing	Mpa 400	1 min	Yes	855

* Modified from Dubey.[391]
[a] Undiluted household ammonia.
[b] Aldesol, a solution for disinfection, contains 5 g benzalchoniumchloride, 6 g glutaraldehyde, and 8 g gloxal in 100 g of solution.
[c] Hibisept tincture contains 0.5 g chlorhexidine gluconate in 70% ethanol in 100 ml of tincture.
[d] Izosan-G granulate contains 99 g of sodium dichloroizicyanurate-dihydrate in 100 g granulate.

drinking water in British Columbia, Canada,[116] and Brazil.[236] In the Canadian outbreak oocysts were not detected in drinking water taken from the reservoir after the outbreak,[710] but viable oocysts were detected in rectal contents (sample A) of a wild trapped cougar (*Felis concolor vancouverensis*) and in a fecal pile (sample B) in the vicinity of the reservoir.[39] *T. gondii* was isolated once in Brazil from samples taken from small reservoirs on roof tops.[236] Water from roof-top tanks had a lot of particulate matter making filtration difficult. To recover viable *T. gondii* from the Brazilian

outbreak, we filtered water through membranes, and then fed these filters to *T. gondii*-free pigs and chickens; both pigs and chickens developed *T. gondii* infection.[236]

1.4.2 Transmission by Tissue Cysts

Tissue cysts are an essential part of the life cycle of *T. gondii.* They are the end stage of the parasite waiting to be eaten by animals, including humans. As indicated in Table 1.1, Weinman and Chandler[1348] first suggested that humans might become infected through the ingestion of undercooked meat and Desmonts et al.[249] provided evidence for it in an experiment with children in a Paris sanitorium (uncooked meat was being fed with the belief that it helped recovery from tuberculosis). They compared the acquisition rates of *T. gondii* infection in children before and after admission to the sanitorium. The 10% yearly acquisition rate of *T. gondii* antibodies rose to 50% after adding two portions of barely cooked beef or horse meat to the daily diet and to a 100% yearly rate after the addition of barely cooked lamb chops. Since the prevalence of *T. gondii* is much higher in sheep than in horses or cattle this illustrated the importance of carnivorism in transmission of *T. gondii.* Another outbreak was circumstantially linked to eating raw mutton at a party in Brazil in 1993. There were 17 cases of symptomatic acute toxoplasmosis, sixteen (94.5%) of which presented with fever, headache, myalgia, arthralgia, and adenopathy (cervical or cervical/axilar). One patient presented a clinical picture of chorioretinitis. All patients had specific antibodies evidencing acute phase of toxoplasmosis.[110] One of the patients was also reported to have transmitted toxoplasmosis to her nursing child.[109] Kean et al.[758] described toxoplasmosis in a group of medical students who had eaten undercooked hamburgers and clinical toxoplasmosis and blindness were linked to the ingestion of undercooked pork in Korea.[174] Symptomatic toxoplasmosis in a family in New York City was circumstantially linked to eating rare-cooked lamb.[893]

Viable tissue cysts have been found in tissues of naturally infected animals, particularly in pigs and sheep, and also in wild game (see later chapters). They are extremely rare in cattle. Tissue cysts have been found to persist in edible tissues of live animals for months (Table 1.4), and considering the number of tissue cysts in 50 g of infected samples from a single infected food animal (Table 1.4), the whole carcass could infect many people.

There are numerous reports tracing toxoplasmosis to the consumption of infected meat, and some serological surveys incriminate meat more strongly than cats as a source of human infection.[302] As would be expected, the prevalence of *T. gondii* infection is greater in abattoir workers and in others who handle raw meat than in the general population. Meat grinders and butchers' knives may be contaminated from an infected carcass or piece of meat. There is a bizarre report of a woman developing a fatal *T. gondii* infection apparently after being savagely stabbed with a butcher's knife.[502]

Serological or parasitological surveys based on slaughterhouse samples do not provide a true assessment of risk to humans because storage and post harvest treatments of meat can kill some *T. gondii*. For example, nearly half of the pork and a substantial amount of the chicken in retail meat in the United States is injected with salts and water.[396] Some of the salt treatments (labeled as "enhanced" meat) kill *T. gondii* tissue cysts.[656] Further, most of the retail chicken sold in the United States is frozen, which also kills *T. gondii*. We are not aware of a risk assessment study in the United States, but in a retrospective study of 131 mothers who had given birth to children infected with *T. gondii,* 50% recalled having eaten uncooked meat.[117] In Europe, ingestion of inadequately cooked meat (lamb, beef, or game) was identified as the main risk.[108,188]

Fortunately, tissue cysts are not resistant to conventional cooking[312] at 60°C or higher. Based on a time and temperature curve, shown in Figure 1.26, tissue cysts are killed when the meat reached an internal temperature of 66°C. For advice given to consumers regarding cooking of meat at lower temperatures, one should take into consideration the 3.5 min come-up and come-down time. Microwave cooking is another matter. Because of hot and cold spots and because of the nature of microwave physics, microwave cooking cannot be relied upon to kill *T. gondii* in meat.[873] Salting,

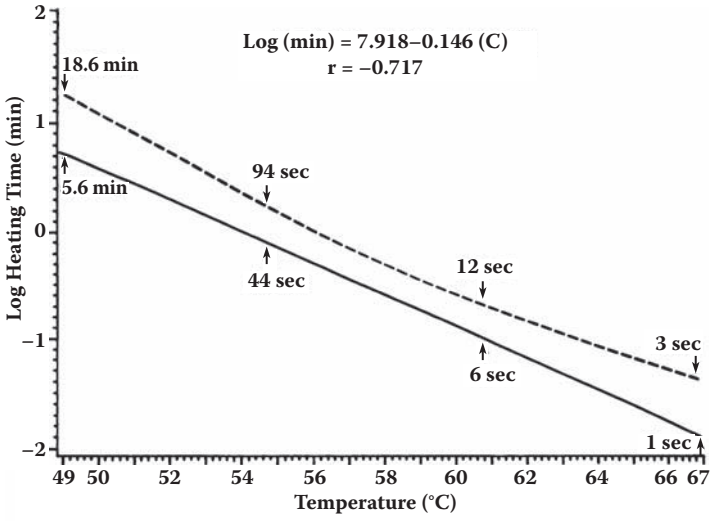

Figure 1.26 Heat killing of *T. gondii* tissue cysts. Linear regression (solid line) and the 99% upper confidence limits (dotted line) of the time required at each temperature for the inactivation of *T. gondii*. Three and one-half minutes representing come-up and come-down times must be added to the times obtained from the equation on the curves. From Dubey et al.[312]

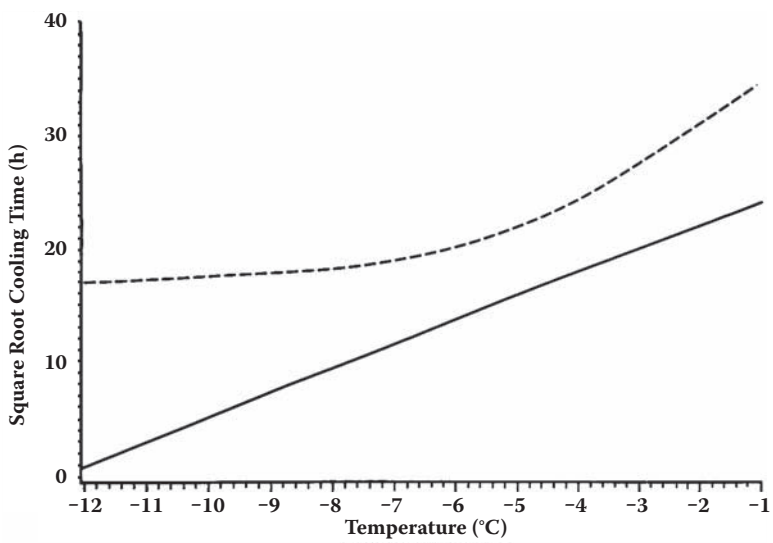

Figure 1.27 Effect of freezing on viability of *T. gondii* tissue cysts**.** The least square linear regression of freezing times and temperatures for the inactivation of *T. gondii* (solid line) expressed by the equation: Square root of time (h) = 26.72 + 16 (°C), with r = 0.77 and the 99% upper confidence interval for individual values (broken line). From Kotula et al.[783]

curing, and pickling procedures do kill tissue cysts,[873] but these procedures have not been standardized universally. Tissue cysts usually are destroyed by freezing (Figure 1.27) at –12°C.[783] In studies shown in Figure 1.26 and 1.27 the data were based on measuring the internal temperature of infected meat of a uniform thickness. Storage of meat at higher temperatures does not kill tissue cysts. They survive storage at 4–6°C for up to 2 months.[716] Some tissue cysts survive for several days after the death of an infected animal, even though its tissues have begun decomposing, and we have isolated viable *T. gondii* from the brain of a naturally infected Hawaiian crow whose carcass flesh had been completely eaten by maggots.[1374]

1.4.3 Transmission by Tachyzoites

The tachyzoite is a delicate organism unable to survive outside the body of its host and is usually destroyed by gastric secretions. It can infect by transplacental transmission from a mother to her fetus, by transfusion of packed leukocytes (ordinary blood transfusion is virtually free from risk), and laboratory accidents. Tachyzoites splashed on the face can enter the cornea of the eye and buccal mucosa.

Although *T. gondii* has been found in the semen of goats, sheep, and man, there is practically no risk of venereal transmission. It has also been found in saliva, but there is no evidence of its being spread by kissing. *T. gondii* infection can be transmitted by transplantation (see Chapter 2) and it may arise in two ways: first, from implantation of organ or bone marrow from an infected donor into a non-immune, immunocompromised recipient and, second, from a non-infected donor into an immunocompromised, latently infected recipient, thereby activating the latent infection. In these cases, both tachyzoites and tissue cysts might be involved, though tissue cysts are more likely. In both instances, cytotoxic and immunosuppressive therapy given to the recipient will increase the chances of active infection.

T. gondii can be found in milk.[283,455,1065] There is little, if any, danger from cow milk and in any case it is generally pasteurized or even boiled. Toxoplasmosis in humans was circumstantially linked to drinking raw goat milk[1032,1101,1129,1199] or human milk.[109] Milk from goats is more easily digested by children than cow's milk. Although transmission by milk is unlikely, there are other pathogens that make drinking raw milk unhealthy.

Prevalence of *T. gondii* in chicken eggs is extremely low and the ingestion of uncooked eggs is not considered an important risk for toxoplasmosis (see Chapter 12). However, eggs should be cooked thoroughly before human consumption due to health risks from other pathogens.

Parasitemia during pregnancy can cause fetal placentitis and spread of *T. gondii* to the fetus. In higher mammals (humans, sheep, goats, pigs), congenital infection occurs mainly if the dam becomes infected during pregnancy. Unlike humans, repeated congenital infection can occur in mice and hamsters and perhaps in other small mammals. Several litters may be infected from an infected mouse or hamster without re-infection from an outside source. Congenitally infected mice can themselves produce congenitally infected mice for ten or more generations.[97] Congenitally infected mice and rats may not develop antibodies to *T. gondii*, probably due to immune tolerance, and viable *T. gondii* has been isolated from naturally infected mice.[344] Although information from congenital infections in these small mammals is not generally relevant to that seen in higher mammals, specific biologic questions may be resolved in these hosts. For example, it is intriguing that in mice infected with avirulent strains and later challenged with a virulent strain, only the avirulent strain crossed the placenta. The strain of mouse, the stage of the parasite, and the route of inoculation can affect the rate of congenital transmission. Reichard and Gross[1088] elegantly summarized animal models of congenital toxoplasmosis.

1.5 EPIDEMIOLOGY

The extent of *T. gondii* infection in cats depends on the availability of infected birds and small mammals (see Chapter 18), which in their turn become infected by ingesting oocysts. One would expect that the prevalence would be higher in cats from rural areas than from urban areas, and in stray than in pet cats (see Chapter 3). There are no data on the relative importance of different intermediate hosts as sources of infection for cats (for prevalence in small mammals and birds, see later chapters). Historically, cats have been associated with domestic animals as an aid to rodent control. *T. gondii* can injure the neural tissues of infected mice and rats, resulting in impaired learning and memory and behavioral abnormalities.[1328,1344] (See Vyas et al. 2007[1328] and references therein for additional information.) *T. gondii*-infected rodents become less neophobic, leading to decreased aversion to the odor of cats. The hypothesis that *T. gondii* can manipulate the behavior of its hosts to its own advantages is fascinating (see Lamberton et al. 2008[794] and references therein for additional information). This behavior might help ensure that rodents would be eaten by cats and the life cycle would be preserved.

Serological surveys comparing infection in persons who had and had not had contact with cats have, however, given equivocal results. Some have found an association; others have not. The reason may lie in the questions asked and the age group surveyed; a neighbor's cat may be as dangerous as the owner's cat, and it is not cat contact that matters, but contact with oocysts in cat feces that is a universal hazard. Feline feces are generally hard and may remain confined to the area of defecation for a long time.

As said earlier, sporulated oocysts survive for long periods under moderate environmental conditions. Whether fruit and vegetables are contaminated with *T. gondii* oocysts in nature is not known, and there are no data on the efficacy of removal of oocysts from different types of fruits.[777]

Humans may acquire toxoplasmosis by petting dogs that have rolled over in infected cat feces,[481,845] and oocysts have been found in dog feces.[1151]

T. gondii infection in man and animals is widespread throughout the world, but varies in different geographical areas of a country. Causes for these variations are not yet known. Environmental conditions may determine the degree of natural spread of *T. gondii* infection. Infection is more prevalent in warm climates and in low-lying areas than in cold climates and mountain regions and in humid areas than in dry areas. This is probably related to conditions favoring sporulation and survival of oocysts in the environment.

Cultural habits and hygiene and not ethnicity or race of people may play a role. For example, in France, seroprevalence of *T. gondii* is high. This is probably related to the cooking and eating habits of the French. Eating raw or undercooked meat is in some measure related to high standards of living and it is more prevalent in the western world. Most Asians cook their meat well. In third world countries, pork is usually cooked well before consumption because of the presence of *Trichinella spiralis* and *Taenia solium*. These differences in cooking habits might account in part for lower prevalence rates of *T. gondii* infection in Africa and Asia than in Europe and America (see Chapter 2). Sources of meat for human *T. gondii* infection may be dependent on infection in local food animals. In the United States, beef has not been found infected with *T. gondii*, while meat from adult sheep (mutton) and horse meat is not eaten by people. Poultry is usually well cooked before consumption. *Trichinella* spp. infection in horses is considered rare because it is a herbivore, yet outbreaks of clinical trichinosis in humans in France were traced to eating uncooked horse meat imported from the United States.[33] If trichinosis is any indication of transmission by meat then many people are more likely to contract toxoplasmosis than trichinosis. The ingestion of lamb is probably an important source of *T. gondii* infection in many countries, especially Europe.[188,768] However, very little lamb is consumed in the United States.[396] The ingestion of undercooked meat (mainly beef) was considered as the main risk for *T. gondii* infection in humans in Serbia.[107,108]

It is not known what proportion of human *T. gondii* infection is due to eating raw or undercooked meat containing tissue cysts and what is due to oocysts on unwashed hands or vegetables; but it seems likely that where cats abound and defecate around and even in houses, as in Costa Rica and Brazil, oocysts will be the main source, whereas in Paris, where cats are better controlled and where much raw or undercooked meat is eaten, tissue cysts will be the main source. It may be possible in the future to differentiate between the two types of infection serologically, based on methods to detect stage-specific antibodies. Risk assessment studies should take into account that *T. gondii* can be transmitted in many ways and cats are everywhere, while results of assessment may vary with questions asked. Good examples of risk assessment studies are given in Frenkel et al.,[546] Weigel et al.,[1346] Jenum et al.,[724] Baril et al.,[71] Nash et al.,[969] and Jones et al.[737]

There are indications of a decline in the prevalence and rate of infection in the United States and Europe (see Chapter 2). Suggested reasons are (1) more hygienic maintenance of piggeries; (2) increased proportion of frozen meat; and (3) health education.

1.6 HOST-PARASITE RELATIONSHIPS

T. gondii usually parasitizes the host (both definitive and intermediate) without producing clinical signs. Only rarely does it cause severe clinical manifestations. Natural infections are probably acquired by ingestion of tissue cysts in infected meat or oocysts from food and water contaminated with cat feces. The bradyzoites from the tissue cysts or sporozoites from the oocyst penetrate the intestinal epithelial cells and multiply in the intestine (Figure 1.28). *T. gondii* may spread locally to mesenteric lymph nodes and to distant organs by invasion of lymphatics and blood. A host may die because of necrosis of the intestine and mesenteric lymph nodes before other organs are severely damaged.[274,1173] Focal areas of necrosis may develop in many organs (Figures 1.29–1.31). The clinical picture is determined by the extent of injury to these organs, especially vital organs such as the eyes, heart, and adrenals. Necrosis is caused by the intracellular growth of tachyzoites. *T. gondii* does not produce a toxin.

The host may die of acute toxoplasmosis, but more often recovers with the acquisition of immunity coincident with the appearance of humoral antibodies. Inflammation usually follows the initial necrosis. By about the third week after infection, tachyzoites begin to disappear from visceral tissues and may localize as tissue cysts in neural and muscular tissues (Figures 1.30 and 1.31). Tachyzoites may persist longer in the spinal cord and brain than in visceral tissues, because immunity is less effective in neural organs; this varies with the strain of *T. gondii* and the host species.

Latent toxoplasmosis may be reactivated by rupture of tissue cysts. When and why tissue cysts rupture is not known. Naturally infected asymptomatic cats can start shedding oocysts in feces as early as 4 days after injection with high doses of corticosteroids,[276] simulating shedding of oocysts 3 days after feeding on bradyzoites by naïve cats.

Why some individuals become ill due to toxoplasmosis whereas others remain well is not fully understood. Age, host species, strain of *T. gondii,* number of parasites, and route of administration may account for some of these differences. New World monkeys and Australian marsupials are the most susceptible to toxoplasmosis, whereas Old World monkeys, rats, cattle, and horses are highly resistant. Evolution, genetics, and ecology may have something to do with susceptibility. New World monkeys live above the ground in trees and thus may never be exposed to *T. gondii* oocysts. Because there were no cats in Australia and New Zealand before settlement by Europeans, marsupials may never have been exposed to *T. gondii* oocysts during the evolutionary process.[302] This increased susceptibility is not related to the number of tissue cysts or oocysts because even a few are lethal to squirrel monkeys. *T. gondii* was (and still is) highly pathogenic to *C. gundi,* a species living in dry and desert regions where survival of oocysts is impeded, hence there is little chance of infection.

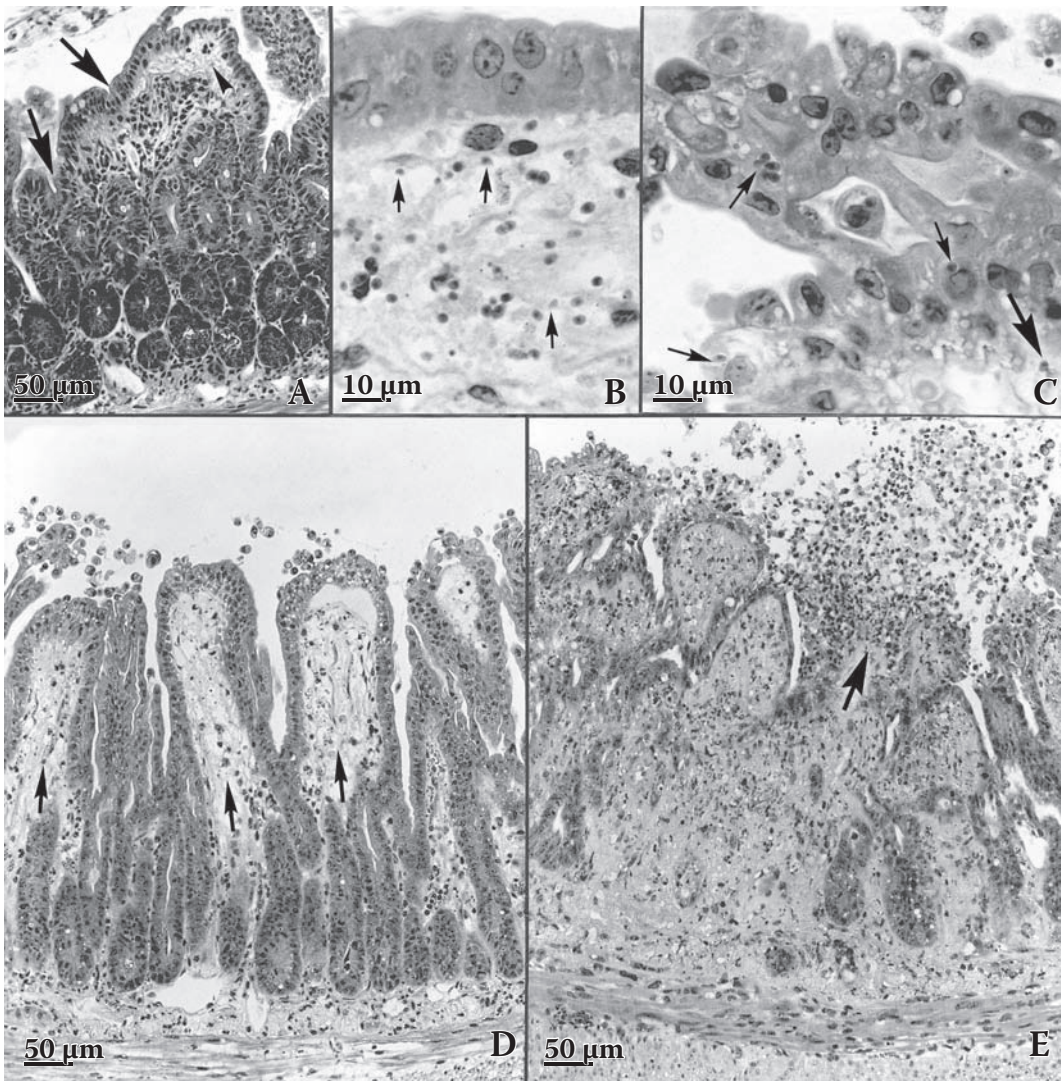

Figure 1.28 Pathogenesis of *T. gondii*-induced enteritis. Lesions in the ileum of mice fed *T. gondii* oocysts. H and E. **A.** Edema (arrowhead) in the lamina propria and fusion of villi (arrow). 24 hr p.i. **B.** Necrosis of lamina propria cells due to tachyzoites (arrows). The surface epithelium is unaffected. 48 hr p.i. **C.** Desquamation of surface epithelium in the lumen and tachyzoites in epithelial cells (small arrows). One tachyzoite (large arrow) is present in the lamina propria. 48 hr p.i. **D.** Groups of villi with necrosis of the lamina propria (arrows). 48 hr p.i. **E.** Focal ulceration of the tips of villi and necrosis extending up to the muscularis mucosa. 5 days p.i. From Dubey et al.[359]

It is unknown whether the severity of toxoplasmosis in immunocompetent humans and livestock is due to the parasite strain, host variability, or other factors. Although there is much speculation about the role of parasite virulence and disease in humans and animals, the host genetics may also be important. Experimentally, oocyst-induced infections are more severe than those induced by tissue cysts and bradyzoites by the natural oral route, irrespective of the dose.[274,302,358,359] Severe clinical toxoplasmosis was reported in humans and was linked epidemiologically to ingestion of *T. gondii* oocysts in food or water.[83,116,236,1274] Little is known concerning the effect of race or geography on clinical toxoplasmosis in humans, except that there is a high prevalence of eye disease in southern Brazil.[595,669,1192]

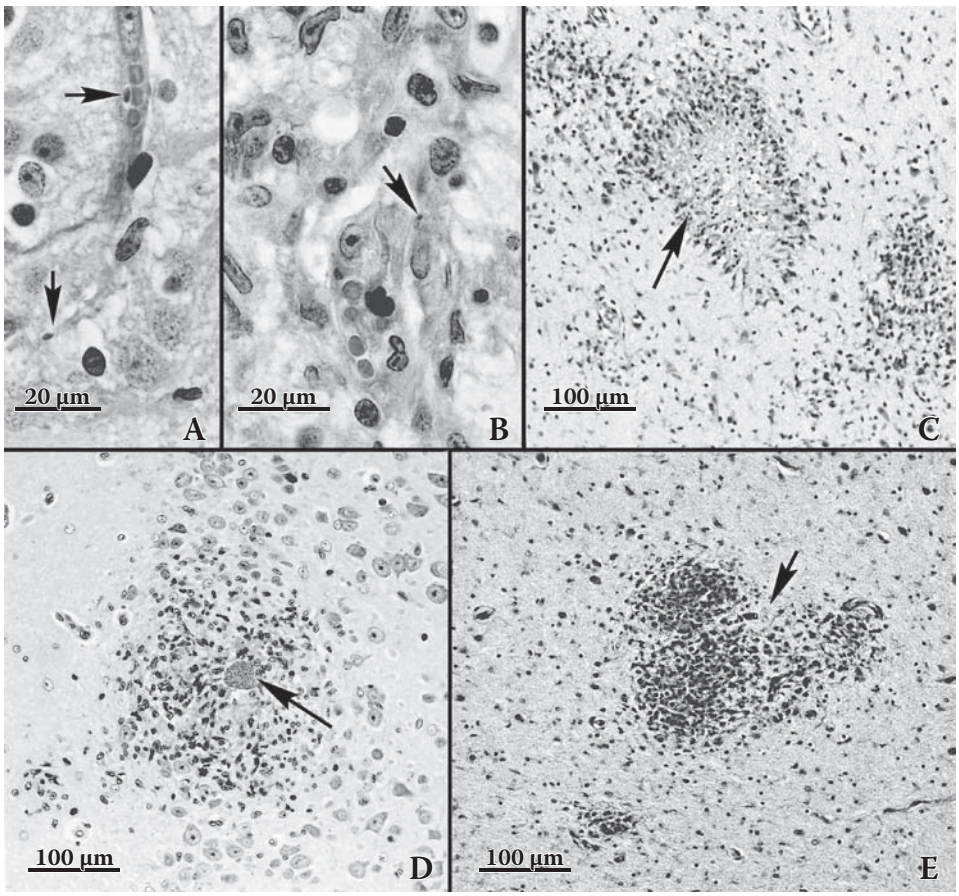

Figure 1.29 Pathogenesis of neural lesions in the brains of congenitally infected kittens. **A**. Two tachyzoites (arrows) in capillary endothelium. **B.** Vasculitis with one visible tachyzoite (arrow). **C.** A focus of central necrosis (arrow) with peripheral gliosis. **D.** A glial nodule surrounds a *T. gondii* tissue cyst. **E.** Perivasculitis and gliosis (arrow) without visible *T. gondii*. From Dubey et al.[351]

Recently attention has been focused on the genetic variability among *T. gondii* isolates from apparently healthy and sick hosts. Circumstantial evidence suggests that certain genetic types of *T. gondii* may be associated with clinical toxoplasmosis in humans in the United States.[111,606,683,761,763] It has been suggested that type I isolates or recombinants of types I and III are more likely to result in clinical toxoplasmosis (see Khan et al.[762,763]), but genetic characterization has essentially been limited to isolates from patients with toxoplasmosis. In France, type II strains are largely predominant in both benign and severe congenital toxoplasmosis suggesting no apparent association between severity of disease and genotype.[15] In immunocompromised patients, the genotype of *T. gondii* strains (type II vs. non-type II) is not associated with the clinical presentation or the severity of infection but is strongly linked to the geographical area of infection: type II strains are common in patients who acquired infection in Europe whereas non-type II strains are more frequently observed in toxoplasmosis acquired outside Europe, especially in sub-Saharan Africa.[18] In humans in French Guiana and Suriname, severe cases of toxoplasmosis in immunocompetent patients have been related to *T. gondii* strains with atypical genotypes.[17,245]

There is very little information regarding the genetic diversity of *T. gondii* isolates circulating in the general human population. Therefore we must be cautious in claiming a linkage between

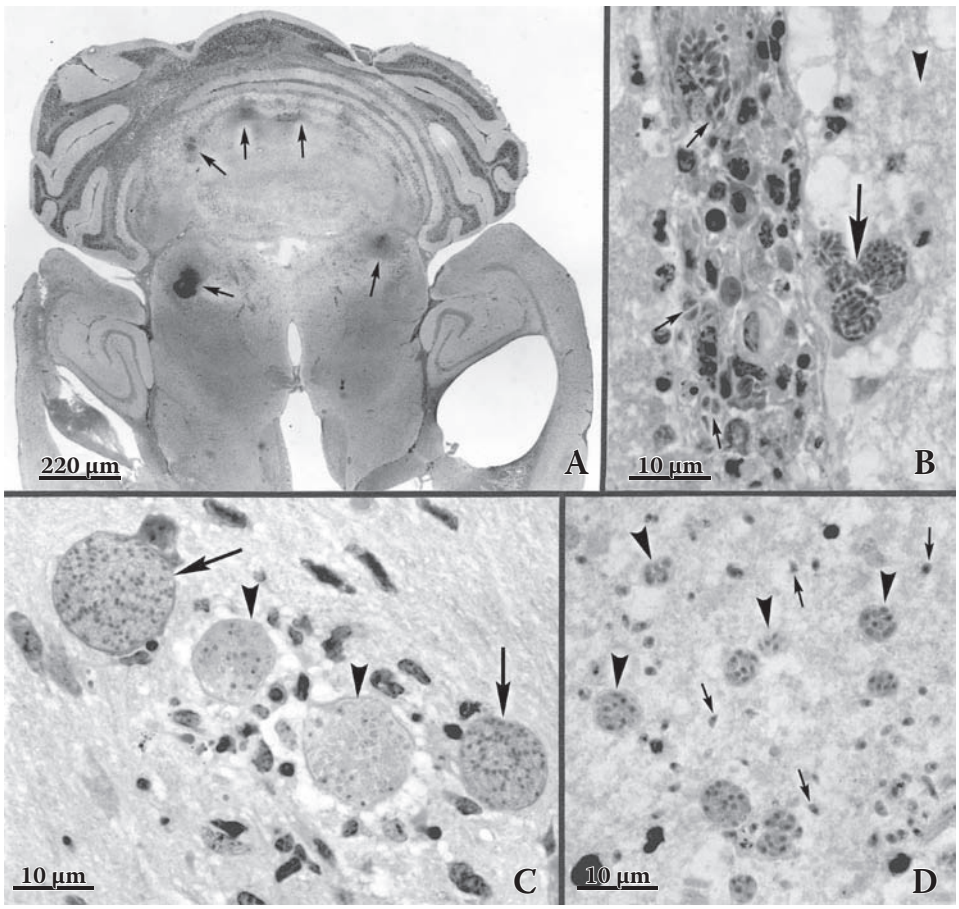

Figure 1.30 *T. gondii*-induced encephalitis. Sections of brain of a mouse 87 days after feeding oocysts. H and E. **A.** Coronal section showing grossly visible areas (arrows) of necrosis in cerebellum and pons. **B.** Necrosis in cerebrum and numerous tachyzoites (small arrows) in a meningeal blood vessel with severe meningitis, a group of extravascular tachyzoites (large arrow), and necrosis (arrowhead) **C.** Four tissue cysts, two of which (arrowheads) are degenerating. **D.** Numerous tissue cysts (arrowheads) and tachyzoites (arrows). From Dubey et al.[363]

parasite genotypes and disease presentations without more complete knowledge of the *T. gondii* genotypes in human populations and the environment. Mouse-virulent strains with atypical genotypes, similar to strains from clinical cases of humans in Brazil,[763] have been found in asymptomatic chickens and cats from Brazil.[384,409,1042] Recently it was suggested that the outcome of congenital infection with *T. gondii* infection may be affected by genetic predisposition.[719]

Distinctions should be made among susceptibility, pathogenicity, and resistance to clinical toxoplasmosis. For example, adult rats, cattle, and horses are resistant to clinical toxoplasmosis. However, there is no difference in infectivity of *T. gondii* to adult rats and mice. To illustrate this, I diluted 10-fold oocysts of the VEG strain (a type III strain) and orally inoculated rats and mice. One oocyst was infective to both rats and mice (Table 1.18). However, 100 oocysts were lethal to mice, but rats survived 100,000 oocysts (Table 1.18). In cattle fed oocysts, *T. gondii* initially multiplied in cattle tissues but was quickly eliminated.[288] Size and weight of the animal species is not the main determinant, because a single oocyst was infective to pigs but 1000 oocysts are needed to infect cats.[353,397] Although viable *T. gondii* has been isolated from tissues of adult horses, there is no confirmed report of clinical toxoplasmosis in horses.[302]

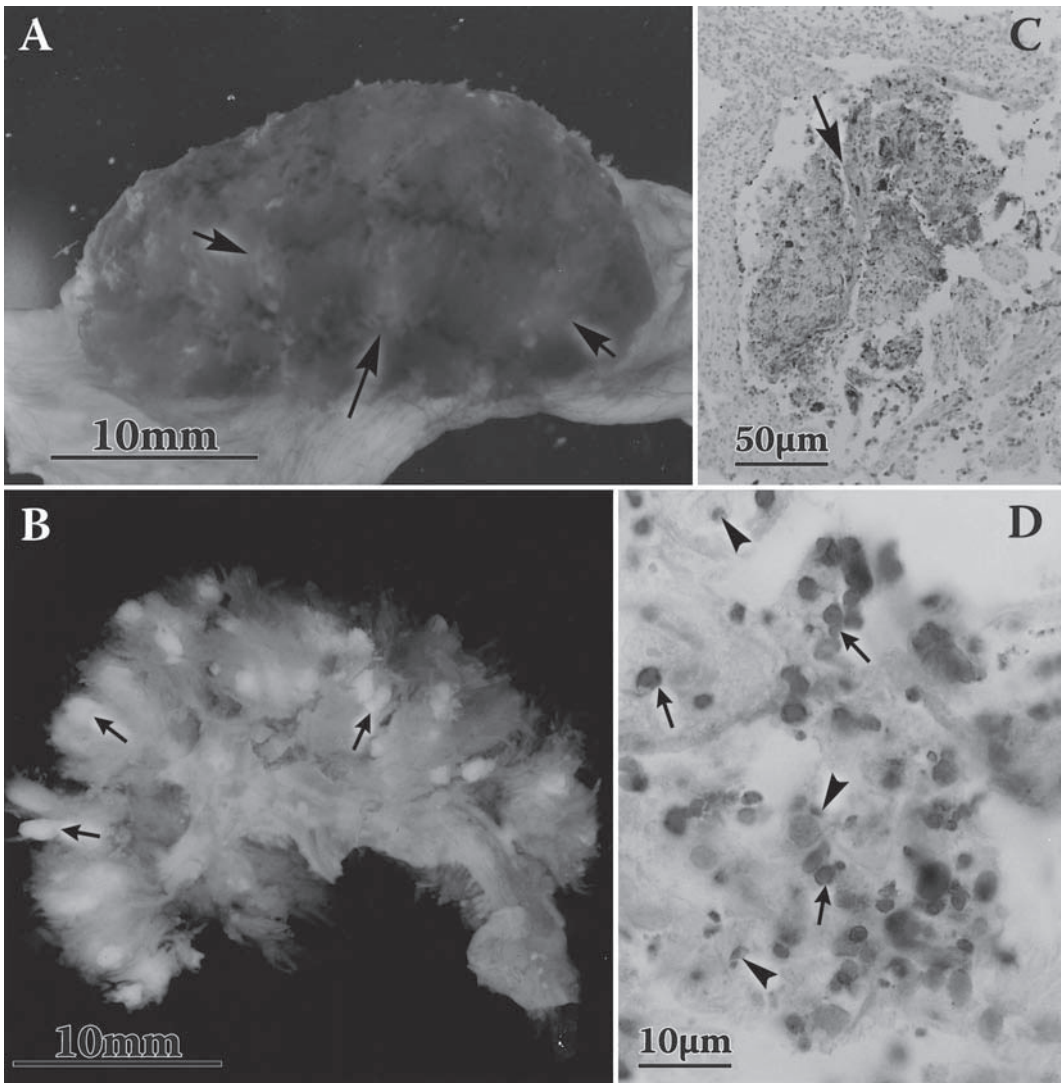

Figure 1.31 (Please see color insert following page 46.) *T. gondii*-induced placentitis in naturally infected goat (A, C, D), and sheep (B). Both of these animals aborted dead fetuses. Placenta in goats and sheep consists of numerous (>300) cotyledons, the site of fetal attachment. *T. gondii* causes necrosis of the cotyledons that can be macroscopic. **A.** Grossly visible foci of necrosis (arrows) in fetal cotyledon. Unstained. **B.** Section of fetal cotyledon. Note foci of necrosis (arrows). Unstained. **C.** Histologic section of fetal cotyledon. Note a focus of necrosis and mineralization. IHC staining with *T. gondii*-specific antibodies. **D.** Higher magnification of the lesion in **(C)**. Note numerous intact tachyzoites and particulate antigen from degenerating tachyzoites. From Dubey and Stewart.[391a]

Pathogenicity of *T. gondii* is closely related to the virulence of the strain and host species. Because of cost, convenience, availability, susceptibility, and extreme rarity of natural infection, mice are generally preferred as an experimental animal model for toxoplasmosis. *T. gondii* strains vary in their pathogenicity to mice. Such differences are most marked in primary isolations since pathogenicity may be increased by frequent passages in a given host.

Adaptation to a given host may, however, differ with different strains of *T. gondii*. For example, the RH strain killed four out of five infected mice on first passage in 17 to 21 days and after only three intraperitoneal (i.p.) passages, and killed all inoculated mice in 3 to 5 days.[1127] The

Table 1.18 Infectivity and Mortality in Rats and Mice Fed Oocysts of the VEG Strain of *T. gondii*[a]

No. of Oocysts	Rats No. Infected	Mice No. Infected
1,000,000	5 (3)[b]	5 (5)[b]
100,000	5	5 (5)
10,000	5	5 (5)
1,000	5	5 (5)
100	5	5 (5)
10	5	5 (2)
1	3	4 (0)
<1	0	0

[a] From Dubey.[352]
[b] Five rats and five mice were fed oocysts in each group; no. in parenthesis are dead animals. Infectivity is based on finding *T. gondii* and specific antibodies.

pathogenicity of this strain has been stable for the last 60 years. On the other hand, with the M-7741 strain, even after 62 passages, mice injected with 100,000 tachyzoites survived for more than 9 days.[279] Until recently, the virulence of strains has been measured by inoculation of *T. gondii* into mice by artificial parenteral routes. Titration of oocysts and tissue cysts in mice by the oral route may provide a better measure of the natural virulence of the strain in nature than by inoculation by parenteral routes. Oocysts of most strains of *T. gondii* are pathogenic for mice.

Certain strains of mice are more susceptible than others, and results within the same strain may also vary. Generally, inbred strains are more resistant to clinical toxoplasmosis and, in part, resistance is genetic. However, there is no indication from the published literature that the susceptibility to toxoplasmosis differs in any breed or race of large animal species or man.

Sex may determine the outcome of *T. gondii* infection in a host. Lymphadenopathy was found to be more prevalent in human males than females under the age of 15 years and more frequent in females than males of 25 years and over, although there was no difference in the rate of infection.[95] These sex-related differences in the prevalence of clinical toxoplasmosis were probably due to the effect of female hormones on the immune system.

Other unknown factors vaguely classified as "stress" may affect the results of *T. gondii* infection in a host. More severe infections are found in pregnant or lactating mice than in non-lactating mice. Concomitant infections may make a host more susceptible or resistant to *T. gondii* infection. Clinical toxoplasmosis in dogs is often associated with canine distemper virus infection.

1.7 DIAGNOSIS

Diagnosis is made by biologic, serologic, and histological methods, or by some combination of these. Clinical signs of toxoplasmosis are nonspecific and cannot be depended upon for a definite diagnosis.

T. gondii can be isolated from patients by inoculation of laboratory animals and tissue culture. The choice of inoculum will depend upon the circumstances. Secretions, excretions, body fluids, and tissues taken by biopsy, such as lymph nodes or muscle tissue, are possible specimens from which to attempt isolation. Cerebral spinal fluid from a child with possible congenital infection and encephalitis or lymph node material from a person with lymphadenopathy are good sources of *T. gondii*. Details of isolation procedures are given in Section 1.11.

Diagnosis can also be made by finding *T. gondii* in host tissue removed by biopsy or at necropsy. A rapid diagnosis may be made by microscopic examination of impression smears stained with Giemsa stain (Figure 1.2A). Well-preserved *T. gondii* are crescent-shaped and stain well with any of the Romanowsky stains. However, degenerating organisms, which are commonly found in lesions, usually appear oval and their cytoplasm stains poorly as compared to their nuclei. Diagnosis should not be made unless organisms with typical structure are seen, because degenerating host cells may resemble degenerating *T. gondii*. In sections, the tachyzoites are usually oval to round. Occasionally, tissue cysts may be found in areas with lesions. IHC staining can confirm the diagnosis within a few hours. The finding of tachyzoites indicates active infection and is diagnostic; finding of tissue cysts may indicate latent infection and is not necessarily diagnostic. Polyclonal antibodies are better than monoclonal antibodies for IHC staining. The specificity depends on the quality of polyclonal antibodies.

Finding *T. gondii* antibody can aid diagnosis. Numerous serologic procedures used to detect humoral antibodies are described in Section 1.11 (Techniques).

Even with all the available serologic tests, the results of examining one positive serum sample only establishes that the host has been infected at some time in the past. A 16-fold rise in titer in serum taken 2 to 4 weeks after the first specimen was tested definitively indicates acute acquired infection, but this may happen within 6 weeks of acquiring infection, and the titer may have reached its maximum before toxoplasmosis is suspected. Antibody titer has no correlation with the severity of signs or symptoms.

Diagnosis of toxoplasmosis specific to each host is discussed in subsequent chapters.

1.8 PROTECTIVE IMMUNITY AND VACCINATION

Host protection against *T. gondii* is complex, involving innate and specific immunity. In the 1940s humoral antibodies were found to kill extracellular but not intracellular tachyzoites.[1125,1128] Over the next 50 years protective immunity was found to be mediated largely by lymphoid immune cells.[534] Interferon gamma is the main cytokine for protection.[580,1249] The role of different immune cells, including CD4 and CD8 cells, cytokines, and antibodies in mediation of immunity to toxoplasmosis has been reviewed by others who are leaders in this field.[143,523,1043,1104,1250]

Rapid humoral responses and recovery of most hosts from *T. gondii* infection indicate that *T. gondii* is highly immunogenic. However, re-infection is possible, even in hosts solidly immune to *T. gondii*.

Infection or vaccination with avirulent strains of *T. gondii* usually induces life-long immunity, but vaccination of human beings with such strains cannot be recommended, as it is not without risk, particularly to the fetus. Vaccination with killed *T. gondii* has been unsuccessful and provides at best only marginal protection. Attenuation of *T. gondii* by irradiation for vaccination is also not practical because the virulence of *T. gondii* is not affected even at high doses of irradiation. Vaccination of human beings with mutants of *T. gondii* is also not practical because there is no guarantee that the mutant would not revert. Fishback and Frenkel,[511] and Schaap et al.[1150] have discussed various strategies for vaccination of intermediate and definitive hosts for immunization. Vaccination of sheep with a live cystless strain of *T. gondii* reduces neonatal mortality in lambs and this vaccine is available commercially (see Chapter 4). To date, there is no vaccine suitable for human use.

It would be desirable to have a killed vaccine for the prevention of *T. gondii* infection in cats that would prevent oocyst shedding. Such a vaccine is not yet available, and in view of the enormous numbers of cats and the difficulty of catching them, its mass use is unlikely. Infection of cats with an oocyst-less strain of *T. gondii* (such as the RH strain) or tachyzoites does not prevent oocyst shedding on challenge.[551] Parenteral inoculation of *T. gondii* in cats leads to oocyst shedding in about half of the inoculated cats. An oral live vaccine using bradyzoites of a mutant strain (T-263) can prevent cats from shedding *T. gondii* oocysts.[544,551,894] Commercial production of this vaccine was discontinued because of the vaccine's need to be kept frozen, its short shelf life, high cost, and lack of interest on the part of cat owners.

1.9 TREATMENT

Sulfonamides and pyrimethamine (Daraprim®) are two drugs widely used for therapy of toxoplasmosis. These two drugs act synergistically by blocking the metabolic pathway involving *p*-aminobenzoic acid and the folic-folinic acid cycle, respectively.[484,1126] These two drugs are usually well tolerated, but sometimes thrombocytopenia and/or leukopenia may develop. These effects can be overcome by giving patients folinic acid and yeast without interfering with treatment, because the vertebrate host can utilize presynthesized folinic acid while *T. gondii* cannot. The commonly used sulfonamides, sulfadiazine, sulfamethazine, and sulfamerazine, are all effective against toxoplasmosis. Generally, any sulfonamide that diffuses across the host cell membrane is useful in anti-*T. gondii* therapy. While these drugs have beneficial action when given in the acute stage of the disease process when there is active multiplication of the parasite, they will not usually eradicate infection. It is believed that these drugs have little effect on subclinical infection, but the growth of tissue cysts in mice has been restrained with sulfonamides.[96] Sulfa compounds are excreted within a few hours of administration; therefore treatment has to be administered in daily divided doses (four doses of 500 mg each) usually for several weeks or months. A loading dose (75 mg in man) of pyrimethamine during the first three days has been recommended because it is absorbed slowly and binds to tissues. From the fourth day, the dose of pyrimethamine is reduced to 25 mg and 2 to 10 mg of folinic acid or 5 to 10 g of baker's yeast is added.[536]

Because pyrimethamine is toxic, some authors use a combination of trimethoprim and sulfamethoxazole as possible alternatives to pyrimethamine and sulfadiazine. Although trimethoprim, like pyrimethamine, is a folic acid antagonist, it has no synergistic effect in combination with sulfamethoxazole against murine toxoplasmosis. Therefore trimethoprim is not recommended against toxoplasmosis.

Certain other drugs, spiramycin, piritrexin, roxithromycin, clindamycin, cyclosporin A, atovaquone, ponazuril,[933] a novel triazine,[958] and others have been found effective in experimentally induced *T. gondii* infection in animals or cell cultures (see McCabe[898]). Spiramycin produces high tissue concentrations, particularly in the placenta, without crossing the placental barrier. Spiramycin, however, has inferior anti-*T. gondii* properties compared with sulfadiazine and pyrimethamine. Clindamycin is reported to give good results, but may cause ulcerative colitis. In summary, little progress has been made to replace sulfadiazine and pyrimethamine in the last 50 years. Unlike other drugs, atovaquone was reported to kill tissue cysts.

1.10 PREVENTION AND CONTROL

To prevent infection of human beings by *T. gondii*, hands should be washed thoroughly with soap and water after handling meat. All cutting boards, sink tops, knives, and other materials coming in contact with uncooked meat should be washed with soap and water because the stages of *T. gondii* in meat are killed by water. Meat of any animal should be cooked thoroughly until internal temperature has reached 66°C[312] before human or animal consumption, and tasting meat while cooking or seasoning homemade sausages should be avoided. Cooking times will vary with the thickness and the type of the cut of meat. Microwave cooking is unreliable for killing *T. gondii*. Freezing meat to an internal temperature of –12°C is effective in killing tissue cysts; freezing meat overnight in a household freezer is effective in killing most tissue cysts.[783] Salting, curing, smoking, and the addition of products to meat to enhance color and taste (enhancing solutions) can have deleterious effect on the viability of *T. gondii* in meat, but there is too much variability in standards for these procedures to make a safety recommendation.[354,658,916] Pregnant women, especially, should avoid contact with cats, soil, and raw meat. Pet cats should be fed only dry, canned, or cooked food. The cat litter should be emptied every day (to prevent sporulation of oocysts), preferably not by a pregnant woman. Gloves should be worn while gardening, while changing cat litter, and while

handling soil potentially contaminated with cat feces. Owners may also be advised to keep dogs away from the cat litter box to prevent ingestion of and passage through of oocysts.[545,1151] Vegetables should be washed thoroughly before eating, because they may have been contaminated with cat feces. Irradiation at 50 krads or high-pressure processing at 400 MPa are effective in killing tissue cysts[294,336,854] as well as oocysts.[348,369, 852] Pregnant women should be aware of the dangers of toxoplasmosis (see Chapter 2).

To prevent infection in cats, they should never be fed uncooked meat, viscera, or bones, and efforts should be made to keep cats indoors to prevent hunting. However, if a choice has to be made, frozen meat is less infective than fresh meat, and beef is less likely to contain *T. gondii* than is horse meat, pork, or mutton. Because cats cannot utilize plant sources of vitamin A, some owners feed raw liver to improve the coat of their cat. This practice should be discontinued because *T. gondii* tissue cysts frequently are found in the liver of food animals, and because cat foods contain most essential nutrients; there is no need to feed raw meat to cats. Trash cans also should be covered to prevent scavenging. Cats should be spayed to control the feline population on farms.

Pigs and other dead animals should be removed promptly to prevent cannibalism by pigs and scavenging by cats. Sheep that have aborted due to toxoplasmosis usually do not have recurrent toxoplasmic abortions, and thus can be saved for future breeding. Fetal membranes and dead fetuses should not be handled with bare hands and should be buried or incinerated to prevent infection of felids and other animals on the farm. Cats should not be allowed near pregnant sheep and goats. Grain should be kept covered to prevent oocyst contamination.

To prevent infection of zoo animals with *T. gondii*, cats, including all wild felids, should be housed in a building separate from other animals, particularly marsupials and New World monkeys. Cats as a rule should not be fed uncooked meat. Dissemination of *T. gondii* oocysts in the zoo should be prevented because of potential exposure of children. Brooms, shovels, and other equipment used to clean cat cages and cat enclosures should be autoclaved or heated to 70°C for at least 10 min. While cleaning cages, animal caretakers should wear masks and protective clothing. Feline feces should be removed daily to prevent sporulation of oocysts.

1.11 TECHNIQUES

1.11.1 Cultivation

T. gondii has not been grown in cell-free media, even though it contains cytochromes, glycolytic, hydrolytic, lipid, and respiratory systems.[49,190,516,524]

T. gondii can be cultivated in laboratory animals, chick embryos, and cell cultures. Mice, hamsters, guinea pigs, and rabbits are all susceptible, but mice are generally used as hosts because they are more susceptible than other species and are not naturally infected when raised in the laboratory on commercial dry food free of cat feces.

1.11.1.1 Tachyzoites

Tachyzoites grow in the peritoneal cavity of mice, sometimes producing ascites, and also grow in most of the other host tissues after i.p. inoculation with tissue cysts, tachyzoites, or oocysts. Virulent strains usually produce illness in mice and sometimes kill them within 1 to 2 weeks. Tachyzoites of a virulent strain can be aspirated from the peritoneal cavity after light anesthesia. Avirulent strains grow slowly in mice, and free tachyzoites of these strains are often difficult to obtain. Immunosuppression with corticosteroids (cortisone acetate, 2.5 mg, injected s.c. twice weekly or dexamethasone phosphate orally 10 μg per ml of drinking water) in out-bred mice or interferon gamma gene knock-out (KO) mice can enhance tachyzoite multiplication. To obtain tachyzoites from the peritoneal exudates (pex),

sick mice should be killed, bled out, and skinned to expose the abdomen. Tachyzoites can be aspirated from mouse peritoneal cavities by injecting 1–5 ml of phosphate buffer saline (PBS) with a 20–22 gauge needle and a 6 ml syringe and gently aspirating the pex.

Frequent, rapid passage of tachyzoites of low virulence may increase virulence. Strains become more virulent for mice after rapid i.p. passage. But with the RH strain, which has been passed twice weekly for many years in mice, the ID_{50} and LD_{50} have been identical for years.[279] Repeated frequent passages of a strain appear to modify some other biological characteristics. For example, old laboratory strains like the RH strain and some lines derived from the Beverley and M-7741 strains no longer produce oocysts after tissue cysts are fed to cats.[542] Loss of oocyst production by mouse passage can perhaps be avoided by storing the oocysts at 4°C. Oocysts can survive up to 27 months at 4°C, but the number of infectious oocysts decreases sharply between 12 and 18 months. There is no change in the virulence of *T. gondii* oocysts stored at 4°C.

T. gondii tachyzoites will multiply in almost all mammalian cell lines in tissue cultures. Spread out cells such as fibroblasts are commonly used. The yield of tachyzoites will vary with the cell line and the strain of *T. gondii*. Mouse-"virulent" strains rapidly destroy the cells while "avirulent" strains grow slowly causing minimal cell damage. The mean generation time of tachyzoites of the RH strain is 5 hours. Unlike passage in mice, passage of tachyzoites in cell culture is not known to alter the virulence of the organism. Striepen and Soldati[1239] provide details of *in vitro* culture, separation, and cryopreservation of *T. gondii* in human foreskin fibroblasts (HFF) for cell biology studies.

Chick embryos can be infected by any route, but the chorioallantoic membrane route is most commonly used. *T. gondii* tachyzoites form lesions on the chorioallantoic membrane like those produced by pox viruses.

Various methods (differential centrifugation gradients, treatment with lectins, filtration through sintered glass filters, nylon wool, glass wool, and digestion) have been described to separate *T. gondii* tachyzoites from host cells with varying success.[247]

1.11.1.2 Tissue Cysts

Tissue cysts are obtained by injecting tachyzoites, bradyzoites, or oocysts into mice. To obtain tissue cysts from mice inoculated with a virulent strain, it is necessary to administer anti-*T. gondii* chemotherapy (sulfadiazine sodium) to prevent death from acute toxoplasmosis before tissue cysts form. The effective dose varies with the virulence of the *T. gondii* strain. Sulfadiazine sodium (available from Sigma) in drinking water at a concentration of 15 mg in 100 ml from day 4 to day 12 of infection is effective in controlling growth of the M-7741 strain of *T. gondii,* but will not control growth of the RH or GT-1 strains of *T. gondii*. For the control of the growth of these strains, 125 mg of sulfadiazine sodium in each 100 ml of drinking water is needed from day 1 to day 21 after infection. Sulfadiazine is insoluble in acidic pH and therefore NaOH should be added to dissolve it in water in case the sulfadiazine sodium is not available. Conversely, with slow-growing strains, it may be necessary to inject immunosuppressive drugs to increase the number of tissue cysts. For maintenance of tissue cysts in mice, these animals should be inoculated s.c. with brain homogenate every 3 to 6 months. Tissue cysts are easily recognized in the mouse brain about 6 to 8 weeks after infection, and their size may vary with the strain of *T. gondii*. They can be released from the tissue by grinding the brain in a mortar and suspending the homogenate in saline. Many tissue cysts remain intact after grinding the brain tissue.

Cornelissen et al.[191] described a method to separate tissue cysts from the brains of mice after suspensions of brain homogenates were run on discontinuous Percoll gradients. According to them, *T. gondii* tissue cysts have a specific gravity of 1.056. This method has been widely used. Tissue cysts can also be separated on discontinuous gradients using 25–30% Percoll[105,1051] or a 20% dextran solution.[552] The degree of success in purifying tissue cysts depends on the host tissue and the amount of blood contamination. To minimize red blood cell and tissue contamination, the mice

should be bled out before harvesting brains and the brain homogenate passed through a 90-μm sieve. Tissue cysts of *T. gondii* are smaller than 90 μm and pass through the sieve. Tissue cysts can be stored in saline at 4°C for a week or so without loss of infectivity.

Tissue cysts can be grown in cultured cells, but the yield is lower than in mice. Most strains of *T. gondii* will spontaneously develop tissue cysts in cell culture with no manipulation. However, the numbers of tissue cysts produced spontaneously is low and manipulation of the cell culture system is needed to increase the numbers of tissue cysts.

Tachyzoite-to-bradyzoite conversion can be induced by implementing external stress on various types of infected cell lines. Many investigators use pH manipulation to induce *in vitro* stage conversion.[1349] Both acidic (pH 6.6) and basic (pH 8.0 to 8.2) manipulations will cause conversion of tachyzoites to bradyzoites. Exposure of extracellular tachyzoites to pH 8.1 medium for 1 hr increases the formation of bradyzoite/tissue cysts. Mitochondrial inhibitors (oligomycin, antimycin A, atavoquone, rotenone, myxothiazol, carbonyl cyanide m-chlorophenyl-hydrazone), temperature stress (40°C), and chemical stress (sodium arsenite) will also induce tachyzoite-to-bradyzoite transformation.[363,448,1286] Tissue cysts grown in cell culture are fragile and it is not easy to separate tissue cysts from bradyzoites, tachyzoites, and host cells.

1.11.1.3 Oocysts

Oocysts can be obtained by feeding tissue cysts from chronically infected mice to *T. gondii*-free cats. It is preferable to use recently weaned (10- to 12-week-old) kittens because they are easy to handle, their bowel movements are more regular, and they are less likely to be naturally infected. For feeding *T. gondii* to cats, some of the brain from an infected mouse should be crushed between a cover glass and glass slide and examined under the microscope to confirm the presence of tissue cysts. The unhomogenized brain may be fed to the cat by placing it at the back of the tongue of the cat. The rest of the mouse carcass may be fed to the cat after cutting it into small pieces or blending it in a chilled blender. Some cats will not eat mice or will vomit when "force fed" even after they have been starved for several days. Such cats can be lightly sedated using ketamine hydrochloride and mouse homogenate and can then be "force fed" by placing the inoculum on the back of the tongue. Swallowing reflexes are unaffected by ketamine hydrochloride. Normally oocysts will appear in cat feces 3 to 10 days after the cat ingests tissue cysts.

Within the laboratory setting, working with infectious oocysts poses the greatest risk of acquiring infection since this stage is environmentally resistant; care must be taken to keep oocysts contained. The primary hazard is through ingestion of oocysts. During the period of oocyst shedding, *T. gondii*-infected cats are housed individually in stainless steel cages in a room with limited access. In our facility, each cage has a 2-kg stainless steel litter pan with a heavy bottom so that cats cannot tip it and a bottom tray to catch the spilled material. Feces are collected daily from each litter pan using disposable gloves and tongue depressors. The litter pan is filled to about one-half the depth with crushed corn cobs as bedding. During the period of oocyst shedding, the floor under and in front of the cages is covered with plastic sheets to catch any material that may be shed by cats; the plastic sheets are replaced daily and used sheets are incinerated. During the period of oocyst shedding, the litter pans and bottom trays are not washed in order to avoid the spread of oocysts. When cats have stopped shedding oocysts, they are killed or transferred to clean cages. The contaminated cages, including bottom trays, food and water dishes, and litter pans, are first run through a steam cage washer (internal temperature of >70°C) to kill oocysts before cages are scrubbed. All bedding and waste from the room housing infected cats are incinerated. Feces are stored in a refrigerator (4°C) to prevent sporulation prior to being processed in a separate building.

All feces for each cat are collected daily from 3 to 21 days after feeding tissue cysts and examined for *T. gondii* oocysts. For initial examination (screening), approximately 10 g of feces from each cat for each day are mixed thoroughly with 5 volumes of sucrose solution (specific gravity 1.15

or higher), poured into a 50-ml tube, and centrifuged at 1,180 *g* (2,000 rpm) for 10 min. A drop of fecal float from the top of the meniscus is examined microscopically for *T. gondii* oocysts between a coverslip and a glass slide.

For collection of large numbers of oocysts, those fecal samples containing a sufficient number of oocysts are mixed with water with a few drops of detergent (household soap works well) and shaken on a shaker until feces are completely broken. The mixture is filtered through gauze and centrifuged in 250-ml bottles at low speed (1,000 rpm, 15 min) without brake. The supernatant is discarded in a bottle and the sediment mixed with water. The bottles are centrifuged two or three times, with the supernatant decanted, and replaced with clean water each time. After this, the final supernatant is decanted, and the sediment mixed with 5 volumes of sucrose solution of 1.15 specific gravity and centrifuged for 10 min at 2,000 rpm in 50-ml tubes with a conical bottom. Most oocysts float to the top of the tube and can be aspirated. However, not all oocysts rise to the top. Therefore the entire supernatant (40–45 ml) can be poured into a 250-ml bottle, mixed thoroughly (but not vigorously shaken to prevent frothing), and centrifuged at 1,500 rpm for 15 min. The sediment containing the oocysts should be washed one more time and then mixed with 2% H_2SO_4 and aerated on a shaker for 7 days at room temperature (20–22°C). In my laboratory, all supernatant and the leftover feces are incinerated and not poured down the drain.

Sporulated oocysts have a lighter density than the unsporulated oocysts. Oocysts can be further cleaned using a cesium chloride gradient. We[1229] modified the procedure of Dumètre and Dardé.[440]

1. After floating oocysts in sucrose solution, add 10 ml of fecal solution (in 2% sulfuric acid) into a new 50-ml falcon tube. Neutralize the acid with 6 ml of 1N NaOH and bring volume to 50 ml with water. Vortex to mix well.
2. Centrifuge fecal sample for 20 min at 1,200 × *g* at room temperature with no brakes.
3. Discard supernatant and resuspend pellet in Tris buffer (TE, 50 mM Tris, 10 mM EDTA, pH 7.2).
4. Prepare stock solution of CsCl (specific gravity = 1.15) by mixing 21.75 g of CsCl with 103.25 ml of TE buffer and prepare gradient as shown in table below:

Solution	Specific Gravity	TE	CsCl (1.150 specific gravity)
A	1.050	30 ml	20 ml
B	1.110	20 ml	30 ml
C	1.125	10 ml	40 ml

5. Pour discontinuous gradient in a Nalgene Oak Ridge (Nalgene: Oak Ridge polycarbonate tubes 29 × 107 mm 50 ml, Cat #: 05-529C). Gradient is formed by carefully underlaying each solution using syringe/needle with a two-way stopcock as illustrated in (Figure 1.32).
6. Slowly add the following in the order listed below into the bottom of the Nalgene Oak Ridge tube (Note: flow rate should 0.5 ml/sec or slower):
   i. 10 ml TE/oocysts suspension sample
   ii. 8 ml of solution A
   iii. 8 ml of solution B
   iv. 8 ml of solution C
7. Centrifuge at 12,000 × *g* 60 min + 4°C with no brakes. For best separation, use a high-speed swinging bucket rotor, but a fixed-angle rotor is OK.
8. Collect oocysts from the opaque-to-white interphase layer (between 1.05 and 1.11 layers) by using a 10-ml pipet. Be mindful and minimize disturbing the gradient and debris when transferring interphase into new 50-ml falcon tube.

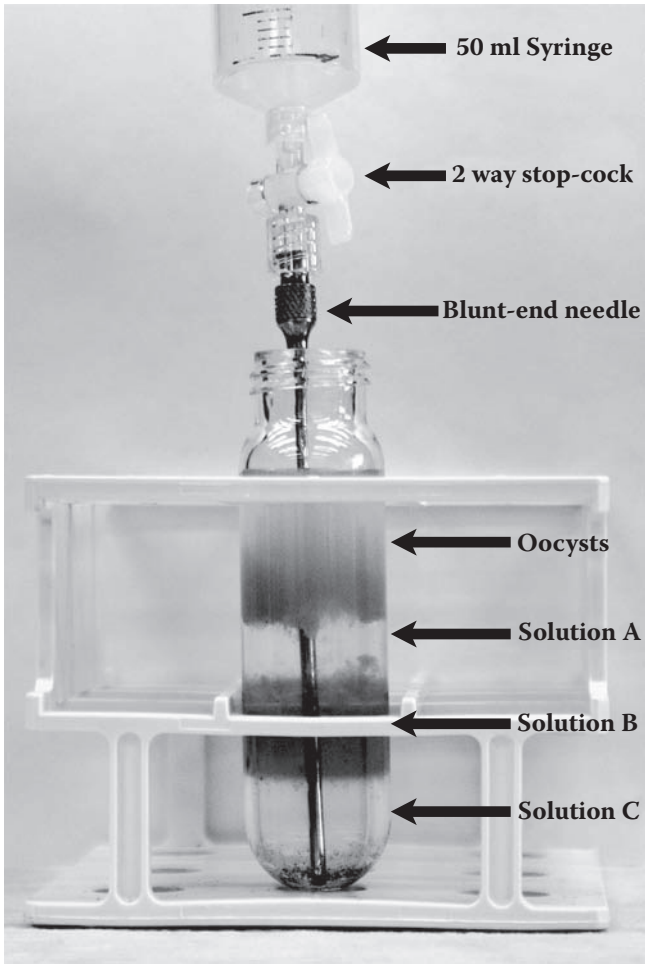

Figure 1.32 (Please see color insert following page 46.) Cesium chloride gradient for separating *T. gondii* oocysts from cat feces. Courtesy of Dr. Eric Villegas.

9. Wash oocysts by adding water up to 50 ml. Centrifuge at 2,000 × *g* for 10 min, at room temperature with no brakes. Carefully aspirate supernatant (do not disturb pellet). Repeat the wash two times.
10. Resuspend oocysts in 2% sulfuric acid for long-term storage.

Sporulated oocysts are stored in capped plastic bottles with a plastic liner under the cap. Oocysts can be stored for one year with minimal loss of infectivity. I make 10-fold dilutions in 2% H_2SO_4 at one time and store these at 4°C and use the desired concentration for inoculations into animals.

Procedures for optimal excystation of *T. gondii* sporozoites have not been published. Bile salts and trypsin, used for coccidian oocysts also excyst *T. gondii*. We have used 5% bovine bile.[179] Although intact oocysts can be excysted,[1220] success rate is better with free sporocysts. Sporocysts can be released from oocysts by vigorously shaking (or vortexing) sporulated oocysts with 500-µm glass beads until 80% of sporocysts have been released. Incubate sporocysts with 5% filtered ox bile at 37°C for 20 min or longer. Sporozoites can be separated from debris by filtration through 3-µm filters.

Work with oocysts is confined to a covered workbench inside a tray lined with plastic-lined absorbent sheets. Incineration and disinfection with boiling water are the most effective means of killing oocysts.

1.11.2 Isolation of *T. gondii*

1.11.2.1 Bioassays of Tissues in Mice

1.11.2.1.1 Body Fluids

For the isolation of *T. gondii* from heparinized blood or cerebrospinal fluid, the samples are centrifuged for 10 min at 2,000 rpm ($400 \times g$), the sediment is suspended in saline, and injected i.p. (s.c. if specimens are not sterile) into mice. Heparinized blood may be inoculated directly.

1.11.2.1.2 Acutely Infected Tissues

For the isolation of *T. gondii* from tissues, the tissues are homogenized in a mortar (or better in a blender), possibly with sterilized sand, and are then suspended in saline. If bacterial contamination is suspected, tissues or fluids can be mixed with antibiotics in 0.9% NaCl solution (saline) to give a concentration of 1,000 units of penicillin and 100 µg of streptomycin per ml (antibiotic saline) without reducing the infectivity. Contaminated specimens suspended in antibiotics are allowed to stand at room temperature for 1 hr. Just before inoculation, the suspension is shaken; when the heavy particles have settled to the bottom, the supernatant fluid is injected s.c., i.p., or both into mice, depending on the circumstances. In my laboratory, we always inoculate mice s.c. with samples suspected of *T. gondii* because it is easier, more volume can be injected, and mice are less likely to die of secondary infections. The infectivity of *T. gondii* by s.c. and i.p. routes was identical in numerous titrations that I have performed.

1.11.2.1.3 Chronically Infected Tissues

The number of *T. gondii* in tissues of chronically infected animals or tissues is low, and tissue cysts are more likely to be found than tachyzoites. Therefore, it may be necessary to use a digestion method to concentrate *T. gondii* in the inoculum. Jacobs et al.[716] found that the tissue cyst wall in isolated tissue cysts is destroyed immediately by pepsin or trypsin, but released bradyzoites can survive up to 6 hr, whereas tachyzoites are killed by pepsin, but not by trypsin. Thus, host tissues can be digested in pepsin or trypsin without affecting the viability of *T. gondii*. Pepsin is preferred to trypsin because acid pepsin digests muscle tissue faster and more efficiently and it kills many bacteria. Moreover, trypsin is more toxic to mice. Therefore, all traces of trypsin should be removed before inoculation into mice, and this process is time consuming. One should remember that a proportion of *T. gondii* are destroyed by pepsin or trypsin as the host tissue is digested. Therefore, homogenized tissue is digested for 60–90 min, allowing enough time for digestion of host tissue without destroying many *T. gondii*. The advantage of trypsin digestion is that it is less deleterious to tachyzoites than pepsin. The final concentration of trypsin in the tissue homogenate should be not more than 0.25%. We routinely use the following procedure.[367] All homogenizations should be performed under a hood to prevent splashing of material on the face of the operator.

1. Trim connective tissue, fat, epithelium (e.g., from tongue) from muscular tissues using nonporous, hard plastic cutting boards, scissors, or disposable razors. Cut muscle into small (1–2 cm) pieces and store in plastic bags or cups.
2. Grind muscle in a blender for 15 sec at low speed without saline. Then add 125 ml of saline and blend at top speed for about 30 sec. Rinse blender with 125 ml of saline and add the washings to the

muscle homogenate. To save expense and time in cleaning the lid of the blender every time to avoid cross contamination, line the lid of the blender with a disposable plastic sheet (commercially available 16.5 × 14 cm sandwich bags are convenient to use).

3. Pour the tissue homogenate into a 1,000-ml wide-mouth plastic jar with a disposable plastic liner. Make two labels for each jar with the type of tissue, and the animal number using a good adhesive tape and water-resistant marker; transferring one of these labels throughout the procedure helps to reduce mislabeling. Homogenates can be left at room temperature for 1–3 hr until all specimens have been processed.
4. To the prewarmed (37°C) homogenate add 250 ml of freshly prepared, prewarmed (37°C) acid pepsin solution (pepsin 5.2 g, NaCl 10.0 g, HCL 14 ml, and distilled water to make 1,000 ml, pH 1.10–1.20. Note in the Dubey[367] paper the concentration of these reagents was printed as half of what should be). Incubate at 37°C in a shaking water bath for 60 min. The source and purity of pepsin used is probably not critical, but porcine stomach pepsin (1:10000 biological activity, Sigma Chemical Co., St. Louis, MO) has been used routinely and successfully.
5. Filter the homogenate through two layers of gauze and centrifuge 250 ml of filtered homogenate in a 250-ml wide-mouth polypropylene centrifuge bottle (Nalgene) at 1,200 × *g* for 10 min.
6. Pour off the supernatant. Depending on the tissue, fatty scum may stick to the rim of the centrifuge bottle. To prevent this, suspend the sediment in 20 ml of phosphate buffered saline (PBS, pH 7.2) using disposable plastic pipettes. Transfer the homogenate in a 50-ml centrifuge tube with a conical bottom. Neutralize the homogenate with 12 to 15 ml of freshly prepared 1.2% sodium bicarbonate (pH 8.3) with phenol red as a pH indicator until the color changes to orange. After mixing, centrifuge at 1200 × *g* for 10 min.
7. Pour off the supernatant and add 5–10 ml of saline that contains 1,000 units penicillin and 100 μg of streptomycin per ml.
8. *T. gondii* stages (tachyzoites, bradyzoites) in tissues are killed by water and by heating to 60°C, and so blenders, cutting boards, and other materials can be cleaned with soap and hot water, then rinsed with cold water and finally with saline before use for the next specimen.
9. Inoculate 1 ml of tissue homogenate subcutaneously into each of 5 to 10 mice (Swiss-Webster weighing 20–25 g) over the back using a 4-cm long 21–23-gauge needle. It is preferable that each mouse is identified with a rodent ear tag (Ear tag size No. 1, National Band and Tag Company, Newport, KY). For additional identification, each mouse can also be marked with a streak of alcoholic picric acid in case the ear tag is lost accidentally.
10. Examine all inoculated mice for *T. gondii* infection.

1.11.2.2 Bioassays of T. gondii *in Cats*

The number of tissue cysts in food animals may be too small to be detectable by bioassays in mice. Therefore, cats have been used to detect viable *T. gondii* in meat because larger volumes of tissues (500 g or more) can be fed to cats than could be assayed in mice (usually 0.25 g). After tissue cysts are ingested, *T. gondii* multiplies extensively in the intestine of the cat, eventually leading to excretion of numerous oocysts in feces, and, therefore, the many-fold amplification greatly facilitates the detection of *T. gondii* in test samples with small numbers of tissue cysts.

1.11.2.3 Examination of Cat Feces for T. gondii *Oocysts*

Proper identification of *T. gondii* oocysts in the feces of cats is also important from a public health viewpoint. Detection of *T. gondii* oocysts is possible using any of the standard fecal flotation techniques. However, the use of salt solutions over 1.18 specific gravity is not recommended because the distortion produced in the oocyst makes identification difficult. The following is an outline of a procedure for floating *T. gondii* oocysts from cat feces. Materials needed are plastic, paper, or glass container (250 ml), tongue depressors, gauze or cheese cloth, Pasteur pipettes, rubber bulb, razor blade, scissors, centrifuge tubes, centrifuge (floating head type), microscope, cover glasses, slides,

container to hold discarded pipettes, sucrose solution of 1.15 specific gravity (sugar 53 g, water 100 ml, liquid phenol 0.8 ml or 2% sulfuric acid).

Procedure:

1. Collect the fecal specimen in a disposable waterproof container. Add enough water to wet the feces. Leave the cup covered for 1 to 2 hr or longer (preferably at 4°C to prevent sporulation), until feces have softened.
2. Emulsify the feces thoroughly before adding the sucrose flotation solution. Slowly add about 5 to 10 volumes of sucrose solution while emulsifying the feces with a tongue depressor.
3. Strain feces through 1–2 layers of cheesecloth or gauze held over a container by means of a rubber band. Discard the gauze and the feces retained on it carefully, so as not to spill the feces. The safest way to remove the gauze is to cut the rubber band with scissors or a razor blade while the other hand holds the gauze and container firmly above the band. Any other method can cause the rubber band to catapult the feces.
4. Pour the strained feces into a 50-ml centrifuge tube until the tube is filled to within 1 to 2 cm of the top.
5. After balancing the tube, centrifuge at about 2,000 rpm (400 × *g*). Top speed in a clinical centrifuge is satisfactory.
6. After 10 min centrifugation, sample 1 to 3 drops from the very top of the tube using a disposable pipette with a bulb. Mount the drop on a glass slide, avoiding aerosols, and cover with a cover slip.
7. Let the slide sit on a flat surface for 1 to 5 min so that the fecal particles are immobilized. Examine with 100× magnification and look for coccidian oocysts, and then further examine with 400× magnifications (Figure 1.33). Unsporulated *T. gondii* oocysts measure 10 × 12 µm and are about one-quarter the size of *Isospora* (also called *Cystoisospora*) *felis* (Figures 1.33 and 1.34), one-sixth to one-eighth the size of a *Toxocara cati* egg, and about twice the size of red blood cells. Because of the small size of *T. gondii* oocysts, they usually lie in a different plane of focus than oocysts of *I. rivolta, I. felis*, and *Toxocara cati* eggs. It is unusual to find both large and small structures in one focus as shown in Figure 1.33. In some cats, only a few *T. gondii* oocysts are shed, so a careful search may be required to find them. It is not unusual to find only 1–5 oocysts in the entire 22 × 22-mm cover glass preparation. Oocysts are easy to spot in sucrose solution at the edges of the cover slip. The threshold for microscopic detection is estimated to be around 1,000 oocysts per gram of feces.

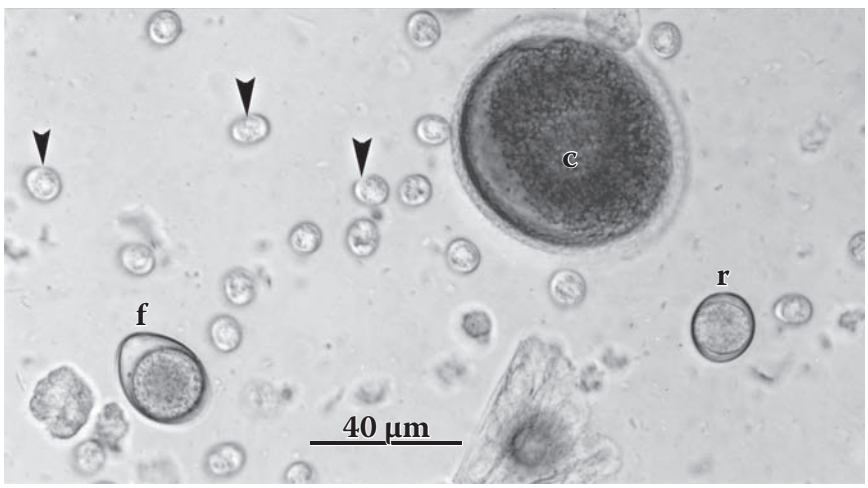

Figure 1.33 Sugar fecal float of cat feces showing unsporulated oocysts of *T. gondii* (T), *Isospora felis* (f), and *I. rivolta* (r).*T. gondii* oocysts are the smallest of all feline coccidia, averaging 10 × 12 µm. *I. felis* oocysts are pear-shaped and average 40 × 30 µm. *I. rivolta* oocysts are ovoid and measure about 25 × 20 µm. All three coccidian oocysts are compared to the size of a *Toxocara cati* (C) egg, the common feline round worm.

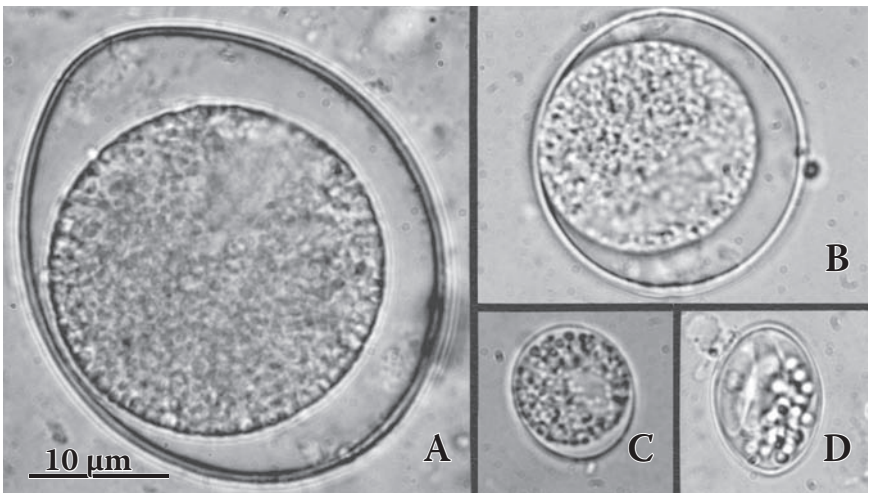

Figure 1.34 Unsporulated oocysts of *I. felis* (**A**), *I. rivolta* (**B**), and *T. gondii* (**C**) compared with sporulated sporocyst of *Sarcocystis muris* (**D**). All feline coccidia, except *Sarcocystis,* are shed unsporulated. Magnification ×1600.

8. Discard all disposable material used for floating feces by burning. Alternatively, boil or autoclave all the material.
9. Fecal flotation should be done the same day feces are collected from cats or the feces should be stored at 4°C. *T. gondii* oocysts are unsporulated and not infectious in freshly passed feces, but sporulate and become infectious within 1 day at room temperature (22–26°C) and aerobic conditions.
10. A presumptive diagnosis of toxoplasmosis can be made if oocysts measuring 10×12 µm are found in cat feces. However, for a definitive diagnosis, feces should be inoculated in mice to differentiate the oocyst from *Hammondia* oocysts. For this, 0.5 ml of the supernatant from the very top of the centrifuge fecal suspension is mixed with 4.5 ml of 2% sulfuric acid in a 30-ml bottle. After capping, the bottle is left at room temperature for 3 to 7 days. Shaking the bottle helps to aerate the oocysts.
11. For inoculation into mice, the sulfuric acid can be removed from stored oocysts by dilution in water and centrifugation or can be neutralized with 3.3% sodium hydroxide (NaOH) containing 2% phenol red as indicator. The NaOH solution should be added drop by drop until the end point is reached, at which time the color changes from yellow to orange.
12. Because *T. gondii* oocysts are pathogenic to humans, mice should be inoculated with care.

1.11.3 Inoculation and Examination of Mice for *T. gondii*

Although all strains of mice are susceptible to *T. gondii* infection, we use out-bred albino female adult mice because they are inexpensive. Although males are usually less expensive than females, we use the latter to avoid fighting and tail biting among competing mice. Mouse inoculation may be by the s.c., i.p., or oral routes. Each of these three methods has its advantages. The i.p. route is most useful for isolating *T. gondii* from sterile specimens. The s.c. route is useful for isolating *T. gondii* from contaminated specimens. The oral route is best for isolating *T. gondii* from feces of cats. About 0.5 to 1 ml of fluid can be inoculated by the i.p. or s.c. route into a 20- to 25-g mouse. To minimize leakage of the inoculum, the needle should be inserted s.c. for at least 1 cm before entry into the peritoneal cavity. Depending upon the virulence of the strain, ascites may develop in the mice in 7 to 14 days after i.p. inoculation. Tachyzoites may be found in the peritoneal fluid or in imprints made from the mesenteric lymph nodes. After oral inoculation, the mice may die within 10 days due to

enteritis and mesenteric lymphadenitis. Imprints of intestinal and mesenteric lymph nodes should be made soon after the mice die or, better yet, from mice killed while moribund. When mice die in the second week, they usually do so from pneumonia and encephalitis, irrespective of the route of inoculation, and imprints of the lungs and brain should be examined for tachyzoites. After 14 days, organisms usually begin to disappear from the visceral organs and *T. gondii* is most likely to be demonstrated in the brain. However, in some strains, *T. gondii* (e.g., the GT-1 strain) persists in the lung for several weeks and is more easily demonstrated there than in the brain. A diagnosis is made by finding *T. gondii* in films of body fluids or tissue imprints stained by one of the Romanowsky methods.

Tissue cysts should be sought in survivors 6 to 8 weeks after inoculation, even though tissue cysts may appear earlier; however, early tissue cysts are small and may be missed. Examination of brain tissue for tissue cysts can be carried out by grinding it with a pestle and mortar, adding saline (1 ml per mouse) slowly, and examining 1 or 2 drops of this saline-brain suspension between a glass slide and cover slip using the low magnification lens of the microscope. *T. gondii* tissue cysts are darker than the brain tissue and are easily seen at 100× magnification. Alternatively, small portions (2–3 mm) of cerebrum may be squashed on a slide under a 22 × 22-mm cover slip using another slide for pressure. The resultant smear should be thin enough to read print through it. Two to three smears from each mouse are examined. It is best to bleed the mice out completely before removing the brains for examination. This prevents contamination of brain with blood, which makes the identification of tissue cysts easier, and the blood is available for serologic examination. A portion of brain should be fixed for sectioning. Certain pollen grains structurally resemble *T. gondii* tissue cysts, and therefore, to avoid erroneous diagnosis, cover glasses, slides, and pestle and mortars should be kept covered when not in use, and identification should always be confirmed on stained smears or sections. Because failure to demonstrate *T. gondii* in mice does not prove lack of infection, antibodies to *T. gondii* should be sought in the sera of inoculated mice. Antibody tests may be done as early as 2 weeks after infection. To obtain blood for serologic examination, anesthetized mice are bled from the orbital sinus. About 0.25 ml of blood can be easily obtained from the orbital sinus without killing the mice. Blood can also be obtained from the tail vein, but the yield is poor and the method is more time consuming. Mice develop antibodies to *T. gondii* between 3 and 70 days after infection, depending upon the strain of *T. gondii*, dose, and stage of the parasite inoculated.[340] Most mice seroconvert to *T. gondii* within 30 days p.i. but occasionally not until 2 mo p.i. We observe mice up to 2 mo p.i.

For *T. gondii* serological diagnosis, we test a 1:25 dilution of mouse serum by the modified agglutination test (MAT) 45 days p.i. Although the MAT is highly specific, diagnosis should be confirmed by demonstration of organisms. Many strains of *T. gondii* produce only a few tissue cysts in out-bred mice. If tissue cysts are not found in brain smears of seropositive mice, homogenized brain should be subpassaged into immunosuppressed or gamma interferon gene knock-out (KO) mice.

1.11.4 Serologic Procedures

1.11.4.1 Detection of Humoral Antibodies

Many serologic tests have been used for the detection of IgG and IgM antibodies to *T. gondii*: Sabin-Feldman dye test (DT), indirect hemagglutination (IHA), indirect fluorescent antibody (IFA), modified agglutination test (MAT), latex agglutination (LA), enzyme-linked immunoabsorbent assay (ELISA), and complement fixation (CF). Of these, IFA, ELISA, and MAT have been modified to detect IgM antibodies. Although antibodies are best preserved at lower temperatures (–20°C or lower) they have survived for 6 months when stored on dried filter papers with silica gel.[984]

1.11.4.1.1 Dye Test

This still remains the definitive test for human toxoplasmosis. More is known of the DT antibodies in man than of any other type of antibodies. This is essentially a complement-mediated neutralizing type of antigen-antibody reaction. In this test, live tachyzoites are incubated with accessory factor (complement from human serum) and the test serum at 37°C for 1 hr and a dye (methylene blue) is added. Tachyzoites unaffected by antibody are stained uniformly with methylene blue. Specific antibody induces complement-mediated cytolysis of tachyzoites and the cytoplasm leaks out. As a result, tachyzoites affected by antibody do not incorporate methylene blue and appear as "ghosts." The titer reported is the dilution at which 50% of tachyzoites remain unstained. It is important that the reports of serological testing should be consistent and comparable. For this reason, the FAO/WHO Collaborating Center for Research and Reference on Toxoplasmosis established in the Statens Seruminstitut, Copenhagen, Denmark, supplies standard anti-*Toxoplasma* sera. Results are expressed in international units (IU). One ampule contains 2,000 IU, corresponding to 2 ml serum, the titer of which is 1,000 IU.

The DT is highly specific and sensitive with no evidence of false results in humans. However, it is expensive, requires a high degree of technical expertise, and is potentially unsafe because of the use of live virulent organisms. The procedure was detailed by Frenkel and Jacobs.[533] Most hosts develop DT antibodies within 4 weeks and titers may remain stable for months or years. Although DT measures antibodies specific to *T. gondii* in many hosts, ruminant sera have substances that give false results unless sera are inactivated at 60°C for 30 min and the test does not work with some avian sera (see Chapter 12).

1.11.4.1.2 Indirect Hemagglutination Test

Soluble antigen from tachyzoites is coated on tanned red blood cells that are then agglutinated by immune serum. Full technical details are given in a serology manual prepared by Palmer et al.,[1022] and commercial kits are available. Although the IHA test is simple in principle, technical variables make its use impractical. Furthermore, IHA detects antibodies later than the DT and titers remain elevated for a long period, so acute infections are likely to be missed by this test. The IHA test is also frequently negative in congenital infections. In animals, titers lower than 1:128 may be nonspecific.

1.11.4.1.3 Complement Fixation Test

It is generally believed that CF antibodies appear later than DT antibodies and the test is positive during acute infection, but this largely depends on the antigenic preparation. This is not a test of choice because of complex procedures and lack of standardization of the antigen and the reagents.

1.11.4.1.4 Modified Agglutination Test

The development of a simple direct agglutination test has aided tremendously in the serological diagnosis of toxoplasmosis in humans and other animals. In this test, no special equipment or conjugates are needed. This test was initially developed by Fulton and Turk[556] and improved by Desmonts and Remington[253] and Dubey and Desmonts[297] who called it the modified agglutination test (MAT). The MAT has been used extensively for the diagnosis of toxoplasmosis in animals. Titers in the MAT parallel those in the DT both in human sera and in animal sera. The sensitivity and specificity of MAT has been validated by comparing serologic data and isolation of the parasite from naturally and experimentally infected pigs.[339,360] In the MAT, sera are treated with 2-mercaptoethanol to

remove non-specific IgM or IgM-like substances. This test detects only IgG antibodies; therefore, it may give false negative results during early stages of acute infection.[424] The MAT is commercially available (Toxo-Screen DA, bioMérieux, Charbonnières Beins, France). The Toxo-Screen DA is the same test as MAT. The results obtained with MAT differ, depending on the preservative used to prepare the antigen. Thulliez et al.[1284] reported that using acetone (AC test) in place of formalin (HS test) can detect IgG present during acute infection. The AC test has been very useful in diagnosis of toxoplasmosis in AIDS patients, and acute glandular toxoplasmosis.[941,1284] The antigen for the AC test is not commercially available at present and methanol is now substituted for acetone (Thulliez, personal communication). Thulliez and I have extensively tested the dynamics of the AC test for detectable IgG antibodies in experimentally infected animals (see later chapters). Although serum is preferred, the test works with blood plasma and even whole blood; hemolysis does not interefere with the test.

Technical details are given by Desmonts and Remington[253] and Dubey and Desmonts.[297] The MAT has also been modified to detect IgM antibodies (see ELISA section). We use the following protocol for MAT.

1. Serum Diluting Buffer
   Dissolve 42.5 g NaCl, 1.54 g NaH_2PO_4 (M.W. 120), and 5.4 g Na_2HPO_4 (M.W. 142) in 900 ml deionized water. Adjust the pH to 7.2. Bring the volume to 1 liter with deionized water. Store in a refrigerator. This is the 5X stock solution. Dilute this stock solution 1:5 to give 0.01 M phosphate buffered saline (PBS) (1 part stock and 4 parts deionized water). PBS should be filtered just before use through a 0.22 µm membrane.
2. Antigen Diluting Buffer
   Dissolve 7.01 g sodium chloride, 3.09 g boric acid, and 2.0 g sodium azide in 900 ml deionized water. Add 24 ml 1N NaOH and adjust the pH to 8.95. Bring the volume to 1 liter. This is the stock solution and can be stored at room temperature. For the working antigen diluting buffer, dissolve 0.4 g bovine serum albumin (BSA) in 100 ml borate buffer. Store at 4°C.
3. Dilute serum samples with serum diluting buffer (no. 1) in small test tubes (1.2 ml in strips of 8 or 12) with a multichannel pipette, starting at 1:25. Microtiter plates may also be used for making serum dilutions.
4. Prepare antigen mixture as follows:
   For each plate, mix 2.5 ml antigen diluting buffer (no. 2), 35 µl 2-mercaptoethanol, 50 µl Evans blue dye solution (2 mg/ml water), and 0.15 ml antigen (formalin-fixed whole parasites).

5. Agglutination is done in U-bottom 96-well microtiter plates. Pipette 25 µl antigen mixture to each well immediately after mixing. Pipette 25 µl serum dilutions into the wells and mix gently with the antigen by repeated pipetting action.
6. A positive control should be included in each plate. The control should have a titer of 1:200, and twofold dilutions from 1:25 to 1:3200 should be used.
7. Cover the plates with sealing tape and incubate at 37°C overnight.
8. Read results using a magnifying mirror. A blue button at the bottom of the well means negative. A clear bottom means positive.

1.11.4.1.5 Latex Agglutination Test

In this test, soluble antigen is coated on latex particles and the pattern of agglutination is observed when the serum to be tested is added. The LA test is commercially available (ToxoTest, Eiken Chemical Co., Tokyo, Japan). It is easy to perform and does not require special training or equipment. Its sensitivity for livestock sera needs to be improved.

1.11.4.1.6 Indirect Fluorescent Antibody Test

In the conventional IFA test, whole, killed tachyzoites are incubated with serum and antibody detection is enhanced by adding fluorescent-labeled antispecies IgG (or whole immunoglobulin) and viewing with a fluorescent microscope. Technical details of this and other tests were given by Carmichael.[155] IFA titers generally correspond with those of the DT, and the test has been standardized in some laboratories. Its disadvantages are the need for a fluorescent microscope and for species-specific conjugates and cross reaction with rheumatoid factor (RF) and antinuclear antibodies (ANA). A modification of IFA (IgM-IFA) was developed to detect IgM antibodies in congenitally infected children because heavier IgM antibodies do not cross the placenta, whereas the lighter IgG antibodies can cross the human placenta. However, the test detects only 75% of congenital infections because of (1) false positive results due to cross reaction with antinuclear antibodies, (2) lack of specificity of reagents due to contamination of IgM conjugates with IgG, (3) delayed antibody synthesis in the child, and (4) suppressive effect of passively transferred IgG. Some of these problems can be overcome by separation of IgM from IgG from RF and ANA by filtration.

1.11.4.1.7 Enzyme-Linked Immunoabsorbent Assay

In the ELISA test, soluble antigen is absorbed on a plastic surface (microtiter plates or slides), and the antigen-antibody reaction is enhanced by the addition of a secondary enzyme-linked antibody-antigen system, and the reaction can be assessed objectively by quantification of the color that develops. ELISA can be automated so that a large number of sera can be examined rapidly in a central laboratory. ELISA and its modifications appear to be the rational tests of the future for serologic diagnosis. It does, however, require an ELISA reader to quantify the color reaction. Numerous modifications of ELISA have been published.

Naot and Remington,[967] and Naot et al.[968] developed an ELISA to detect IgM antibodies (IgM-ELISA) to overcome the pitfalls of IgM-IFA test. In IgM-ELISA, the wells of microtiter plates are coated with IgM antibodies and then *T. gondii* antigen is added. By using an enzyme conjugated with antiserum to *T. gondii,* the presence of the complex between anti-*T. gondii* IgM and *T. gondii* antigen is detected in the form of a color reaction. Franco et al.[525] developed a modification of IgM-ELISA in which *T. gondii* antigen is conjugated to the enzyme instead of antiserum.

1.11.4.1.8 Immunoglobulin M Immunoabsorbent Agglutination Assay Test

Desmonts et al.[254] developed an ingenious modification of IgM-ELISA to eliminate the necessity for an enzyme conjugate while combining it with the agglutination test (IgM-ISAGA). In this test, wells of microtiter plates are coated with antihuman IgM antibodies. After washing, test sera are added to the wells and incubated overnight at 37°C to allow the binding of IgM. The plates are then washed and a suspension of whole tachyzoites (as in the direct agglutination test of Desmonts and Remington[253]) is added to the wells. The pattern of agglutination is observed as in the agglutination test. If the patient's serum has specific IgM, then it binds to the antispecies IgM and will then agglutinate a fixed parasite antigen. If the patient serum is negative for IgM, then *T. gondii* settles at the bottom of the well and gives a negative reaction. This test is simpler, more rapid, and easier to perform than the IgM-ELISA. However, IgM-ISAGA requires large numbers of tachyzoites. To overcome this, Remington et al.[1092] modified the IgM-ISAGA by substituting whole tachyzoites with latex beads coated with soluble *T. gondii* antigen. The type of latex beads used is apparently critical.

1.11.4.1.9 Western Blotting

This test can be used as an aid to conventional serological tests described earlier. In this test, sera are reacted with *T. gondii* antigens on a membrane transferred from a polyacrylamide gel and the resulting banding patterns are matched with known molecular weight controls. This test has been used to distinguish IgG antibodies present in infant versus mother sera.[607]

1.11.4.2 Avidity Tests

The binding (avidity) of *T. gondii* antigen to specific antibodies can change during the course of infection. During the early (acute) stage of infection, avidity values are low and increase with duration of infection. In this test, sera are run with or without treatment with urea (or other protein denaturing agents) and the difference in titers can be used to determine recency of infection. The test can be used with IgG, IgA, and IgE antibodies using different serological procedures, most often ELISA.[51,79,638,1093,1102] However, this test also has a few limitations. *T. gondii*-specific low-avidity IgG-antibodies in pregnant women may persist for months, often during the entire pregnancy, and treatment of *T. gondii* may delay the avidity maturation (Eskild Petersen, personal communication).

1.11.5 Detection of *T. gondii* DNA

T. gondii DNA can be detected by PCR. There are many different markers, but the 35-copy B1 gene[128] and the 300-copy 529-bp element[672] are most frequently used.[1242] The disadvantage of using a single-copy gene target is that the sensitivity is compromised compared to the highly repetitive sequences. However, this can be compensated for to some extent by nested PCR. The 529-bp repeat marker is 10–100 times more sensitive than the B1 marker. The advantage of recently developed, real-time PCR assay is that it also quantifies the parasite DNA. Su and Dubey[1242] have provided detailed information on reagents and their sources, equipment, cost, and stepwise procedure for various methods to detect *T. gondii* DNA. Overall, PCR is very sensitive (it can detect DNA from one tachyzoite), specific, and can provide rapid diagnosis. It is most suitable for clinical specimens. Cross-contamination, however, is a major concern.

From a public health viewpoint, it is necessary to distinguish *T. gondii* oocysts from oocysts of a related coccidium, *Hammondia hammondi,* also present in cat feces. *H. hammondi* is non-pathogenic.[540] Bioassays, currently the only definitive way to detect viable oocysts of these parasites, are expensive, and only a few laboratories in the world have facilities to do them. Although DNA detection is considered highly specific, cross reactivity has been observed between *T. gondii* and *H. hammondi*.[1152] Recently, *H hammondi*-specific primers have been published.[1152]

Detection of DNA from *T. gondii* oocysts may present additional problems because of inhibitors in fecal matter and difficulty of releasing DNA from the oocysts.[211,936,1133,1153,1159] Salant et al.[1133] were able to detect DNA from five *T. gondii* oocysts in feces experimentally contaminated with oocysts. However, Dabritz et al.[211] did not find DNA in feces of three naturally infected cats that contained *T. gondii*-like oocysts. Schares et al.[1153] used the following procedure. This protocol consists of lysis steps utilizing freezing and thawing cycles, treatment with saline saturated buffers, proteinase K and cety-trimethyl ammonium bromide. The method is partially based on the recommendations of Zhao et al.[1395]

Reagents:

1. PBS: 300 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.7 mM NaH_2PO_4.
2. Sodium hypochlorite, aqueous solution, ≥4% as active chlorine (Aldrich 23,930-5).
3. OOC-lysis buffer (pH 9.5): 600 mM EDTA, 1.3% (v/v) N-lauroylsarcosine, 2 mg/ml proteinase K.

4. OOC-CTAB buffer: 2% (w/v) cetyl-trimethyl ammonium bromide, 1.4 M NaCl, 0.2% (v/v) mercapthoethanol, 20 mM EDTA, 100 mM tris(hydroxymethyl)aminomethane.
5. phenol/chloroform/isoamyl alcohol (25/24/1).
6. (Sigma P3803).
7. 4 M NaCl.
8. 96% (v/v) ethanol.
9. 70% (v/v) ethanol.

Procedure:

1. Wash oocysts four times by centrifugation (1,100 × *g*, 7 min, without the use of the brake) in 15 ml PBS in a 15 ml centrifugation tube.
2. Incubate oocyst pellet and the remaining contaminants (up to 0.5 ml) in 2 ml 5.75% sodium hypochlorite (30 min, 37°C).
3. Add double-distilled H_2O up to 15 ml.
4. Centrifuge supernatant in a 15 ml tube (7 min, 1,100 × *g*, without the use of the brake) and mix the pellet with PBS. Wash the pellet three times with PBS (7 min, 1,100 *g*, without brake).
5. After a last centrifugation, re-suspend the pellet in 1 ml PBS, transfer into a 1.5 ml reaction tube and spin down (7 min, 1,100 × *g*, without brake).
6. Carefully remove as much of the supernatant as possible and apply three freezing (10 min, –20°C)/thawing (2 min, room temperature) cycles to the pellet.
7. Re-suspend the pellet in 100 µl OOC lysis buffer (45 min, 65°C).
8. Add 400 µl OOC-CTAB buffer (60 min, 60°C).
9. Mix with 500 µl phenol/chloroform/isoamylalcohol (25/24/1) by inverting 50 times. Centrifuge for 7 min at 13,000 × *g*.
10. Transfer the supernatant to a fresh tube and mix again with 500 µl phenol/chloroform/isoamylalcohol (25/24/1) by inverting 50 times. Centrifuge for 7 min at 13,000 × *g*.
11. Transfer the supernatant to a fresh tube and add 0.04 volumes of 4 M NaCl and 2–3 volumes of –20°C cold 96% (v/v) ethanol to precipitate DNA (keep at least 20–30 min at –20°C).
12. Centrifuge for 15 min at 13,000 × *g*. Decant the supernatant.
13. Wash the pellet using 70% (v/v) ethanol and centrifuge for 15 min at 13,000 × *g*.
14. Discard the ethanol solution and air dry the pellet.
15. Resolve DNA in double distilled water for at least 12 hr at 4°C.
16. Use 2.5–10 µl aliquots for PCR.

1.11.6 Immunohistochemical Staining

Formalin-fixed, paraffin-embedded tissues can be used in this technique.[837] Although *T. gondii* antigen can be detected even 1 year after fixation in 10% formalin, fixation for short periods (24 hr) is recommended. Sections are cut 4 to 5 µm thick, deparaffinized, and dehydrated in ethanol in the usual way. It is necessary to use grease-free clean slides, otherwise the sections detach from the slide; also an addition of chrome-gelatin in the water bath helps adherence of sections to the slide. Polyclonal rabbit-anti *T. gondii* antibodies are better than monoclonal antibodies. Although rabbits can be infected with tachyzoites, bradyzoites, or oocysts, the latter are preferred because oocysts can be cleaned of fecal matter and treated with 5.25% sodium hypochlorite solution (Clorox) to remove any host material. The IHC technique can be used to distinguish tachyzoites from bradyzoites. An antibody produced in rabbits against BAG1 (formerly BAG5) antigen of *T. gondii*[896] has proved very useful in tracing the development of bradyzoites in tissues.[359] This anti-BAG1 antibody is stage, but not parasite, specific; it does not react with tachyzoites but will react with bradyzoites not only of *T. gondii,* but also *Neospora, Besnoitia,* and *Sarcocystis.*

We carry out the following procedure:

1. De-paraffinize slides in old xylene I for 30 min. Discard xylene I. Pour xylene II into xylene I container. Soak 5 min.
2. Pour fresh xylene into xylene II. Soak slides in fresh xylene for 5 min.
3. Soak in 100% ethanol I for 5 min. Discard ethanol I. Pour ethanol II into ethanol I container. Soak 5 min. Pour fresh 100% ethanol into ethanol II container. Soak another 5 min.
4. Soak in 95% ethanol for 5 min.
5. Quench endogenous peroxidase in 3% H_2O_2 in methanol for 15 min (20 ml 30% hydrogen peroxide and 180 ml methanol).
6. Soak in 80% ethanol for 5 min.
7. Soak in 70% ethanol for 5 min.
8. Soak in 50% ethanol for 5 min.
9. Soak in saline for 5 min.
10. Soak in warm saline (37°C) for 5 min.
11. Pepsin digestion: incubate slides in 0.4% pepsin in 0.01 N HCl for 15 min at 37°C (0.8 gm pepsin, 0.333 ml 6 N HCl, 200 ml saline, prewarmed to 37°C for 30 min).
12. Soak in 0.75% BRIJ-PBS for 5 min twice (2.5 ml 30% BRIJ 35 per liter PBS).
13. Block nonspecific binding with 0.5% Na caseinate in BRIJ-PBS for 10 min.
14. Drain and without rinsing apply the properly diluted primary antibody to the tissues for 30 min at 37°C.
15. Soak slides in BRIJ-PBS for 5 min. twice.
16. Treat tissues with Dako EnVision Rabbit peroxidase solution at 37°C for 30 min.
17. Soak slides in RIJ-PBS for 5 min twice.
18. Apply Dako AEC substrate chromogen solution to slides at 37°C for 15 min.
19. Collect chromogen in hazard waste bottle, and soak slides in BRIJ-PBS for 5 min.
20. Rinse slides in running tap water for 2 min. Rinse in de-ionized water for 2 min. twice.
21. Counterstain with Mayer's Hematoxylin for 1 min.
22. Wash slides in running tap water for 1 min.
23. Soak slides in Scott's tap water substitute ($MgSO_4$ $7H_2O$ 20 gm, $NaHCO_3$ 2 gm, water 1 liter) for 1 min.
24. Soak in running tap water for 1 min. Soak in de-ionized water for 2 min twice.
25. Apply Crystal Mount and incubate slides overnight at room temperature.
26. Apply Permount and cover slip, if desired.

1.11.7 Cryopreservation

T. gondii tachyzoites, bradyzoites, or sporozoites can be preserved by freezing by techniques described for other coccidia. *T. gondii* survives freezing better in dimethylsulfoxide (DMSO) than without it. It is advisable to start with a high number (100,000 or more per ml) in the inoculum because not all organisms survive freezing. For preserving *T. gondii*, suspend organisms in tissue culture medium (TCM), mix with DMSO, and slowly freeze in liquid nitrogen (LN). For this, prepare a stock solution of 16% bovine serum albumin in TCM (solution A) and 50% concentration of DMSO in TCM (solution B). Mix equal volumes of solutions A and B to produce solution C. Finally, mix equal volume of solution C with equal volume of the *T. gondii* in the TCM (thus, the final concentration of DMSO is 12.5%) and let the mixture incubate for 30 min at room temperature. Freeze samples in a freezing box. We use Nalgene Cryo 1°C Freezing Container 18 place with isoproponyl alcohol to achieve a –1°C/min rate of cooling (Nalgene Cat. No.5100-0001). Store the box at –70°C overnight and then transfer frozen vials to LN. In case such a freezing box is not available, a practical method of doing this is to immerse the sealed vial of tachyzoites in 95% ethanol, store at –70°C overnight, and the next day, transfer the frozen vial to LN. For experimentation, thaw tachyzoites quickly (37°C water bath, 1 min), and inoculate into mice or cell culture (mice are better if the number of viable *T. gondii* is expected to be low). I have revived *T. gondii* up to 12 years after storage in liquid nitrogen.

The survival of intracellular tachyzoites in LN is better than extracellular but free bradyzoites and sporozoites survive better than intact tissue cysts and oocysts. I routinely cryopreserve *T. gondii*-infected tissues on their first (primary) isolation to minimize the effect of sub-passage in the laboratory. For this, after ascertaining the presence of *T. gondii* in biopsy or infected mouse tissue (most often lung), the tissue is ground in PBS, diluted with cryopreserving medium and processed as with infected cell culture. For cryopreservation of bradyzoites, bradyzoites are liberated from tissue cysts by digestion in pepsin for 5–10 min, depending on the tissue, neutralized with sodium bicarbonate, centrifuged, and mixed with CM.

1.12 SAFETY CONCERNS AND PRECAUTIONS WHILE WORKING WITH *T. GONDII*

All three stages of *T. gondii* (oocysts/sporozoites, tachyzoites, and bradyzoites) are infective to humans. All persons working with *T. gondii*, particularly women of childbearing age, must be made aware of the potential hazards, and must be trained and proficient in the practices and techniques required for handling such material safely.

All persons working with *T. gondii* should be encouraged to enroll in the Institutional Occupational Medical Surveillance Program and tested for antibodies to *T. gondii* before handling live parasites. They should sign a written document that they are aware of the potential pathogenicity of *T. gondii*. Serologically negative women of childbearing age who might become pregnant should receive extensive counseling about the potential risks to the fetus. At-risk employees (immunocompromised) who choose not to be exposed should be provided with alternative assignments in a work area where viable *T. gondii* organisms are not being handled. Pregnant women should not work with *T. gondii* or in areas where viable organisms are handled. For more information on *T. gondii* and toxoplasmosis, visit the CDC's Division of Parasitic Diseases fact sheets at www.cdc.gov/ncidod/dpd/parasites/index.htm, the Public Health Agency of Canada's Material Safety Data Sheets at www.phac-aspc.gc.ca/msds-ftss/, and the Biosafety in Biomedical and Microbiological Laboratories (most current edition) at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Seek medical attention if *T. gondii*-infected materials (irrespective of the stage of the parasite) are ingested or splashed in the eyes. Notify your supervisor, and the Safety, Occupational Health and Environmental Staff, as soon as practical.

Wear protective clothing, gloves, goggles (especially for those who do not wear glasses), and masks while handling potentially infected tissues. Contaminated clothing should be autoclaved and washed on site; disposable clothing should be incinerated.

All disposable items including plasticware, absorbent towels, gloves, etc. should be disposed of in a biohazard bag and incinerated or autoclaved and disposed of in an autoclave dumpster. Infected animal carcasses should be incinerated. All cat feces and any other materials coming in contact with infected cat feces must be autoclaved or incinerated.

Use only Luer-lock syringes to inoculate *T. gondii* into animals or for passage into cell culture. Avoid putting plastic sheaths on the needle during or after inoculation; instead dispose of the syringe and the needle in Sharp-type biohazard containers. Homogenize infected tissues only in safety hoods so that the operator's face is not exposed. In case of spills on the face, thoroughly wash face and eyes; *T. gondii* is infectious orally (including tachyzoites) and via intact conjuctiva. Needle pricks are the most common source of laboratory infection. In case of needle prick, squeeze the prick site under running water and wipe with alcohol. Inform the supervisor and seek medical attention. Fever and hyperemia at the site of needle prick are indications of infection. There is no non-pathogenic strain of *T. gondii* with respect to human infection and care must be taken while working with any strain of this parasite.

No special precautions are necessary for housing and handling mice infected with *T. gondii* tachyzoites and bradyzoites; the parasite is not transmitted from mouse to mouse (unless eaten) or to humans. Special precautions are needed, however, for inoculation and handling of oocysts. For inoculation of mice with clean oocysts, the subcutaneous route is less hazardous than the oral. After oral inoculation, few oocysts pass through the mouse gut intact and remain infective to humans as well as to other animals. This passive excretion of oocysts depends upon the gut emptying time and the diet. For s.c. inoculation the oocysts should be deposited under the skin over the back using a 22-gauge 4-cm-long needle; the needle should not be pulled out straight (to prevent leakage of the inoculum from the site of inoculation). A biohazard sign should be posted on the mouse cages inoculated with oocysts and all bedding and cages should be autoclaved up to 10 days to provide a biweekly cleaning cycle. Animal caretakers should wear gloves and face masks while working with oocyst-infected mice or other animals at least for 10 days from the time of oocyst inoculation.

Educational material or a current review on toxoplasmosis should be kept handy in case of laboratory accidents. Laboratory acquired infections were reviewed by Herwaldt.[651]

CHAPTER 2

Toxoplasmosis in Humans (*Homo sapiens*)

2.1 SEROLOGIC PREVALENCE

T. gondii infection is widespread among humans and its prevalence varies widely from place to place. Dubey and Beattie[302] summarized *T. gondii* prevalences before 1988 and Tenter et al.[1271] did so for surveys 1989–2000. Selected reports in pregnant women or women of childbearing age are given in reference 889a and in Table 2.1. In these surveys the prevalences of human infection ranged from a low of 4% in Korea to a high of 92% in Brazil. Sample size, age, and serological techniques may account for some of the differences in the reported prevalences. Infections are found on all continents, including Antarctica. In general, seroprevalences are higher in Latin America than in North America and East Asia. It is of interest that the magnitude of antibody titers in some South American countries is higher than that in the United States, perhaps related to the probability of repeat infections.[543a] The DT antibody titers in the general population in El Salavador,[1091] Costa Rica,[543a] and six other countries in Latin America were higher than a survey in the United States. The serological test and the cutoff titer used are major considerations in any survey, and, as indicated in Chapter 1, DT titer may decline to the extent that antibodies are detectable only in undiluted serum. In the Costa Rican survey titers of 1:8 or lower accounted for 3% and lower than 1:64 accounted for 14% of the samples. Considering the bioassay data in pigs (see Chapter 6) there is no reason why these low DT titers are nonspecific. Infection is, on the whole, more common in warm climates and in low-lying areas than in cold climates and mountainous regions. This is probably related to conditions favoring sporulation and survival of oocysts. During the last decade, prevalences in general have decreased (Figure 2.1). More data on decreasing seroprevalences are available from countries that have established programs for screening of pregnant women.[55,87,781] Berger et al.[87] summarized data on French women of five different ages (20, 25, 30, 35, and 40 years old) from 1995 to 2003 and concluded that prevalence decreased in all five age groups. The greatest decline was in 20-year-olds (17.4% decrease from 37.6% in 1995 to 31.0% in 2003), and lowest in 40-year-olds (13.1% decrease from 65.4% in 1995 to 56.8% in 2003). These studies were based on 13,459 women in 1995 and 15,108 in 2003 tested by ELISA, and data were pooled from different laboratories. Another example of this decreasing prevalence is given in Table 2.2. A population-based National Health and Nutrition Examination Survey (NHANES) found a decrease in the age-adjusted *T. gondii* prevalence in U.S.-born persons 12–49 years old, from 14.1% in 1988–1994 to 9% in 1999–2004, a seroprevalence of 11% in U.S.-born women 15–44 years old in 1999–2004, and a seroprevalence of 28.1% in foreign-born women in 1999–2004.[738] These population-based surveys are expensive and difficult to conduct in countries with a large population. The constant influx of immigrants with different ethnic backgrounds and cultures in a country such as the United States also affects seroprevalence estimates. In North America (most data are from the United States) there is an upsurge of infection during adolescence, whereas in Central and South America there is a steady rise during childhood.[426,652,734] There are only a few reports of seroprevalences in young children. In one study of 500 3–10-year-old

Table 2.1 Seroprevalence of *T. gondii* Antibodies in Pregnant Women or Childbearing Age,[a] General Population,[b] or Others[c]

Country	No. of People	% Positive[d]	Ref
Argentina[a]	3,049	59	555
Australia	10,207	35	1333
Austria[a]	167,041	43	55
Bahrain[b]	4,739	22	1263
Bangladesh	286	39	52
Belgium	25,137	57	121
Brazil	10,468	61	1089
Brazil	32,512	92	507
Cameroon	421	49	890
Canada[b]	917	60	914
Chile[b]	76,317	37	187
Colombia	955	46	1118
Congo[a]	2,897	60	883
Costa Rica[b]	1,234	76	43
Cote d`Ivoire[a]	1,025	60	6
Croatia[a]	2,778	46	1236
Cuba	5,537	71	598a
Czech Republic	3,392	37	649
Democratic Republic of São Tomé and Príncipe	499	75	692
Denmark	24,989	28	814
Djibouti[c]	108	43	165
Egypt	600	27	59
Ethiopia[c]	170	80	1368
France	158,001	61	723
Gabon	767	71	964
Germany	5,670	39	88
Greece	5,532	29	37
Grenada, West Indies	534	57	57
Guatemala	550	44	1198
India	23,094	24	256
Indonesia[b]	1,693	70	1272
Iran	576	34	487
Ireland[c]	1,276	13	1267
Israel	213	21	527
Italy	3,426	22	239
Jamaica	1,604	57	1067
Japan[c]	56	5	966
Jordan	280	47	745
Korea	899	4	1123
Libya	369	47	756
Madagascar	599	84	820
Malaysia	200	49	983
Mayotte[b]	871	84	743
Mexico	439	8	30
Moldavia	571	44	199

Table 2.1 Seroprevalence of *T. gondii* Antibodies in Pregnant Women or Childbearing Age,[a] General Population,[b] or Others[c] (Continued)

Country	No. of People	% Positive	Ref
Morocco	2,456	51	462
Nepal	345	55	1081
Netherlands[b]	7,521	41	781
New Caledonia[a]	2,416	57	122
New Zealand	500	33	952
Niger[b]	371	18	744
Norway	35,940	11	724
Pakistan[b]	640	6	1021
Panama[b]	326	32	1216
Papua New Guinea	197	18	774
People's Republic of China	235	11	861
Philippines[b]	1,169	19	757
Poland	3,734	59	1039
Qatar[b]	1,625	30	2
Republic of Benin	211	54	1108
Saudi Arabia[b]	150	47	1144
Sénégal	109	36	973
Sierra Leone[c]	93	43	1115
Singapore	120	17	1372
Slovakia	656	22	1240
Slovenia	21,270	34	864
Spain[a]	6,454	30	720
Sudan	487	34	469
Sweden	3,094	14	482
Taiwan	426	31	834
Tanzania	849	35	265
Thailand	1,200	13	173
Trinidad and Tobago, West Indies	450	35	1084
Tunisia[a]	3,288	64	82
Turkey[a]	2,586	70	1269
Uganda[c]	130	54	857
United Arab Emirates	1,503	23	217
United Kingdom	13,000	8	24
United States[e]	23,217	38	1170
Venezuela[b]	254	37	262
Vietnam	300	11	127
Yugoslavia[a]	1,157	77	106
Zambia[c]	376	7	1399

d = number rounded off.
e = See Table 2.2.

Guatemalan children, antibodies were found in 37.8%; seroprevalence increased from 25% in 3-year-olds to 45% in 5-year-olds.[736] Souza et al.[1217] found marked differences in seropositivity among 608 school children from two localities, Bonsucesso (166 children) and Jacarepaguá (442 children) and two age groups (6–8-year-olds, and 12–14-year-olds) within the city of Rio de Janeiro, Brazil.

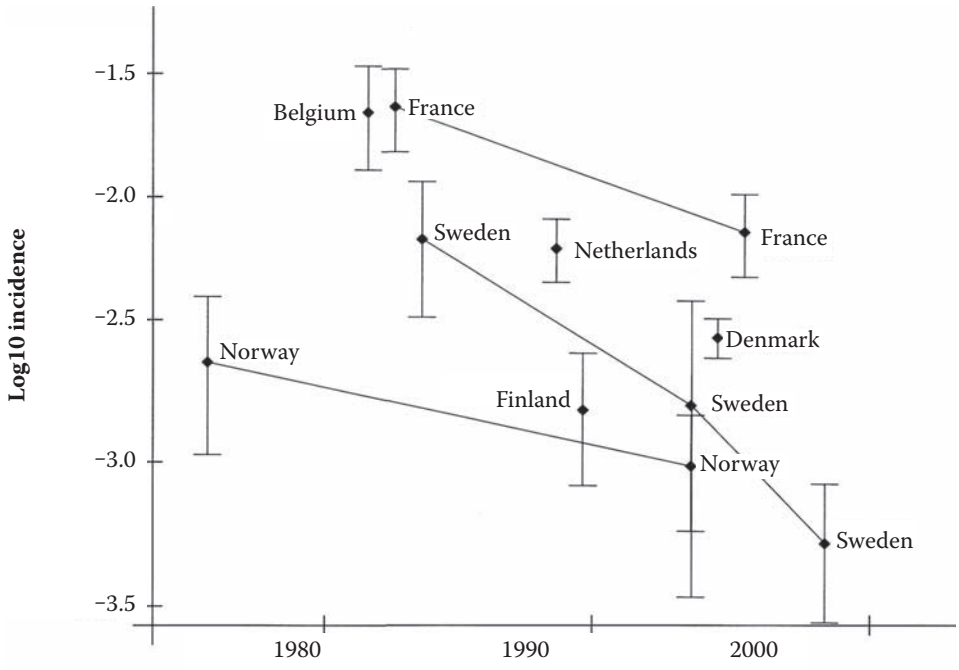

Figure 2.1 Decreasing seroprevalences of *T. gondii* based on the paper by Welton and Ades.[1354] Courtesy of Tony Ades, Eskild Petersen, and Ruth Gilbert.

Overall, 68.4% of 608 children were seropositive (IFA, 1:16), but none had IgM antibodies; 36.5% of 81 6–8-year-olds and 70.4% of 81 12–14-year-olds in Bonsucesso and 64% of 222 6–8-year-olds and 84.5% of 220 12–14-year-olds in Jacarepaguá had *T. gondii* antibodies. Results indicate a very high rate of postnatal transmission of *T. gondii* during childhood. Recently, using the same test (IFA, cutoff 1:16) Lopes et al.[865] found that 46.4% of 4–11-year-old children from Paraná, Brazil had *T. gondii* antibodies. Frenkel et al.[546] found that 72 of 571 (12.6%) of children in Panama City seroconverted between 1 and 6 years of age.

Table 2.2 Selected Reports of *T. gondii* Seroprevalence in Humans in the United States[a]

Year Sampled	Age Group	Source of Sera	No. Tested	Test	% Positive	Ref
1962	young adult	Military recruits	2680	DT	14	494
1989	young adult	Military recruits	2,862	MAT	9.5	1210
1988–1994	age-adjusted ≥12 yrs old	NHANES	17,658	ELISA[d]	22.5	734
1999–2000	age-adjusted 12–49 yrs[b]	NHANES	4234	ELISA[d]	15.8	735
1999–2004	age-adjusted 12–49 yrs[c]	NHANES	15,960	ELISA[d]	10.8	738

[a] Modified from Dubey and Jones.[426]
[b] Women 12–49 yrs, 14.9%.
[c] Women 15–44 yrs, 11%.
[d] Platelia Toxo-G enzyme immunoassay kit.

The prevalence of infection varies among ethnic groups, but this is considered due to sanitary and cooking habits rather than to genetic differences. Most surveys have shown a higher rural than urban prevalence, but some showed no difference, and some found higher prevalence in subjects from cities than rural areas.[27,71,302,724,725] An excessive prevalence has been found in people who have had frequent contact with soil and animals.[302] Of relevance here are outbreaks of infection among children who played in or ate soil contaminated by cat feces.[1232] Prevalence of *T. gondii* infection was generally higher at low altitudes and in wet areas than in mountains and dry areas. *T. gondii* infection is also common in abattoir workers, waste pickers, and garbage handlers.[28,302] However, lower seroprevalences in abattoir workers than in pig farmers have also been reported.[1169] Higher infection rate in pig farmers might be associated more with contact with contaminated soil than handling of pigs.[1347]

2.2 CLINICAL INFECTIONS

2.2.1 Primary, Postnatally Acquired Toxoplasmosis

T. gondii is one of the best adapted of all parasites, being able to persist for long periods in the body of its hosts—probably for the entire lifetime of its hosts. Tissue cysts have been found at autopsy from patients who died of unrelated causes and in organs removed surgically.[1090,1168] *T. gondii* has even been isolated from the blood of healthy people or those who died of unrelated causes.[927]

In an immune-competent person, *T. gondii* rarely causes illness, but is much more likely to do so in an immune-compromised person. In immune competent persons illness usually begins with listlessness, fatigue, headache, excessive sweating, and muscle and joint pains; there may be slight fever and, sometimes, a maculopapular rash. These manifestations may last for one or several weeks and then subside.[664] General malaise and listlessness may persist for months. Because these symptoms are common in other diseases (e.g., influenza, viral syndromes, mononucleosis, etc.); acute toxoplasmosis is more often unrecognized than recognized. Suspicion of toxoplasmosis is most likely to be aroused when (as is generally the case) one or more of these symptoms are accompanied by enlargement of lymph glands, particularly at the angle of the jaw, in the pre- and post-auricular regions, and behind the sternocleidomastoid muscle (Figure 2.2). Glands in the axillary and inguinal regions in the hila of the lungs and in the mesentery are sometimes also involved. The lymph nodes average 1 to 2 cm in diameter, are discrete, and tender to the touch, but not usually painful; they never suppurate. Lymphadenopathy may persist for months (e.g., when the author J.P. had toxoplasmosis, his lymph nodes were enlarged for more than 6 months). Lymphadenopathy is the most commonly recognized form of toxoplasmosis and if any manifestation can be said to be characteristic, this is it.[302,897] Clinical manifestations have been reported to be more common in boys than in girls and in young women than in young men—possibly for hormonal reasons.[98]

Uncomplicated lymphadenopathy due to *T. gondii* is rarely serious but it may cause a protracted illness followed by a lengthy convalescence. It is part of the differential diagnosis of a mononucleosis syndrome that can be caused by Epstein-Barr virus (EBV), Cytomegalovirus (CMV), or *T. gondii*. Lymphadenopathy is often accompanied by myalgia, which may proceed to myositis or dermatomyositis or by fatigue preceding myocarditis or pericarditis, pneumonitis, hepatitis, polyneuritis, hemolytic anemia, and nephritis.[302,938,939] Skin lesions may be found and usually take the form of a maculopapular rash; erythema nodosum has also been reported. Virtually all organs may be involved (Table 2.3).

The relative frequency of various manifestations of postnatally acquired toxoplasmosis in three outbreaks linked to ingestion or inhalation of oocysts is shown in Table 2.3. Some of the patients were ill enough to be admitted to the hospital, but none died. However, disastrous consequences can occur in pregnant women. For example, 3 of the 37 patrons of an Atlanta outbreak were pregnant. One of these women aborted in her first trimester and viable *T. gondii* was isolated from the fetus's amniotic fluid.[285,1274a] In the Brazilian waterborne outbreak 408 patients were examined for

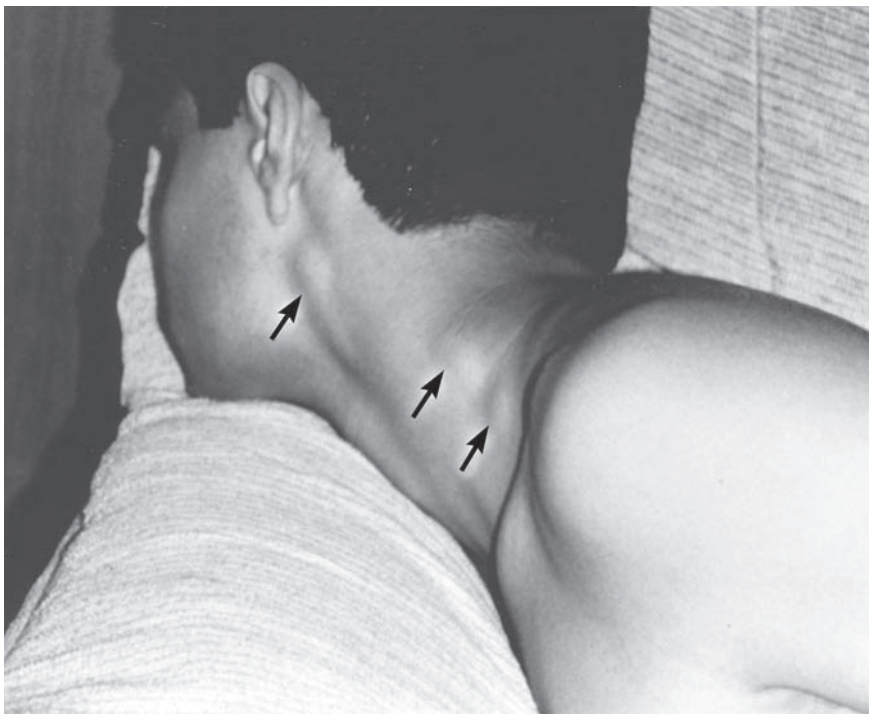

Figure 2.2 Enlarged cervical lymph nodes (arrows) in a 19-year-old boy with acquired toxoplasmosis.

Table 2.3 Frequency of Symptoms in Outbreaks of Toxoplasmosis in Adult Humans[a]

Location	Atlanta, Georgia	Panama	Paraná, Brazil
Ref	**1274**	**83**	**236**
No. of Patients	**35**	**35**	**155**
	Patients with Symptoms (%)		
Fever	94	90	82
Lymphadenopathy	88	77	75
Headache	88	77	87
Myalgia	63	68	80
Stiff neck	57	55	NR
Anorexia	57	NR[b]	69
Sore throat	46	NR	NR
Arthralgia	26	29	61
Rash	23	0	7
Confusion	20	NR	NR
Earache	17	NR	NR
Nausea	17	36	38
Eye pain	14	26	NR
Abdominal pain	11	55	NR

[a] From Dubey.[436]
[b] NR = not reported.

eye lesions and IgG and IgM *T. gondii* antibodies (Silveira,[1193] and personal written communication); 18 had typical lesions of retinochoroiditis (15 unilateral, 3 bilateral) and 24 had atypical superficial retinitis lesions. Ten women seroconverted during pregnancy; six babies were born with congenital toxoplasmosis, four with ocular lesions, one with neurological signs. One woman had tetra-eye involvement—both of her eyes and both eyes of her infant were affected (Belfort Rubens, personal communication).

Another outbreak of human toxoplasmosis epidemiologically linked to drinking water from a water reservoir was initially identified because of the screening of pregnant women. Lymphadenitis was found in 51 of these 100 patients.[116] An unusual finding, not previously recognized, was finding choroidoretinitis in 20 of these 100 adults; both eyes were affected in 1 patient.[116,129] Although data on congenital toxoplasmosis in this outbreak have not been published, at least one had a congenitally infected baby with symptoms (McLeod et al.[903a]; McLeod, personal communication). The clinical spectrum documented in Table 2.3 was possible because many patients became ill at the same time and because these outbreaks were recognized early in the course of infection.

Small outbreaks of toxoplasmosis were reported by others and these have been summarized previously.[302,584,1207] Of these, two reports from the United States[758] and Korea[174] are recalled here because of their unusual nature. Five medical students developed symptoms of acute toxoplasmosis characterized by headache, fever, lymphadenopathy, myalgia, and splenomegaly during the second week after eating rare hamburgers at a university snack bar.[758] These five students were unrelated and lived and ate separately—except one evening when they were in a hurry to attend a talk by the famous heart surgeon (Dr. Christian Barnard), and the hamburgers were made in a hurry. Fortunately, only one of them was female, and she was not pregnant. In the Korean episode, in two separate incidents eight adults developed lymphadenopathy and ocular disease after feasting on raw or undercooked pork; one of them became blind even after chemotherapy.[174] Diagnosis of outbreaks of toxoplasmoisis based solely on serology or symptoms is often difficult and uncertain. [265,1021a.]

Although most cases of acquired toxoplasmosis reported were in adolescents or adults, perhaps one of the most serious cases known was in an asymptomatic 6-year-old boy from Cincinnati, Ohio (Sabin[1127]). This child with initials of R.H. was hit with a baseball bat on October 22, 1937. He developed a headache two days later and convulsions the day after. He was admitted to the hospital on the seventh day but without obvious clinical signs. Except for lymphadenopathy and enlarged spleen, nothing abnormal was found. He then developed neurological signs and died on the thirtieth day of illness. The brain and spinal cord were removed for histopathological examination and bioassay. Because of the suspicion of polio virus infection (Dr. Sabin was an expert on polio virus) a homogenate of cerebral cortex was inoculated into mice for possible virus isolation. *T. gondii* was isolated from the inoculated mice and this isolate was given the initials of the child and became the famous RH strain. Only small lesions of nonsuppurative encephalitis were found microscopically in the brain of this child, without any calcification. This child most likely had acquired *T. gondii* infection recently and the blow to the head was coincidental and unrelated to the onset of symptoms.

Ocular disease is probably the most common involvement of acquired toxoplasmosis.[461] Until the 1980s, most of *T. gondii* retinochoroiditis was thought to be congenital.[669,670] Ophalmologists from Brazil first reported retinochoroiditis in multiple siblings.[1191] These findings have now been amply confirmed.[129] In the Canadian outbreak, 20 of 100 patients had acquired retinochoroiditis.[116,129] Acute toxoplasmic retinochoroiditis results in pain, photophobia, tearing, and loss of vision. Lesions tend to recur with progressive loss of vision over time, especially when the lesions are near the central structures of the eye.[669,670,1262] As many as 1.26 million persons in the United States may have ocular toxoplasmosis (based on the 2000 census), and an estimated up to 2% of persons with *T. gondii*.[669] One should also remember that *T. gondii* was first found in eyes that had been removed from patients suspected to have tumors or tuberculosis.[1358] Ocular toxoplasmosis was the most frequent cause of unilateral visual loss among 582 patients at two eye clinics in The Netherlands.[1119] In this study, ocular toxoplasmosis was thought to be postnatally acquired in children.

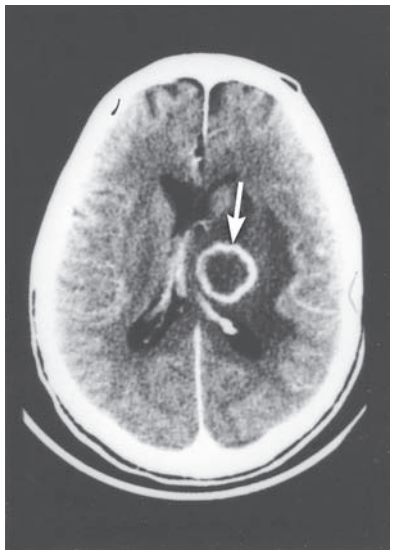

Figure 2.3 Toxoplasmic encephalitis demonstrated by computerized tomography (CT) scan of brain. Patient infected with HIV. Intravenous contrast material shows ring enhancing lesion. This lesion was biopsied because of its large size and displacement of brain tissue, not typical of toxoplasmic encephalitis in AIDS patients. Biopsy demonstrated proliferating *T. gondii* tachyzoites. Courtesy of John J. Warren, Department of Laboratory Medicine, University of Seattle, Washington.

The most dangerous of the complications of toxoplasmosis are found in patients whose immunity has been depressed by malignancies and anti-tumor therapy or the acquired immune deficiency syndrome (AIDS). Toxoplasmosis ranks high on the list of diseases that lead to the death of patients with AIDS.[867–869,1076] Clinically, patients may have headache, disorientation, drowsiness, hemiparesis, reflex changes, and convulsions, and many become comatose. By using a CT scan, lesions (Figure 2.3) were localized in decreasing order of frequency in cortico-medullary, white matter, basal ganglions, cortex, and posterior fossa.[1095] Macroscopically, unilateral or bilateral areas of discoloration indicative of necrosis (Figure 2.4) and hemorrhage are present.[92,1095] Massive hemorrhage in a 41-year-old AIDS patient caused a fatal automobile accident.[622] Microscopically, encephalitis involving many areas is the predominant lesion and the difference may vary depending on whether the patient has received anti-*T. gondii* therapy. In untreated patients, the lesion involves a central area of necrosis with degenerating organisms, surrounded by an inflammatory zone with edema, perivascular infiltration of inflammatory cells, and hemorrhage. *T. gondii* are more numerous in this peripheral zone surrounding healthy and inflamed tissue (Figure 2.5). The lesion may be small or the size of a tennis ball and the contents may vary from fluid to solid, and as many as 100,000 or more tachyzoites may be present in 1 ml of fluid or tissue. Micro-abscesses are more common in treated patients. In active lesions, numerous tachyzoites are found destroying host tissue. In subacute cases and treated patients, glial nodules predominate. Patients should be treated empirically for *T. gondii* encephalitis based on clinical and neurological findings and presence of *T. gondii* antibodies, because a specific diagnosis may not be possible without a biopsy, which is now rarely indicated. Pneumonia, other disseminated systemic disease, retinochoroiditis, and orchitis can be seen, but are not as common as *T. gondii* encephalitis in HIV-infected persons.[1245]

In most AIDS patients, the disease occurs from reactivation of latent *T. gondii* infection because of immunosuppressive effects of the HIV infection. Reactivation of latent infection

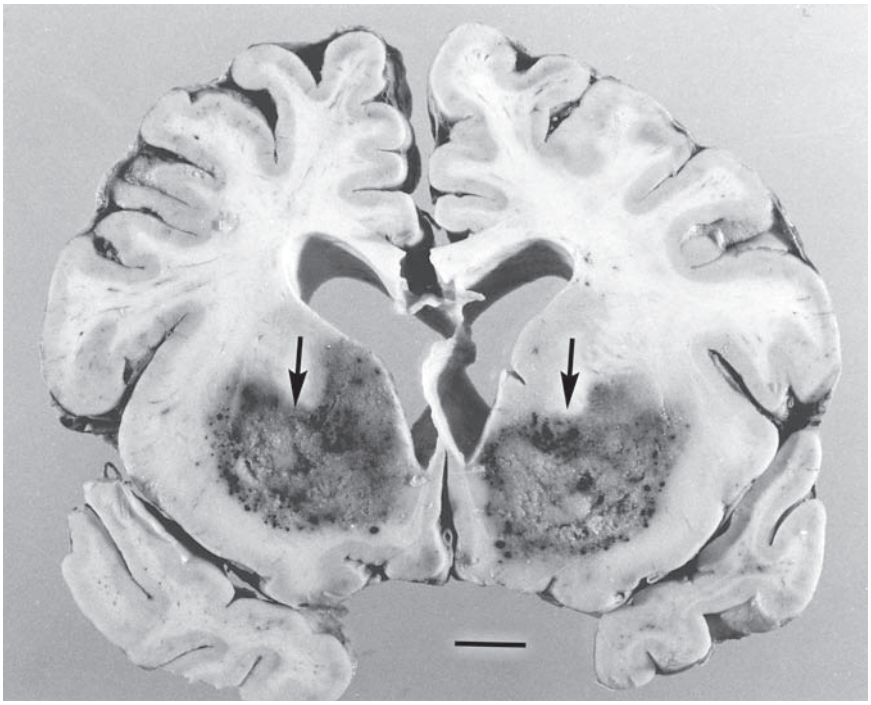

Figure 2.4 Bilateral areas of necrosis (arrows) due to toxoplasmosis in basal ganglia and thalamus of a 67-year-old man treated for Hodgkin's disease. (Courtesy of Drs. N. R. Ghatak and D. R. Sawyer.) From Dubey and Beattie.[302]

occurs when persons become severely immunosuppressed, with the highest risk occurring when the CD4+ T-lymphocyte count drops below 50 cells per microliter.[733,820a,867,869,1064,1373] The incidence of toxoplasmic encephalitis in AIDS patients has decreased since prophylaxis and highly active antiretroviral therapy became widely used against *T. gondii*.[1,674,712]

Transplantation of infected organs or transfusion of infected leukocytes can initiate fatal infection in a seronegative recipient receiving immunosuppressive therapy.[162,302,1096,1201,1376] Transplantation of non-infected organs and leukocyte transfusion can also activate latent infection in a seropositive recipient receiving immunotherapy. Ordinary blood transfusion is virtually free from danger, but transfusion of packed leukocytes and transplantation of bone marrow has caused toxoplasmosis. It seems that the danger of transplanting an organ from a seropositive donor into a seronegative recipient is greater than that of transplanting an organ from a seronegative donor into a seropositive recipient. Rogers et al.[1112] reported fatal toxoplasmosis in two renal allograft recipients who received kidneys from the same cadaveric donor.

As stated above, malignancies or immunosuppressive treatment of malignancies can reactivate latent toxoplasmosis. Toxoplasmosis has been reported most commonly in patients treated for Hodgkin's disease. Untreated Hodgkin's disease is rarely associated with clinical toxoplasmosis. A variety of malignancies, including lymphoma, leukemia, and myeloma, can reactivate toxoplasmosis, but there are also rare reports of toxoplasmosis associated with solid tumors.[711,1376] The involved organs (in decreasing order) are brain, spinal cord, eye, heart, lungs, and, rarely, disseminated infection.

Although generalized, fatal toxoplasmosis is most often found in immune-depressed patients, it can sometimes be found in patients with no obvious defect of immunity.[221,245,755]

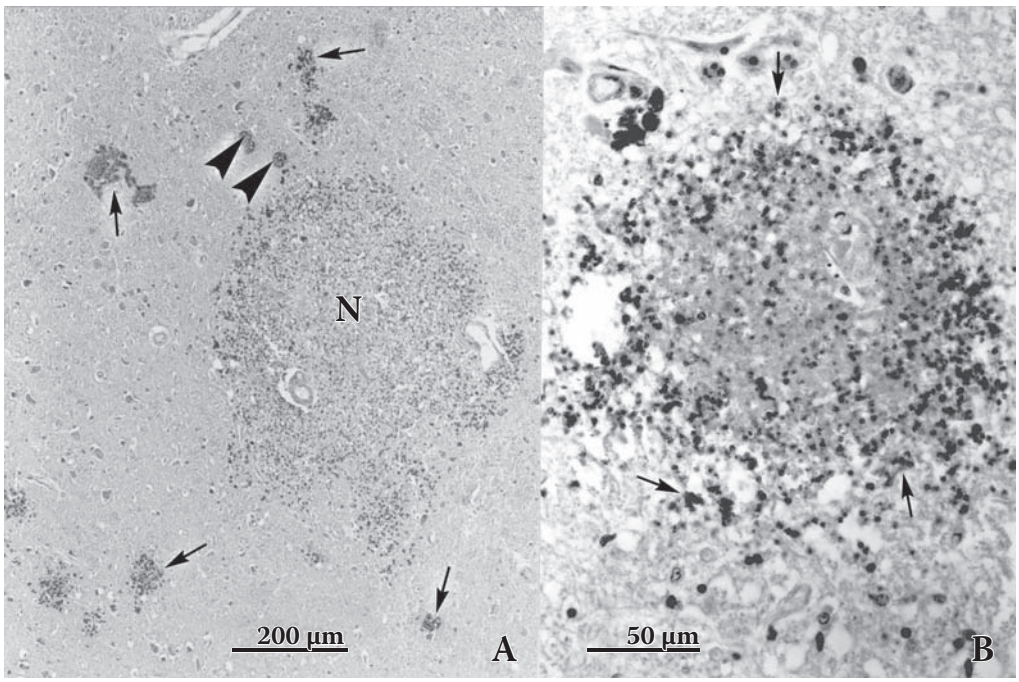

Figure 2.5 Necrosis associated with *T. gondii* in an AIDS patient. Immunohistochemical stain with anti-*T. gondii* serum. **(A)** Central area of necrosis (N), two tissue cysts (arrowheads), and several satellite lesions (arrows). **(B)** Higher magnification of one of the satellite lesions. Note hundreds of tachyzoites (arrows, all black dots) at the periphery of the necrotic lesion.

The effects of asymptomatic chronic *T. gondii* infection on human behavior and well-being in general are difficult to evaluate with respect to a cause–effect relationship; *T. gondii* seropositivity has been linked to reaction time,[635] tendency for accidents,[513] behavior,[515,792] and mental illness.[125,514,649a,979,1382]

2.2.2 Secondary, Prenatally Transmitted (Congenital) Toxoplasmosis

When a pregnant woman acquires *T. gondii* infection, she may transmit it transplacentally to her fetus. This happens usually when a woman is newly infected during pregnancy. Rarely, transmission occurs in women infected just before pregnancy or during chronic infection (Remington et al.[1094]; Elbez-Rubinstein et al.[465] and references cited therein). In addition, in immunosuppressed women, reactivation of an infection acquired before pregnancy can lead to congenital toxoplasmosis, but is rare. The risk of congenital infection is lowest when maternal infection is in the first trimester (10–15%) and highest when infection occurs during the third trimester (60%–90%).[446,522,1094] If maternal infection occurs early in pregnancy, it results in fewer infected babies, but they are more severely affected than the greater number of infected babies born when infection is acquired later in pregnancy. The highest risk to the fetus is when infection is acquired between the 10th and 24th week of gestation.[91,213,251,252,446,1068,1094] As seen in Table 2.4, most of this information on transmission of congenital toxoplasmosis is derived from studies in France. In their pioneering study, Desmonts and Couvreur[251,252] reported congenital toxoplasmosis in 210 children born at a hospital in Paris;

Table 2.4 Rate of Congenital Transmission According to Gestational Age of Maternal Seroconversion*

Gestational Age (wks)	n = 489	n = 187	n = 2081	n = 603	n = 1438
<16	9.1	3.9	3.7	6	15
16–28	28.2	17.1	16.5	40	44
>28	59.3	53.1	28.9	72	71
Ref.	**255**	**1068**	**667**	**446**	**1260**

* No. of women who seroconverted during pregnancy.

approximately 26% were subclinically infected at birth. Only about 10% were clinically affected—6% mildly and 4% severely—and up to 3% died in the neonatal period. In a subsequent study from the same hospital, Daffos et al.[213] reported clinical outcome in 148 infected fetuses of 2,030 mothers who seroconverted during pregnancy. Based on ultrasound examinations they found that 48%, 12%, and 3% of fetuses had cerebral ventricular dilatations when mothers became infected in early (<16 weeks), middle (17–23 wk), and late (after 24 weeks) of gestation, respectively.[213] A recent multicenter European study of 255 congenitally infected children indicated that gestational age at the time of seroconversion in mothers was correlated with cerebral lesions but not retinochoroiditis.[603] In this study, 51 of 255 infants had one or more lesions, and 9 had both intracranial and ocular lesions. Of these, 4 of 55 children died (at 13 mo, 11 mo, 3 mo, and 7 days of age). The mother of the baby that died at 1 week of age had seroconverted between 5 and 31 days of gestation and she had received spiramycin prophylaxis from 32 weeks until delivery.[603] The frequency and severity of clinical disease in congenitally infected children in France and Austria has decreased dramatically in the last decade, perhaps as a result of improved early detection and treatment (see Remington et al.[1094]).

Fortunately about 60–70% of babies born of infected mothers escape infection.[446, 589] The severity of toxoplasmosis in the fetus or the infant is not related to the degree of symptoms of *T. gondii* infection in the mother, but to the trimester in which infection is acquired. In one study that retrospectively examined risk factors among women who gave birth to infected children, 52% could not recall being sick.[117] In another study from a hospital in Lyon, France, of 603 women who seroconverted during pregnancy only 36 (5%) had clinical symptoms.[446] In 161 of these 603 women (504 were treated for toxoplasmosis), infection was transmitted to their fetuses; 5 of them aborted, 3 were stillborn. Most of the 153 live-born children were followed for 54 mo after birth; 41 (27%) developed clinical signs (33 choroidoretinitis, 14 intracerebral calcification, 8 had combinations of signs) and one child died when 8 days old.[446]

An estimate of the incidence of clinically manifest prenatal toxoplasmosis may be obtained in three ways: first, from reports of observed cases; second, from calculations based on the infection rate during pregnancy, and third, from screening of babies at birth. These rates will vary among countries, and data before 1988 were summarized by Dubey and Beattie.[302] More information is available from countries that have screening (prenatal or postnatal) programs (Table 2.5). Prenatal screening is based on testing mothers for *T. gondii* seroconversion during pregnancy, and postnatal screening is often sampling of cord blood or heel pricks of the newborn combined with phenylketonuria (PKU) testing (Guthrie cards). However, most of these data were based on prevalence at birth without a follow-up period. As discussed later, most children infected with *T. gondii* in utero develop clinical manifestations several years after birth. In Table 2.5, initial data from screening studies are listed; at that time only a few follow-up studies on infected children were undertaken. Subsequently many of these children were followed clinically for 4 or more years, and data were included in the study reported by SYROCOTT.[1260] Of 691 congenitally infected children from Europe, Brazil,[66a,975a]

Table 2.5 Congenital of *T. gondii* Infection in Newborn Children Based on Prenatal or Postnatal Screening[a]

Country	No. Screened	Infected Children	Symptomatic Children[b]	Ref
Australia	18,908	3	0	1333
Austria	?	34	3 (ICC 2, RC 3)	591
	?	57	8 (ICC 4, RC 8)	1260
Brazil	>140,914	> 53	20 (ICC 17, RC 15)	66a, 592, 975a, 1260
Colombia	?	8	3 (RC 3)	577, 1260
Denmark	89,873	27	5 (hydrocephalus and RC1, ICC and RC1 ICC and retarded 1, blind and retarded 1)	591, 814
	262,912	55	12 (ICC 5, RC 2, ICC and RC 4, hydrocephalus, ICC, and RC 1)	1155
Hungary	17,735	0		1256
Finland	16,733	4	2 (ICC and RC 1), no additional cases in 5-yr follow-up	796
France	30,768	36	7 (RC 7, CNS1)	1059
	?	396	65 (ICC 24, RC 46)	1260
Italy	28,247	2	0 at birth, new RC in 3 in 1-yr follow up	1303
	?	15	3 (ICC 3, RC 3)	1260
Netherlands	28,049	12	3 (ICC 1,RC 3)	591, 1260
Norway	35,940	11	1 (RC with loss of vision)	725
	?	17	6 (ICC 4, RC 3)	1260
Poland	>27,516	20	5 (ICC 5, RC 1)	1037, 1038, 1038a,1260
Sweden	35,000	3	1 (ICC, RC 1)	481a, 482, 1260
United States	>635,000	50+5	19 (14 CNS, 9 RC), 1–6 yr follow-up in 39 children, new eye lesions in 4	610, 611

[a] Modified from Petersen[1049] and reference 1260.
[b] Retinochoroiditis (RC), ICC (intracerebral calcification), CNS-central nervous system signs.
? = not stated.

Colombia,[595a] and the United States, 24% had at least one clinical manifestation, 185 had ocular lesions, and 13% had intracerebral calcification.[1260]

Transplacental infection can lead to a wide variety of manifestations in the fetus and infant including spontaneous abortion, stillbirth, and a live infant with severe disease, but most children are asymptomatic at birth. Although *T. gondii* may sometimes cause sporadic abortion, there is no evidence that it causes habitual abortion. Most severe cases of prenatally acquired toxoplasmosis were the first to be reported with the predominant manifestation of encephalomyelitis. Historically, the first confirmed case of congenital toxoplasmosis was in an infant girl who was delivered full term by Caesarean section on May 23, 1938, at Babies Hospital, New York.[1369] The girl developed convulsive seizures at 3 days of age and lesions were noted in the maculae of both eyes through an ophthalmoscope. She died of toxoplasmosis when 1 month old and an autopsy was performed. At post-mortem, brain, spinal cord, and right eye were removed for examination. Free and intracellular *T. gondii* were found in lesions of encephalomyelitis and retinitis and viable *T. gondii* was isolated in animals inoculated with tissues from the girl.

Hydrocephalus is the most dramatic but rare symptom of congenital toxoplasmosis (Figure 2.6 and 2.7). It is produced as a result of host reaction to the parasite. The initial response in the brain to

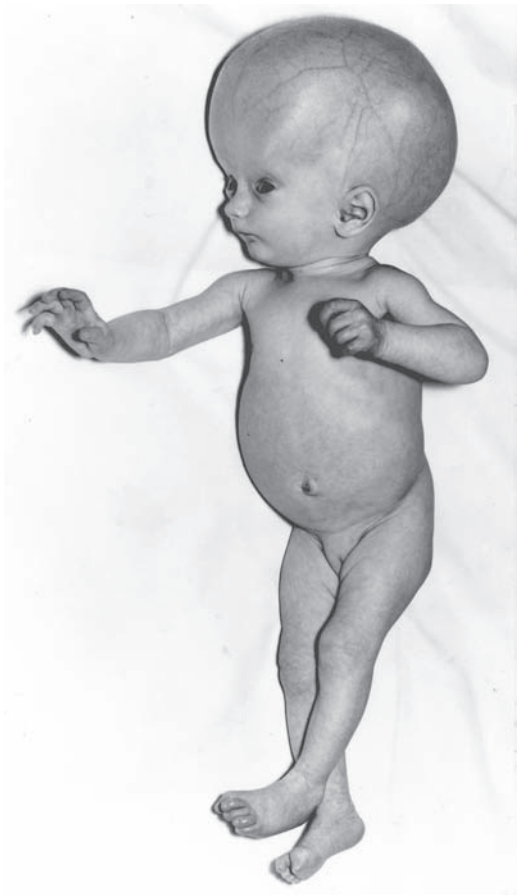

Figure 2.6 Hydrocephalic and spastic baby. From Dubey and Beattie.[302]

T. gondii proliferation is the formation of microglial nodules. Vascular involvement produces areas of necrosis over which the meninges are often inflamed. The greatest damage is to the subendymal tissue of the walls of the lateral ventricles. This becomes desquamated; sloughs block the aqueduct and lead to dilatation of the lateral ventricles and hydrocephalus.[532] Calcification takes place in foci of necrosis (Figure 2.7). In the early 1950s Dr. Albert Sabin proposed a much-quoted triad of signs: hydrocephalus or microcephalus, intracranial calcification, and retinochoroiditis (Figures 2.8 and 2.9). This triad has been useful in drawing attention to prenatal toxoplasmosis. A better understanding came from the work of Eichenwald,[456] who found asymptomatic and clinical toxoplasmosis in many children in the 1950s in Austria. Although the study by Eichenwald[456] was from selected cases before treatment (prenatal and postnatal) of infected children became a routine, it demonstrated that toxoplasmosis can cause serious illness in children.[302]

As stated earlier, the most common manifestation of prenatal toxoplasmosis is ocular disease, sometimes presented as microphthalmy (Figure 2.6), cataracts, strabismus or nystagmus, and even total blindness.[48,903b] In the newborn, the lesions may be active, usually bilateral, and involve the macula. In active ocular toxoplasmosis, there is pain, redness, photophobia, loss of vision,[48,812] and what has been described as "dancing eyes."[1094] Retinochoroiditis (Figure 2.8) is the underlying etiology in most cases. *T. gondii* proliferation in the retina causes necrosis of retinal layers through which yellowish areas of choroid may be visible. If choroid is destroyed, the whitish sclera may be

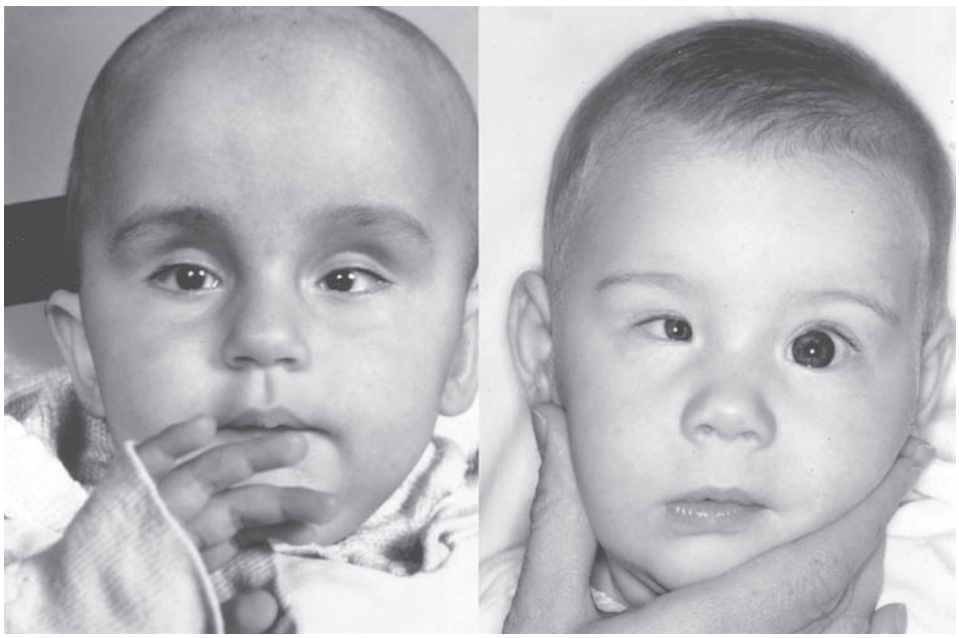

Figure 2.7 Congenital toxoplasmosis in children. Hydrocephalus with bulging forehead (left) and microophthalmia of the left eye (right). (Courtesy of Dr. J. Couvreur.)

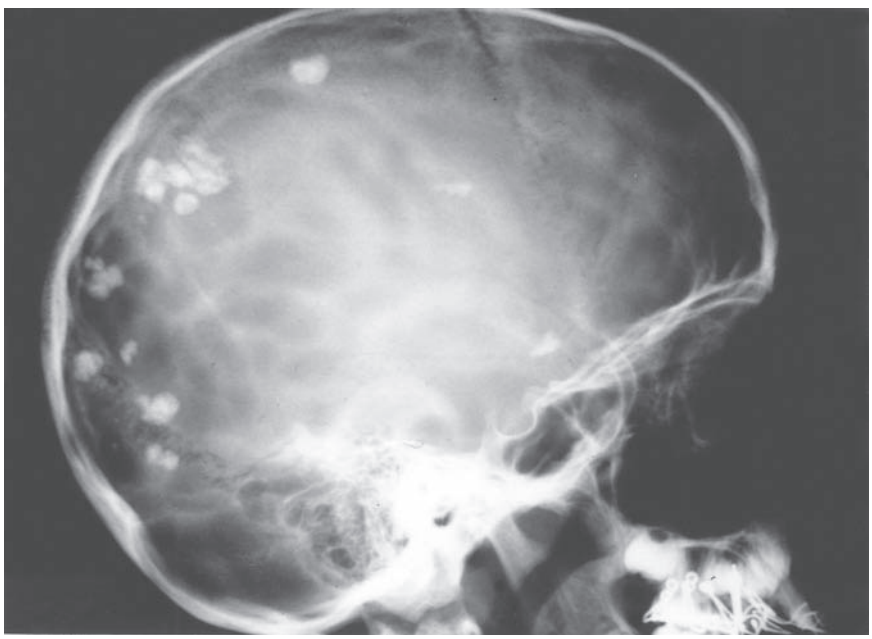

Figure 2.8 Intracerebral calcification discovered fortuitously in a 10-year-old girl, on a dental panoramic x-ray requested by a dentist. The girl had unilateral retinochoroiditis and an IQ of 80. (Courtesy of Dr. J. Couvreur.)

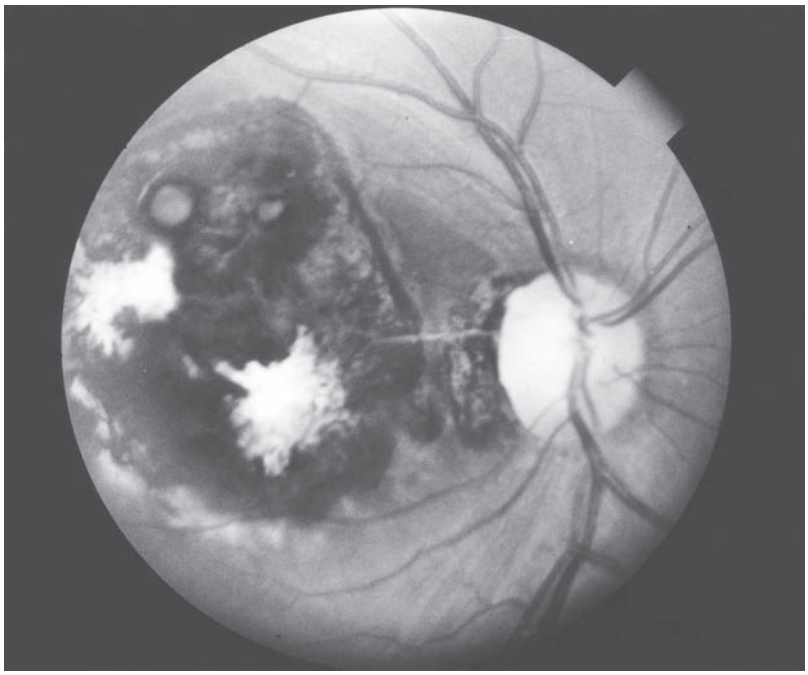

Figure 2.9 Congenital toxoplasmosis. Retinochoroiditis in the macula of the left eye. (Courtesy of Dr. R. Belfort Jr.)

visible. This picture, however, may be clouded by vitreous exudate. In older children acute retinal lesions are usually unilateral and smaller than the optic disc. Acute lesions appear soft and like cotton or wool and have indistinct borders, whereas the older lesions are whitish grey and sharply outlined. Retinal edema may cause blurred vision. Lesions may be reactivated from time to time, and old and new lesions may be found side by side.[669] Inflammation of the anterior uvea, iris, ciliary body, cataract, and optic atrophy can occur.[195]

Most prenatal infections are subclinical at birth. Disease, if present in the neonatal period, is likely to be severe, invariably with neurological signs, and often with signs of generalized infection. Such patients rarely recover without serious sequelae. Disease appearing in the first few months of life is usually less severe and is manifested by nystagmus, convulsions, bulging fontanelle (Figure 2.6), and abnormal increase in skull circumference. Such patients sometimes develop normally.

It is likely that many cases of prenatal toxoplasmosis are missed because of difficulty in diagnosis. Couvreur et al.[194], who diagnosed prenatal toxoplasmosis in 210 babies aged 0 to 10 months, found premature birth, intrauterine growth retardation, or both in 17%, hyperbilirubinemia in 10%, hydrocephaly or microcephaly in 9%, intracranial calcification in 11%, and retinochoroiditis in 22%. Infection was fatal in 2 of the 210, severe in 10%, mild without neurological signs in 34%, and subclinical in 55%. It is noteworthy that over half of the babies born with *T. gondii* infection had no clinical manifestations.

An important question concerns the subsequent fate of subclinically infected babies. Many of them develop retinochoroiditis, although it may not manifest until later in childhood, or even in adult life.[302,446,530,592,610,903b,1056,1057] Eleven of 120 congenitally infected children (many with obvious symptoms) recruited in a treatment program died within 4 years in spite of treatment.[903] The percentage of retinochoroiditis and mental retardation due to toxoplasmosis is unknown because of

difficulty in diagnosis. The clinical picture may vary in twins, with monozygotic twins having similar signs and dizygotic different presentations.[195,1052] In dizygotic twins, one twin can have severe disease whereas the twin mate may be uninfected. Severe clinical toxoplasmosis has been reported in triplets.[1365]

2.3 DIAGNOSIS

2.3.1 Lymphoglandular Toxoplasmosis

If a specimen of glandular tissue has been removed by biopsy, the diagnosis of toxoplasmosis can be assumed from its histological appearance.[1062] It is hyperplastic and has prominent germinal centers containing phagocytosed nuclear debris. Numerous aggregations of epithelioid histiocytes are present, but the normal architecture of the node is preserved. The subcapsular and trabecular sinuses are distended by monocytoid B cells.[449,897,1068,1149] The diagnosis is confirmed if *T. gondii* is found in stained sections, but this rarely happens. A better chance of success follows staining of the section by the immunohistochemical method or by demonstration of *T. gondii* DNA by PCR. Diagnosis can be made certain by isolating the parasite in mice or tissue cultures inoculated with the tissue. The enlargement of these peripheral lymph nodes is probably immunological and only a few *T. gondii* are present even in acute infection. Most commonly, however, diagnosis is solely serologic, resting on the development of *T. gondii* antibodies de novo or their significant increase in titer at repeated examinations as discussed in Chapter 1. The MAT testing with HS (formalin-fixed) or AC (acetone or methanol-fixed) antigens, and avidity tests can aid in determining how recent the infection was.[941,942] Serologic diagnosis can alleviate the need for biopsy. Parasitemia is rarely detectable by the time lymph nodes are palpable.

2.3.2 In the Immune Deficient Patient

Patients immunosuppressed by AIDS, and underlying malignancy, or by anti-tumor or transplant chemotherapy, may fail to develop significant immunity. In such cases an MAT is preferred, because it detects IgG antibodies.[248,1284] As immunodeficient patients often develop encephalitis (Figure 2.7) and brain abscesses; a computerized tomographic brain scan should be used to locate the lesions. Material taken by ordinary or needle biopsy for histologic examination and inoculation into mice or tissue culture or for PCR can establish diagnosis, but this is rarely done now. Isolation from blood or CSF can be successful.

2.3.3 In Ocular Toxoplasmosis

The appearance of the fundus of the eye is characteristic and will arouse suspicion of ocular toxoplasmosis. The diagnosis can be substantiated using serologic tests. However, in congenital infection these are unsatisfactory because retinochoroiditis usually results from infection in fetal life, and by the time it is suspected, perhaps years later, antibody titers will have fallen to levels common in the general population. Many older patients who had histologically confirmed toxoplasmosis had low levels of dye test antibodies (a titer of 1:16), and in one patient antibodies were demonstrable only in undiluted serum.[715] In cases due to postnatal infection, help will be obtained from tests to look for a rise in IgG antibody titers and tests for IgM and low avidity antibodies.[129,942] A comparison of titers of antibodies in the aqueous with those in the blood serum, after equilibration for total immunoglobulin content, may help by showing local synthesis of antibodies in the eye.[250,766] Immunoblots distinguishing intraocular from serum antibodies have been reported.[773] Demonstration of antigen

in the aqueous is possible, but only for a week or so after infection. However, such tests are not routinely performed. Nowadays a combination of local antibody synthesis and PCR tests are being performed in the ocular fluids (aqueous or vitreous) of patients presenting with severe atypical sight-threatening intraocular inflammation.[230]

2.3.4 In the Pregnant Woman

Serologic diagnosis of toxoplasmosis acquired during pregnancy is difficult and may be considered under two headings: first, testing of individuals and, second, mass screening. One reason for individual testing is to detect *T. gondii* infection acquired during pregnancy. Georges Desmonts in Paris, France, initiated mass screening in the 1960s when he was looking at seroconversion in women during pregnancy and the transmission of *T. gondii* to the fetus (Desmonts and Couvreur[251,252]). At about the same time Otto Thalhammer initiated a similar screening program for pregnant women in Austria.[1275,1276] A neonatal serological screening based on blood obtained by heel stick of the baby was initiated in Massachusetts in the 1980s.[610] Although many issues related to the cost and benefit of screening and treatment in pregnancy and in newborns remain controversial, these programs have raised awareness of the seriousness of toxoplasmosis in the general public.[426,903b]

For screening during pregnancy, the woman's serum is tested early in pregnancy for specific *T. gondii* IgG and IgM antibodies. The timing and frequency of testing are important. Testing should start as soon as possible in gestation, ideally in the first trimester for optimal medical intervention to prevent fetal damage. Testing should continue monthly or every 2–3 months, and if possible this should continue 4–6 weeks after the delivery.[942,1048] The objective of serological testing is to determine whether the woman became infected with *T. gondii* recently or in the distant past. This is not easy using one test. Luckily, several serological tests are available (see Chapter 1) to do this, including IgG, IgM, IgE, and IgA antibody tests. The IgM and avidity tests in particular are often used to assess whether infection was acquired recently or in the distant past.[965] Montoya and Remington[942] provided a guide for interpretation of serologic testing and the reasons for confirmation of results in a *Toxoplasma* Reference Laboratory. IgM antibodies appear early in acute infection and generally precede the onset of IgG antibodies but may persist for more than 18 months. High avidity antibodies appear 12–16 weeks after infection. If the IgG test is positive (no matter how low the titer), IgM is negative, and high avidity antibodies are present, this means that the woman has been infected before pregnancy, there is negligible risk to her fetus. If the IgG test is positive and the IgM test is also positive, the tests should be repeated in 3 weeks' time in a reference laboratory and expert advice sought for further testing of mother and the fetus. If the IgG titers have not risen and high avidity antibodies are present, infection may be assumed to have occurred before pregnancy, and there is little risk to the fetus. If, however, the IgG titer has risen, infection has probably taken place around the time of conception and there is a slight risk to the fetus. If the IgG and the IgM results are both negative, the woman is susceptible and the tests should be repeated every 4–6 weeks to see if either or both become positive. In the event the woman has been infected during pregnancy, as confirmed by IgG, IgM, avidity, and IgA testing the fetus is at high risk, and should be tested further. When the woman delivers, her child should be examined and, if found to be infected, clinically or subclinically, should be treated.

Another reason for individual testing is that the development of lymphadenopathy during pregnancy, in the event a high IgG titer (>1000) accompanied by a positive IgM result, makes the diagnosis of active *T. gondii* infection probable. If the IgG titer is <1,000, a second test should be carried out 2–3 weeks later. If infection is proved, advice of experts in the field should be sought regarding treatment.[942,1094]

2.3.5 In the Fetus

If the mother has been infected during pregnancy, the question may arise as to whether the pregnancy should be terminated. Before undertaking this, it is important to decide if the fetus is infected and if damage to the fetus has occurred. This can be done by repeated ultrasound examinations to detect enlargement of cerebral ventricles, a CT scan for cerebral calcification, demonstration of *T. gondii* antibodies in the fetal blood, isolation of *T. gondii,* or demonstration of *T. gondii* DNA by PCR from fetal blood or from amniotic fluid. A positive PCR correlates with active toxoplasmosis and is more sensitive than other diagnostic tests[79,1049]; a negative test does not exclude infection. Sampling at 18 weeks of gestation is optimal to demonstrate *T. gondii* DNA in amniotic fluid and a quantitative PCR can be a guide for parasite load.[1113] Quality control for PCR is a major concern, and confirmation with other methods should be considered before making life and death decisions. Diagnosis by these methods has prevented needless terminations of pregnancy.

2.3.6 In the Baby

Diagnosis may be made by serological examination, by bioassay of cord or peripheral blood into mice or tissue cultures, and by detection of *T. gondii* DNA. Placental tissue may also be used, but failure to isolate *T. gondii* does not rule out prenatal infection nor does success unequivocally prove it. In the event of death, stillbirth, or abortion, autopsy material should be examined. When a mother is known to have been infected during pregnancy and the baby shows some or all of Sabin's triad of signs, diagnosis presents no difficulty. Often, however, it is not known if the mother was infected during pregnancy. Serologic tests are important, both on the mother's blood to see if she has been recently infected, and on the baby's blood serum or cord serum. Antibodies in the last two, however, may merely have been transferred from a mother with longstanding latent infection and may persist for months. Evidence of active infection of the baby may be obtained by demonstrating IgM and IgA antibodies in its serum, because they do not cross the placenta. However, the gestational age and suppressive effect of maternal IgG and type of serologic test used can affect results.

Very often signs and symptoms are not apparent until weeks, months, or even years after birth. In such cases, assurance may be obtained from a primary fall in IgG antibody titers, indicative of the waning of maternal antibodies, followed by a secondary rise in these titers and, perhaps also, appearance of IgM antibodies, indicating the child's own production of antibody.[1332] Usually, passively transferred maternal IgG disappears by 12 months of age but in children given anti-*T. gondii* therapy, synthesis of IgG may be delayed until the second year of life. Immunoblots distinguishing maternal and neonate IgG antibodies have been reported.[980,1049,1093]

As the performance of serologic tests for *T. gondii* infection presents difficulties, and the interpretation of the result is not always easy, it is desirable that the examinations should be made in specialized laboratories.

2.4 TREATMENT

As stated in Chapter 1, pyrimethamine and sulfonamides still remain the drugs of choice for treating toxoplasmosis. Another drug, spiramycin, is also widely used in Europe as a prophylactic during pregnancy, but has not been approved by the Food and Drug Administration for use in the United States (it is available as an expanded access research drug). As explained in Chapter 1, these drugs act on the tachyzoites of *T. gondii*, rather than on bradyzoites in tissue cysts. Hence, while they

may control active infection, they cannot eliminate chronic infection. Pyrimethamine may depress hematopoiesis, and for this reason should be accompanied by folinic acid or fresh brewers' yeast; which is an inexpensive source of folinic acid, but equally effective. It is also considered teratogenic, and for this reason some authorities prefer not to use it in early pregnancy.[942] Sulfonamides may cause skin rashes and thrombocytopenia. These side effects are not reasons to withhold these drugs, but rather to watch the patient carefully while they are administered. Another drug sometimes used is clindamycin, but it can cause pseudomembranous colitis.

Uncomplicated lymphoglandular toxoplasmosis usually does not call for treatment, but patients with active toxoplasmosis involving vital organs and immunocompromised patients should be treated immediately. Ocular toxoplasmosis is difficult to treat. Pyrimethamine and sulfadiazine were found helpful in 60–70% of cases, and clindamycin is reported to have ameliorated attacks; if pseudomembranous colitis results it can be controlled by vancomycin. Clindamycin can be injected directly in the eye in sight-threatened patients.[1211] Corticosteroids may have to be used to reduce inflammation, but should never be given alone. The effectiveness of antiparasitic treatment for chronic ocular toxoplasmosis has formally not yet been demonstrated.[1119,1234a] Treatment failure may be related to late timing of treatment and due to the fact that currently available drugs cannot reach the parasite stage within ocular tissue cysts. Recently successful treatment has been described by off-label use of intravitreal injections with clindamycin.[1211]

Some success has been achieved in treating prenatally infected babies, depending on the severity of disease at birth.[610,611,903b,1094] Women found to have been infected during pregnancy should be treated not for themselves, but to prevent transmission of infection to their offspring, or to treat fetal infection.[903b]

Although pyrimethamine and sulfa drugs are widely used for treating toxoplasmosis their doses will vary when they are used in pregnancy, children, and immune suppressed individuals.[903b] A schedule of treatment given in Table 2.6 is only meant as a general guide, and specialists should be consulted for dosages, schedules, and toxicity. For further information on treatment and management of toxoplasmosis in humans. see McCabe,[898] Montoya and Rosso,[940] Remington et al.,[1094] Petersen and Liesenfeld,[1048] and Montoya and Remington.[942]

2.5 PREVENTION

General control methods are discussed in Chapter 1. On an individual basis, prevention of *T. gondii* infection in pregnant women and immunosuppressed patients is discussed here.

2.5.1 Immunosuppressed Patients

Before being given immunosuppressive treatment, patients should be serologically tested and, if devoid of *T. gondii* antibodies, be treated. This is particularly desirable in the case of patients receiving transplants of organs. As there will never be time to carry out serologic tests on all donors, they must all be considered potentially dangerous. There will, however, be time to test the recipients, and if seronegative, they should certainly be given prophylactic treatment. Nowadays most immunosuppressed patients receive trimethoprim-sulfamethoxazole to prevent *Pneumocystis* pneumonia, which at the same time is prophylactic for *T. gondii* infection.[69] The decision to give prophylaxis to all patients receiving transplants should consider the seroprevalence in the general population in a given country and the organ to be transplanted. In a recent survey of two large hospitals in New York City, there was no case of toxoplasmosis among 596 patients who received cardiac transplant and trimethoprim-sulfamethoxazole prophylaxis for pneumocystosis.[69]

Table 2.6 Treatment Schedule for Toxoplasmosis

I	**General**
	Pyrimethamine + sulfadiazine+ folinic acid. Pyrimethamine: 0.5 to 2 mg/kg/day, loading dose for 2 days. Sulfadiazine: 50 to 100 mg/kg/day in two divided doses. Folinic acid (leukovorin calcium): 2 to 20 mg (or 5 to 10 g baker's yeast) twice weekly during pyrimethamine treatment. Treatment given until symptoms subside.
II	**Prophylactic Treatment during Pregnancy**
	Treatment to be started as soon as prenatal diagnosis is made. Spiramycin, 1 gm every 8 hr, without food, before the 18th wk of pregnancy or until fetal infection confirmed by amniocentesis and thereafter pyrimethamine and sulfadiazine treatment. Pyrimethamine, 50 mg each 12 hr for 2 days, then 50 mg once daily. Sulfadiazine 75 mg/kg, then 50mg/kg each 12 hr, maximum 4 gm. Folinic acid 1–20 mg daily. Continue treatment until term (see Remington et al., 2006[1094] for further details).
III	**Congenital Toxoplasmosis in Infant (see Remington et al., 2006)[1094]**
	Pyrimethamine: Loading dose: 1 mg/kg orally each 12 hr for 2 days, then 1 mg/kg per day for 2–6 mos, then thrice weekly + sulphadiazine: 50 mg/kg per day orally (2 divided doses) + folinic acid: 10 mg orally thrice weekly during and for 1 wk after pyrimethamine therapy. Treatment given for 1 yr or more, depending on clinical signs. Corticosteriods (predisone, 0.5 mg/kg each 12 hr, to reduce inflammation in active chorioretinitis), if necessary.
IV	**Ocular Toxoplasmosis**
	Pryimethamine 75 mg/day and sulfadiazine 2 g daily, or Clindamycin 300 mg orally, 4 times daily. Coricosteroids: only if inflammation present. Prednisone or methylprednisone 1 to 2 mg/kg/day in 2 divided doses.
V	**AIDS Patients**
	A Acute Toxoplasmic Encephalitis
	Pyrimethamine 200 mg oral initially and then 75–100 mg orally 4 times daily + Sulfadiazine 1–2 grams orally, 4 times daily. or Pyrimethamine + clindamycin 60 mg orally or intravenously 6 hourly. or Pyrimethamine + dapsone 100 mg orally 4 times daily. or Pyrimethamine + azithromycin 1200–1500 mg orally 4 times daily. or Pyrimethamine + clarithromycin 1 gram orally 2 times daily.
	B Maintenance Treatment
	Doses of pyrimethamine and sulfadiazine reduced to half or less of those given for treating acute toxoplasmosis and the treatment continued for life. Folinic acid should be given daily. Prophylactic treatment in AIDS patients with antibodies to *T. gondii*. Trimethoprim–sulfamethoxazole (80 mg trimethoprim and 400 mg sulfamethoxazole in a single-strength tablet, 1 tablet a day), widely used for the prophylaxis and treatment of *Pneumocystis carinii* infections thought to reduce onset of toxoplasmic encephalitis.

2.5.2 Prophylactic Treatment during Pregnancy

Prevention of infection of the fetus by prophylactic treatment of the mother depends on the delay that occurs between maternal infection and its transmission to the fetus. It is also hoped that if infection is already present in the fetus, treatment may limit its ill effects. Treatment is begun as soon as possible during the prenatal period. In Austria, treatment is by spiramycin before the week of

pregnancy and thereafter by pyrimethamine and sulfonamide; in France, it is by spiramycin alone. As indicated earlier, this subject is controversial.

However, a recently reported large European multicenter cohort study found no evidence that prenatal treatment with either spiramycin or sulfonamide combined with pyrimethamine had an effect on maternal transmission.[590] The relative risk of mother-to-child transmission of *T. gondii* compared to Lyon, France (women treated) was 1.24 in Austria (women treated), 0.59 in Denmark (no treatment), and 0.65 in The Netherlands (50% treated, 50% not treated).[590] A meta-analysis of 22 European cohorts found weak evidence that treatment started within 3 weeks of seroconversion reduced mother-to-child transmission compared with treatment started after 8 weeks or more.[603,1260] One current practice is to start a woman on spiramycin if she becomes newly infected during pregnancy in the first or early second trimester, and then perform amniocentesis to detect fetal infection. If fetal infection is detected, then medication is switched to pyrimethamine and sulfadiazine.[939] Pyrimethamine or sulfadiazine may be used initially in the late second and third trimesters when acute infection is detected. However, it is important to note that maternal screening and treatment are not without risk.[63] Each country needs to evaluate the cost of screening of pregnant women, cost of treating congenitally infected children, and human suffering based on resources and prevalence in the general population.[426,903b,1172]

Where it has been carried out with thoroughness, persistence, and determination, as in France, education appears to have contributed to a reduction in the incidence of *T. gondii* infection during pregnancy. It should be included in the instructions given in antenatal clinics and by obstetricians and midwives dealing with individual patients. Individual instruction given in person is likely to be most effective, but should be supplemented by booklets printed in the various languages of the patients and by videos in the waiting rooms of antenatal clinics. Prevention and controls are discussed in detail in Chapter 1.

ACKNOWLEDGMENT

I appreciate the generous advice of Drs. Ruth Gilbert, Jeffery Jones, Aize Kijlstra, Eskild Petersen, and Louis Weiss**.**

CHAPTER 3

Toxoplasmosis in Domestic Cats and Other Felids

3.1 DOMESTIC CATS (*FELIS CATUS*)

3.1.1 Natural Infections

3.1.1.1 Serologic Prevalence

T. gondii antibodies have been found worldwide (Table 3.1). The seropositivity increases with the age of the cat, indicating postnatal transmission of *T. gondii*.[7,302] Antibodies to *T. gondii* have been detected in most cats after weaning (6 to 10 weeks). It is possible that in some young cats in Table 3.1, the low antibody titers represented maternally transferred antibodies. Maternally transferred antibodies disappear in the cat by 12 weeks of age.[275,345,1008]

Prevalence of *T. gondii* infection varies according to the life style of cats. It is generally higher in feral cats that hunt for their food than in domestic cats. Much is dependent on availability of food.[244] Seroprevalence to *T. gondii* varied among countries, within different areas of a country, and within the same city (Table 3.1). The reasons for these variations are many, and no generalizations should be made. For example, low seroprevalence (7.3–11%) in Bangkok, Thailand, was attributed to the life style of the cats surveyed. Most (95%) people in Thailand are Buddhist, and killing any pet (or any life) is sinful. There are approximately 15,000 cats (30 × 500 temples) that live permanently around Buddhist temples, and these cats are fed by the public or monks, mainly a diet of cooked fish and rice.[1245a]

In a study of *T. gondii* seroprevalence in an urban population of domestic cats in Lyon, France,[7] the seroprevalence was only 18.6% of 301 cats, approximately half the prevalence in other surveys in Europe (see Table 3.1). Prevalence was the highest when the weather was hot (>32°C) and moist (rain > 22 mm), or moderate and drier (rain 20 mm). Rarity of rodents in the area (due to regular rodent poison control) and feeding of cats by people were considered as possible factors for this reduced prevalence.

Economic underdevelopment and poor hygiene in a country were not the determining factors for low seroprevalence of *T. gondii* in cats in Durango, Mexico.[27a,434] Seroprevalence was 21% of 105 cats among those sampled in 2007,[27a] and only 9.3% in 150 cats sampled from the same sources a year later[434]; all the cats were tested at a cutoff of 1:25 in MAT and all sera were tested by the same person. This low seroprevalence was attributed to low *T. gondii* prevalence in the local small animal population; *T. gondii* was not isolated by bioassays of 249 rats, 127 mice, 69 squirrels, and 66 opossums.[434] In conclusion, factors affecting the prevalence of *T. gondii* in cats are not fully understood and need further investigation.

Concurrent infections with certain feline pathogens can affect *T. gondii* infections in cats. *Bartonella* spp. are bacterial zoonotic pathogens that can cause cat scratch disease, endocarditis,

Table 3.1 Seroprevalence of *T. gondii* Antibodies in Domestic Cats (*Felis domesticus*) (1988–2008)*

Location	Reference	Test	No Exam	% Pos.	Cutoff Titer	No with Terminal Titers of:						Notes
						2–10	16–20	25–40	50–64	128	≥256	
Argentina	Fernández et al., 1995	IHA	169	19.5	32			14	3	8	8	a,b
	Venturini et al., 1995	IFA	68	25	8	17						
	Venturini et al., 1997a,b	IFA	100	27	8							a,f,g,s
Australia												
Tasmania	Milstein and Goldsmid, 1997	LAT	18	50	64				9			s
Melbourne	Sumner and Ackland, 1999	ELISA	103	39	NS							a,b,c
Austria	Edelhofer and Aspock, 1996	IFA	456	48.2	40			21	12	29	158	p,s
Bangladesh	Samad et al., 1997	LAT	24	33.3	64		2				6	s
Belgium (Ghent)	Dorny et al., 2002	MAT	346	70.2	40			69			174	b,d,f,s
	de Craeye et al., 2008	IFA	567	24.9	50							a,p,se
Brazil												
Amazon	Cavalcante et al., 2006b	MAT	63	87.3	25			2	2	7	44	
Paraná	Garcia et al., 1999	IFA	163	73	16		12		17		90	b,p
	Dubey et al., 2004	MAT	58	84.4	20			4	1	14	30	s
Rio de Janeiro	Netto et al., 2003	IHA	41	24.4	NS							a,b,g,s
	Mendes-de-Almeida et al., 2007	IHA	118	72	16		85					a,b,d,f,s
	Lucas et al., 1999	IFA	248	17.7	16		12		14		18	a,b,c,p.s
São Paulo	da Silva et al., 2002	MAT	502	26.3	20		10	40	73		9	a,b,p,s
	Meireles et al., 2004	ELISA	100	40	NS							s,se
	Pena et al., 2006	MAT	237	35.4	25		9	10	12	8	53	s
	da Silva et al., 2002	MAT	100	19	16		7		8		4	se
	120a	IFA	400	25	64				16	14	70	p,s
	1016	IFA	28	53.5	64			1			14	p,s
Canada (Ontario)	Quesnel et al., 1997	IHA	25	12	64		23					d,f,g
Chile	Stutzin et al., 1989	IHA	27	85.2	16		23					
Valdivia city	Ovalle et al., 2000	IFA	97	33	16		7		6		19	a,b
Colombia	Londoño et al., 1998	IFA	28	89.3	NS							p
	Dubey et al., 2006	MAT	170	35.8	20	25	10	7	4	8	23	s
Czech Republic	Svoboda et al.,1988	DT	620	48.7	4	141	32	25	3	3		g,p
	Svobodová et al., 1998	IFA	390	61.5	10	20	11	29	31	39	110	a,b,d,f,p,s
	Sedlák and Bártová, 2006, 2007	IFA	286	44.1	40			126				p

Egypt												
Giza, Cairo, Kaluba	634a	LAT	97	57.7	64				12	7	25	p,s
Cairo	Aboul-Magd et al., 1988	IFA	177	58.8	64				54	23	27	p,s
Gharbia	Abu-Zakham et al., 1989	IHA	114	18.4	64				12	7	25	p,s
France (Lyon)	Afonso et al., 2006	MAT	301	18.6	40			56				a,c,s
Galápagos (Isabela Island)	Levy et al., 2008	ELISA	52	63	64							d,e,f
Germany	Knaus and Fehler,1989	DT	267	59.9	16							s,p
	Tenter et al., 1994	ELISA	306	45	NS							p,s
	Hecking-Veltman et al., 2001	ELISA	300	65.6	NS							a,s
Guatemala (Petén region)	Lickey et al., 2005	MAT	30	53	32				16			d,f,p
Hungary	Hornok et al., 2008	IFA	330	47.6	20				126		31	a,b,h,p,s
Iran												
	Haddadzadeh et al., 2006	IFA	100	63	32			3	6	13	31	a,b,p,s
Kashan	Hooshyar et al., 2007	IFA	50	86	20		10	5	9	11	8	b,s
Sari	Sharif et al., 2009	LAT	100	40	1	10	2	4	2	6	8	a,b,s
Israel (Jerusalem)	Salant and Spira, 2004	ELISA	1062	16.8	80							a,b,c,p,s
Italy												
Veneto	Natale et al., 2007	IHA	189	69.3	200			131				a,s
Verona	D'Amore et al., 1997	MAT	490	33.3	64				163			a,b,d,s
Rome	Macri et al.[813]	MAT	115	38	20		7	6	5	6	24	
Japan												
Hokkaido and Okinawa	Maruyama et al., 2003	LAT	1447	5.4	64				78			a,b,c,d,e,f,p
Hyogo	Khin-Sane-Win et al., 1997	LAT	231	19	64				44			a,b
Kanto	Fujinami et al., 1983	IHA	171	47.1	64							
Saitama	Furuya et al., 1993	LAT	726	10.5	32			17	115	19	42	a,b
Saporo and Tokyo	Kimbita et al., 2001	ELISA	193	20.7	NS							
Saitama	Huang et al., 2002	ELISA	192	21.9	64				42			se
Saitama	Huang et al., 2004	LAT	179	18.4	64			33				p,se
Various areas	Oikawa et al, 1990	IFA	335	25.7	4							
	Nogami et al., 1998	LAT	800	6	32				8	12	28	a,p,s
	Maruyama et al., 1998	LAT	471	8.7	64				41			a,b,d,e,f,p

(*continued*)

Table 3.1 Seroprevalence of *T. gondii* Antibodies in Domestic Cats (*Felis domesticus*) (1988–2008) (Continued)

Location	Reference	Test	No Exam	% Pos.	Cutoff Titer	No with Terminal Titers of: 2–10	16–20	25–40	50–64	128	≥256	Notes
Kerguelen Islands (French territory)	Afonso et al., 2007	MAT	276	51.1	40							
Korea (South)	Sohn and Nam, 1999	WB	198	13.1	200			26				s,se
	Kim et al, 2008	LAT	174	8.1	32			14				b,s,se
	Lee et al., 2008	PCR	106	47.2								
Malaysia	Chandrawathani et al., 2008	IFA	55	14.5	200						8	
Mexico												
Colima	Garcia-Márquez et al., 2007	ELISA	80	28.8	NS							a,b.c,p
Durango	Alavarado-Esquivel et al., 2007	MAT	105	21	25			3	4		15	a,b,c
	Dubey et al., 2009a[434]	MAT	150	9.3	25			8		2	6	a, s
Guadalajara	Galván Ramirez et al., 1999	ELISA	24	70.8	NS				6	1	10	p
Mexico City	Besné-Mérida et al., 2008	ELISA	169	21.8	NS							a,b,c
Pakistan	Shahzad et al., 2006	LAT	50	56	16		15		7	3	3	a,b,c,p,s
Panama (Panama City)	Frenkel et al., 1995	MAT	241	45.6	1	110						
People's Republic of China												
Beijing	Yu et al., 2006	ELISA	128	14.1	NS							
	Yu et al., 2008	ELISA or LAT	335	14.9	NS							a.b,p
Guangzhou	Chen et al., 2005	ELISA	114	23.7	NS							
	Dubey et al., 2007a	MAT	34	79.4	20			1	1	3	22	s
Hebei	Yuan et al., 2004	ELISA	75	57.3	NS							
Hubei	Chen, 2001	ELISA	105	31.4	NS							
Shanghai	Lu et al., 1997	IHA	142	38.1	64							
Shandong	Fu et al., 1995	IHA	200	46	64							
Guangdong	Shen et al., 1990	IHA	47	2.1	64				1			

Poland	Śmielewska-Łoś and Pacoń, 2002	LAT	200	52.5	64				25	31	49	a,b,c,d,f,s,p
	Michalski and Platt-Samoraj, 2004	MAT	17	70.6	20			12				
Portugal (northeastern)	Lopes et al., 2008	MAT	204	35.8	20		3	18			55	a,b,c,d,f,p,s
Puerto Rico (Mona Island)	Dubey et al., 2007b	MAT	19	70.3	20	2			1	4	9	s
Romania	1285a	LAT	50	42	NS							p,s
Singapore	Chong et al., 1993	LAT	772	30.3	64			37	56	44	82	a,b
Slovak Republic	Ondrejka et al., 2007	ELISA	164	18.9	NS							d,f,s
Spain	Miró et al., 2004	IFA	585	32.3	80				81	64	73	a,b,p,s
	Montoya et al., 2008	IFA	592	26	80					154		s,p
Barcelona	Gauss et al., 2003	MAT	220	45	25			26	57		16	a,b,c.d,f,p,s
Andalusia	Millán et al., 2009	MAT	25	50.2	25							
Balearic Island	917a	MAT	59	84.7	25			1		4	45	s
Sweden	Uggla et al., 1990	ELISA	244	42	NS							
	Ljungström et al., 1994	IFA	60	46.6	25			27			31	se
Taiwan	Lin et al., 1990	KELA	117	7.7								d, f
	Tsai et al., 1997	LAT	202	5.5	32			4	1	5	1	p,s
Thailand	Sukthana et al., 2003	DT	315	7.3	16		23					c,s
Bangkok	Sriwaranard et al.,1981	IHA	40	57.5	256						23	g,p
	Jittapalapong et al., 2007	LAT	592	11	64				5	15	45	b,s
Turkey												
Ankara	Inci et al., 1996	DT	65	43	16		12				7	a,p
	Özkan et al., 2008	DT	99	40.3	4		21		16		3	a,b,p,s,se
Nigde	Karatepe et al., 2008	DT	72	76.4	16		29		16		10	b,s

(*continued*)

Table 3.1 Seroprevalence of *T. gondii* Antibodies in Domestic Cats (*Felis domesticus*) (1988–2008) (Continued)

Location	Reference	Test	No Exam	% Pos.	Cutoff Titer	No with Terminal Titers of:						Notes
						2–10	16–20	25–40	50–64	128	≥256	
United States												
California	Dabritz et al., 2007a	ELISA	194	24.2	64			147	8	5	34	a,b,se
		IFA	194	36.1	16		3	5	14	9	39	
	Miller et al., 2008	IFA	5	60	320						3	
Colorado	Hill et al., 2000	ELISA	206	23.6	64				43			s,p
Florida	Luria et al., 2004	ELISA	553	10.8	NS							d,e,f,s
Florida, Georgia, Ohio	Lappin et al., 1992	ELISA	124	74.2	NS				92			d,f,g,h,p
Georgia	Lappin et al., 1989	ELISA	188	60.7	NS				114			p,g
Hawaii	Danner et al., 2007	ELISA	67	37.7	64							a,b,d,f,s
Illinois	Dubey et al., 1995	MAT	391	68.3	25			33	36	8	198	s
Iowa	Smith et al., 1992	MAT	74	41.9	32			31				s
	Hill et al., 1998	MAT	20	80	32			16				a,b,s
Maryland	Witt et al., 1989	IFA	585	15.2	32			28	11	12	32	a,b,d,f
Midwestern zoos	de Camps et al., 2008	MAT	34	29.4	25			10				
North Carolina	Nutter et al., 2004	MAT	176	50.6	25						5	d,e,f,s,p
Ohio	Dubey et al., 2002	MAT	275	48	25			24	37		72	s,p
Oklahoma	Rodgers and Baldwin, 1990	LAT	618	22	16		135					a,b,d,f,p
Pennsylvania	Dubey et al., 2009b[437c]	MAT	210	19.5	25			4	9	7	21	a,s
Rhode Island	DeFeo et al., 2002	MAT	200	42	25			17	26	41		a,b,s,p
Nationwide	Vollaire et al., 2005	ELISA	12,628	31.6	NS							g
West Indies												
Grenada	Asthana et al.,2006	MAT	40	35	25			7	4		3	c
	Dubey et al., 2009c[437b]	MAT	176	28.9	25			5	8	4	28	p,s
St Kitts	Moura et al., 2007	MAT	106	84.9	25			23	34	18	12	p,s
	430	MAT	96	10	73.9	6	6	7	3	10	39	s

*Modified from Jones and Dubey.[739] For references, see this paper unless stated otherwise.
a = age, b = sex, c = risk assessment, d = FIV, e = *Bartonella* spp., f = FeLV, g = clinical, h = feline infections peritonitis (FIP), p = pet, s = stray, bold = significant, se = serological comparison, t = biossay.

and several other syndromes in humans. Feline immunodeficiency virus (FIV) is a retrovirus related to human immunodeficiency virus (HIV), and is known to cause immunosuppression in cats. Feline leukemia virus (FeLv), is related to human leukemia virus, and can also cause immunosuppression in cats. It has been reported[268a,1366] that both the seroprevalence and the magnitude of *T. gondii* antibodies were higher in FIV-infected cats. A review of *T. gondii* and concurrent infections with other feline pathogens in asymptomatic cats (Table 3.2) concluded that there is no critical evidence for any association among these infections.[437b]

It is not possible to compare results in Table 3.1 because of the sample size, age of cats, and the serological tests used. There is little information available comparing different tests in sera from naturally exposed cats (Table 3.3). I am not aware of any validation of serological tests in cats using isolation of the parasite as a guide.

Table 3.2 Reports of *T. gondii* and Other Concurrent Infections in Naturally Exposed Cats[a]

Country	No. of Cats	*T. gondii* (Test, Titer)	*Bartonella* spp.	FIV	FeLv	Reference[b]
Belgium	346, stray	70.2 (MAT, 40)	ND	11.3	3.8	Dorny et al., 2002
Grenada, West Indies	75, pets 101, feral	30.6 (MAT, 25) 27.7 (MAT, 25)	50.6 52.4	8 21.8	0 0	Dubey et al.[437b]
Guatemala	30, stray	53 (MAT, 32)	ND	3	17	Lickey et al., 2005
Japan	471, pets	8.7 (LAT, 64)	9.1	23.8 (16 of 67)	8.9	Maruyama et al., 1998
	1,447, pets	5.4 (LAT, 64)	8.8	9.6 (107 of 1,088)	2.9	Maruyama et al., 2003
Poland	200, stray	52.5 (LAT)	ND	0	0	Śmielewska-Łoś and Pacoń, 2002
Portugal	207, pets	76 (MAT, 20)	ND	14.6 (16 of 108)	ND	Lopes et al., 2008
Spain	220, pets	45 (MAT, 25)	ND	4 (6 of 149)	4.6	Gauss et al., 2003
St Kitts, West Indies	96, feral	63.5 (MAT, 20)	ND	15.6 (15 of 96)	0	Dubey et al.[430]
United States	585[c]	15.2 (IFAT, 32)	ND	2.4	3 of 34 selected samples	Witt et al., 1989
	592[c] (195, stray, 345, pets)		11.8 12.2	ND	ND	Childs et al., 1994
	618, pets	22 (LAT, 64)	ND	10	15	Rodgers and Waldwin, 1990
	117, pets, stray, sick	7.7 (ELISA)	ND	0.9	7.6	Lin et al., 1990
	124, sick (uveitis)	74.2 (ELISA)	ND	12.9	12.1	Lappin et al., 1992
	553, stray	10.8 (ELISA)	33.6	5.2	3.3	Luria et al., 2004
	76, pets 100, stray	34 (MAT, 25) 63 (MAT, 25)	75 93	4 5	1 4	Nutter et al., 2004
	67, stray	37.3 (ELISA)	ND	8.8	16.2	Danner et al., 2007
	210, pets	19.5 (MAT, 25)	25.7	ND	ND	Dubey et al.[437c]

[a] From Dubey et al.[437b]
[b] For references see reference.[437b]
[c] Same group of cats.

Table 3.3 A Comparison of Different Serological Tests for the Detection of *T. gondii* Antibodies in Naturally Exposed Cats

Country	No. of Sera	Percent Positive						Ref
		MAT	IFA	ELISA	LAT	IHA	DT	
Argentina	68	ND[a]	25 (1:8)[b]	ND	13.2 (1:8)	ND	ND	1313
Belgium	567	ND	24.9 (1:50)	84.1 (1:50)	ND	ND	ND	234
Brazil	100	ND	ND	40 (1:100)	ND	17 (1:16)	ND	910
	63	87.3 (1:25)	87.3 (1:25)	ND	ND	ND	ND	159
Italy	115	38 (1:20)	38 (1:20)	ND	ND	ND	ND	813
Japan	192	ND	ND	21.9 SAG2	20.3 (1:64)	ND	ND	687
	179[c]	ND	ND	18.4	20.1	ND	ND	688
Korea	174[d]	ND	ND	16.1 (1:32)	8.1 (1:32)	ND	ND	770
	198[e]	ND	ND	13.2 (1:500)	12.6 (1:32)	ND	ND	1214
Sweden	60	46.6 (1:40)	46.6 (1:40)	ND	ND	ND	ND	862
Turkey	99	ND	34.3 (1:16)	ND	ND	ND	40.3 (1:4)	1020
United States	15[f]	ND	ND	100	33 (1:64)	46.6 (1:64)	ND	801
	194	ND	30.9 (1:16)	24.2 (1:64)	ND	ND	ND	210

[a] ND = Not done.
[b] Serum dilution.
[c] 22.9% were positive by immunochromatographic test.
[d] 13.2% were positive by PCR.
[e] Sera were initially tested by Western blots, and then compared in LAT and ELISA. See the original paper for details.
[f] IgM pos.

3.1.1.2 Prevalence of Viable T. gondii *in Tissues of Cats*

T. gondii was isolated from tissues of naturally infected cats by bioassay in mice or cats (Table 3.4). This parasite was more prevalent in muscles than in the brain of cats (see Table 1.4, Chapter 1).

3.1.1.3 Clinical Infections

3.1.1.3.1 Confirmed Clinical Reports

Cats of any age or breed and either sex can die of toxoplasmosis. Reports of fatal toxoplasmosis in the last 20 years are summarized in Table 3.5. Clinical signs among 100 cats included fever, anorexia, dyspnea and polypnea, jaundice, indications of pain or discomfort on abdominal palpation attributable to hepatitis or pancreatitis or discomfort from interference with respiration. Signs attributed to neural involvement (hypothermia, partial or total blindness, increased affectionate behavior, stupor, incoordination, atypical cry, ear twitch, circling, torticollis, head bobbing, anisocoria, and seizures) were also reported. Additional findings were cutaneous limb lesions, consisting

Table 3.4 Isolation of *T. gondii* from Tissues of Naturally Infected Cats

Country	No. Exam	No. Positive	Percent Positive	Tissues Inoculated	Ref
Brazil	54	37	68.5	B, H, S	394
	71[a]	47	66.1	B, H, S, T	1041
Canada	2	2	100	B, H, M	429
Colombia	116	15	12.9	B, H, T	401
Czechoslovakia	150 dead cats	17	11.3	B, L, S	1252
Iran	50	2	4	B	675
Mexico	8[a]	5	62.5	B, H	434
People's Republic of China	27[a]	17	60.3	B, H, T	412
Puerto Rico (Mona Island)	19	12	60.3	B, H, M	408
Spain	103[a]	42	40.8	B	937, 937a
United States	2	2	100	B	390
West Indies (St Kitts)	10[a]	7	70	B, H, T	430

[a] Seropositive.
[b] B = brain, H = heart, L = liver, M = skeletal muscle, S = spleen, T = tongue.

of dermal and subcutaneous nodules and ulceration, signs of joint pain and lameness attributable to periarticular inflammation, ocular disease including aqueous flare, hyphema, velvety iris (iritis), retinal hemorrhages, mydriasis, anisocoria, and slow pupillary light reflex.[328]

Among 100 cases of confirmed toxoplasmosis from one hospital, 36 had generalized infection, 26 had pulmonary lesions, 16 were abdominal, 12 were hepatic, 7 were neurologic, 4 ocular, 2 cutaneous, 1 pancreatic, and 1 cardiac.[328] Although any organ may be involved, pneumonitis is the most common finding and it can be rapidly fatal.

3.1.1.3.2 Oocyst Shedding and Clinical Signs

Little is known of the clinical status of cats found shedding *T. gondii* oocysts. As yet, there is no confirmed report of oocyst shedding by a clinically ill cat. In the study reported by Dubey and Carpenter[328,330] of 100 fatal cases, there were 3 instances of enteroepithelial stage of *T. gondii*. In two instances, *T gondii*-like oocysts were found in the feces of kittens that were 2 to 3 weeks old. The other instance was a 14-month-old cat that had severe enteritis and *T. gondii* schizonts in the intestine, but oocysts were not identified. The fourth case was a 3-year-old cat that died despite anti-toxoplasmic treatment. It had *T. gondii*-like oocysts in histologic sections of its intestines, and the diagnosis was confirmed by IHC staining[309]; oocysts could not be identified as *T. gondii* because bioassay was not performed.

3.1.1.3.3 Concomittent Infections

Among 100 histologically confirmed cases of toxoplasmosis in naturally infected cats, 1 cat had a drug reaction causing hypoxia, 1 thiamine deficiency, 2 lymphoma, 1 lymphosarcoma, 2 feline viral panleukopenia, 4 bacteremia, 1 salmonellosis, and 3 cats had positive results for feline leukemia virus antigen.[328]

Experimentally, coinfection with FIV can aggravate acute toxoplasmosis and cause immunos uppression.[223,822,825,828–830] However, FIV infection in latently experimentally infected cats did not reactivate toxoplasmosis and dually infected cats remained clinically normal.[802] Concurrent FeLV infection also did not affect the outcome of *T. gondii* infection in cats.[1033] In naturally infected cats, the interaction of FIV and *T. gondii* infection is intriguing because both the seroprevalence and the magnitude of *T. gondii* antibodies were higher in FIV-infected cats.[1366] Moreover, FIV-infected cats had higher prevalence of IgM *T. gondii* antibodies than IgG antibodies.[805,991] Since these initial

Table 3.5 Histologically Confirmed Clinical Toxoplasmosis in Naturally Infected Cats (1988–2008)

Country	No. of Cats	Age	Main Organs	Immuno	Ref
Australia	2	2 wk	Brain, heart	Yes	307
	2	4 yr, 7 yr	Brain, lung	No	1197
	1	6 yr	Abdominal	No	1097
	1	8 yr	Lung, carcinoma	No	520
	1	5 yr	Lung	No	859
Denmark	5	5 wk, 9 wk,10 wk, 1yr, 1yr	Liver, lung, spleen	Yes	650
Italy	1	9 yr	Skin	TEM, PCR, Immuno	35
	1	12 yr	Brain granuloma	MRI, Immuno	487a
Japan	1	16 yr	Skin	Immuno	1025
New Zealand	2	?	Lung, adrenal		1281
Slovakia	2	10 wk, 4.5 mo	Brain, lung	No	1252
Turkey	3	3, 6, 8 mo	Lung	TEM	637
United States	1	3 yr	Hepatitis	Immuno	309
	1	8 yr	Skin	Immuno, TEM	860
	100	2 wk–16 yr (mean 4 yr)	86 cats primary,14 cats complications[a]	Immuno	328
	1	10 yr	Myelitis, FIV	Immuno	639
	3	4, 8, 12 yr	Renal transplants, multiple organs	ND	90
	1	5 yr	Lymphoma, liver, spleen, lung, bone marrow	Immuno, TEM	722
	1	10 yr	Pancreatitis	Immuno	444
	1	12 yr	Pneumonia, needle biopsy		1143
	1	5 yr	Pneumonia, bronchoaleveolar lavage		126
	1	8 yr	Cystitis, following renal transplant	Histology, serology	986
	1	4 yr	Neurological	Histology, immuno	710a

[a] 1 cat drug reaction causing hypoxia, 1 thiamine deficiency, 2 lymphoma, 1 lymphosarcoma, 2 feline viral panleukopenia, 4 bacteremia, 1 salmonellosis, and 3 cats had positive results for FeLV antigen.

papers there have been many serological surveys (see Table 3.1) documenting the exposure to both infections but clinical or epidemiological significance is unknown and not important. I am aware of only one case of clinical toxoplasmosis in an FIV-infected cat.[639] This 10-year-old male cat had severe necrotizing myelitis with intralesional *T. gondii*.[639]

3.1.1.3.4 Cases of Presumed Toxoplasmosis

Reports of presumed toxoplasmosis are summarized in Table 3.6.

3.1.1.3.5 Ante-Mortem Diagnosis

Clinical signs, laboratory findings, serologic examinations, and response to anti-toxoplasmic treatment can aid in the ante-mortem diagnosis of toxoplasmosis. A high persistent fever, unresponsive to penicillin and streptomycin, is a frequent finding in toxoplasmosis. Although toxoplasmosis

Table 3.6 Presumed or Ante-Mortem Diagnosed Case of Toxoplasmosis in Cats

Country	No. of Cats	IgM Titer	IgG	Biopsy	Treatment	Ref
Nigeria[a]	10,1.5–3.5 yr old Siamese cats, all ill at one time, diarrhea	Yes	Yes	ND[b]	Some cats treated with clindamycin	999
United States	73				Ocular toxoplasmosis suspected, based on serology and treatment	167
	92				Ocular toxoplasmosis suspected, based on serology and treatment	804
	1, 2 yr old male weight loss, fever, retinochoroiditis	1:256	1:2,048	ND	Clindamycin, 8.3 mg/kg, tid, clinical improvement	800
	2	Yes	Yes		Inflammatory intestinal disease, treatment	1050
	3	ND	Yes		Suspected based on serology	1224

[a] Other diseases not ruled out.
[b] ND = no data.

is often included in the differential diagnosis of neurologic disease, cats rarely have obvious neurologic signs. Most of the cats have thoracic or abdominal signs.

Ocular fundic examination should be a routine part of the examination of febrile cats. Multifocal iridocyclochoroiditis was the most common lesion (81.8%) of the 22 cats with ophthalmitis in the study reported by Dubey and Carpenter.[328] Of the ten cats with histologically demonstrable *T. gondii* in the eyes, multifocal iridocyclochoroiditis was observed in nine cats. These findings are contrary to the location of ocular lesions in human beings. In human beings, *T. gondii* is found mainly in the retina and not in the choroid. Considering the clinical findings in cats of our study, all ten cats with proven *T. gondii* ophthalmitis also had generalized toxoplasmosis, and ophthalmitis was bilateral in five of six cats in which both eyes were studied.[328] In four cats, only one eye was examined histologically. Findings in some studies have suggested that uveitis may be more common in cats with clinical or subclinical toxoplasmosis than was believed. The data are based on the evidence of local synthesis of *T. gondii* antibodies and/or release of *T. gondii* antigen in aqueous humor and response to clindamycin treatment.

T. gondii should be looked for routinely in smears obtained from tracheal aspirates and from thoracic or peritoneal effusions. Biopsies of palpably large abdominal organs or cutaneous nodules that are detected during a physical examination may provide diagnostic information. However, diagnosis should be confirmed by other tests. Staining with specific antisera to *T. gondii* can confirm the diagnosis. Detection of *T. gondii* DNA in tissues or fluids can aid diagnosis.[937a,1154]

Serum biochemical analysis, radiographic examination, and other laboratory tests can aid antemortem diagnosis of toxoplasmosis. Although asthma induces peribronchial markings similar to

those seen with toxoplasmosis on radiography, asthma also induces bronchial gland hyperplasia, smooth muscle hyperplasia of the tunica media of pulmonary arteries, and diffuse hyperplasia of all smooth muscles including those of alveolar ducts. *Aelurostrongylus abstrusus* lungworm infection has similar findings to those in asthma; however, eggs and larvae are observed in alveoli in scattered foci. Although high serum amylase activity is indicative of pancreatitis, normal values do not exclude the existence of severe pancreatitis. Determination of serum alkaline phosphatase activity, bilirubin urea nitrogen, serum creatinine, and serum calcium concentrations appeared to have little value for diagnosing feline toxoplasmosis.[328] Urine abnormalities that were frequently associated with feline toxoplasmosis were high specific gravity, mild proteinuria, hyaline casts, hyperbilirubinuria, and urobilinogenuria. The specific gravity often was high because of high fever, low water and food intake, and sometimes from dehydration from vomiting or diarrhea. Icteric plasma because of hepatic necrosis was frequent. Partial or, less often, complete obstruction of the common bile duct caused by pancreatitis contributed markedly to the hyperbilirubinemia and hyperbilirubinuria.[328]

Diffuse, poorly demarcated, radio-dense pulmonary foci, considered characteristic of toxoplasmosis in febrile cats, are not pathognomonic of *T. gondii* because similar findings can be seen in febrile cats with hematogenous bacterial pneumonia and in febrile cats with asthma or severe cases of lungworm infection. Determination of specific antibodies to *T. gondii* can aid in the diagnosis of clinical toxoplasmosis. Several serologic tests are available for detection of feline *T. gondii* antibodies. The dye test is not very sensitive for cats fed tissue cysts.[304] In cats with confirmed toxoplasmosis, dye-test results were negative in 6 of the 15 cats tested,[328] and viable *T. gondii* has been isolated from tissues of naturally infected cats without demonstrable dye test antibodies.[285] Thus, lack of detection of antibody by the dye test or any other test cannot be relied on to rule out clinical toxoplasmosis. Of the 10 cats tested for *T. gondii* antibodies, via the IFA test, 8 had antibodies (1:256) detected. More is being learned of the MAT and ELISA tests for the detection of *T. gondii* antibodies in experimentally and naturally infected cats, and are best suited for the detection of IgM antibodies. Serologic examination can only rule in the diagnosis of toxoplasmosis, but cannot rule out the diagnosis of toxoplasmosis. Use of serologic examination is limited to cases in which the tests can be performed repeatedly on the same animal during illness. Determination of locally synthesized antibodies or *T. gondii* antigen or DNA in the eye can aid diagnosis of ocular toxoplasmosis.[130,168,662,808–810,827,915]

3.1.1.3.6 Treatment

The synergistic effect of sulfadiazine or sulfamerazine and pyrimethamine is to interfere with the biosynthesis of folinic acid by *T. gondii*. This combination of drugs is believed to be more effective than sulfadiazine/trimethoprim. The sulfonamide dosage is 15 mg/kg of body weight, oral, every 6 hours, for at least 2 weeks (see Chapter 1). Proper patient hydration is important to prevent sulfonamide crystal formation in renal tubules. The pyrimethamine dosage is 0.5 to 1.0 mg/kg, oral, every 24 hours. Pyrimethamine is available as a 25-mg tablet, which is in excess of that required for treating a cat.[399] Pyrimethamine and trimethoprim are bitter and can induce frothing when administered orally to cats. For parenteral administration, pyrimethamine can be dissolved in 85% lactic acid or 5% acetic acid, refrigerated, and diluted with 0.9% NaC1 solution as needed. Sulfadiazine also can be administered parenterally. The sodium salt of sulfadiazine is soluble in water. Hematologic examinations should be performed before and during treatment to evaluate bone marrow function. Pyrimethamine should not be given if marrow suppression becomes marked as indicated by low WBC values. If pyrimethamine must be stopped before 2 weeks, folinic acid should be given at a dosage of 1 mg/kg, by injection or orally, every 24 hours. Clindamycin hydrochloride (25 to 50 mg/kg) i.m. every 8–12 hours) also has been used to treat toxoplasmosis in cats. Treatment for toxoplasmosis should be started as soon as a probable diagnosis is established because the disease

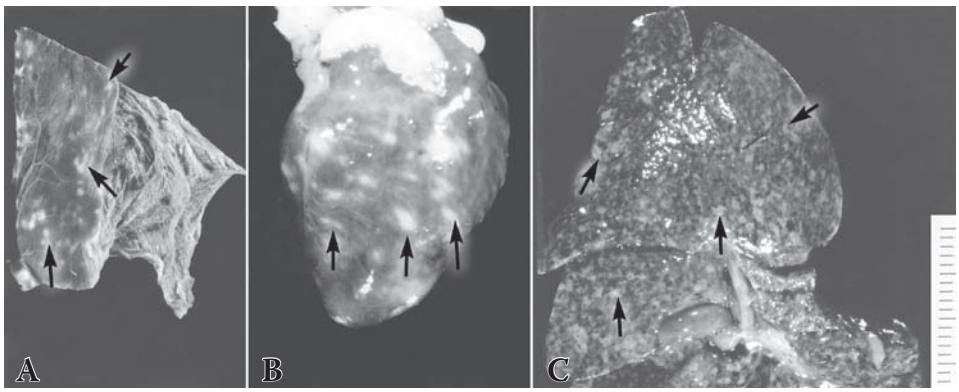

Figure 3.1 Multifocal areas of grossly visible necrosis (arrows) in allantochorion of placenta (A), heart (B), and liver (C). From Dubey et al.[351]

is usually acute or subacute with half of the deaths occurring within 2 weeks of the illness. Good personal hygiene should be practiced when treating ill cats. Tachyzoites may be shed in vomitus, feces, urine, skin exudates from ulcers, and possibly from oral secretions or exudates. Care should be taken when performing necropsies on cats suspected to have toxoplasmosis.

3.1.2 Experimental Infections

3.1.2.1 Pathogenesis

Cats can be easily infected with *T. gondii* in the laboratory. The age of cats, the stage of the parasite, and the route of inoculation can influence the outcome of toxoplasmosis. Newborn kittens can develop acute toxoplasmosis and die from it. Passively transferred antibodies have no apparent effect on the outcome of toxoplasmosis. Cats older than 3 months of age rarely show any clinical signs following oral infection with tissue cysts. However, cats of any age may die or develop severe disease following parenteral inoculation with tachyzoites, bradyzoites, or oocysts.[223,225,302,636] Cats infected during pregnancy can develop placentitis, and congenitally infected kittens can develop severe toxoplasmosis (Figures 3.1, 3.2), including ophthalmitis.[345,954,1066,1146]

As stated in Chapter 1, bradyzoites are highly infective for cats; they have shed millions of oocysts after ingesting few bradyzoites.[377] Although cats can be infected by being fed tachyzoites, it is not always successful.[397] Oocysts are nonpathogenic for cats; cats fed millions of oocysts remained asymptomatic and 1,000 oocysts were needed to infect cats.[398,548] Whether this is due to inefficient excystation of sporozoites in the gut of the cat or due to other factors is not known.

Administration of large doses of corticosteroids can aggravate clinical toxoplasmosis in experimentally infected cats.[276,1147] A study reported aggravation of clinical toxoplasmosis in cats inoculated with *T. gondii* and treated with clindamycin.[225]

3.1.2.2 Immune Responses

Serological responses of cats after oral infection with tissue cysts were studied with MAT, IHA, DT, and ELISA. The cats were bled sequentially starting 7 days p.i. and up to 6 yr p.i.[341] The MAT was found to be most sensitive.[342] Cats seroconverted 10 days p.i. and high titers persisted even after 6 years. Reports of cellular and humoral responses in experimentally infected cats can be found in other papers.[130,168,223,224,304,342,345,687,688,771,797,798,806–808,811,826-830,960,1005–1008,1010,1146,1196,1270]

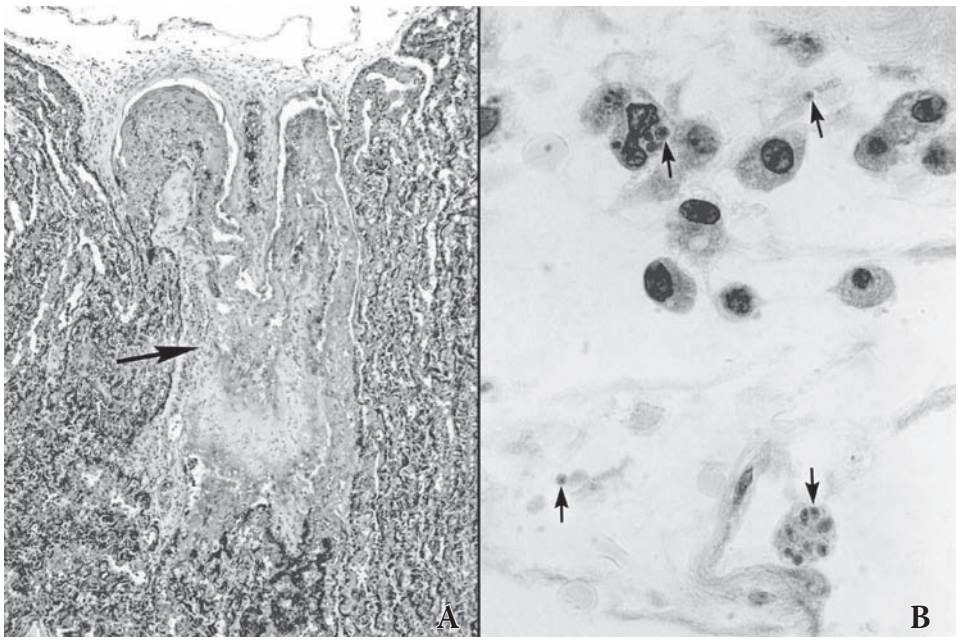

Figure 3.2 **(A)** Section of placental labyrinth of an experimentally infected kitten. The normal lamellar architecture of the allantochorion is disrupted by a large focus of necrosis (arrow) and mixed leukocytic infiltration. **(B)** Higher magnification showing edema and macrophages expanding the allantochorion. Note both free and intracellular tachyzoites (arrows). From Dubey et al.[351]

3.2 OTHER FELIDS

3.2.1 Serologic Prevalence

Serologic prevalence was higher in captive versus free-range cats (Table 3.7). Among the surveys listed in Table 3.7, the study reported by Silva et al.[1185] is noteworthy. This planned survey of 71 zoological parks and 15 breeding centers included nearly all zoos and breeding centers in Brazil. All 865 neotropical felids in 540 enclosures were bled and serologically tested. In addition, a risk assessment study was conducted.[1187] Feeding meat frozen at –12°C for 7 days was considered the most practical strategy to reduce *T. gondii* infection in these animals. Although *T. gondii* tissue cysts are killed instantly at –12°C,[783] internal temperatures in freezers for storing meat vary a great deal, and some were reported to have internal temperature of only –2°C.[233] Therefore, the recommendation for freezing meat for 1 week before feeding to cats seems prudent.

3.2.2 Isolation of Viable *T. gondii* from Feral Felids

Little is known of isolates of *T. gondii* from wild felids. Walton and Walls[1334] isolated viable *T. gondii* from the brains of 1 of 16 bobcats killed at Fort Stewart Military Reservation, Georgia. The low isolation rate may be because only a small amount of brain was inoculated into three mice. Dubey et al.[393] tested six bobcats that were killed in Albany County, Georgia. Five of these bobcats were seropositive (Table 3.7); their hearts were digested in pepsin and homogenates were inoculated into mice. Viable *T. gondii* was isolated from the hearts of all five seropositive bobcats;

Table 3.7 Seroprevalence of *T. gondii* in Wild Felids

Species	Location	Reference	Test	No. Exam	% Pos	Cutoff Titer	No. with Titers of: <10	16–20	25–40	50–64	128	≥256	Notes
Panthera spp.	Brazil	Silva et al., 2001	MAT	2	100	20				2			c
		1101a	IFA	2	100	100							
P. tigris (tiger)	Thailand	Thiangtum et al., 2006	LAT	18	27.8	64				5			c
	United States												
	Florida	Lappin et al., 1991	ELISA	4	75	64				3			c
	Various areas	Spencer et al., 2003	IFA	11	63.6	50				3	1	3	c
P. t. Altaica (Amur tiger)	Midwestern zoos	de Camps et al., 2008	MAT	18	27.8	25							c
P. leo (lion)		Penzhorn et al., 2002	IFA	53	92	20		49					fr
	Brazil	Silva et al., 2001a	MAT	27	51.8	20			9	4		1	c
		1101a	IFA	3	100	NS							
	Southern Africa	Cheadle et al., 1999	IFA	41	90.2	50				37		6	fr
		Spencer and Morkel, 1993	ELISA	66	98	NS							fr
		Penzhorn et al., 2002	IFA	42	100	20		42					fr
	Thailand	Thiangtum et al., 2006	LAT	7	14.3	64				1			c
	United States												
	Florida	Lappin et al., 1991	ELISA	2	100	64				2			c
	Midwestern zoos	de Camps et al., 2008	MAT	22	54.5	25			12				c
	Various areas	Spencer et al., 2003	IFA	10	80	50				1	4	3	c
	Zimbabwe	Penzhorn et al., 2002	IFA	21	100	20							fr
		Hove and Mukaratirwa, 2005	MAT	26	92.3	25			24				fr
P. pardus (leopard)	Brazil	Silva et al., 2001a	MAT	3	100	20				2		1	c
	Botswana	Penzhorn et al., 2002	IFA	1	100	20							fr

(*continued*)

Table 3.7 Seroprevalence of *T. gondii* in Wild Felids (Continued)

Species	Location	Reference	Test	No. Exam	% Pos	Cutoff Titer	No. with Titers of: <10	16–20	25–40	50–64	128	≥256	Notes
	Southern Africa	Cheadle et al., 1999	IFA	4	75	50							c
	South Africa	Penzhorn et al., 2002	IFA	7	86	20		6					fr
	Thailand	Thiangtum et al., 2006	LAT	19	15.8	64				3			c
	United States												
	California	Spencer et al., 2003	IFA	1	100	50					1		c
	Florida	Lappin et al., 1991	ELISA	3	66.6	64							c
	Midwestern zoos	de Camps et al., 2008	MAT	1	100	25			1				c
P. onca (jaguar)	Brazil	Silva et al., 2001b	MAT	212	63.2	20							
		1101a	IFA	3	100	NS							
	French Guiana	Demar et al., 2008	MAT	1	100	4000						1	fr
	Thailand	Thiangtum et al., 2006	LAT	3	33.3	100				1			c
	United States												
	Various areas	Spencer et al., 2003	IFA	2	100	50				2			c
	Midwestern zoos	de Camps et al., 2008	MAT	1	100	25			1				c
P. uncia (snow leopard, panthera snow)	United States												
	Midwestern zoos	de Camps et al., 2008	MAT	14	35.7	25							c
	Thailand	Thiangtum et al., 2006	LAT	1	100	64				1			c
Lynx spp.													
L. rufus (bobcat)	Canada												
	Québec	Labelle et al., 2001	MAT	10	40	25			1	3			fr
	Mexico	Kikuchi et al., 2004	LAT	6	66.6	64				4			fr

	United States												
	California	Kikuchi et al., 2004	LAT	52	50	64				26			fr
		Riley et al., 2004	LAT	25	88	64			22				fr
		Miller et al., 2008	IFA	3	100	320						3	fr
	Georgia	Dubey et al., 2004	MAT	6	83.3	25					5		fr
	Kansas, Missouri	Smith and Frenkel, 1995	DT	2	50	8	1						fr
	Pennsylvania	Mucker et al., 2006	MAT	131	83	25		8		79		22	fr
	Various areas	Spencer et al., 2003	IFA	3	333	50					1		c
L. canadiensis (lynx)	Canada												
	Québec	Labelle et al., 2001	MAT	106	44	25				41			fr
	United States												
	California	Spencer et al., 2003	IFA	1	100	50				1			c
L. lynx (Eurasian lynx)	Brazil	Silva et al., 2001a	MAT	1	100	20				1			c
	Canada	Philippa et al., 2004	ELISA	5	20	NS			1				c
	Quebec	Labelle et al., 2001	MAT	106	44	25			12	35			fr
	Sweden	Ryser-Degiorgis et al., 2006	MAT	207	75	40			29	23		88	fr
L. pardinus (Iberian lynx, polecat)	Spain	Sobrino et al., 2007	MAT	27	81.5	25		1		3	8	10	fr
		Millán et al., 2009	MAT	26	80.7	25							
		154a	MAT	129	62.8	25				7	31	91	
		Roelke et al., 2008	IHA,LAT	57	44	64.2				21			fr
L.caracal (*Caracal caracal)*	United States												
	California	Spencer et al., 2003	IFA	1	100	50					1		
	Midwestern zoo	de Camps et al., 2008	MAT	4	50	25							
Acinonyx spp.													
Acinonyx jubatus (cheetah)	Southern Africa	Cheadle et al., 1999	IFA	23	43.4	50				10			c

(*continued*)

Table 3.7 Seroprevalence of *T. gondii* in Wild Felids (Continued)

Species	Location	Reference	Test	No. Exam	% Pos	Cutoff Titer	No. with Titers of: <10	16–20	25–40	50–64	128	≥256	Notes
	Thailand	Thiangtum et al., 2006	LAT	1	100	64				1			c
	United States												
	Various areas	Spencer et al., 2003	IFA	9	77.7	50					2	5	c
	Florida	Stover et al., 1990	IHA	16	68.7	64					6	5	c
	Midwestern zoos	de Camps et al., 2008	MAT	22	27.3	25			6				c
Felis spp.													
F. concolor	Brazil	Silva et al., 2001b	MAT	172	48.3	20			41	42			c
		1101a	IFA	5	100	NS							
(cougar, puma, or Florida panther, mountain lion)	Canada	Kikuchi et al., 2004	LAT	23	34.8	64				8			fr
		Philippa et al., 2004	ELISA	15	7	NS							c
	Central and South America	Kikuchi et al., 2004	LAT	83	32.5	64							fr
	Mexico	Kikuchi et al., 2004	LAT	12	16.7	64				2			fr
	United States	Kikuchi et al., 2004	LAT	320	19.1	64				61			fr
	California	Paul-Murphy et al.,1994	LAT	36	58	32			21				fr
		Spencer et al., 2003	IFA	42	25.5	50				2	4	4	c
		Miller et al., 2008	IFA	26	92.3	40			4	3	4	13	fr
	Florida	Roelke et al., 1993	ELISA	38	9	48				5			fr
		Lappin et al., 1991	ELISA	6	83.3	64				5			c
	Midwestern zoos	de Camps et al., 2008	MAT	8	62.5	25			5				c
	Various areas	Spencer et al., 2003	IFA	5	60	50					3		c

F. c. vancouverensis	Canada											
	Vancouver Island	Stephen et al., 1996	IHA	5	100	40	2	3				fr
		Aramini et al., 1998	MAT	12	92	25		8		3		fr
F. chaus (jungle cat)	Brazil	Silva et al., 2001a	MAT	2	100	20		2				c
F. euptilurus (Amur leopard cat)	United States Midwestern zoos	de Camps et al., 2008	MAT	1	100	25			1			c
F. margarita (sand cat)	United Arab Emirates	Pas and Dubey, 2008b	MAT	6	100	25			1	5		c
			LAT	4	75	64				3		c
F. manul (*Otocolobus manul*), Pallas cat	Austria	Basso et al., 2005	MAT	8	100	40				8		c
	United States											
	Colorado	Kenny et al., 2002	LAT	4	100	32		15				c
	Midwestern zoos	de Camps et al., 2008	MAT	5	20	25						c
	Ohio	Swanson, 1999	ELISA	14	79	NS	13					c
	Oklahoma	Ketz-Riley et al., 2003	KELA	6	100	2048				6		c
	Wisconsin	Dubey et al., 1988	MAT	3	67	64						c
F. lynx	United States											
	Alaska	Zarnke et al., 2001	MAT	255	15.3	25	10	10	9	10		fr
F. silvestris (wild cat)	United Kingdom	Yamaguchi et al., 1996	IHA	45	62	16						fr
	Spain	Sobrino et al., 2007	MAT	6	50	25			1	2		fr

(*continued*)

Table 3.7 Seroprevalence of *T. gondii* in Wild Felids (Continued)

Species	Location	Reference	Test	No. Exam	% Pos	Cutoff Titer	No. with Titers of: <10	16–20	25–40	50–64	128	≥256	Notes
F. s. gordoni (Gordon's cat)	United Arab Emirates	Pas and Dubey, 2008a	MAT	36	86.1	25				2	5	17	c
F. serval (*Leptailurus serval,* serval)	Brazil	Silva et al., 2001a	MAT	2	100	20				2			c
	United States												
	Florida	Lappin et al., 1991	ELISA	2	50	64							c
	Various areas	Spencer et al., 2003	IFA	3	33.3	50					1		c
F. temmincki (Asian golden cat, golden cat)	Thailand	Thiangtum et al., 2006	LAT	8	12.5	64							
F. viverrinus (fishing cat)	United States Midwestern zoos	de Camps et al., 2008	MAT	4	25	25			1				c
	Thailand	Thiangtum et al., 2006	LAT	27	22.2	64				6			c
Oncifelis spp.													
O. geoffroyi (Geoffroy's cat)	Bolivia Chaco	Fiorello et al., 2006	ELISA	8	25	NS							fr
	Brazil	Silva et al., 2001b	MAT	12	75	20			1	8			c
O. colocolo (Pampas cat)	Brazil	Silva et al., 2001b	MAT	8	12.5	20						1	
Leopardus spp.	Bolivian Chaco	Fiorello et al., 2006	ELISA	10	100	NS							fr
L. pardalis (ocelot)	Brazil	Silva et al., 2001b	MAT	168	57.7	20			38	58			c
		1101a	IFA	5	80	NS							
	United States Alabama	Spencer et al., 2003	IFA	1	100	50					1		c

L. tigrinus (oncilla)	Brazil	Silva et al., 2001b	MAT	131	51.9	20	25	43				c
L. wiedii (margay)	Brazil	Silva et al., 2001b	MAT	63	55.5	20	9	26				c
	United States											
Neofelis nebulosa (clouded leopard)	California	Spencer et al., 2003	IFA	2	50	50			1			
	Midwestern zoos	de Camps et al., 2008	MAT	7	14.3	25		1				c
	Thailand	Thiangtum et al., 2006	LAT	16	12.5	64			2			c
Herpailurus yogouaroundi (jaguarundi)	Brazil	Silva et al., 2001b 1101a	MAT IFA	99 1	45.9 100	20 NS	15	29			1	c
	United States											
	Florida	Lappin et al., 1991	ELISA	1	100	64						c

Modified from Jones and Dubey.[739] For references see this paper, unless stated otherwise.
c= captive.
fr= freerange.

Table 3.8 Clinical Toxoplasmosis in Wild Felids

Animal Species	Country	Clinical Data	Serology	Histopathology	Remarks	Ref
Cheetah (*Acinonyx jubatus*)	S. Africa	Captive, litter of 3	ND	Peritonitis, encephalitis, tachyzoites found	Co-infection with FIP	1308
	United States	Captive, 8 cubs and 2 mothers affected, 4 cubs died	IHA,IFA positive	Not reported	Treated with sulfa and pyrimethamine	150
	UAE	16 wk-old	IgM and IgG positive	Generalized toxoplasmosis	*T. gondii* DNA found by PCR, IHC-positive	863
Lion (*Panthera leo*)	Nigeria	Two 4 and 6 mo-old cubs in the same cage	DT and IHA positive	Pneumonitis with intralesional tachyzoites	Oocysts found in feces	996
Bob cat (*Lynx rufus)*	United States	One wk-old cub	ND	Hepatis, myocarditis, encephalitis	Tachyzoites and tissue cysts, probably congenital	296
	United States	Six mo-old	MAT titer 1: 40960	Meningoencephalitis	Diagnosis confirmed by IHC.	1209
Sand cats (*Felis margarita*)	UAE	Seven of 8 kittens from 2 litters died	ND	Hepatitis, myocarditis	Diagnosis confirmed by IHC. Queens had high MAT titers before pregnancy	1028
Pallas cats (*Felis manul*)	United States	One adult female and several kittens	IHA titers up to1: 16,777,216	Generalized toxoplasmosis	*T. gondii* isolated from a queen	1100
	United States	13 kittens from various zoos	ELISA	Generalized toxoplasmosis	Clinical and histopathology	1258
	United States	50% mortality in zoos		Generalized toxoplasmosis	Clinical and histopathology	1259
	United States	6 yr-old	ND	Generalized toxoplasmosis	Diagnosis confirmed by IHC	300
	United States	Several kittens and young adults	ELISA	Generalized toxoplasmosis	Histopathology, PCR	760
	Austria	Several kittens	MAT, high titers	Generalized toxoplasmosis	Histopathology, oocysts shed, *T. gondii* isolated	73

these isolates were cryopreserved for genetic studies. Miller et al.[926] isolated viable *T. gondii* in cell cultures seeded with brain homogenates from one bobcat and two mountain lions (*Felis concolor*) from California; all three strains were cryopreserved for genetic characterization.

Demar et al.[246] isolated a mouse-virulent *T. gondii* strain (GUY-2004-JAG) from the heart of a wild jaguar (*Panthera onca*) accidently killed in French Guiana; the strain was genetically characterized.

The only other viable isolates now available are from Canadian cougars. During the Canadian waterborne outbreak of toxoplasmosis in humans,[116] *T. gondii* was isolated from the feces (sample A, cougar 562) of two naturally infected cougars from Vancouver Island, British Columbia, Canada.[39,40] Sample A was obtained from the rectum of a 1.5-year-old male that was killed; it had a serum MAT titer of 1:500. Sample B was obtained from watershed as fecal scat sample. Mice orally inoculated with oocysts from both cougars developed toxoplasmosis and the strains were cryopreserved in liquid nitrogen. These strains were recently designated[429]; the strain from sample A as TgCgCa1 and the strain from sample B as TgCgCa2. Genetic typing suggests that *T. gondii* strains from sample A and B might be from the same animal.[429]

3.2.3 Clinical Toxoplasmosis

Reports in bobcats, lions, Pallas cats, and sand cats are summarized in Table 3.8.

3.2.3.1 Pallas Cats

The Pallas cat (*Felis manul manul*) is highly susceptible to toxoplasmosis. It is the size of a large domestic cat but with distinctive round eyes and vertical bars on the fur. The natural habitat of Pallas cats is the high mountains of Tibet, western Siberia, Turkestan, western China, and Mongolia. Unlike humans, Pallas cats infected with *T. gondii* before pregnancy can repeatedly transmit *T. gondii* to their kittens.[73,1100] Up to 50% of kittens born in captivity have died of acute toxoplasmosis. The high susceptibility to toxoplasmosis is thought to be due to immunodefiency.[760a] Treatment with clindamycin or diclazuril was partially effective as prophylatic in kittens.[73,1259]

Nothing is known of *T. gondii* infection in Pallas cats in the wild. There are two other subspecies of Pallas cats, *F. manul ferruginea* in central Asia and *F. manul nigripecta* in Kashmir, Tibet, and Nepal, but there is no report of *T. gondii* infection in these animals.

3.2.3.2 Sand Cats

The Sand cat (*Felis margarita*) is a small desert felid weighing 2–3 kg. It is found in sand and stone deserts ranging from the north of Africa to Asia, with the Arabian Peninsula as its center of distribution. It is well adapted to living in arid areas and in areas where temperature changes are extreme with temperatures ranging from 0 to 58°C. The Sand cat is mainly nocturnal, spending the day in a shallow burrow or under vegetation.[1028] Like the Pallas cat, *T. gondii* infection prior to pregnancy does not prevent transplacental transmission of the parasite. Congenitally infected kittens can die of acute toxoplasmosis.[1028]

CHAPTER 4

Toxoplasmosis in Sheep (*Ovis aries*)

4.1 NATURAL INFECTIONS

4.1.1 Serologic Prevalence

Antibodies to *T. gondii* have been found in sheep worldwide (Table 4.1). The prevalence of antibodies in ewes was more than twice that in lambs, but results were dependent on the age of lambs sampled.[441,504,601,874,1077,1120] Seroprevalence was shown to increase with age, reaching 95% in 6-year-old ewes in some flocks,[306] suggesting that animals acquire infection postnatally. In general, most sheep acquired infection before 4 years of age, but one-third of old ewes were still seronegative in highly endemic flocks.[306] Prevalence was also higher in ewes on farms where epizootics of abortions were reported.[306] Seroprevalence in intensively managed sheep was lower than in semi-intensive management.[3,1077,1114,1148]

There is little information on the rate of seroconversion in sheep under different management systems, except in a study reported by Lundèn et al.,[874] in which a flock of sheep in Sweden was followed serologically for 6 years. Most sheep became infected in autumn. An interesting finding was that 39% of 136 ewes became seropositive while only 6% of lambs seroconverted.

A few studies statistically analyzed risk factors associated with *T. gondii* seropositivity in sheep.[3,144,775,1077,1200,1319] In a report from Serbia, ewes from state-owned flocks had a higher seropositivity than ewes from privately owned flocks.[775,1319] In a survey of 1961 sheep from 62 farms in southern Italy, the presence of cats on the farm, using surface water for drinking water, and farm size were factors associated with seropositivity.[1319] A study from Spain found the presence of cats and abortion history as risk factors associated with *T. gondii* infection in sheep and goats; 64 of 541 (11.8%) had antibodies in MAT at serum dilution of 1:64 (however, this study did not separate data for goats and sheep).[881]A study from Mexico found that altitude and farm size affected infection rates; prevalence was higher at low altitudes and on large farms.[144]

There is a thorough cross-sectional serological survey of sheep in Italy using serum samples of ten adult sheep from each of 117 farms.[558] Additionally milk samples (all ten samples were pooled for each farm) were also tested for *T. gondii* DNA. *T. gondii* DNA was found in only 4 milk samples.[558]

The data presented in Table 4.1 are not strictly comparable among different locations because of the use of different serological tests and different cutoff values used to determine seropositivity. For example, most authors used 1:16 as cutoff in IFA, but some used 1:200[891] or 1:256.[989] The year of sampling might also have influenced the results. For example, seroprevalence had declined more than half in samples compared a decade later.[1178]

Table 4.1 Seroprevalence of *T. gondii* in Sheep

Location	Reference	Test	No. Exam	% Pos.	Cutoff Titer	No. with Terminal Titers of:						*Notes
						2–10	16–20	25–40	50–64	128	≥256	
Argentina	West et al., 1998	IFA	29	41.4	64				23			a
Austria	Edelhofer and Aspöck, 1996	IFA	4079	66.4	40			2708				a
Bangladesh	Samad et al., 1993b	LAT	56	64	8	6			12	6	12	a
	Samad et al., 1993a	LAT	17	17.6	64	2			1	1	1	b
Brazil												
Bahia	Gondim et al., 1999 a	LAT	240	18.8	64				45			a
Federal District	Ueno et al., 2009	IFA	1028	38.2	64							a,c
Paraná	Zonta et al., 1987–1988	IHA	662	18.2	64				106		15	a
	Freire et al., 1995	IFA	370	47.8	64				177			a,e,f
	Garcia et al., 1999	IFA	228	51.8	64		62		22		96	a,c,e,f
	Romanelli et al., 2007	IFA	305	51.5	64				157			a,c,e,f
	de Moura et al., 2007	IFA	157	7	64				2		9	
Pernambuco	da Silva et al., 2003	IFA	173	35.3	16		48		1		12	
Rio Grande do Norte	Clementino et al., 2007	ELISA	102	29.4	NS							a,e,f
	Soares et al., 2009	IFA	409	20.7	64							a,d.f
Rio Grande do Sul	da Silva and Langoni, 2001	IFA	522	7.7	16		19		15		6	b,e,h
		IFA	173	35.3	16		61					
	Silva and de la Rue, 2006	IFA	123	39	20							a,g
		IHA	123	21.1	16		19		15		6	a,e
Rondônia	Cavalcante et al., 2004	IFA	141	46.8	64				66			a
São Paulo	Oliveira-Sequeira et al., 1993	IFA	177	22.5	16		31					a,g
	da Silva et al., 2002	IFA	100	23	16		3		15		5	g
	Meireles et al., 2003	ELISA	200	31	NS					62		b
	Figliuolo et al., 2004	IFA	597	34.7	64				38	19	150	a,e
Bulgaria	Ragozo et al., 2008	MAT	495	24.2	25			35	21	30	34	b,e,f,h
	Prelezov et al., 2008	IHA	380	48.2	80	10	31	67	46	18	11	a
Burkina Faso	Deconinck et al., 1996	IHA	65	23	64							
Cameroon	Achu-Kwi and Ekue, 1994	LAT	211	31.8	64				67			a,e,f
Canada	Waltner-Toews et al., 1991	ELISA	3872	57.6	NS				2246			a,c,d
Chile	Gorman et al., 1999	IFA	408	28	16			114				a,g

Côte d'Ivoire (Ivory Coast)	Deconinck et al., 1996	IHA	62	68	64						
Czech Republic	Hejlíček and Literák, 1994	DT	886	55	4						
	Sedlák and Bártová, 2007	IFA	24	4	40			1			
	Bártová et al., 2009[78]	ELISA	547	59	NS						a
Djibouti	Deconinck et al., 1996	IHA	183	13	64						
Egypt	El Ridi et al., 1990	IHA	17	29	NS						
	El-Ghaysh and Mansour, 1994	MAT	102	49	49			49			a,d,g
	32	IHA	100	39	64			18	9	6	b
	Shaapan et al., 2008	MAT	300	43.7	25		131				b,d,g
Ethiopia	Bekele and Kasali, 1989	IHA	899	22.9	64	131		81		125	b
	Negash et al., 2004	MAT	116	52.6	NS			61			a,g
	Deconinck et al., 1996	IHA	94	26	NS						
France	Dumètre et al., 2006	MAT	93 ewes	65.6	20		16	5		40	b,h
			164 lambs	22	20	10	8	2		16	b
	Halos et al.[623]	MAT	343 lambs	17.7	6						b
			83 adults	89	6						
Haute-Vienne	Rozette et al., 2005	MAT	141 lambs	20.5	20		84				
			65 ewes	59.1	20		44				
Germany	Seineke, 1996	ELISA	1122	33	NS			383			
Ghana	Van der Puije et al., 2000	ELISA	732	33	NS				242		
India	Verma et al., 1988	IHA	164	8	16		10		3		d
	Dubey et al., 1993	MAT	88	22.7	25		14	5		9	a
	Mirdha et al., 1999	IHA	40	25	18	6		2	2		a
Punjab	Sharma et al., 2008	ELISA	186	3.8	NS						a

(*continued*)

Table 4.1 Seroprevalence of *T. gondii* in Sheep

Location	Reference	Test	No. Exam	% Pos.	Cutoff Titer	No. with Terminal Titers of:						*Notes
						2–10	16–20	25–40	50–64	128	≥256	
Iran												
Ahvaz	Hamidinejat et al., 2008	ELISA	150	72.6	NS							a,g
Khoozestan	Hoghooghi-Rad and Afraa, 1993	LAT	138	13.8	8	2		5	8	5	1	b
	Hashemi-Fesharki, 1996	LAT	2209	24.3	8							a,g
		IHA	1102	24.6	64					271		a,g
Ardabil	Ghazaei, 2005	ELISA	200	30	NS			60				b
Mazandaran	Sharif et al., 2007	IFA	588	35	16		106	88	12			b
	Zia-Ali et al., 2007	LAT	105	20.9	8	10	4	2	2	3	1	b,h
Ireland	O'Brien and Geraghty, 1990	IHA	837	55.6	256						456	a
Israel	Shkap et al., 1992	IFA	259 (1979–1981)	72	16		21		26		140	
		IFA	372 (1985–1990)	30	16		15		35		43	
Italy	Sindoni et al., 1989	MAT	169	13.61	16				23			a
	Balbo et al., 1991	ELISA	1531	13	NS							a
	Masala et al., 2003	IFA	7194	28.4	200				2043			a,d
	Fusco et al., 2007	IFA	1170	28.5	100						333	a,c
Sicily	Vesco et al., 2007	ELISA	1876	49.9	NS				936			a,d,e
Central Alps	Gaffuri et al., 2006	LAT	1056	78	32			821				
Sardinia	1392a	IFA	422	53.4	40							a,d
Jordan	Harps, 1993	LAT	559	20.6	16							a
Lithuania	Stimbirys et al., 2007	ELISA	354	42.1	NS							a,e
Malaysia	Rajamanickam et al., 1990	IHA	106	22.6	64				24			a
Mexico	Garciá-Vaźquez et al., 1990	IFA	495	30	16		40	27	21	10	52	a
	Cruz-Vazquez et al., 1992	IFA	702	37.9	16		266					a,c
	Caballero-Ortega et al., 2008b	ELISA	351	29.1	NS					102		a,c,e,f
Morocco	Benkirane et al., 1989	LAT	304	29	64				88			a,d
	Sawadogo et al., 2005	ELISA	261	27.6	NS			72				b

Netherlands	Cremers et al., 1991	ELISA	40	65	NS			26			
Niger	Weitzman et al., 1991	LAT	70	14	NS			10			a
	Deconinck et al., 1996	IHA	77	19.5	64						
Norway	Skjerve et al., 1998	ELISA	1940	16.2	NS	314					a,c
Pakistan	Zaki, 1995	LAT	40	3	64						
Peru	Reif et al., 1989	IHA	211	27	64			57			a,d
Poland	Górecki et al., 2008	IFA	41	53.6	8						a
	Michalski and Platt-Samoraj, 2004	MAT	20	55	40						
Saudi Arabia	Hossain et al., 1987	IHA	210	11	64			14		9	b
	Amin and Morsy, 1997	IHA	100	39	64			18	9	6	b
	Sanad and Al-Ghabban, 2007	IFA	397	52.2	NS						b,g
	El-Metenawy, 2000	IHA	150	3.3	128				5		d
Senegal	Pangui et al., 1993	IFA	190	46.3	16	87					a,e,g
	Deconinck et al., 1996	IHA	52	11.5	64						
Serbia	Klun et al., 2006	MAT	511	84.5	25			432			c,e,f
Slovakia	Kovâčovâ, 1993	DT	1939	10	4						
South Africa	Abu Samra et al., 2007	IFA	600	5.6	64		33				a,b,c,g
Spain	Moreno et al., 1991	IFA	550	34.9	40		125		44	23	a,g
	Marca et al., 1996	IFA	2306	42.7	40		209	100	188	489	a,g
	Mainar-Jaime and Barberán, 2007	MAT	203	40.4	40				20	63	a,d,g
Sweden	Lundén et al., 1992	ELISA	704	19	NS			83			a,d
Switzerland	1377	ELISA	150	53	NS						b
Turkey	Zeybek et al., 1995	LAT	1050	14.6	64			154			a
	Oz et al., 1995	IHA	42	9.5	NS			4			
	Mor and Arslan, 2007	ELISA	460	95.7	NS		440				
	Acici et al., 2008	DT	95	49.5	16			38	6	3	a,e,f
Samsun	Babür et al., 1997	DT	62	88.7	16	33		16		6	
	Altintaş, 1996	DT	603	31.1	16	130		34		23	a
Ka Yseri	Inci et al., 1999	DT	154	33.8	16	30		14		8	a

(*continued*)

Table 4.1 Seroprevalence of *T. gondii* in Sheep

Location	Reference	Test	No. Exam	% Pos.	Cutoff Titer	No. with Terminal Titers of:						*Notes
						2–10	16–20	25–40	50–64	128	≥256	
Afyon	Çiçek et al., 2004	DT	172	54.6	16		42		33		19	a
Nigde	Karatepe et al., 2004	DT	110	50.9	16		29		17		10	a,d
Istanbul	Öncel and Vural, 2006	ELISA	181	31	NS				25			a,c,e,f
Kars	Mor and Arslan, 2007	ELISA	460	95.7	NS							a
	Dumanli et al., 1991	IHA	295	27.7	32			46	33		3	a
	Oz et al., 1995	IHA	259 (aborted)	25.5	64							
			42 (normal)	9.5	64							
Yozgat	Babür et al, 2001	DT	152	45.4	16							
Yalova	Öncel et al., 2005	DT	63	66.6	16							
UK-Scotland	Johnston, 1988	LAT	909	26.2	512						131	a
Uruguay	Savio and Nieto, 1995	IFA	526	32.5	8	32	23	25	25	31	67	a,d,g
	Freyre et al., 1999	MAT	1613	28.7	64						463	a
United States												
New York	Dubey and Welcome, 1988	MAT	592	73.8	50			32	57		348	a,d,e
North central	Dubey and Kirkbride, 1989a	MAT	1564	65.5	64				233		790	a,d
Northeastern	Malik et al., 1990	ELISA	654	58.6	NS							b,g
Michigan	Underwood and Rook, 1992	NS	63	60	NS						42	a,d
Maryland, Virginia	Dubey et al., 2008	MAT	Lambs 383	27.1	25			20	21	20	43	b,h
Zimbabwe	Pandey and van Knapen 1992	ELISA	216	6	NS							b,e,g
	Hove et al., 2005	IFA	23	47.8	50				7	1	2	a

Modified from Dubey.[437a] For references, see this paper.
* a = farm, b = abattoir, c = risk factors, d = abortion, e = age, f = sex, g = serological test compared, h = isolation of *T. gondii*.

4.1.2 Comparison of Different Serological Tests for Detecting *T. gondii* Antibodies in Latently Infected Sheep

To my knowledge, there is no validation of any serological test for the detection of *T. gondii* infection in sheep using isolation of the parasite to verify data. Available data on different serological tests in naturally infected sheep are summarized in Table 4.2. Some data are comparing parasite isolation results with seropositivity in MAT. *T. gondii* was isolated from 8 of 30 (26.6%) ewes from

Table 4.2 Comparisons of Different Serological Tests for Detection of *T. gondii* Antibodies in Naturally Exposed Sheep

Country	No. of Sera	% Positive (Cutoff Value)					Reference
		MAT	IFA	IHA	LAT	ELISA	
Brazil	177	ND	17.5(1:16)	22.5(1:16)	ND	ND	Oliveira-Sequeira et al., 1993
	100	27 (1:16)	23 (1:16)	ND	ND	ND	Silva et al., 2002
	123	ND	39 (1:20)	20.3 (1:16)	ND	ND	Silva and de la Rue, 2006
Chile	408	ND	28 (1:16)	12 (1:16)	ND	ND	Gorman et al., 1999
Egypt	102	49 (1:40)	50 (1:40)	ND	ND	46	El-Ghaysh and Mansour, 1994
	300	43.7 (1:25)	37(1:64)	ND	ND	41.7	Shaapen et al., 2008
Ethiopia	116	52.6	ND	ND	ND	56	Negash et al., 2004
Iran	150	69.3 (1:80)	ND	ND	ND	72.6	Hamidinejat et al., 2008
Senegal	190	ND	46.3	ND	ND	55.2	Pangui et al., 1993
Serbia	180	90.6 (1:25) 75.6 (1:50) 57.2 (1:100)	ND	ND	ND	68.3 [b] 35[c]	Klun et al., 2007
Saudi Arabia	397	ND	ND	41.8	23.4	ND	Sanad and Al–Ghabban, 2007
South Africa	600	ND	5.6 (1:64)	ND	ND	4.3[a,b]	Abu Samra et al., 2007
Spain	550	35.1 (1:40)	34.9 (1:40)	ND	ND	ND	Moreno et al., 1991
	203	40.4 (1:40)	ND	ND	ND	36.9[a]	Mainar-Jaime and Barberán, 2007
	2306	35.2 (1:40)	33.7 (1:40)	ND	ND	ND	Marca et al., 1996
Sweden	58	36.2 (1:40)	36.2 (1:40)	ND	ND	ND	Ljungdtröm et al., 1994
Uruguay	526	ND	33.6 (1:16)	34.2 (1:16)	ND	ND	Savio and Nieto, 1995
Zimbabwe	107	ND	ND	5.6 (1:64)	ND	2.8	Pandey and van Knapen, 1992

Modified from Dubey[437a]. For references see this paper.
ND = No data
[a] CHECKIT-Toxo test, IDEXX test kit (Dr BommeliAG, Switzerland).
[b] low, 30% cutoff.
[c] high,100% cutoff.

France with MAT titers of 1:20 or higher, from 1 of 8 ewes with titers of 1:80, and from 7 of 11 ewes with titers of 1:160 or higher.[441] In our studies, *T. gondii* was isolated from 53 of 68 (77.9%) seropositive lambs, from 6 of 13 (46.1%) lambs with MAT titers of 1:50 and 1:100, and from 47 of 54 (87%) lambs with titers of 1:200 or higher. The parasite was not isolated from one lamb with MAT titer of 1:25 or from 44 lambs with a MAT titer of <1:25.[423] These data taken collectively suggest that a MAT titer of 1:100 or above would be indicative of a persistently infected animal.

In one report[886] excellent correlation was found between MAT and IFA at a dilution of 1:80 of 2,306 sera; 42.7%, 33.7%, 29.3%, and 21.2% were positive at dilutions of 40, 80, 160, and 320, respectively, by IFA and 38.1%, 35.2%, 34.8%, and 33.7 % were positive at the same dilutions, by MAT.

Various ELISA methods using crude, fractionated, or recombinant antigens have been used to detect *T. gondii* antibodies in ovine sera. One study compared ELISA based on crude and recombinant antigens and found varying degrees of specificity based on naturally and experimentally infected sheep.[1270] Another study[145] reported a good correlation between their in-house *T. gondii* crude antigen ELISA and Western blots in 103 naturally exposed sheep; 91% of the samples showed the same result in both tests. The highest sensitivity was with immunoblots while specificity was higher with ELISA. They also reported on the value of an avidity ELISA to investigate the duration of infection in sheep.

Standardization of ELISA is a major problem because only a few kits are commercially available for use in animals. One ELISA kit (CHECKIT, Table 4.2) has been tested against MAT.[776,880] Klun et al.[776] compared the sensitivity and specificity of the CHECKIT ELISA using two cutoff values (30% and 100%) with the MAT at 1:25, 1:50, and 1:100 dilutions using sera from 180 sheep; these sera were selected from 511 sera tested in a general survey (see Table 4.1). Overall agreement was best (sensitivity of 58.3%, and specificity of 96.1%) with the MAT at a 1:100 dilution and the ELISA at a high cutoff value. They concluded that the ELISA and MAT compared favorably under specific conditions. Mainar-Jaime and Barberán[880] also found good correlation between MAT (at a 1:40 dilution) and ELISA optical density values of 30% or higher.

4.1.3 Isolation and Genetic Characterization of *T. gondii* from Tissues of Naturally Exposed Sheep

Viable *T. gondii* has been recovered from tissues of persistently infected sheep (Table 4.3). In addition, other attempts were made to isolate *T. gondii* from sheep. Using a variety of techniques[197,468,749,1018] *T. gondii* was isolated from sheep using a bioassay in cats. In this assay, cats fed minced mutton from samples pooled from 40 sheep in Egypt were found to shed *T. gondii* oocysts.[468] *T. gondii* bradyzoites were not observed by microscopic examination of digests of 5 g muscle samples from 40 sheep, although 65% of sheep were seropositive.[197] Viable *T. gondii* was isolated from five slaughtered sheep and seven aborted lambs in Denmark but did not provide additional details.[749] *T. gondii* was isolated from the hearts of two healthy lambs in England; no details were given.[1018] Wyss et al.[1377] detected *T. gondii* DNA in tissues of 6% of 150 sheep in Switzerland.

Little genetic typing has been performed on *T. gondii* isolates from sheep. Owen and Trees[1018] found that DNA amplified directly from the placentas of 13 aborted sheep from 10 widely separated farms in the United Kingdom and 2 isolates from the hearts of lambs from an undefined location were all type II, based on the SAG2 locus. Jungersen et al.[749] reported that 11 isolates of *T. gondii* from Denmark (6 from aborted lambs, 5 from healthy sheep) were type II. The results from the United Kingdom and Denmark are of interest because there was no difference in genotype based on health (abortion) status of the animals. Dumètre et al.[441] found that all eight *T. gondii* isolates from adult sheep from France persistently infected with *T. gondii* were clonal type II, using the SAG2 locus and five satellite markers (TUB2, TgM-A, W35, B17, B18). Using the same markers as

Table 4.3 Isolation of Viable *T. gondii* from Tissues of Naturally Infected Sheep

Country	Type	No Exam.	No. Positive (%)	Tissues Bioassayed	Isolate Designation	Genetic Data	Reference
Brazil	NS	136	5 (3.6) [b]	Brain	No	No	Spósito Filha et al., 1992
	NS	40 [a]	10 (25)	Brain, diaphragm	None	No	da Silva and Langoni, 2001
	Lambs, adults	82[a]	16 (19.5)	Brain, heart, diaphragm	TgShBr1-16	No	Ragozo et al., 2008
France	Adults	30	8 (26.6)	Heart	Fr2-2005-*Ovi ari*1-6, Fr2-2006-*Ovi ari*1-2	Yes	Dumètre et al., 2006
	315 lambs, 82 adults	397	48(5.4)	Heart or diaphragm	No	Yes	623a
Iran	Adults	105	4 (3.8)	Brain	No	Yes	Zia-Ali et al., 2007
United States	Lambs	68	53 (77.9)	Heart	TgShUs1-53	Yes	Dubey et al., 2008

Modified from Dubey.[437a] For references, see this paper, unless stated otherwise.
[a] Seropositive.
[b] Authors state that they found additionally tissue cysts in H and E stained sections of mice inoculated with brains of 15 sheep.
NS = not stated.

Dumètre et al.,[441] Zia-Ali et al.[1396] found that of the four isolates of *T. gondii* from adult sheep in Iran, two isolates were type II and two were type III.

Dubey et al.[423] found 15 genotypes among 57 *T. gondii* isolates using 10 PCR-RFLP markers, suggesting high genetic diversity of the parasite in lambs from Maryland, Virginia, and West Virginia. Phylogenetic analysis indicated that the clonal type II lineage and its closely related genotypes accounted for 68% (39 of 57) of the isolates. Type III lineage accounted for 14% (8 of 57) of the strains and was the second most prevalent genotype. The identification of unique alleles in several loci including SAG1 in two isolates, c22-8 one isolate, and PK1 for one isolate, indicates that the genetic makeup of the nonclonal genotypes are quite diverse. Most of the nonclonal genotypes have a combination of alleles of types I, II, and III from different loci. It is not clear if those are simply recombinants from genetic crosses of clonal type I, II, and III strains, or are diverged lineages. In summary, published data indicated that type II was the predominant lineage of the strain isolated from sheep. Interestingly, no type I isolate of *T. gondii* has been found in sheep to date.

Recently, Halos et al.[623a] reported that of the 46 isolates of *T. gondii* genotyped from sheep in France, 45 were Type II and 1 was Type III.

4.1.4 Abortion and Lamb Losses Due to Toxoplasmosis

T. gondii has been recognized as one of the main causes of infective ovine abortion in New Zealand, Australia, the United Kingdom, Norway, and the United States.[302] Actual losses in lambs due to toxoplasmosis are difficult to estimate because (1) the disease is usually sporadic, (2) only a small number of aborted lambs are submitted for diagnosis, (3) those submitted may be inadequately examined, (4) unsuitable material may be sent for diagnosis, (5) the serologic test may not be specific, and (6) toxoplasmosis does not produce clinical disease in the ewe, so this disease does not alarm the farmer as much as other bacterial and viral infections.

Table 4.4 Reports of *T. gondii*-Induced Abortion in Sheep*

Country	No. of Fetuses Exam	% Positive	Diagnostic Methods: Other Causes Investigated	Diagnostic Methods: IHC[a]	Diagnostic Methods: PCR[b]	Diagnostic Methods: Fetal Serology	Reference
Germany	47	10.6	Yes	Yes	Yes	No	Steuber et al., 1995
Italy	582	11.1	No	No	Yes	No	Masala et al., 2003
	366	18.1	Yes	No	Yes	No	Masala et al., 2007
Spain	53	16.9	Yes	No	Yes	Yes	Hurtado et al., 2001
	173	23.1	No	Yes	Yes	Yes	Pereira-Bueno et al., 2004
United States	556	13.7	Yes	Yes	No	Yes	Dubey et al., 1990
	1201	17.5	Yes	Yes	No	Yes	Dubey and Kirkbride,1990

*Modified from Dubey.[437a] For references, see this paper.
[a] IHC = immunohistochemistry.
[b] PCR = polymerase chain reaction.

Table 4.4 summarizes reports of abortion in sheep as a result of *T. gondii* infection in different countries in the last 20 years. *T. gondii* or *T. gondii* DNA was detected in up to 23% of 173 aborted fetuses submitted to one diagnostic facility in Spain.[1044] Of these, 106 fetuses were examined by all three techniques: histology, fetal serology, and PCR. *T. gondii* was detected in 37 (34.9%) of 106 fetuses by one or more of the techniques: 4 by all three tests, 7 by PCR and serology, 2 by histology and serology, and 24 by one test (6 by histology, 1 by PCR, and 17 by serology). Of the two serologic tests used, there was a 100% agreement between IFA (titer 1:32) and ELISA (50 IU). *T. gondii* aborted fetuses submitted for examination were in their mid (60%) or last (40%) term of gestation.[1044]

In two surveys, *T. gondii* DNA was found by PCR in the tissues of aborted sheep in Sardinia, Italy.[891,892] In the first survey (1999–2002), *T. gondii* DNA was found in 271 of 2,471 (11.1%) fetuses; in 42 of 133 (31.5%) placentas; 76 of 496 (15.3%) muscle samples; 63 of 500 (12.6%) brains; 32 of 420 (7.6%) abomasi; 34 of 479 (7%) livers; and 24 of 394 (6%) spleens.[891] In the second survey of sheep from 98 farms (2003–2005), they found *T. gondii* DNA in 53 of 292 (18.1%) fetuses; in 10 of 76 (13.1%) placentas; 34 of 274 (12.4%) muscle samples; 33 of 275 (12.0%) brains; 21 of 250 (8.4%) abomasi; 22 of 268 (8.2%) livers; and 18 of 230 (7.8%) spleens.[892] In both surveys, DNA detection was the highest using muscle and brain samples of the fetuses. Several researchers from the UK[605,1294,1356] reported on the value of detection of *T. gondii* DNA in aborted specimens, especially from autolyzed fetuses that are unsuitable for histologic examination.

In addition to the information presented in Table 4.4, there are other reports of abortion in sheep linked to *T. gondii* infection. Toxoplasmosis abortion was reported in a small flock in Denmark; 9 of 15 ewes aborted and the diagnosis was confirmed in 5 fetuses using histological techniques or by bioassay in mice.[1277] Verma et al.[1318] isolated *T. gondii* from 3 of 77 aborted fetuses in India. *T. gondii* was identified by IHC staining in five ovine fetuses from five flocks in Hungary; in four of these there was also concurrent *Chlamydophila abortus* infection.[1261] Presumptive *T. gondii* abortion was found in a Canadian flock based on history and serology of the dam, and the finding of placentitis in a fetus.[1352] Toxoplasmosis was diagnosed in a flock of sheep in the United States that had a 27.2% lamb mortality; placental lesions characteristic of toxoplasmosis and *T. gondii* antibodies were found in all 11 aborted fetuses examined.[1300]

Johnston[732] suggested that *T. gondii* may cause barrenness in sheep, but the evidence was based on serology. Although fetal serology and DNA detection are useful aids, histopathology is essential to establish a cause–effect association, because *T. gondii* can be passively transmitted transplacentally and fetuses can die of other causes.

Detection of *T. gondii* antibodies in fetal fluids or serum is useful in diagnosis of ovine abortion. Arthur and Blewett[47] examined fetal fluids from 171 aborted fetuses from 55 flocks in Scotland and found that an IFA titer of 1:256 was diagnostic of exposure to *T. gondii*. Trees et al.[1287] examined 478 aborted ovine fetuses in England and found *T. gondii* antibodies in 40.4% by the MAT, in 40% by the dye test, in 37.4% by IFA, and 29% by LAT using a dilution of 1:16 in all tests.

It has been reported that 7–32% of abortion outbreaks in New Zealand sheep from 1973 to 1989 were caused by *T. gondii* with higher rates in the 1980s.[615,1014] *T. gondii* has been suspected as a cause of ovine abortion in Morocco,[85] Egypt,[634] Turkey,[1012a] and Uruguay[553,1148] based on dam serology.

4.1.5 Subclinical Congenital Transmission

The rate of subclinical congenital transmission has not been documented, because only a few animals are tested once a diagnosis has been established. An example where subclinical congenital transmission of *T. gondii* was documented in sheep is provided in the paper of Dubey and Kirkbride.[308] In this study, a flock of 80 Hampshire ewes were pastured in 1987 on a farm in South Dakota.[308] The 80 ewes produced 144 lambs of which 30 were stillborn. Toxoplasmosis was confirmed in 11 of 30 aborted dead lambs based on fetal serology and IHC examination. *T. gondii* antibodies (MAT) were found in 68 of 114 (59.6%) lambs 3–4 months after birth; 2 had titers of 1:64, 1 of 1:256, 12 of 1:1024, and 53 had titers of 1:4096. These high titers are indicative of an immune response initiated by an active infection and not due to passive immunity acquired through suckling of colostrum. Eight of these lambs with MAT titers of 1:1,024 or higher were slaughtered when they were 7 months old. *T. gondii* was isolated from the leg muscles of eight lambs, from the tongues of seven lambs, from the chops of seven lambs, and from the hearts of three using a bioassay in mice with 100-g samples of each tissue.[306] Serological examination of the ewes 2–3 weeks after lambing revealed that 56 of 80 ewes had MAT titers of 1:64 or more, indicating a high rate of infection in ewes.[308]

4.1.6 Prenatal, Postnatal, and Repeat Transmission of *T. gondii*

Until recently, the prevailing view was that most sheep acquire *T. gondii* infection after birth. Although exact data are not available it is thought that less than 2% of sheep become congenitally infected with *T. gondii*, and less than 4% of persistently infected sheep transmit it in the next generation.[141,142,302] Evidence for these conclusions is based on three older studies[631,959,1341] and one recent study.[1106] Hartley,[631] and Watson and Beverley[1341] studied experimentally infected ewes. Of 38 ewes infected with *T. gondii* during a previous pregnancy, all but 1 had uninfected lambs; *T. gondii* was isolated from only 1 placenta.[631] Of 26 ewes inoculated with *T. gondii* in previous pregnancy, 24 had uninfected live lambs, 1 aborted twins, and 1 was barren. *T. gondii* was isolated from the brain of the aborted lamb.[1341] Of 178 lambs born to 135 persistently naturally infected ewes, none had precolostral *T. gondii* antibodies; the placenta of 1 of these was infected with *T. gondii*.[959] Recent studies by a group of researchers from Scotland[141,1106] supported these findings that congenital transmission of *T. gondii* from ewes persistently infected with the parasites is infrequent. Their observations were based on a flock of 46 Scottish black ewes; 31 of these were seropositive and 15 were seronegative for *T. gondii*[141,1106] (both papers discuss the same data). Progeny of these ewes and placental tissue were tested using histopathology, PCR, pre-colostral lamb serology, along with clinical outcome to determine the presence of *T. gondii*. The seropositive ewes delivered 43 live and 6 dead lambs, but none of the lambs was infected with *T. gondii* based on histopathology, and there were no findings of *T. gondii* using DNA analysis or the presence of *T. gondii* antigen and/or intact tachyzoites in IHC staining of tissues. Antibodies were not found by IFA in fetal fluids from the dead lambs and/or in precolostral sera from all but two live twin lambs using Western blot. Thus at most, only 1 of the 31 (3.2%) naturally infected ewes had transmitted the infection transplacentally.

The seronegative ewes produced 24 live uninfected lambs. In conclusion, all four studies discussed above reached the same conclusion that congenital transmission of *T. gondii* from ewes persistently infected with the parasite may occur but is very infrequent.

Recently, a series of papers was published from a group of researchers from England.[445,950,951,1362] These authors proposed that repeat transplacental transmission of *T. gondii* may be more common than previously believed. However, all the evidence they presented was based on the detection of *T. gondii* DNA by PCR. In the first paper, placental and fetal tissues from a flock of 88 Suffolk cross sheep from Worcestershire were tested for *T. gondii* DNA.[445] They detected DNA in the placenta of 37 of 70 sheep with birth of live lambs, indicating a 42% congenital transmission. *T. gondii* DNA was detected in 17 of 18 placental and fetal tissues, from the brains of 15, and hearts of 14.[445] Similar findings were reported in the second paper,[950] a study of two pedigree Charollais sheep flocks in Cheshire and a Suffolk flock. In these three flocks 4.5–18.9% of lambs aborted with a 91% *T. gondii* infectivity based on PCR.[1362] More interestingly, 65% of live lambs had evidence of *T. gondii* DNA based on placental or cord samples. These three flocks were geographically separated. The higher abortion rate observed in the Charollais as compared with the Suffolk flock raises the important question of breed susceptibility,[1362] although abortion may be caused by several other agents, e.g., *Chlamydophila abortus*, so it is important to do a differential diagnosis on abortion material submitted for examination. In the third paper, abortion and *T. gondii* infection were associated with different families of Charollais sheep within a flock.[950] In this study, abortion data in 765 ewes from 27 families in one flock were analyzed. The abortion rate varied 0–100%.[950] In total, tissues of 155 aborted lambs were tested for *T. gondii* DNA. The frequency of *T. gondii* DNA-positive lambs varied from 0 to 100%; however, again the group did not look for the presence of other abortifacient agents in this study. In the fourth paper by these authors,[951] further observations were conducted on the Charollais sheep flock reported on earlier by Morley et al.[950] In this study, 29 ewes were selected based on whether they had two or more lambings during the 2000–2003 seasons. Of these 29 ewes, 9 (31%) produced *T. gondii*-positive progeny over two successive lambings. Of the 35 lambs from these 9 ewes, 12 were born alive and 22 were aborted or mummified; more importantly 33 of the 35 lambs were PCR-positive.[951] A major shortcoming of these studies is that their conclusions are based solely on *T. gondii* DNA detection; they have not demonstrated *T. gondii*-associated lesions using conventional histopathology, which would have helped to establish a cause–effect relationship, nor do they look for the presence of other abortifacient agents.

4.2 EXPERIMENTAL INFECTIONS

In the last 20 years, several authors reported on experimental studies of ovine toxoplasmosis using different strains of *T. gondii*. Results from these studies are summarized below.

4.2.1 Clinical Disease in Sheep Orally Inoculated with Oocysts

Sheep can be easily infected by feeding them sporulated *T. gondii* oocysts. Most of the studies performed were in the UK, using the M1 or M3 isolates of *T. gondii* originally isolated from sheep. The data from these studies are summarized in Table 4.5 in order to facilitate future studies with respect to experimental dosages used to infect sheep. In my opinion, even a few live oocysts could infect sheep; pigs fed as few as one oocyst developed patent *T. gondii* infection (see Chapter 6). Lambs (14 months old) fed 20 oocysts each became infected,[134] and ewes given 100 oocysts produced congenitally infected lambs.[772] Before dosing sheep it is advisable to verify infectivity of oocysts by bioassays in mice, because some oocysts may not be viable. This may be the reason that

Table 4.5 Experimental Toxoplasmosis in Sheep Orally Inoculated with Oocysts

T. gondii Isolate	Dose	Type	Remarks	Reference
M1	2,000, 12,000	20 (10+10), 91–94 days gestation	Fever, abortion, dead lambs. Antibody titers compared in IFA, IHA, and LAT	Buxton et al., 1988; Trees et al., 1989
TS1, 2 Nigerian isolates	10,000	9 (3 per strain) pregnant ewes, 3 rams	Fever, diarrhea, abortion, dead lambs *T. gondii* in semen and milk	Aganga et al., 1988
M1	2,000, 200, 20	59 ewes	All 19 ewes fed 2000 oocysts became febrile, and became immune, only 3 of 10 ewes fed 200 oocysts seroconverted. Ewes seroconverted starting 14 days p.i. 20 oocysts were not infective	McColgan et al., 1988
M1	2000	13 ewes at 91 day gestation	Fever, abortion, dead lambs Ewes seroconverted starting 14 days p.i.	Buxton et al., 1989
M3	**A**. 20, 70, 200, 700, 2,000	14 mo-old lambs fed graded doses	All lambs developed fever and antibodies to *T. gondii*	Buxton et al., 1991
	B. 2,000	20, 80–90 days gestation ewes	Fever, abortion, dead lambs Ewes seroconverted starting 14 days p.i.	
TS1	100	17, 105 days to late gestation	Fever, dead congenitally-infected lambs	Kirkbride et al., 1992
M3	2,000	34, 90 days gestation	Fever, abortion, dead lambs Ewes seroconverted satating 14 days p.i.	Buxton et al., 1993a
Not given	2,000	4 pregnant ewes	Fever, abortion	Samad and Clarkson, 1995
M1		21, 90 days gestation	Fever, abortion, mummification, dead lambs, live lambs Seropositive at 21 days p.i. Sera tested using recombinant ELISA	Coughlan et al., 1995; Owen et al., 1998a
	1,500 (in 3 doses of 500)	ewes	Direct PCR found sensitive for diagnosis of congenital toxoplasmosis	
M1	2,000	18 ewes at 80–90 days gestation	Fever, abortion, resorption, live	Owen et al., 1998b
			Congenitally-infected lambs	
			PCR found sensitive for diagnosis	
M3	1,000–100,000	12, 9-mo-old Grey face	Fever, parasitemia between 3 and 10 days p.i., seroconversion starting 12 days in ELISA and IFA	Esteban-Redondo and Innes, 1998
			Tissue cysts not found by histology; DNA found in tissues	
M3	2,000	10, adult sheep	Fever, parasitemia, viable *T. gondii* found in ovine tissues by bioassays in mice	Esteban-Redondo et al., 1999
M3	2,000	30, 89 days gestation	Fever, dead congenitally-infected lambs	Buxton et al., 1993b
M3	200	30, 89 days gestation	Fever, dead congenitally-infected lambs	Buxton et al., 1996
P	200,000	3, rams	*T. gondii* detected in semen	Lopes et al.[793]

Modified from Dubey.[437a] For references see this paper, unless stated otherwise.

in the study reported by McColgan et al.,[899] only three of ten sheep fed 200 oocysts became infected. However, there may be differences in infectivity of oocysts for hosts with one simple stomach (such as pigs) versus ruminants with four stomachs.

Sheep given an oral dose of viable oocysts developed fever and occasionally showed some respiratory distress, but recovered by 14 days p.i. (Table 4.5). None of the sheep died as a result of administration of an oral dose of *T. gondii* oocysts. Some ewes aborted nonspecifically during the acute phase (second week) of infection, probably due to pyrexia and hormonal deregulation.[12,529,1017,1288] Ewes fed 100 or more oocysts at mid gestation aborted or produced dead lambs, usually 4 weeks p.i. In one study even ewes infected in the last trimester produced dead lambs.[772]

4.2.2 Immunological Responses

Sheep can develop very high levels of *T. gondii* antibodies during acute infection and high IgG antibodies can persist for months or years.[302] Trees et al.[1288] followed the kinetics of IgG antibodies by three tests (IFA, LAT, IHA) and IgM (by fractionation) antibodies in 20 sheep fed oocysts and drew the following conclusions. A titer of 1:16 in LAT was considered non-specific because this titer was found in pre-inoculation samples, and titers increased eightfold after feeding of oocysts to sheep. In general, antibodies were detected later by IHA as compared with IFA. *T. gondii*-specific IgM antibodies peaked at 3 weeks p.i. and preceded an IgG response. Similar kinetics for a *T. gondii*-specific IgG response in sheep orally infected with oocysts were found using an ELISA.[193,479,480,899] Similar results were obtained in sheep parenterally inoculated with tachyzoites.[1040] In sheep inoculated intravenously with the RH strain tachyzoites, IgM was detected by 1 month p.i. and persisted 3 months p.i. Re-infection did not increase the antibody titer.[899] Tenter et al.[1270] reported that two sheep fed 5,000 or 50,000 oocysts developed antibodies detectable by ELISA by 14 days p.i. Sheep inoculated intraperitoneally with RH strain tachyzoites developed antibodies detectable by immunoprecitation by 24 days p.i.[1317]

As *T. gondii* is an obligate intracellular parasite, protective immunity in sheep, similar to the situation in other host species, involves cellular responses, and most of the information in this regard is derived from studies by a group of researchers from Scotland using sheep infected with the S48 vaccine strain.[138,705,706,1337–1339] Using a technique involving chronic cannulation of the efferent duct of a lymph node draining the site of infection, this group was able to monitor the development of immune responses *in vivo* over the course of an acute infection with *T. gondii*. Immune cells from the infected animals were also used in functional studies to examine their anti-parasite activity. They concluded that T-cells (CD4+, CD8+) and IFN-*Y* were critical in recovery from a primary infection with *T. gondii*.

4.2.3 Detection of *T. gondii* in Tissues of Sheep Fed Oocysts

Earlier studies in my laboratory using an oral dose of 10,000 oocysts indicated that *T. gondii* can persist for more than 5 months in tissues of asymptomatic sheep; 50 g of tissues were digested and bioassayed in mice (see Chapter 1). Esteban-Redondo and Innes[479] and Esteban-Redondo et al.[480] compared several methods to detect *T. gondii* in ovine tissues. Sheep (four for each dose) were fed 1,000, 10,000, or 100,000 oocysts and killed 6 weeks p.i.[479] Samples of all major organs were collected to allow analysis for the presence of *T. gondii* using a range of different techniques. Samples of all tissues were fixed in formalin and studied histologically. In addition, approximately 2 g of brain, heart, skeletal muscle, and peripheral blood monocytes from 1–14 days p.i. were tested for *T. gondii* DNA. At 6 weeks p.i., *T. gondii* DNA was detected in 8 of 12 sheep. It was more readily detected in the samples from the group of sheep receiving the highest dose of oocysts and the parasite more frequently detected in the brain and heart than muscle samples. *T. gondii* was not detected using histological techniques.[479] Similar results were found in the second study by these authors.[480]

In this study, sheep were examined at 6 weeks and 6 months after feeding 1000 or 100,000 oocysts. *T. gondii* DNA was detected more frequently from tissues of sheep fed the higher dose, and more readily from the brain and heart than muscle. As a comparison, the authors performed a similar experiment in cattle and did not detect *T. gondii* DNA from any of the tissues of cattle fed a similar number of *T. gondii* oocysts as those used to dose sheep.[480]

4.3 DIAGNOSIS OF *T. GONDII*-INDUCED ABORTION

The following account is from Dubey and Beattie,[302] unless referenced otherwise. *T. gondii*-induced abortion can occur in ewes of all ages, although maiden ewes are most affected. Abortion occurs mainly in ewes that acquire infection during pregnancy and less than 1% of ewes abort due to toxoplasmosis in subsequent pregnancies. The ewes themselves are generally clinically normal at the time of abortion. Occasionally, there is difficulty in parturition, probably because of uterine inertia due to dead fetuses. Lambs may be mummified, macerated, aborted, stillborn, or may be born weak or die within a week of birth. Those that survive the first week generally grow normally to adulthood and produce *T. gondii*-free lambs. Ewes that have aborted due to *T. gondii* return to heat and conceive normally. No positive correlation between abortion due to toxoplasmosis and permanent infertility has been established.

Histologic, cytologic, and serologic examinations and animal inoculations can be used to diagnose abortion due to toxoplasmosis. There are no pathognomonic macroscopic lesions in the fetus; however, macroscopic lesions are seen in about half of *T. gondii*-infected placentas. *T. gondii* produces characteristic necrosis in placental cotyledons.

The main changes in the placenta are focal inflammation and necrosis of the fetal cotyledons; intercotyledonary areas are normal. Lesions may vary from micro- to macroscopic. The characteristic lesions consist of white flecks or multiple white, chalky nodules up to 2 mm in diameter and are found in approximately half of the confirmed cases. These foci may be sparse or dense and may occur in any plane of the cotyledon (Figure 4.1). Foci may be confluent, and not all cotyledons are affected to the same degree. Therefore, it is necessary to wash cotyledons thoroughly in saline solution to expose the deeper lesions that may otherwise be overlooked.

The earliest lesions are necrosis of mesenchymal cells in villi, and edematous fetal villi with infiltrations of mononuclear cells associated with hyperplasia and focal coagulative necrosis of the trophoblastic epithelium. Older lesions consist of foci of caseous necrosis involving fetal and maternal villi which may show mineralization. Tachyzoites are present, usually in small numbers. They are seen more easily in early lesions in mesenchymal cells and the trophoblasts adjacent to foci of necrosis. In older lesions, tachyzoites may be seen at the edge of caseous necrotic lesions where they are often undergoing degeneration. Tissue cysts may be found occasionally.

In the fetus, *T. gondii*-induced gross lesions are generally nonspecific (anasarca, excessive fluids in cavities, etc.) and are possibly related to impaired nutrition and intrauterine death. Rarely, small circumscribed discrete chalky nodules up to 1 mm in diameter are found in the liver. The white matter of the cerebral hemispheres sometimes contains scattered chalky-white foci up to 2 mm in diameter. Occasionally, multiple minute hemorrhages are seen in the white matter. Liver rupture has been reported in 25% of lambs with congenital toxoplasmosis. In twins, only one fetus may be affected, or both may be affected, and fetuses from ewes with diseased placentas may escape infection.

More lesions are found in the brain than in any of the other organs of fetuses examined. Characteristic lesions are seen in the brain of at least 90% of prenatally infected fetuses and consist of foci of leukoencephalomalacia and gliosis. The malacic lesions are often quite extensive and are most frequently seen in the anterior cerebral periventricular white matter. They consist of focal myelin loss, axonal swelling, and degeneration. Older lesions may show some peripheral gliosis and central calcification. Focal chronic inflammatory lesions may be seen at any level of the brain and

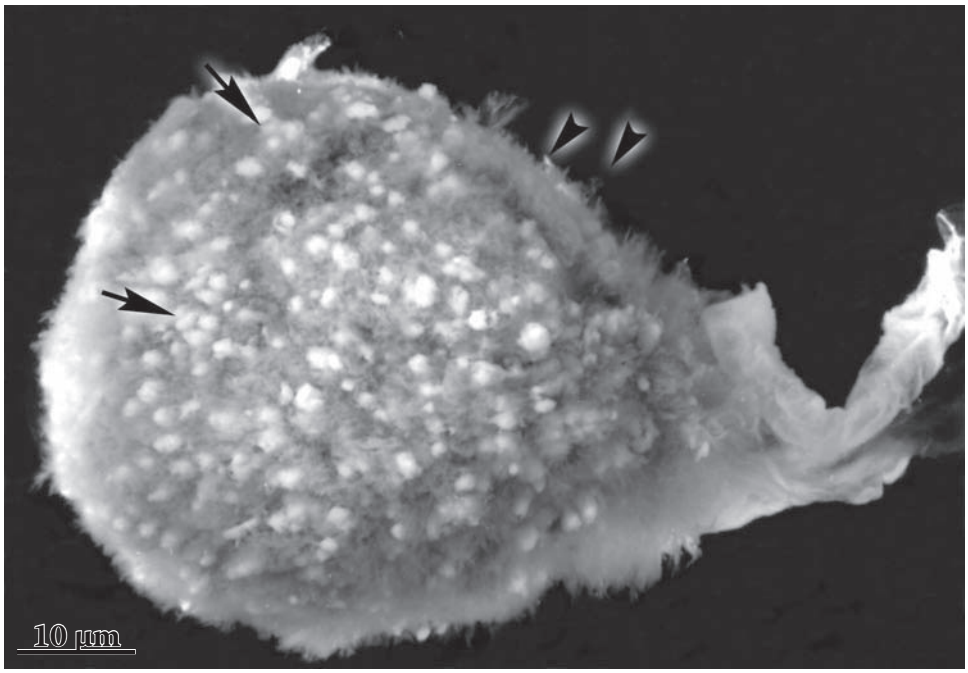

Figure 4.1 Cotyledon of aborted fetus from a naturally infected sheep. There are numerous yellowish-white areas of discoloration. Nearly all cotyledons from this fetus had similar lesions. Individual (arrowheads) and groups of villi are necrotic (arrows). This cotyledon was rinsed with saline solution and photographed under saline to avoid highlights. From Dubey et al.[293]

may show central caseation and, rarely, calcification. Occasionally, mild to moderate nonsuppurative meningoencephalitis is seen. Tissue cysts are sometimes seen adjacent to foci of gliosis. Very rarely, tachyzoites are seen in the vascular endothelium.

The typical hepatic lesions are focal granulomas, some of which have central necrosis. Focal infiltrations with mononuclear cells are seen in different parts of lobules, but *T. gondii* is rarely seen in sections of liver. Myocarditis and pneumonitis are other lesions. Undisputed lesions of toxoplasmosis have not been reported in sheep older than 2 weeks.

It is more important to recognize the type of lesions than to find *T. gondii,* because the organism may be difficult to observe in the placenta, particularly when only degenerating *T. gondii* tachyzoites are present. Degenerating host cells are often mistaken for tachyzoites of *T. gondii*. Tissue cysts may occasionally be found in tissue sections. Occasionally, *T. gondii* can be recognized in impression smears made from fetal placenta, lungs, or brain.

A presumptive diagnosis of ovine toxoplasmosis can be made by finding characteristic lesions in fetal placenta and/or fetal brain. Finding *T. gondii* antibodies in fetal fluids or serum from presuckling lambs is also diagnostic because maternal antibodies normally do not cross the ovine placenta. Both the heart blood-serum and other body fluids can be used to look for *T. gondii* antibodies. However, the absence of *T. gondii* antibody does not preclude the possibility of toxoplasmosis, because the development of fetal antibody is dependent on the age of the fetus at the time of infection and the period between infection and examination. The fetal antibodies can be measured by the DT or MAT test and the IFA test. IHA and LA tests are less sensitive for detecting antibodies in ovine fetuses.[295,1287] Determination of antibody in sera of lambs that have already had colostrum is of no value in the diagnosis of congenital toxoplasmosis because antibodies are transferred to the lamb via colostrum. Because the level of antibody is several times greater in colostrum than in ewe

serum, a higher antibody titer in the serum of the lamb than in that of the dam is also of no diagnostic value. Passively transferred *T. gondii* antibodies disappear in lamb sera by 3 months of age.

The isolation of *T. gondii* from placental and fetal tissues by mouse inoculation can confirm the diagnosis and is particularly helpful in recovering organisms from autolyzed tissues that may be unsuitable for histologic examination. However, in autolyzed tissues, *T. gondii* may have autolyzed along with host tissue. Therefore, failure to isolate does not necessarily mean no *T. gondii* infection. Placenta and fetal brain are the best tissues for isolating *T. gondii*. If neither tissue is available, skeletal muscle (e.g., leg of the fetal lamb) can be used, but as diagnosis may take 6 to 8 weeks, this method is impractical for most diagnostic laboratories.

The fluorescent antibody technique can also be used to recognize *T. gondii* antigen in the fetal placenta or tissues and is quicker than conventional histologic examination. However, it is difficult to obtain specific reagents. The IHC staining is useful in recognizing *T. gondii* tachyzoites in formalin-fixed tissues.

Serologic examinations of aborted ewes may be helpful in indicating that the flock had been exposed to *T. gondii*. However, the presence of *T. gondii* antibodies is not diagnostic because of the high prevalence of infection in sheep and because the titer may remain high (>512), even into the next breeding season. Moreover, even non-pregnant ewes have high titers. The search for a four fold rising antibody titer in the ewe as an indication of acute infection is not practical because sheep reach peak antibody titers within a few weeks of infection. The search for IgM antibody titers in aborting ewes as an indication of recency of infection is not likely to be rewarding because IgM titers decline a few weeks after infection. However, the absence of *T. gondii* antibody can generally exclude toxoplasmosis because by the time abortions are likely to be detected, the antibody would have peaked.

4.4 PATHOGENESIS

It is now firmly established that *T. gondii* causes early embryonic death and resorption, fetal death and mummification, abortion, stillbirth, and neonatal death, largely dependent on the stage of pregnancy at which the ewe becomes infected. After the ingestion of oocysts, *T. gondii* multiplies in the intestinal submucosa and associated lymph nodes and quickly spreads to other organs by the lymph and blood. Ewes may have diarrhea, respiratory distress, and nasal discharge, but usually recover by 14 days p.i.

Although no firm data are available, it is unlikely that infection of the ewe at the time of mating or shortly after mating leads to abortion. In pregnant ewes tachyzoites circulate in the blood during the first 2 weeks after the ingestion of oocysts and infection of the fetus occurs at the end of the period of parasitemia. It takes approximately 2 weeks for the ovine ovum to be fertilized and implanted. Thus, the earliest effects of toxoplasmosis could be expected after implantation. Review of the experimental data suggests that *T. gondii* can be lethal to a 1-month-old fetus while a fetus as old as a 50 days (less than 100 g) can be resorbed. Abortion is likely to be the result of infection of the ewe during mid-pregnancy (60 to 90 days), whereas infection during the last month of pregnancy is likely to produce only mild disease in lambs. Analysis of abortion data also indicated that ewes that abort become infected during mid-pregnancy. A period of 4 weeks generally elapses between the time of inoculation of the ewe with *T. gondii* and abortion, although a few ewes abort between 7 and 14 days after ingesting oocysts, due to pyrexia.

The cause of abortion in toxoplasmosis is not well understood. Lesions in fetal tissues are not extensive, and some lambs associated with severely damaged placentas are born healthy. *T. gondii* causing deregulation of hormonal balance is a possibility. Why abortions due to *T. gondii* in some countries are more common than in others is not clear. Experimentally, the breed of sheep, strain of *T. gondii*, or the numbers of organisms inoculated in ewes do not markedly affect the results.

4.5 REDUCING LOSSES IN SHEEP DUE TO TOXOPLASMOSIS THROUGH PROPHYLAXIS

4.5.1 Vaccine

A live vaccine (Toxovax) is commercially marketed in the UK and New Zealand for reducing losses to the sheep industry from congenital toxoplasmosis.[135,138] This vaccine was initially developed in New Zealand,[990,1359,1361] and further efficacy studies were conducted by Buxton and his colleagues in Scotland. The vaccine consists of a modified strain (S48) of *T. gondii* originally isolated from an aborted lamb in New Zealand. By repeated passages in mice for many years, the strain has lost the capacity to form tissue cysts and oocysts. The commercial vaccine consists of live cell culture-grown tachyzoites that have a shelf life of 10 days. It is recommended to be given 3 weeks before mating. One subcutaneous injection of this 2-ml suspension induces protective immunity for at least 18 months.[138] After subcutaneous inoculation, S48 tachyzoites multiply locally, producing parasitemia and fever. Tachyzoites are controlled by the host immune response as soon as 10 days p.i. and are not demonstrable by bioassays at 6 months p.i.[134,135,137,138,1359] Vaccinated sheep develop humoral and cellular immunity involving CD4 and CD8 T cells and IFN-*y*.[705,706,1338–1340] The mechanism of this persistent immunity in the absence of demonstrable live *T. gondii* is most intriguing and merits further research.

The search for a non-infectious vaccine should continue because of shortcomings of the live vaccine—a short shelf life and safety concerns for those handling it. Identifying new methods of delivery and characterization of the protective antigens should continue.[133,875,1235]

4.5.2 Prophylactic Treatment

Prophylactic treatment of ewes with monensin has been reported to reduce fetal mortality due to toxoplasmosis.[132] Ewes given daily doses of 16.8 or 27.9 mg of monensin per head had fewer abortions than unmedicated ewes. Both groups were challenged with 2,000 or 12,000 *T. gondii* oocysts at 91–94 days of pregnancy; lamb mortality was 16.7% in ewes fed monensin versus 55.2% mortality in lambs from ewes not fed monensin. Similar results were obtained by feeding sulfamezathine, pyrimethamine,[136] or decoquinate.[139,1141] However, feeding another ionophore, lasalocid, did not prevent fetal losses due to toxoplasmosis.[772]

CHAPTER 5

Toxoplasmosis in Goats (*Capra hircus*)

5.1 NATURAL INFECTIONS

5.1.1 Serologic Prevalence

Antibodies have been found in goats worldwide (Table 5.1). In a seroprevalence study of *T. gondii* infection on 2,445 adult goats from 94 dairy goat farms in Sardinia, Italy, IgG antibodies were found in 12.3% and IgM antibodies in 5.6%.[891] The seroprevalence was underestimated because the sera were diluted 1:200 for IgG and 1:20 for IgM determination. In another study, seroprevalence increased with age, indicating postnatal transmission.[1013] A low seropositivity (IHA titer 1:64) was reported in 58 (4.8%) of 1,200 goats from Inner Mongolia, China.[1243] *T. gondii* antibodies were observed in 11.8% of 541 sheep and goats from Spain, but the data were not separated by species.[881]

As with serologic surveys in other animals, little information is available on the comparative efficacy of different serologic tests in detection of antibodies specific to *T. gondii* (Table 5.2). Opel et al.,[1013] using 185 sera from 14 farms in New Zealand, found that 83.8% of titers were within 1 serum dilution and 96.8% were within 2 dilutions. Bahia et al.[66] reported that *T. gondii* antibodies dried on filter papers remained stable for 45 days at room temperature and for 6 months at 4ºC, provided humidity was stable.

5.1.2 Isolation of Viable *T. gondii* from Tissues of Goats

T. gondii was isolated from tissues of goats in Brazil.[157,1078,1227] Spósito Filha et al. isolated it from the diaphragms of 3 of 95 goats; one isolate was mouse virulent.[1227] Cavalcante et al.[157] obtained two *T. gondii* isolates (G1, G2) from the hearts of 2 of 169 goats in the State of Ceará, Brazil. For this, 20 g of myocardium were digested in pepsin and bioassayed individually into two mice each. One of these isolates (G1) was virulent for mice. *T. gondii* was not isolated from ten seropositive goats in Italy.[948]

Ragozo et al.[1078] isolated *T. gondii* from 143 goats slaughtered for human consumption. Tissues of 26 goats with MAT titers of 1:25 or higher were selected for bioassays in mice. From each animal, samples of brain and heart (group A, total 50 g), masseter, and diaphragm (group B, total 50 g) were pooled, and digested in pepsin, and homogenates were inoculated into ten mice. Viable *T. gondii* was isolated from 12 goats; isolation was higher (41.7%) from pooling of heart and brain than from skeletal muscle (33%), and 3 isolates were from both groups of tissues. Ten of these 12 isolates killed all infected mice.[1078]

Table 5.1 Seroprevalence of *T. gondii* in Goats

					No. Terminal Titers of:							
Country	**Test**	**No. Exam**	**%Pos**	**Cutoff Titer**	**<10**	**16–20**	**25–40**	**50–64**	**128**	**≥256**	**Notes**	**Ref**
Argentina	LAT	386	15	16		22	6	6	7	17	a	1312
Austria	IFA	687	68.7	40							a	452
Bangladesh	LAT	15	26.7	128					1	3	a,d	1138
	LAT	33	36.4	64				9	3		a	1139
	LAT	306	12.1	64				10	6	21	b	1140
Botswana	IHA	345	10.1	32			35				d	101
Gaborone	IHA	1799	30	64				540			d,f	1176
Brazil												
Bahia	LAT	439	28.9	64				127			a	597
	IFA	373	16.4	16		49	61	61	104	98	a,g	1301
Various Areas	MAT	143	32.2	25			18	14	7	7	a,b,e	1078
Ceará	ELISA	2362	25.1	NS							a,c	158
	IFA	169	5.9	16		10					b,e f	157
Minas Gerais	IFA	372	36.8	16		49		45		42	a,g	879
	IFA	169	68	16			115				a,f	64
	IFA	200	58	NS								65
	IFA	164	67.1	16							a,f	66
	IFA	767	45.7	64							a,c,f	155a
	IHA	174	19	64							a,f,g	506
Paraíba	IFA	306	24.5	64				19	7	49	b,g	491
Paraná	IFA	282	44.7	64				54		72	a,f,g	269
	IFA	153	30.7	64		20		21		26	a,g	1166
Pernambuco	IFA	213	40.4	16		64		5		17	a	205
Rio de Janeiro	IFA	202	15.8	16		32					a	202
Rio Grande do Norte	IFA	366	30.6	64				4	12	96	a,c	41
	IFA	381	17.1	64				4	8	53	a,c	824

São Paulo												
	IFA	442	14.5	16		9		16		39	a	882
	ELISA	200	17	NS							a	909
	MAT	100	11	16		8		3			a,f	204
	IFA	394	28.7	64				6	7	100	a,c,g	505
	IFA	923	23.4	16							a,c	933a, 1231
Bulgaria	IHA	364	59.8	10	8	8	23	37	43	99	a	1069
Chile, Juan Fernandes Island	IHA	12	75	60		9						1241
Croatia	MAT	79	13.9	20		11					a,g	1083
	MAT	100	4	20		4					b	
Czech Republic	CFT	247	20.2	8	50						a	858
	CFT	405	37.3	8							a,f	1202
	DT	54	61.1	4							a	642
	IFA	4	25	40				1				1161
Djibouti	IHA	35	31.4	64				11			a	243
	IHA	176	21	64				37			b	
Egypt												
Zagazig	IHA	14	28.6	16							b	463
Various areas	IHA	48	35.4	64				4	5	3	a	1083a
Ethiopia	IHA	753	11.6	64				87		.	a,b	80
	IHA	133	19.5	64				26				243
	MAT	58	24.1	NS							a,f	974
	MAT	641	74.8	20			.				a,c	1273
France	ELISA	80	46	NS				37				161
Germany												
Hannover	LAT	305	17.1	64							a,d,f,g	629
Ghana	ELISA	526	26.8	NS					141		a,c,g	1305
Greece (Crete)	ELISA	1538	30.5	NS		183		140		146		957
India	MAT	95	68.4	25			16	39		10	a	331
	IHA	107	19.6	18		14	4				a	930
	MAT	165	41.2	64				11	16	31	a,b,f	99

(*continued*)

Table 5.1 Seroprevalence of *T. gondii* in Goats (Continued)

					No. Terminal Titers of:							
Country	**Test**	**No. Exam**	**%Pos**	**Cutoff Titer**	**<10**	**16–20**	**25–40**	**50–64**	**128**	**≥256**	**Notes**	**Ref**
Iran	LAT	530	20	8	102						a	633
	IHA	108	18.5	64				20			a	633
	ELISA	200	15	NS			30				a	585
Fars	IFA	599	14	64				56	16	12	a	50
Mazandaran	IFA	400	30	16				.	.		a	1174
Italy												
Sicily	MAT	167	61.7					103				1195
Sardinia	IFA	2445	12.3	200						302	a,d	891
Malaysia	IHA	107	17.8	64				19			a	1082
	MAT	400	35.2	40			141				a	268b
	IFA	200	35.5	200						71	b	162a
Mexico	IFA	211	44	16		15	12	9	8	48	a	575
	ELISA	707	3.2	NS							a	567
Netherlands	LAT	189	47	NS			89				a,c	38
	LAT	22	90.9			1		2		17	a,d	905
New Zealand	LAT	kids 88	7	64				74			a,f,g	1013
		yearlings 65	23	64								
		adults 145	37	64								
Norway	DT	160	5	8						8	a,d	471
People's Republic of China	IHA	1200	4.8	64				58				1243
	IHA	202	6.9	64				14				686
Poland	MAT	142	61.9	40								915a
La Réunion Island	ELISA	395	25.3	NS							NS	1111
Saudi Arabia	IHA	25	8	64				2			b	676
	IHA	100	28	64				20		8	b	32
	IHA	56	3.6	128						2	a	460
	LAT	290	19.3	32			56				b,f	1142
Senegal	IHA	144	3.5	64				5			a,b	243
Spain: Grand Canary Island	ELISA	1052	63.3	NS							a	1110

Sri Lanka	MAT	139	22.3	40				2	29	a,b	268
Thailand	LAT	631	27.9	64			176			a,g	727
Tunisia	IFA	656	38.4	16				252			561
Turkey											
Çankiri	DT	38	63.2	16	15		7		2		62
	DT	247	36.8	16	47		30		12	a,c	26
Nigde	DT	46	41.3	16	16		2		1		754
Uganda	ELISA	784	31	NS		240				a	102
United States											
Northwest	MAT	1000	22.1	40	153				68	a,g	315
Tennessee, Kentucky, Georgia	IHA	108	51.9	64						a,f	1032
	MAT	72	65.3	100						a,f	1032
Venezuela	IHA	438	5.94	64			10		16	a,g	981
West Indies, Tobago	LAT	171	38.5	64			3	4	59	c,d	114
Zimbabwe	ELISA	156	4.5	64						a,f	1023
	IFA	312	68.6	40			70	98	46	a	679

a = farm, b = abattoir, c = risk factors, d = abortion, e = isolation of viable *T. gondii,* f = serological test comparision, g = age.

Table 5.2 Comparisons of Different Serological Tests for Detection of *T. gondii* Antibodies in Naturally Exposed Goats

Country	No. of Sera	% Positive (Cutoff Value)					Ref
		MAT	IFA	IHA	LAT	ELISA	
Brazil	169	ND	68 (1:16)	ND	ND	70 (1:16)	64
	100	11 (1:16)	8 (1:16)	ND	ND	ND	204
	169	ND	5.9 (1:16)	ND	ND	4.7	157
	174	ND	19.5 (1:64)	19 (1:64)	ND	19.5 (1:64)	506
	282	23.1(1:64)	44.7 (1:64)	ND	ND	ND	269
	767	ND	46 (1:64)	ND	ND	43	155a
Ethiopia	58	24.1 (NS)	ND	ND	ND	25.9 (NS)	974
India	165	41.2 (1:64)	ND	32.1 (1:64)	ND	ND	99
New Zealand	185	ND	28.1 (1:64)	ND	32.3 (1:64)	ND	1013
Saudi Arabia	290	ND	51.7	40.7	19.3	ND	1142
Zimbabwe	53	ND	ND	ND	7.5 (1:64)	7.5 (NS)	1023

ND = no data, NS = not stated.

5.1.3 Clinical Infections

Earlier reports of abortion and neonatal mortality in goats from Australia, New Zealand, India, and the United States were summarized by Dubey and Beattie.[302] Goats appear to be more susceptible to clinical toxoplasmosis, and even adult goats have died of acute toxoplasmosis.

Diagnosis of abortion in livestock, including goats, is difficult. A 50% diagnostic rate is considered a success. In a well-equipped diagnostic facility in California, etiology was determined in 47% of 211 cases of caprine abortions.[934] *T. gondii* was identified as a cause of abortion in 7 (3%) of these 211 submissions; the fetuses had encephalitis and placentitis with intra-lesional tachyzoites. Diagnosis was confirmed by IHC staining.[934]

Pescador et al.[1046] examined six aborted fetuses, stillborn, and weak newborn goats in Rio Grande do Sul, Brazil. *T. gondii* was demonstrated by IHC staining in several tissues that had degenerative lesions. In five other goats *T. gondii* DNA, but not parasites, was found in caprine tissues. The six dams had IFA titers of 1:512–2048. This was apparently the first report of *T. gondii*-induced caprine abortion in Brazil.

Masala et al.[891,892] reported distribution of *T. gondii* DNA by PCR in tissues of aborted goats in Sardinia, Italy. In the first survey (1999–2002) *T. gondii* DNA was found in 6.4% (23 of 362) of fetuses, in 6 of 12 placentas, 7 of 81 brains, 3 of 41 spleens, 5 of 80 muscles, and 2 of 79 livers. In the second survey (2003–2005), they found *T. gondii* DNA in 3 of 23 (13%) fetuses from nine farms.

Presumptive clinical caprine toxoplasmosis was reported from Bangladesh, West Indies, People's Republic of China, Norway, and the Czech Republic. Bari et al.[70] described lesions suggestive of toxoplasmosis in ten fetuses submitted for diagnosis at the Bangladesh Agricultural University, Mymensingh, but the parasite was not isolated nor demonstrated convincingly in tissues. Engeland et al.[471] surveyed abortions among 1439 dairy goats from 22 herds in Norway. Eight goats in one herd were seropositive by the dye test; six had titers of 1:1024, and two had titers of 1:256. *T. gondii* was not isolated from one fetus and isolation was not attempted on others because of decomposition. Šlosárková et al.[1202] found abortion and iodine deficiency in goats serologically positive to *T. gondii* in the Czech Republic. Borde et al.[114] found association between *T. gondii* seropositivity and abortion on a goat farm in Tobago, West Indies.

An interesting episode of toxoplasmosis was reported in a small Scottish goat herd and the owners.[1199] Four of six adult does had DT antibodies in titers of 1:15 to 1:320 and all gave birth to dead or weak kids. Viable *T. gondii* was isolated from the brain and mesenteric lymph nodes of one

weak kid and milk from a doe, 49 days post delivery. One 13-year-old son of the owner developed evidence of acute toxoplasmosis; the parents were seronegative. Patton et al.[1032] reported a similar episode in the United States. A mother who drank unpasteurized goat milk developed toxoplasmosis in her early pregnancy and delivered a premature baby that died of toxoplasmosis.

5.2 PATHOGENESIS AND EXPERIMENTAL INFECTIONS

5.2.1 Earlier (pre-1988) Observations

Two or three decades ago, I studied several aspects of caprine toxoplasmosis in goats orally inoculated with either 10, 100, 1,000, or 100,000 oocysts, and these findings were summarized in Dubey and Beattie.[302] Salient features are restated here because to my knowledge such studies on pathogenesis of toxoplasmosis in livestock species have not been reported.

Clinical signs were dose dependent.[284,305] All goats became febrile (40°C or higher) within 2 to 5 days p.i., and the fever lasted 2 to 10 days. Goats fed 100 or more oocysts were lethargic, had reduced appetite, were dyspneic, and half of them had diarrhea. Several goats fed 1,000 or more oocysts died between 7 and 20 days p.i. None of the 10 goats given 10 or 100 oocysts died; all were pregnant before inoculation of *T. gondii*. Of 17 pregnant does given either 10 oocysts (4 does), 100 (6 does), or 1,000 (7 does), all aborted or had kids infected with *T. gondii*. The stage of gestation affected the outcome of pregnancy. Of the three does inoculated with *T. gondii* at 51, 68, and 79 days after breeding, all had retained or resorbed fetuses. Of the 14 remaining does infected at 83 to 130 days of gestation, one aborted, ten had at least one stillborn kid, and three had live but infected kids. The earliest abortion occurred 9 days p.i.

In 26 goats fed *T. gondii* oocysts of the GT-1 strain, parasitemia occurred 4 to 8 days p.i., lasting 3 to 10 days. Encystment of *T. gondii* occurred during the second week and the parasite persisted 441 days p.i. (last tested). Of six goats killed 335 to 441 days p.i. *T. gondii* persisted in the liver, thigh muscles, heart, and diaphragm of all six, brain of five, and kidneys of three (see Chapter 1, Table 1.3). Thus, *T. gondii* encysted in the liver of goats with the same frequency as in muscles. *T. gondii* was excreted in goat semen and milk but in small numbers.

T. gondii invaded goat placentas as early as 9 days p.i. and fetal tissues 15 days p.i. Titrations of placenta from goats 18, 49, and 62 days p.i. showed increasing numbers of *T. gondii* per gram of placenta, indicating that *T. gondii* may multiply longer in placental tissue than in other tissues of goats. *T. gondii* was widely distributed in tissues of congenitally infected kids; of the 20 congenitally infected kids examined, *T. gondii* was isolated from the leg muscles, brain, heart, and lungs of 18, spinal cord of 15, liver of 14, diaphragm of 12, and spleen and kidneys of 10. *T. gondii* persisted in several tissues of congenitally infected kids; in four animals killed 138, 141, 170, and 235 days after birth *T. gondii* was demonstrated in liver, leg muscles, and brain of all four, heart of three, and diaphragm and kidneys of two. Thus, from a practical point of view, either a leg or the head of an aborted kid could be sent to the diagnostic laboratory for attempted isolation of *T. gondii*.

Serum samples from eight pregnant goats inoculated with 10 to 1,000 oocysts at 83–102 days of gestation and from the kids born from them were analyzed in the DT, MAT, and LAT. Six of these does were observed for over 1 year, during which time they had given birth twice. All does developed DT and MAT antibody titers of 2,048 or higher within 29 days p.i. and such high titers persisted through the second pregnancy. Thus, serologic examination of the doe alone is not reliable for the diagnosis of *T. gondii*-induced abortion in goats. On the other hand, all congenitally infected kids bled before ingesting colostrum had DT or MAT antibody titers of 2,048, indicating the usefulness of the serologic examination of the fetus in the diagnosis of abortion. No antibody was found in the sera of uninfected kids born to *T. gondii*-infected does. The passively acquired antibody from

colostrum declined by 3 months and disappeared by 5 months of age. Thus, all specific antibody found in adult goats is likely to be actively acquired.

5.2.2 Recent (post-1988) Studies

5.2.2.1 Abortion and Protection

Obendorf et al.[994] reported an important study in 42 pregnant cashmere-type goats. Eleven of them (group A) had aborted due to toxoplasmosis after an experimental challenge during prior pregnancy and the remaining 31 (group B) were uninfected with *T. gondii*. All goats were challenged orally with 10,000 *T. gondii* oocysts at 90 days of gestation. Goats in group B produced 31 kids, most of which aborted or were stillborn. In contrast, goats from group A produced 22 viable kids not infected with *T. gondii*. This study demonstrates protective immunity to *T. gondii* abortion in goats.

Chartier and Mallereau[166] provided evidence that the commercial Toxovac S48 live vaccine can provide protection in goats. Two groups of pregnant goats (group A, n=17, vaccinated; group B, n=16, unvaccinated) were fed 10,000 *T. gondii* oocysts at 90 day of gestation. Twenty-five percent of group A goats aborted or gave birth to stillborn kids whereas none of the group B goats aborted.

Goats fed oocysts often develop fever and can abort during the second week p.i., before *T. gondii* has had time to multiply in the placenta, probably due to pyrexia.[302,1116] Hormonal deregulation associated with *T. gondii* multiplication in placental trophoblasts probably causes abortion 2 weeks p.i.[470,1392]

Experimental studies indicate that goats may abort due to toxoplasmosis more than once,[287] and stage of gestation during primary infection may affect the outcome of pregnancy. Seven of eight goats inoculated with *T. gondii* tachyzoites aborted or had weak kids, one goat infected at 100 days gestation had an infected but healthy kid, and two goats inoculated at 121 and 133 days delivered uninfected kids.[1324] Repeat transplacental transfer of *T. gondii* occurred in one goat.[1324]

5.2.2.2 Serological Responses

Vitor et al.[1323–1326] and Bahia et al.[65] reported the dynamics of *T. gondii* in does and their fetuses. Goats inoculated with tachyzoites and bled sequentially seroconverted between 5 and 12 days p.i. using IHA, Western blots, and ELISA. Parasitemia occurred intermittently 5 to 64 days p.i.[1326] Antibodies to *T. gondii* were detected in fetal fluids, depending on the fetal age.[1325] Sella et al.[1166] reported similar findings. *T. gondii* was excreted occasionally in urine, saliva, and milk of goats.[1323] Recently, Conde de Felipe et al.[184] showed that goats develop IgM antibodies as early as 1 week p.i. and 29–35 kDa proteins gave a more specific response than whole crude tachyzoite lysate. Bahia et al.[65] found that 9 M urea was better than 3 and 6 M urea as dissociating solution for avidity tests to discriminate acute and chronic infection in goats; avidity values increased 100 days p.i.

CHAPTER 6

Toxoplasmosis in Pigs (*Sus scrofa*)

6.1 DOMESTIC PIGS

6.1.1 Natural Infections in Domestic Pigs

6.1.1.1 Serologic Prevalence

Surveys based on the presence of antibodies in blood sera have reported a worldwide distribution of *T. gondii* (Table 6.1). Most of these studies were based on convenience samples collected from slaughtered pigs. Prevalence of *T. gondii* varied dramatically among the classes of pigs surveyed (market pigs versus sows, indoor pigs from biosecure housing systems versus free-range or organic pigs). In the United States, the pigs sold for fresh pork consumption (feeder pigs, market pigs) are mostly raised indoors in well-managed facilities to prevent access to rodents and cats. In these well-managed facilities, prevalence of *T. gondii* has declined drastically in the last decade (Table 6.1). In a statistically valid population-based nationwide survey conducted in 1983–1984, seroprevalence was 23% in market pigs and 42% in breeder pigs (sows).[319] When pigs from these same areas were tested in 1992, prevalence had dropped to 20.8% in breeders and 3.1% in finisher pigs.[344] The institution of a National Animal Health Monitoring System (NAHMS) in the United States for swine now allows periodic surveillance of pigs for microbial infections. The prevalence of *T. gondii* in four NAHMS swine surveys in 1990, 1996, 1998, and 2006 showed a steady decline (Table 6.1). The prevalence of *T. gondii* in pigs is also influenced by management systems. In poorly managed nonconfinement systems, prevalence in pigs was as high as 68% (Table 6.1). Reports of *T. gondii* prevalence in pigs in People's Republic of China were summarized by Zou et al.[1386]

The higher seroprevalence in sows compared with market-age pigs is epidemiologically relevant with respect to transmission of *T. gondii*; market age pigs are sold for use in fresh, unprocessed pork products whereas meat from breeding sows is usually processed (such as sausages, salami, etc.); processing kills or reduces *T. gondii* in pork. In the 1990s seroprevalences also decreased in pigs under intensive managements in some European countries. For example, in Austria, seroprevalence of 14% in 1982 decreased to 0.9% in 1992.[451] The recent trend of rearing pigs outdoors (organic farming) in European countries is likely to increase seroprevalence in pigs in The Netherlands.[767–769,908,1304] The definitions for organic and free-range (FR) pigs provided by Kijlstra et al.[767] are repeated here. There are three classifications of pigs in The Netherlands. "Finishing pigs" are housed indoors, usually on concrete floors, are fed regular pig feed, and come from intensive farms. Free-range pigs, on the other hand, are allowed access to outside enclosures and have straw bedding; they too are fed regular pig feed. Organic pig raising is controlled by a set of regulations crafted by the European Union (EU regulation 2092/91), which stipulate access to the outside, straw bedding, and organic pig feed, which often contains the same plant ingredients as regular pig feed,

Table 6.1 Seroprevalence of *T. gondii* Antibodies in Domestic Pigs

					No. with Terminal Titers of:							
Country	**Test**	**No. Exam**	**% Pos.**	**Cutoff Titer**	**2–10**	**16–20**	**25–40**	**50–64**	**128**	**≥256**	**Notes**	**Ref**
Argentina	IFA	109*	63.3	64				57		12	b	1009
	MAT	230	37.8	25			87				a,b	1315
Austria	IFA	*2238 (1982)	12.2	16		197		41		36	b	451
	IFA	†113 (1982)	43.4	16		21		18		10	b	
	IFA	*2300 (1992)	0.8	16		14		4		0	b	
	IFA	†46 (1992)	4.3	16		2					b	
	CFT	3917	3.8	8					148		a,e	1074
Bangladesh	IHA	200*	20	64						40	b	103
Brazil												
Amazon	MAT	80	37.5	25			2	3	2	23	a,f	159
Minas Gerais	IFA	198	90.4	16		65	77			55	a,e	613
Paraíba	IFA	130	36.2	64				8	7	32	a	60a
Paraná	IFA	117	8.54	64				6		4	b	235
	MAT	304	7.2					8		14	a	207a
	IFA	267	24	64				36		28	a	568
Rio Grande do Sul	IHA	200*	18	64				14	2	20	a	608
São Paulo	ELISA	300	9.6	NS				29			a,f	1244
	MAT	286*	17	25	3	3	3	41			b,d	270
São Paulo and Pernambuco	MAT	759	1.32	64				6		4	a,c,f	153
Canada	MAT	1443†	9.4	40			36	136			a	1206
	LAT	2800*	8.6	32			154	56	22	8	b,e	559
Ontario	ELISA	6048*	0.74	NS							a,c	1063
Chile	DT	1474	28.1	16		324		51		39	a,c,f	1265
Costa Rica	IFA	496	43.8	20		130	51				a,e	42
Czech Republic	DT	2616	5.9	4	140	11	3				b,f	641
	DT	259	0.4	4							b,f	1327
	IFA	20	20	64				4				1161

Germany	ELISA	1005	20.5	NS						a,c,d	493
	ELISA	2041†	18.5	NS						a,e,f	214
	ELISA	1500†	9.3	NS						a	215
	ELISA	4999†	*4.1	NS						a	232
Ghana	ELISA	641	40.6	NS						a,e,f	44
Indonesia	LAT	208	6.25	64			6	4	3	b	707
Italy	IFA	90	64.4	40		49		4	6	a,c,e	582
	ELISA	3472	10.4	NS						a,c	1321a
Korea	ELISA	482	15.1	NS			73			a	726
Malaysia	IHA	122	15.6	64			24				1082
Mexico	ELISA	1203	8.9	NS						a	567
The Netherlands	ELISA	23,348	2.1	NS						b	86
	ELISA	994*	1.8	NS		18				b,e	1307
	ELISA	1,009†	30.9	NS		312				b,e	
	ELISA	2796*	3	NS		85				b,c	908
	ELISA	265€	0.38	NS				1		a,c	1304
	ELISA	178**	5.62	NS				10		a,c	
	ELISA	402‡	2.74	NS				11		a,c	
	ELISA	406	10.9	NS							769
	LAT	660‡	1.2	64						f	767
		635**	4.7	64						f	
		621	0	64						f	
Panama	IFA	290€	32.1	20	15	31	33	14		b	192
People's Republic of China	ELISA	338	23.6	NS						f	200
	IHA	338	9.5	NS			32			f	
	IHA	816	10.4	64			85				833
	IHA	831	16.9	64			24	4	113	b	1398a
	IHA	525	20.19	64			106				686
Peru	ELISA	96	32.3	NS			35			a,f	1244
	WB	137	27.7	NS			38			b,e	1124
Poland	ELISA	925	36.4	100			173	67	97	a	72
	MAT	106	26.4	40						b,f	1222

(*continued*)

Table 6.1 Seroprevalence of *T. gondii* Antibodies in Domestic Pigs (Continued)

					No. with Terminal Titers of:							
Country	**Test**	**No. Exam**	**% Pos.**	**Cutoff Titer**	**2–10**	**16–20**	**25–40**	**50–64**	**128**	**≥256**	**Notes**	**Ref**
Portugal	MAT	333	15.6	20		7	12	33			b,d	240
Serbia	MAT	605	28.9	25			67	53	14	41	c,e	775
Sweden	MAT	60	50	40				4	4	22	a,f	862
	ELISA	695*	3.3	NS				23			b	876
	ELISA	110†	17.3	NS				19			b	
Switzerland	ELISA	100*	1.0	NS								1377
	ELISA	100†	27.0	NS								1377
Taiwan	LAT	3880	27.7	NS			1073				e	164
	LAT	111*	28.8	32			6	10	9	7	b	490
	LAT	395*	10.1	32			18	14	6	2	a,c	1289
Uruguay	DT	601	70.2	16		422					e	550
United States												
Georgia	WB	152	16.4	NS				25			b,e	1124
Hawaii	MAT	509	48.5	25			26	146		75	a	324
Illinois	MAT	5080†	20.8	25			1057				a,e	1345
	MAT	1885*	3.1	25			59				a,e	
	MAT	4252*	2.3	25			36	18	12	31	a	344
	MAT	2617†	15.1	25			121	85	53	136	a	
Iowa	MAT	273†	14.3	32			39				a	1208
	MAT	1000†	22.2	20		35	21	50	32	84	b,d	340
Maryland	ELISA	48**	25	NS							a	418
	MAT	48**	70.83	25	1	4	7	3	8	11	a,d	

Massachusetts	MAT	55*	92.7	10	1	3	2		21	22	a,d	383
Midwest	ELISA	616*	4.1	NS							c	581
NAHMS[$]	ELISA	2029*	4.78	NS							a	1397
	ELISA	587*	10.05	NS								
	MAT	3479†	20	32			679				a	1034
	MAT	3473	19	32			659					685
	MAT	4712*	3.2	32			173				a	1035
		3236†	15	32			488				a	
	MAT	8086†	6	32			487				a	1036
		5720*	0.9	32			49				a	
	ELISA	6,238	2.65	NS							a,f	659
Nationwide	MAT	11229*	23	25		1544		680		360	b	319
		613†	42	25		77		121		56	b	
New England states	MAT	1897	47.4	25			138	515		247	a,c	563
North Carolina	MAT	2238*	0.58	32			13				a	227
Tennessee	MAT	3841†	36	32			1130				a,c	56
Vietnam	MAT	587	27.2	25			32	34	33	61	a,b,e	694
Zimbabwe	MAT	97	9.3	25			5	4				677
	IFA	238*	19.7	50							f	678
	IFA	70†	35.1	50							f	

a = farms, b = abattoir, c = risk factors, d = isolation, e = age, f = test camparison, NS = not stated, *market, †sows, ‡organic, **free-range, €intensive.
$NAHMS = National Animal Health Monitoring Systems.

but is grown on farms that do not use artificial chemical fertilizers or pesticides. Other differences on organic pig farms include greater living space provided for organic pigs than on intensive farms, clipping teeth and cutting tails are prohibited, animals are weaned at a later age, and use of antibiotics and drugs are restricted. The waiting time after use of antibiotic or drug treatments is twice that of regular pig farms. Organic slaughter pigs are allowed only one synthetic drug or antibiotic treatment in their lifetime. If the pig requires more extensive treatment, it loses its status as an organic pig and must be sold as a (cheaper) regular pig. Because of the bovine spongiform encephalitis (BSE) crisis in the EU, farmers there are forbidden to feed their pigs products of "animal" origin.[767]

Kijlstra et al.[767] found that 8 of 660 (1.2%) organic pigs, 30 of 635 FR pigs, and none of 621 conventional pigs in biosecure facilities in The Netherlands had antibodies to *T. gondii*. In this study, 13 (72%) organic farms were able to raise *T. gondii*-free pigs. Interviews with farmers indicated that both FR and organic farmers kept cats for rodent control. The central issue affecting the seroprevalence is exposure of pigs to oocysts and infected rodents, not the system of management.[232,769,1346] There is general agreement that infection in pigs will not be prevented unless contact with oocysts and rodents is prevented.

In addition to the data in Table 6.1, *T. gondii* antibodies were detected by ELISA in 42 of 807 (5.2%) meat juice samples from pigs in Sweden[876] and 12 (0.5%) of 2094 in the United States.[396]

6.1.1.2 Serological Tests Comparison on Sera from Naturally Infected Pigs

Results in many of the serological surveys in Table 6.1 are not comparable because of differences in serological techniques and cutoff values used. Only a few studies used more than one test for these serological surveys. Tamayo et al.[1265] reported 28.1% and 30.1% seropositivity using the DT and IHA, respectively, in 1,474 pig sera (1:16 dilution) from Chile. Chang et al.[164] found antibodies to *T. gondii* in 27.5% by LAT (cutoff 1:32) and 47.1% by ELISA of 3,880 pigs from Taiwan. Ljungström et al.[862] found 50% seroprevalence using 1:40 serum dilution both in the IFA and MAT in 60 pig sera from Sweden. Hirvelä-Koski[663] reported a better correlation between ELISA and IFA than ELISA and IHA on a select group of 100 pig sera from Finland; the 100 sera were selected out of 1,849 slaughtered pigs on the basis of ELISA testing. Cavalcante et al.[159] found *T. gondii* antibodies in 37.5% of 80 pigs from the Amazon region in Brazil by MAT (titer 1:25) and in 43.7% of the same pigs by IFA (titer 1:64). Damriyasa et al.[215] in Germany found 18.5% *T. gondii* seropositivity by ELISA and 16.5% by IFA (cutoff 1:16). Kijlstra et al.[769] compared different tests on pig sera. They initially tested all 1,916 sera by LAT (1:64), and then LAT-positive sera were tested by IFA (1:50). To test the validity of the IFA they tested 28 (15 seropositive, 13 seronegative) by DT and immunoblots. There was a perfect correlation between the DT and IFA and results were verified by immunoblots.

6.1.2 Demonstration of *T. gondii* in Tissues of Naturally Infected Pigs

Viable *T. gondii* was isolated from tissues of pigs collected from abattoirs or farms (Table 6.2). The success of isolation varied greatly, probably due to bioassay procedures. Bioassay in cats increased the isolation rate because much larger volumes of tissues (500 g or more) can be bioassayed in a cat as compared with mice. Selection of tissues by screening of donor pigs for *T. gondii* antibodies before bioassay increased the efficiency of isolation versus bioassay of all tissues, irrespective of antibody status in the donor pig.

Among the studies listed in Table 6.2, Dias et al.[259] attempted to isolate *T. gondii* from 13 of 149 pork sausages in Brazil. The sausages were made fresh with an unknown contact time with salt (<2 hr) and 50 g samples were digested in pepsin and bioassayed in mice. Viable *T. gondii* was isolated from 1 sample; for the 12 other samples, mice inoculated with sausage digests developed *T. gondii* antibodies in IFA (cutoff 1:16), but viable parasites were not found.

Table 6.2 Isolation of Viable *T. gondii* from Tissues of Naturally Infected Pigs

Country	Type	No. Exam.	No. Positive (%)	Tissues Biossayed	Isolate Designation	Genetic Data	Ref
Argentina	Market	109	14 (12.8)[b]	Diaphragm	No	No	1009
Austria	Market	253	1 (0.4)[b]	Brain, heart, diaphragm	No	No	451
Brazil	Market	28[a]	7 (25)	Heart, brain, tongue	Yes	Yes	270
	Market	12	6 (50)[b]	Brain	No	No	528
	Sausages	149	13 (8.7)[b] (see text)	Sausage	No	No	259
Czech Republic	Market	2447	29 (1.1)[b]	Brain, diaphragm	No	No	641
Portugal	Market	37[a]	15 (40.5)	Heart, brain	TgPiPr1-15	Yes	240
United States	Market	38[a]	14 (36.8)[b]	Heart	TgPgUs1-14	Yes	418; 1311
	Sows	1,000	170 (17)[b]	Heart	TgPg Us15-99	Yes	340; 1311
	Market	300	29 (9.6)[c]	Heart	TgPgUs100-128	Yes	1311
	Market	55	51 (92.7)[c]	Heart, tongue	TgPg Us129-179	Yes	383; 817; 1311
	Retail meat	2,094	7 (0.3)[c]	Loin	TgPgUs180-182	Yes	396

[a] Seropositive.
[b] Isolation in mice.
[c] Isolation in cats.

Serological or parasitological surveys based on abattoir samples do not provide a true assessment of risk to humans, because storage and other post-harvest treatments can reduce or kill *T. gondii*. For example, nearly half of the pork in retail meat in the United States is injected with salts and water.[396] Some of the salt treatments (labeled as "enhanced" meat) kill *T. gondii* tissue cysts.[656] Dubey et al.[396] conducted an extensive survey of retail pork sold in the United States for the prevalence of viable *T. gondii*. Based on a sampling plan designed to estimate prevalence within 0.5% of 2,094 unfrozen pork samples were obtained from 28 metropolitan statistical areas. Each sample consisted of 1 kg of boneless pork. In total, 100 g of each of 2,094 samples was bioassayed in cats; *T. gondii* was isolated from 7 of the 2,094 samples.

In addition to individual samples bioassayed (Table 6.2), Wikerhauser et al.[1357] isolated viable *T. gondii* by bioassay in mice from three of ten pools of diaphragms from adult pigs in Yugoslavia; there were 10–14 diaphragms in each pool. Freyre et al.[550] isolated *T. gondii* from diaphragms pooled from several pigs in Uruguay. Gajadhar et al.[559] did not isolate viable *T. gondii* from the hearts of any of 2,800 pigs in Canada; however, 8.6% of the pigs were seropositive.

In addition to the demonstration of viable *T. gondii* in pork listed in the above discussion, *T. gondii* DNA was found in pork samples from Brazil and Japan. da Silva et al.[206] reported *T. gondii* DNA by PCR in 19 of 70 (27.1%) sausages from 55 establishments from São Paulo, Brazil, and Belfort-Neto et al.[81] found *T. gondii* DNA in 34% of 50 diaphragms and 66% of 50 tongues from pigs from abattoirs from Erechim, Brazil. Zakimi et al.[1388] reported that 57 of 101 lymph node samples from pigs from an abattoir on Okinawa Island, Japan, contained *T. gondii* DNA; this is an unusually high prevalence of *T. gondii* in a lymphoid tissue from chronically infected pigs (for that matter in any other animal species).

6.1.2.1 *Genotyping of* T. gondii *Isolates from Pigs*

Pigs are considered the most important meat source of *T. gondii* for humans in the United States and efforts are being made to genetically compare *T. gondii* isolates from pork with those from humans to get an understanding of transmission. In my laboratory, a total of 182 *T. gondii* isolates (designated TgPgUs1-182) from domestic pigs from various sources in the United States were genotyped[432,1311] using 10 PCR-RFLP markers (SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico). Nine genotypes (#1–9) were recognized from the 182 *T. gondii* isolates. Most isolates (56%, 102) were clonal type II (genotypes #1 and #2) while 27% (49) were clonal type III (genotype #3) strains. Genotype #4 had type II alleles with the exception of type I alleles at loci Apico and L358. Eight isolates (genotype #5) from Iowa had a combination of alleles I, II, and III at different loci. The remaining six isolates were divided into genotypes #6–9 and had a combination of different alleles. Eight of the nine genotypes were previously reported in different animal species and geographical regions. In conclusion, along with the predominance of clonal type II and III strains, a few diverse, previously unrecognized *T. gondii* lineages were found circulating in domestic pigs.

Using the SAG2 PCR-RFLP marker, dos Santos et al.[270] found that of the seven *T. gondii* isolates from pigs from Brazil, two isolates were type I and five were type III. de Sousa et al.[240] genotyped 15 *T. gondii* isolates from pigs from Portugal; 11 were type II, and 4 type III, using SAG2 2 and 6 microsatellite markers.

As stated earlier, da Silva et al.[206] and Belfort-Neto et al.[81] directly amplified *T. gondii* DNA from pork samples from Brazil. Using nested PCR and SAG2, da Silva et al.[206] reported that of 19 *T. gondii* DNA samples from sausages, 14 were type I and 5 were type III. However, recent studies using 10 RFLP markers and sequencing have revealed that unlike in other countries, *T. gondii* isolates from a variety of animals in Brazil are not clonal and type II is rare or absent.[432] Belfort-Neto et al.[81] reported that of the DNA samples directly amplified from pork, one was type I at SAG2, and type III at BTUB, SAG3, and GRA 6.

6.1.2.2 *Validation of Serological Tests for* T. gondii

At the request of the U.S. National Pork Producers Council of America, a validation study was performed on tissues from 1,000 unselected sows from a sausage plant in Osceola, Iowa in 1989–1992.[340] Homogenate of 100 g from heart tissue was digested in pepsin, and inoculated into ten mice. Thus, 10,000 mice were inoculated with pig digests. Additionally, pig sera were screened with MAT and 183 hearts were bioassayed in cats. Sera were retrospectively tested for *T. gondii* antibodies using IHA, LAT, and ELISA; the IHA and LAT were performed using commercial kits and the ELISA was based on crude *T. gondii* tachyzoite lysate.[339] In total, *T. gondii* was isolated from 170 pigs (Table 6.2), 50 by bioassay in mice, 58 by bioassay in mice and cats, and 62 by bioassay in cats only. Isolation of *T. gondii* generally increased with antibody titer (Table 6.3). It is noteworthy, however, that 29 of 170 isolates were from pigs considered to be seronegative (MAT <1:20); 17 of these pigs were found to have MAT titer of 1:10 on further testing.[340] None of the 108 isolates obtained by bioassay in mice killed the mice on initial passage. Judging from the mouse infectivity data, it appears that only a few *T. gondii* were present in the inocula. Of the 10 mice inoculated with each of 108 infected pig hearts, the percent frequency of *T. gondii* positive mice was 32.4 (1/10 mouse positive), 12 (2/10 mice positive), 14.8 (3/10 mice), 7.4 (4/10 mice), 1.8 (5/10 mice), 3.7 (6/10 mice), 9.2 (7/10 mice), 0.9 (8/10 mice), 6.4 (9/10 mice), and 11.1 (10/10 mice positive). In other words, only in 12 of 108 cases all 10 inoculated mice developed *T. gondii* infection. The sensitivity and specificity of different serological tests were calculated to be, respectively, 82.9% and 90.29% for MAT, 29.4% and 98.3% for IHA, 45.9% and 96.9% for LAT, and 72.9% and 85.9% for ELISA based on the isolation of viable *T. gondii*.[339]

Table 6.3 Antibody Titer (MAT) and Isolation of Viable *T. gondii* from Hearts of 1,000 Sows by Bioassays in Mice and Cats[a]

MAT Titer	No. of Sows	No. of Isolates	% Positive
<20	778	29	3.7
20	35	13	37.1
40	21	8	38.1
80	50	30	60
200	32	24	75
400 or higher	29	22	75.8

[a] From Dubey et al.[340]

Gamble et al.[565] further evaluated MAT and ELISA on sera from 274 market-age pigs naturally exposed to *T. gondii*; 70 pigs were bioassay positive and 204 were bioassay negative by tests performed in cats. The ELISA used in this study was based on whole tachyzoite-coated plates in the kit marketed by Safe-Path Laboratoies.[565] Good correlation was obtained between ELISA and MAT; of the 70 bioassay-positive pigs, 60 were positive by MAT at a cutoff of 1:25, and 67 were positive at a cutoff of 1:10; 62 were positive by serum ELISA. Of 204 bioassay negative pigs, 193 were negative in MAT (cutoff 1:25), giving a specificity of 94.6% at a cutoff of 1:25, and 98.0% were negative at a cutoff of 1:10. Use of tissue fluids in the ELISA yielded a poor result; only 40 of the 70 bioassay-positive animals gave positive values.

Similar results were obtained with another study with 55 market pigs from a farm in Massachusetts.[383] In this study, attempts were made to isolate *T. gondii* from 55 pigs, irrespective of antibody status. Hearts (30 pigs, batch 1) or hearts and tongue (25 pigs, batch 2) were fed to cats; 51 of 55 cats shed *T. gondii* oocysts. In the first batch, all 30 pigs had MAT antibodies of 1:100 or higher and all cats fed their tissues shed oocysts. In the second batch, 21 of 25 cats fed pig tissues shed oocysts; the 4 bioassay-negative pigs had MAT titers of <1:10. It is of interest that two bioassay-positive pigs had MAT titers of 1:10 and 1:20 and these were also seropositive by immunoblot.[383]

Overall, whole tachyzoite ELISA and MAT gave good results, but some infected pigs were seronegative. Until now, recombinant ELISA, including p30 antigen, has not performed better than an ELISA using a crude tachyzoites lysate.[565] Omata et al.[1009] isolated *T. gondii* from 14 of 109 swine diaphragms; 5 of 12 with IFA titers of 1,024 or higher; 7 of 57 had titers of 64 to 256, and 2 of 40 had titers of 16 or lower.[437d]

6.1.3 Clinical Toxoplasmosis

Severe clinical toxoplasmosis in pigs is considered rare and reports prior to 1988 have been summarized.[291,302] Since 1988, there have been reports of clinical toxoplasmosis in weaned pigs[823,998,1351] and in neonatal pigs.[164,594,628,788,1279]

Okamoto et al.[998] reported simultaneous outbreaks of acute toxoplasmosis on five farms in Japan, perhaps associated with feed contaminated with oocysts. On a farm in Austria, 13 of 80 feeder pigs became ill.[1351] Clinical signs included anorexia, fever, dyspnea, limb weakness, and seven deaths. Three pigs that died and two surviving pigs were examined histopathologically. *T. gondii* was found in lesions and the diagnosis was confirmed by IHC. All seven pigs had high antibody titers (1:65,536). Epidemiological investigation suggested that pigs probably became infected by ingesting food contaminated with oocysts.[1351] Liao et al.[823] reported clinical toxoplasmosis in two sows from the People's Republic of China but the cause of death of the sows was not ascertained (J. P. Dubey, personal communication).

Kumagai et al.[788] reported neonatal toxoplasmosis in pigs. Seven piglets were born to one of seven sows on a farm in Japan; four of these piglets were stillborn. Three of the seven piglets had gait abnormalities and died before they were 15 days old. On necropsy, the piglets had evidence of encephalitis, pneumonitis, and lymph node necrosis. The diagnosis was confirmed immunohistochemically, and by finding antibodies to *T. gondii*.[788]

Haritani et al.[628] reported congenital toxoplasmosis in four stillborn piglets and one live piglet in Japan. Encephalitis and *T. gondii* were identified in all five piglets. Four of these animals also had pneumonia, and two had hepatic necrosis.

Chang et al.[164] diagnosed toxoplasmosis abortion in 20 of 120 swine on eight farms in Taiwan during June 1987 to July 1988. Degenerative changes associated with tachyzoites were found in placentas and fetal tissues, but they did not provide quantitative data.

Neonatal toxoplasmosis in pigs in Brazil was reported by Giraldi et al.[594] They found *T. gondii* in tissues of 2 aborted fetuses, 6 stillborn, and 10 piglets after birth and in histological sections of brains from 15 piglets, hearts of 13 piglets, lungs of 12 piglets, livers of 11 piglets, retinas of 10 piglets, and spleens of 5 piglets.

A seropositive (LAT titer 1:64) sow in Thailand gave birth to three stillborn and six live piglets.[1279] The piglets had signs of dyspnea and bloody diarrhea. One live and one stillborn piglet were examined at necropsy. Toxoplasmosis was diagnosed in both piglets based on finding of tachyzoites in impression smears of the lungs. Antibodies (LAT titer 1:64 or higher) were found in all three suckling piglets, five of six growing piglets, one boar, and one of four sows on the farm.

Venturini et al.[1314] found a low rate of congenital *T. gondii* infection in stillborn piglets in Argentina; IFAT antibodies (titer 1:20) were detected in 15 of 738 fetal fluids, and 10 of these were also positive by MAT (1:25 titer).

Recently Kim et al.[770a] reported an outbreak of clinical toxoplasmosis in adult sows in a larger herd in Jeju Island, Korea. Affected sows were febrile, anorectic, had neurological signs, and few aborted. *T. gondii* was found histologically in tissues of four adult sows and five aborted littermate piglets.

6.1.4 Risk Assessment

Risk assessment studies are summarized in Table 6.4. Management of pigs varies a great deal within a region and within countries. An extensive study of risk factors for transmission of *T. gondii* on pig farms in Illinois was undertaken by Weigel et al.[1346] In the first phase, at least 30 sows on each of 123 farms were tested for *T. gondii* antibodies and telephone interviews were conducted with producers to assess risk factors; higher seroprevalence in sows was associated with cat access to sows. In the second part of this study, all the risk factors were assessed on 47 farms by actual visits to the farms and examination of domestic and wildlife sources of *T. gondii* infection. The presence of seropositive juvenile cats and higher seroprevalence of *T. gondii* infection in house mice were the two main risk factors for *T. gondii* infection in pigs on these 47 farms.[1346] Vaccination of cats on eight of these farms with a live T-263 vaccine to prevent oocyst shedding apparently reduced seroprevalence of *T. gondii* infection in pigs, although the experiment lacked proper controls.[894,895]

Meerburg et al.[908] and Kijlstra et al.[769] investigated farm epidemiology of toxoplasmosis on organic swine farms in The Netherlands. In the first study, 2,796 pigs from 41 farms were tested for *T. gondii* antibodies at slaughter; *T. gondii* were found in 3% of pigs.[908] Based on analysis of responses to a questionnaire sent to farmers, the number of cats present and feeding of goat whey were positively associated with seroprevalence in pigs.[908] In the second study,[769] three of the organic farms with a rodent problem were studied further. During a 4-month period, 215 rodents were trapped on these farms; *T. gondii* DNA was detected in heart or brain tissue of 11 of 71 rodents from

Table 6.4 Risk Assessment Studies on Toxoplasmosis in Pig

Country	No. of Pigs	No. of Farms	Method	Main Findings	Ref.
Germany	117 farms, mixed	Farms selected out of Federal State of Hesse	Personal interviews	Piglet production versus pedigree breeding identified as risk	214
Italy	Slaughterhouse data	274 farms	Slaughterhouse data and interviews with farmers	Source of water, altitude, number of pigs on farm	1321a
The Netherlands	2,796	41 organic	Questionnaire	Cats and goat whey	908
	100–400, mixed	3 organic	Rodent control	Rodent control dramatically reduced prevalence	769
Spain	23 farms	On-farm epidemiology	Personal interviews	Outdoor housing and the presence of cats	567a
United States	Mixed	120 mixed	Questionnaire	Cat access, rodents	1346
	Mixed	47 mixed	Farm visits, all wildlife tested	Juvenile cats, house mice	
	Sows	107 select farms	Questionnaire; 107 of 303 farms replied	Outdoor housing and size of the farm	56
	Mixed	1 farm	Genotyping of *T. gondii* isolates from pigs and wildlife	Infection originated from pigs	817

farm 1, 1 of 5 from farm 2, and 0 of 25 rodents from farm 3. A total of 406 pigs from these three farms (121 from farm 1, 201 from farm 2, and 84 from farm 3) were tested for *T. gondii* antibodies at slaughter. Overall, the seropositivity on these three farms decreased from 10.9% to 3.3% by the end of the rodent control study. However, the prevalence increased again after the rodent control had stopped. These studies indicate a positive role of rodent control in the prevention of *T. gondii* in pigs on organic farms in The Netherlands.[769] The source of *T. gondii* infection (oocysts, tissue cysts) is at issue because organic pigs can be raised *T. gondii*-free.[1158]

Pigs can be infected from a variety of sources, including wild and domestic animals in the immediate proximity of pig barns. Lehmann et al.[817] used genetic and ecological methods to study transmission on a pig farm in the United States. They isolated viable *T. gondii* from pigs and other domestic and wild animals on the farm and genetically compared isolates from pigs and from animals at different locations on the farm. The results indicated that the transmission of *T. gondii* was higher near the pig sties than in the surrounding areas in terms of strain composition and risk of infection. They concluded that *T. gondii* infection in pigs most likely originated in the pig barn and not from outside the barn.

Recently, Villari et al.[1321a] found that origin of pigs (foreign versus born locally), age of the pigs, management, number of pigs on the farm, and source of water were the main factors for *T. gondii* seroprevalence in pigs in Sicily, Italy. Seroprevalence in market age pigs was 7% and the highest compared to neighboring countries. The altitude of the farm and the number of animals were also important risk factors; seroprevalence was lower on farms at higher altitudes (>200 meters) and on farms with less than 50 pigs on the farm.

Using the 2006 NAHMS data, Hill et al. [659] found that rodent control and carcass disposal methods affected the seroprevalence in pigs on 185 swine production facilities in 16 states of the United States. Burial or composting of dead pigs on the farm was a risk factor for increased seroprevalence, as was the lack of professional rodent control services on the farm.

6.2 EXPERIMENTAL INFECTIONS IN DOMESTIC PIGS

Studies have shown that pigs can be infected by inoculation of tachyzoites and by ingestion of oocysts or tissue cysts.[291,302,1364] Data on pigs fed oocysts are summarized in Table 6.5. Certain aspects of these infections will be reviewed.

6.2.1 Clinical

Weaned pigs fed oocysts or tissue cysts generally developed weight loss, anorexia, fever, but generally recovered by 3 weeks p.i., irrespective of the *T. gondii* isolate.

6.2.2 Immunity

6.2.2.1 Humoral Responses

Pigs inoculated with any infectious stage of *T. gondii* produced humoral antibodies detectable by various serological tests.[34,163,337,350,564,572,574,740,747,748,831,835,1196] Pigs inoculated i.v. with tachyzoites developed IgM antibodies by 8–10 days p.i. and IgG by 10–17 days p.i.[747,835] Pigs inoculated with *T. gondii* tachyzoites, tissue cysts, or oocysts developed specific antibodies detectable by using four types of ELISA and cross reactivity was found when sera of pigs infected with various microbial pathogens of pigs were tested, except *Sarcocystis miescheriana*; low cross reactivity was found in sera from three of nine pigs infected with *S. miescheriana*.[835] However, this cross reactivity between *T. gondii* and *S. miescheriana* was not found using the MAT.[360] Lin and Hung[831] also did not detect cross reactivity between *T. gondii* antigens and other swine pathogens using the avidin–biotin ELISA, but they did not test for antibodies to *Sarcocystis* infection.

Pigs fed even a few oocysts responded serologically, and specific response was verified by parasite isolation. *T. gondii* was isolated from tissues of 13 of 14 pigs fed 10 oocysts and 17 of 28 pigs

Table 6.5 Experimental Toxoplasmosis in Pigs Fed Oocysts

Strain, Dose	No. and Age	Period Days	Main Observations	Ref
GT1, oocysts,1,000	4, 4–5 mo	125–875	Persistence of viable	298
Me49, 1,000	4, 4–5 mo	103–759	*T. gondii* in tissues	
TC2, 1,000	4, 4–5 mo	103–822		
TS2, 1,000	4, 4–5 mo	103–782		
Unknown, 1,000 or 10,000	3, 6-wk old	9 wk	Irradiation of meat, 1 pig died day 8 p.i.	1357a
GT1 or PT1, 1,000	17, pregnant	32–92	Congenital toxoplasmosis	316; 310
Ten strains mixed, 10,000–100,000	8, 3–5 mo	64	Protection, biology	318
GT1,100,000	4	28–471	Persistence of viable	840
			T. gondii in tissues, protection	
GT 1, 80,000	2	48	Persistence of viable	1061
			T. gondii in tissues, protection	
VEG, 10	13, 2–3 mo	69–97	Infectivity dose	350; 353
VEG, 1	16, 2–3 mo	22–99		
SSI, 1,000	9, 8 wk	84–102	Disease in general	1364; 835
SSI, 10,000	10, 8 wk			
VEG, 40,000	30, 7 wk		Protection, biology	570; 572; 574; 1291
AS-28, 50,000	8, 4 mo	47	Diagnostic	1378a

fed 1 oocyst.[353] Pigs fed 1 or 10 oocysts developed antibodies to *T. gondii* by 3 weeks p.i. by MAT, but seroconversion as measured by IHA and LAT was delayed and titers remained low.[350]

6.2.2.2 Protective Immunity

Pigs develop good protective immunity as evidenced by recovery from infection with viable *T. gondii.* The cellular basis of this Th1-related cytokine response has been characterized in pigs fed oocysts.[229,1215] However, pigs can exhibit fever, diarrhea, dyspnea, and even death depending on the dose and the age of the pig. The production of proinflammatory cytokines such as interferon gamma during the acute phase of infection is thought to be responsible for this pathogenic effect.[1215] Limited studies using partially genetically defined miniature pigs fed oocysts indicated no effect of host MHC in mediation of protective immunity.[369]

Pigs inoculated with vaccines containing live modified *T. gondii* strains, mutant strains, or irradiated oocysts developed protective immunity. The RH strain of *T. gondii,* originally isolated from a child in 1938, is one of the most pathogenic parasites in mice; but not in pigs. After intramuscular inoculation of 100,000 tachyzoites, pigs develop a febrile response, and viable parasite can be recovered up to 14 days p.i. but not later.[318,337] Pigs developed good protective immunity in the absence of demonstrable viable parasites as evidenced by failure to recover viable *T. gondii* in five of eight pigs following a lethal challenge with oocysts.[337] The age of pigs, route of inoculation, and the line of RH strain might affect these results because 3-day-old nursing pigs inoculated intravenously with another line of RH strain died of acute toxoplasmosis.[1061] However, nursing pigs inoculated intravenously with a temperature-sensitive mutant of the RH strain remained asymptomatic and had fewer tissue cysts when challenged with oocysts.[1061]

Pigs fed *T. gondii* oocysts irradiated with 0.4 kGy cesium developed protective immunity in the absence of demonstrable viable *T. gondii* and humoral antibodies.[369] Pigs vaccinated with irradiated oocysts remained clinically normal following a lethal oocyst challenge but developed tissue cysts; thus, re-infection with *T. gondii* was not prevented.

Attempts are being made to develop noninfectious vaccines for toxoplasmosis in pigs with some success. Garcia et al.[570] reported that pigs vaccinated with crude rhoptry proteins developed partial protection based on reduction in tissue cyst numbers in pigs challenged with oocysts. Pigs vaccinated intradermally with a GRA1-GRA7 cocktail DNA vaccine developed antibodies and cellular immunity.[740]

6.2.3 Comparison of Diagnostic Methods

Hill et al.,[657] Garcia et al.,[573] Tsutsui et al.[1291] and Yai et al.,[1378a] compared histopathology, bioassay, and PCR for the detection *T. gondii* bradyzoites and concluded that PCR and histopathology were insensitive for the detection of tissue cysts. Yai et al.[1378a] detected *T. gondii* by bioassay in mice from tissues of 4 pigs and by PCR from 5 pigs; 8 pigs were fed oocysts and killed 47 days p.i. The differences in sensitivity of different diagnostic techniques discussed above are probably due to the biology of *T. gondii* infection in pigs. The serological tests (MAT, ELISA) assayed antibodies directed against systemic dissemination of infection and the persistence of antibodies for a long time. The PCR methods detected parasite proteins in a very small amount (< 1g) of host tissue; the concentration of *T. gondii* in pigs is very low (1 tissue cyst in 25–100 g of tissue). The histological method was the least sensitive because in a 5 µm histological section, less than 10 mg of host tissue is examined. Hill et al.[657] compared the efficacy of serum serology, tissue extract serology, real-time PCR, nested PCR, and direct PCR for the detection of *T. gondii* in pork using samples from 25 naturally infected pigs from a farm, 10 experimentally infected pigs, and 34 retail meat samples, and then ranked detection methods in the following descending order of sensitivity: serum ELISA (test sensitivity 100%), serum MAT (80.6%), tissue fluid ELISA

(76.9%), real-time PCR (20.5%), semi-nested PCR (12.8%), and direct PCR (0%). Neither ELISA nor MAT reliably detected antibodies in frozen and thawed muscle samples.[657]

Garcia et al.[573] compared PCR, bioassay, and histopathology in ten pigs fed *T. gondii* oocysts and killed 60 days later; *T. gondii* was detected in 55.1% of 98 muscle samples by bioassay in mice, in 16.6% of 150 muscle samples by PCR, and in 0 samples by histopathology. These authors also found *T. gondii* DNA in three of ten aqueous humor samples from these pigs.[574]

Tsutsui et al.[1291] compared distribution of *T. gondii* in commercial cuts of pork by bioassays and PCR in ten pigs 59 days after feeding them oocysts; *T. gondii* was found in 67.5% by bioassays in mice and 22.5% of samples by PCR.

6.2.4 Congenital Toxoplasmosis

A review of published data suggests that it is difficult to produce congenital toxoplasmosis consistently in swine.[302] The reasons for failure and success are not well understood. The stage of *T. gondii*, route of inoculation, stage of gestation, and breed of sow may account for some of these variations. Some of these factors are reviewed.

Dubey and Urban[316] fed 1,000 oocysts to 17 sows at 32–92 days of gestation (Table 6.5). Six sows produced transplacentally infected fetuses; one aborted an infected fetus 17 days after ingestion of oocysts. Many fetuses were mummified. Examination of sera before and after birth indicated that antibodies were not transmitted across the placenta and colostrum-derived antibodies disappeared by 3 months of age. Placentas and fetal tissues from two of these litters of pigs were selected for detailed histopathological examination.[310] Sow 1 was fed oocysts at 60 days of gestation and killed 49 days later; it had eight dead fetuses, of which one was mummified. Sow 2 was fed oocysts at 45 days of gestation and killed 62 days later (gestational term 120 days); it had 11 fetuses of which 4 were mummified, 2 were dead, and 5 were live.

Lesions and *T. gondii* were found in seven fetuses from sow 1 and three fetuses from sow 2. The predominant lesion was necrotizing placentitis, with numerous tachyzoites in trophoblast cells lining the areole in the placenta. Of the ten fetuses with toxoplasmosis, lesions were present in the brains of eight, hearts of seven, spinal cords of six, lungs of four, skeletal muscle of four, livers of three, tongues of three, kidneys of one, and eye of one. An unusual finding was that tachyzoites were observed histologically but not by bioassays, indicating that in some fetuses, tachyzoites died with host cells. The myocarditis was characterized by multifocal necrotizing myocarditis, mineralization, and tachyzoites in myocytes.[310]

Using inbred minipigs, Jungersen et al.[748] found that some *T. gondii* strains were more pathogenic than others. They inoculated ten pregnant sows intravenously with 30,000 tachyzoites of five *T. gondii* strains (two per sow). Four sows inoculated with SVS P14 and SVS Fox 2 *T. gondii* strains aborted fetuses 10–11 days p.i.; abortion occurred presumably due to pyrexia in the dam. Generalized toxoplasmosis was found in three fetuses from two sows inoculated with SVS 014 strain and fetuses from one of two sows inoculated with the SSI 119 strain; sows were killed 48 days p.i. The two sows inoculated with NED strain were killed 49 days p.i.; in one sow, fetuses were not infected and in the second sow one fetus had localized infection in the allantochorion.

6.2.5 Persistence and Distribution of *T. gondii* in Tissues

Persistence was investigated in 16 pigs fed oocysts of one of the four *T. gondii* strains.[298] *T. gondii* persisted as long as 875 days p.i.; *T. gondii* was recovered from brains of 12 pigs, hearts of 11 pigs, tongues of 10 pigs, and diaphragms of 6 pigs. The parasite was isolated from all commercial cuts of pork, including, bacon, ham, spare ribs, tenderloin, shoulder picnic, and Boston butt. It is noteworthy

Table 6.6 Seroprevalence of *T.gondii* in Feral Pigs

Country	Test	No. Exam	% Pos.	Cutoff Titer	No. with Terminal Titers of: 2–8	16	32	64	128	≥256	Ref
Austria	IFA	364	17.9	16							450
	IFA	269	19.3	NS							453
Brazil	MAT	306	4.5	16		10		3		1	517a
Czech Republic	DT	124	15	4							647
	IFA	565	26.2	40			40	40	27	41	76
France	MAT	148	45.9	6	14	28	26				1059a
Germany	IFA	130	25	80							877
Japan											
Kukamoto	LAT	90	4.4	64			3	1			1177
Iriomote Island	LAT	108	5.6	64		92	10	2	3	1	985
Slovak Republic	ELISA	320	8.1	NS							36
Spain	MAT	517	38.4	25			71	111		3	578
	MAT	91	36.3	25							1122a
United States											
Georgia	MAT	170	18.2	25			14	15		2	361
South Carolina	MAT	257	34.2	32			88				260

a = test comparison

that *T. gondii* persisted in the liver of a pig 759 days p.i. and the kidney of a pig 875 days p.i. Of six 50-g samples bioassayed, *T. gondii* was isolated from five.

6.3 TOXOPLASMOSIS IN WILD PIGS

Seroprevalences are summarized in Table 6.6. Seroprevalence varied from 4 to 34%. Dubey et al.[361] found a very low seroprevalence (0.9%, 11 of 1,264) in pigs from Ossabaw Island, which is a remote island off the coast of Georgia. There is a virtual absence of any type of cat on this island. The low prevalence was attributed to grain that was imported from mainland United States for feeding pigs; it was hypothesized that the grain could have been contaminated with *T. gondii* oocysts or infected migratory birds might have transported *T. gondii* with them. The 11 sera found positive by MAT (titers 1:20–640) were also seropositive by the dye test and LAT, and 10 of 11 were also positive by the IHA. By comparison, seroprevalence in feral pigs on the mainland was high; MAT antibodies were found in 31 (19.2%) of 170 feral pigs. The seroprevalence in pigs increased with age, indicating postnatal transmission of *T. gondii* in feral pigs.

Feral pigs in the United States are hunted for food, and the consumption of improperly cooked pork can be a source of human infection. Recently, Richomme et al.[1059a] found MAT (titer 1:24) antibodies in 17.6% of 148 wild boars from France. They also isolated viable *T. gondii* from the tissues of 21 pigs with MAT titers of 1:6 out of a total of 60 pigs; all isolates were Type II.[1059a]

CHAPTER 7

Toxoplasmosis in Dogs (*Canis familiaris*)

7.1 NATURAL INFECTIONS

7.1.1 Serologic Prevalence

T. gondii antibodies have been found in canine sera worldwide (Table 7.1). The table also includes data from dogs admitted to hospitals for undiagnosed illness. In general, seroprevalences increased with age, indicating postnatal infection. Seropositivity was higher in older dogs[832] and in dogs from rural areas.[241] In a survey of 658 dogs from Taiwan, Lin[832] found IgM antibodies in 2%. Yarim et al.[1380] reported prevalence of different classes of serum globulins in dogs from Turkey. Additionally, Lee et al.[816] detected *T. gondii* DNA in sera of 46.3% of 138 dogs from Korea.

Little is known of the validity of serological tests using isolation of the parasite as a standard. Most of the studies on serologic test comparison for *T. gondii* infections in dogs were from Brazil (Table 7.2). The incentive for these studies is cross-reactivity with the related parasite of dogs,[1184] *Neospora caninum,* which was misdiagnosed as *T. gondii* until 1988.[301] These two parasites share common antigens but cross reactivity in naturally infected dogs is difficult to evaluate because of dual infections.[838,1184] In addition to Table 7.2, Hejlíček et al.[645,646] from the Czech Republic used the DT and CFT to compare data from dogs (Table 7.1). Macrì et al.[813] using a cutoff of 1:20 found *T. gondii* antibodies in 45% by MAT and 56% by IFA in 114 dogs.

7.1.2 Isolation of *T. gondii* from Tissues of Naturally Exposed Dogs

As part of ongoing studies on genetic characterization of *T. gondii,* viable *T. gondii* was isolated from tissues of dogs sent to my laboratory from Brazil, Colombia, Mexico, Vietnam, and Sri Lanka (Table 7.3); PCR-RFLP genotype data are given in papers describing the isolates. In addition, da Silva et al.[207] and Brandão et al.[119] had isolated 18 strains of *T. gondii* from dogs in Brazil. As indicated earlier (Chapter 1, Table 1.4), *T. gondii* was isolated more frequently from muscles than the brain.

In addition to Table 7.3, Svoboda et al.[1252] isolated *T. gondii* from tissues of 1 of the 101 dogs necropsied routinely in the Czech Republic. Schares et al.[1151] obtained two viable *T. gondii* isolates (TG-dgGER 1, 2) from 2 of 24,089 dogs from Germany, confirming that dogs can ingest *T. gondii*-infected cat feces and these oocysts remain viable after passage through the gut of the dog.

7.1.3 Clinical Infections

7.1.3.1 Clinical Reports

Since Mello[912] in 1910 described the first fatal case of toxoplasmosis in a dog in Italy there were numerous such reports until 1988; these were summarized by Dubey and Beattie.[302] In the last 20

Table 7.1 Seroprevalence of *T. gondii* in Dogs

					No. with Terminal Titers of:							
Country	**Test**	**No. Exam**	**% Pos.**	**Cutoff**	**2–8**	**16**	**32**	**64**	**128**	**≥256**	**Notes**	**Ref**
Austria	IFA	242	26	NS			63				p,s	1336
Brazil												
Mato Grosso	IFA	61	88.5	40							p	1142a
Minas Gerais	ELISA	369	30.3	NS				112			p,s	929
	IFA	22	40.9	16		1		3		4	i,p,s	119
	IFA	243	47.3	16		62		35		11	p,s	612
	IHA	40	22.5	64				1	2	6	c,t	1182
	IFA	212	59.9	16		25	17	16	26	43	s,p,t	1189
	IFA	218	52.7	64				115			a,b,p,s	146a
Paraíba	IFA	286	45.1	16		16	23	40	28	22	a,f	60
Paraná	IFA	254	76	16		40		44		109	a,c	531
	IFA	540	8.1	64				16		28	p	207c
	IFA	189	84.1	16		50		61		48	s	569
	MAT	134 (rural)	34.3	25			28	17	1			241
	IFA	24	20.8	16				1		4	s	1114
	IFA	312	23.4	16		27		27		19	a,b,f,s	970
Pernambuco	IFA	170	57.6	64				98			s,p	508
Rondônia	IFA	157	76.4	16		4	12	8	14	82	a,b,f	152
São Paulo	IFA	276	46	40			40	51	17	16	c,p,s,t	266
	MAT	610 (urban stray)	31.6	25			96	91	6		s,t	241
	MAT	500 (urban owned)	5.2	25			20	6			p,t	241
	IFA	203	36	40			73				s,t	653
	IFA	80	32.5	16		11		11		4	c,f	231
	IFA	100	18	16		4	8			6	s,t	204
	ELISA	200	50.5	NS				101			s,t	910
	IFA	111	22.5	16		8		5		12	c,i,s	207
	MAT	118	35.8	20		10	6	5	5	16	i,s	410
	IFA	204	25.5	16		52						1190

	IFA	204	36.8	16		8		5	14	48	p,s	582a
	IFA	47	63.8	8								1135
	IFA	108	23.1	64				10		15	f,p,s	120b
	IFA	90	65.5	16		4	11	20	9	15	p,s	1016
	IFA	100	56	16		32		19		5	s	147
Chile	IHA	31	9.7	16		3					p,s	1241
Colombia												
Bogotá	MAT	309	16.8	20		20	6	17	3	6	i,s	406
Czech and Slovak Republic	DT	1839	34.7	4	564	59	11	5			p	645
	CFT	1839	12.7	10	223	10	1					
Czech Republic	DT	1903	9.8	4	185	1					p	646
	CFT	1903	3.7	10	70							
	IFA	413	25.9	40			107				p,s	1160
	IFA	135	53	40			71					1161
	IFA	240	30.4	40				33	27	15	c	486
Israel	IFA	220	35.5	32			22	25	17	14	c,p,s	67
Italy	MAT	114	42	20		7	6	5	6	24	p	813
Malaysia	IFA	145	26.9	64				39			b,e,s	177
	IFA	135	9.6	200						13	s	162a
Mexico												
Durango	MAT	101	51.5	25			27	11	5	9	p,s	405
	MAT	150	45.3	25			32	22	5	9	i,s	434
People's Republic of China	ELISA	534	24	100							a,b	1384
Poland	MAT	43	46.5	40			7		7	6	c	785
Sri Lanka	MAT	86	67.4	20		8	4	10	22	14	i,s	414
Sweden	ELISA	303	23	100							p,s	1299
Taiwan	LAT	51	19.6	32			10				a,f,p	488
	ELISA	658	7.9	20		15	9	8	1	7	p,s	832

(*continued*)

Table 7.1 Seroprevalence of *T. gondii* in Dogs (Continued)

					No. with Terminal Titers of:							
Country	**Test**	**No. Exam**	**% Pos.**	**Cutoff**	**2–8**	**16**	**32**	**64**	**128**	**≥256**	**Notes**	**Ref**
Taipei	LAT	179 (domestic)	15.6	32			19	3	3	3	p,s	489
		110 (stray)	39.1	32			20	7	9	7		
	LAT	1412	20.1	32			122	75	28	59	p	1290
Thailand (Bangkok)	LAT	427	9.4	64				14	17	10	s	728
Turkey												
	DT	70	68.5	16		8		26		14	p	701
	IFA	119	21	64							p,s	1380
Ankara	DT	116	62	16		3						54
Kocaeli province	DT	116	69.8	16		57		13		11	p,s	1194
United States												
Kansas	MAT	229	25	25		40	11			6	p	838
Vietnam	MAT	42	50	20		6	7	2	2	4	a,p	411
West Indies												
Grenada	MAT	107	48.5	25		17	19	7	0	9	p,s	422

a = age, b = sex, c = clinical, e = co-infection, f = risk factors, i = isolation of *T. gondii*, p = pet, s = stray, t = test comparison.

Table 7.2 A Comparison of Different Serological Tests for *T. gondii* Antibodies in Naturally Exposed Dogs in Brazil

	% Positive				
No. of Sera	**MAT**	**IHA**	**IFA**	**ELISA**	**Ref**
276	ND	ND	46 (1:40)	62.5	266
203	ND	ND	35.9 (1:40)	81.2	653
200	ND	38	ND	50.5	910
40	ND	22.5 (1:64)	35 (1:64)	30	1182
100	19 (1:16)	ND	18 (1:16)	ND	204
212	ND	ND	59.9 (1:16)	70.2 (1:32)	1189

ND = not done.

years there have been only a few such reports for two main reasons. First, most pet dogs are now vaccinated against canine distemper virus (CDV) infection. The CDV lowers resistance to preexisting *T. gondii* infection and the dog succumbs to a combined infection. Second, a new disease, neosporosis caused by *N. caninum,* was discovered in 1988.[301]

Among recent reports of clinical canine toxoplasmosis summarized, concurrent CDV predominated (Table 7.4). The 13 cases reported by Dubey et al.[303] were part of a retrospective study of all cases of toxoplasmosis-like illness at one hospital from 1948 through 1987. Only three cases were primary toxoplasmosis, seven had concurrent CDV, one had lymphoma, one had congenital mitral stenosis, and one had ehrlichiosis. One of these three dogs with primary toxoplasmosis had orchitis with numerous tachyzoites in testis[303] (Figure 7.1). The six cases of toxoplasmosis from Switzerland occurred during an outbreak of CDV.[454] Vaccination with live attenuated CDV vaccine may aggravate canine toxoplasmosis. Twenty pups in four litters of working Kelpie dogs died following vaccination with a live canine vaccine containing the CDV and infectious hepatitis virus.[389] Three pups were autopsied. *T. gondii* tachyzoites (no tissue cysts) were found associated with lesions of myocarditis, nephritis, pneumonitis, hepatitis, adrenalitis, and non-suppurative encephalitis. Numerous tachyzoites were seen in renal tubules.[389] The pups probably became infected postnatally by eating *T. gondii* in uncooked mutton fed to them. The dog following renal transplantation developed fatal pneumonia with intra-lesional tachyzoites; kidneys were not affected.[90] Castillo et al.[160] suggested that toxoplsmosis can cause thyroiditis in dogs but did not provide definitive evidence.

Table 7.3 Isolation of *T. gondii* from Tissues of Naturally Exposed Dogs

Country	**No. Examined**	**No. of Isolates**	**Tissues[c]**	**Mouse Virulence**	**Isolate Designation**	**Genotype Data**	**Ref**
Brazil	36[b]	19	B,H	5 virulent	TgDgBr1-19	Yes	410
Colombia	43[b]	20	B,H,T	7 virulent	TgDgCo1-20	Yes	406
Mexico	28[b]	3	B,H	Not virulent	TgDgMx1-3	Yes	434
Sri Lanka	50[b]	23	B,H,T	Not virulent	TgDgSl1-24	Yes	414
Vietnam	21[b]	8	B,H,T	6 virulent	TgDgVi1-8	Yes	411

[a] Neurological signs.

[b] Seropositive.

[c] B = brain, H = heart, T = tongue.

Table 7.4 Clinical Toxoplasmosis in Naturally Infected Dogs 1988–2008

Country	No.	Main Findings	Diagnosis	Ref
Belgium	1	Ataxia, choroidoretinitis, optic nerve neuritis	Serology, treatment	1306
Canada	1	Ophthalmitis	Serology, treatment	1371
New Zealand	2	Encephalitis	IHC	1031
Switzerland	6	Concurrent distemper virus infection	IHC	454
United States	13	3 primary, 10 concomitant	IHC	303
	1	Hepatitis	IHC	1099
	1	Pneumonia following renal transplant	Tachyzoites in tracheal lavage	90
	1	Cutaneous, disseminated	IHC	1342
	1	Keratoconjunctivitis	IHC	1259a
	1	Neurological	IHC	402

The individual cases reported by Rhyan and Dubey[1099] and Wolfer and Grahn[1371] are of interest. An adult dog without evidence of CDV was diagnosed with massive hepatic necrosis; numerous tachyzoites and tissue cysts were present (Figure 7.2). Ophthalmitis involving anterior and posterior segments of both eyes was diagnosed in a 9-year-old dog[1371] on the basis of lesions and serology. The dog had high IgM without IgG, indicating acute infection. The dog subsequently also developed IgG antibodies. The uveitis resolved with treatment with clindamycin (10 mg/kg body weight, orally for 4 weeks, and corticosteroids systematically given intermittently).

I am not aware of any confirmed report of congenital toxoplasmosis in dogs. Iglmanov[700] reported fatal toxoplasmosis in five of seven pups born to a bitch in the former USSR; it is most likely that the pups died of neosporosis. Šmielewska-Łoś et al. [1203] in Poland detected *T. gondii* DNA in the liver and brain of one of five aborted fetuses from four bitches. Recently, viable *T. gondii* was isolated from a 14-day old naturally infected pup in Australia.[30a]

7.1.3.2 Diagnosis

Toxoplasmosis resembles neosporosis. Clinical signs, serology, DNA detection by PCR, and IHC staining can aid diagnosis.[399,547,1154,1380] Limb paralysis, particularly hind limb hyperextension,

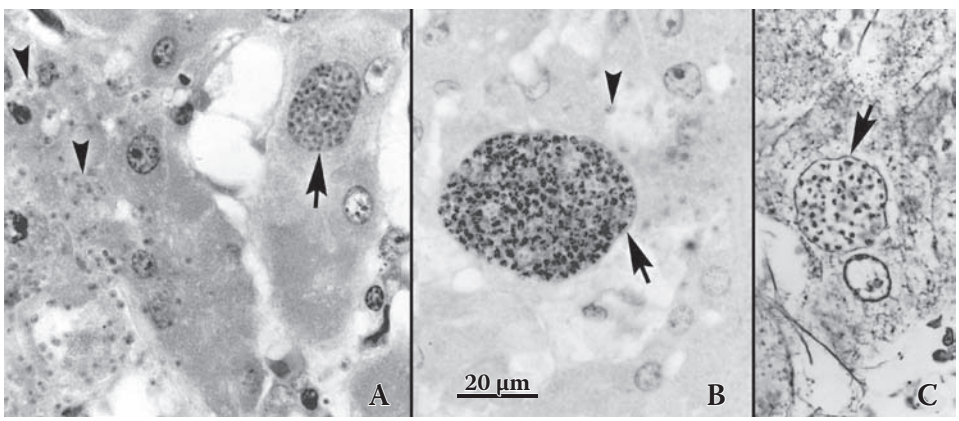

Figure 7.1 *T. gondii* tachyzoites (arrowheads) and tissue cysts (arrows) in sections of hepatocytes in the liver of a naturally infected dog. **(A)** H and E. **(B)** PASH. **(C)** Silver stain. Note the thin tissue cyst wall (arrows) faintly stained with H and E and PASH, but stained distinctively by silver stain. Tachyzoites are oval and faintly stained by PASH. From Rhyan and Dubey.[1099]

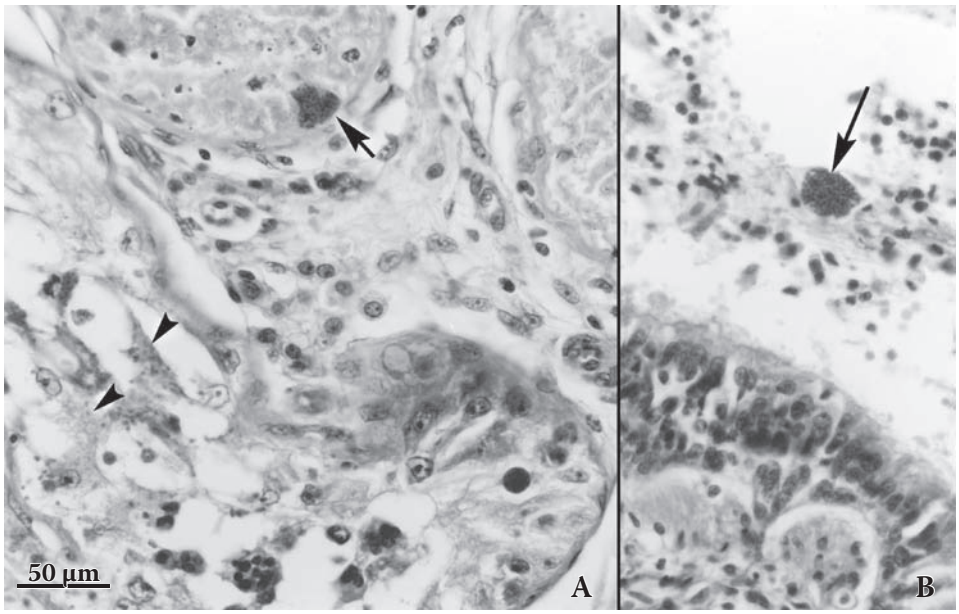

Figure 7.2 *T. gondii* in sections of testis of a naturally infected dog. H and E stain. **(A)** Note individual (arrowheads) and groups of tachyzoites destroying seminiferous tubules. **(B)** A tissue cyst in the lumen of epididymis. PASH. From Dubey et al.[303]

is a hallmark of canine neosporosis. Neosporosis affects limbs usually 3–6 weeks after birth and not all littermates simultaneously. Differential diagnosis is discussed by Dubey and Lappin.[399] As with many other hosts, cutoff values and efficacies for diagnosis for different serological tests have not been established.[1189,1380] Dubey et al.[387] reported dermatitis in a dog with *T.gondii*-like organism.

7.2 EXPERIMENTAL INFECTIONS

Dogs inoculated parenterally with large doses of *T. gondii* tachyzoites sometimes became sick and seroconverted within 2 weeks p.i.[266,1183] IgM antibodies were detected as early as day 7 p.i. and lasted for 3 weeks.[1183] However, studies using natural oral routes of infection indicate that dogs are resistant to clinical toxoplasmosis.[302,843] I am not aware of any confirmed report of congenital toxoplasmosis in dogs. However, there is some experimental evidence for it.[120,120c]

CHAPTER 8

Toxoplasmosis in Cattle (*Bos taurus*)

8.1 NATURAL INFECTIONS

8.1.1 Serologic Prevalence

Serum antibodies to *T. gondii* have been found in cattle in many surveys worldwide (Table 8.1). Actual prevalence rates are likely to be lower than indicated because of problems with the specificity of the tests used.[302]

Little is known concerning specificity and sensitivity of serological diagnosis of *T. gondii* infection in cattle because several tests that are used to diagnose toxoplasmosis in humans give erratic results with cattle sera,[289] and it is difficult to verify specificity using naturally infected cattle. The dye test, which is the most specific test for humans, gives false or erratic results with cattle sera.[289,326] Among all serological tests evaluated, a titer of 1:100 or higher in the MAT appears to be indicative of *T. gondii* infection in cattle.[326]

8.1.2 Demonstration of Viable *T. gondii* in Cattle Tissues

Attempts to isolate *T. gondii* from cattle tissues have been generally unsuccessful.[302] Viable *T. gondii* was isolated from a beef cow in my laboratory.[320] A beef herd in Maryland suffered *Neospora caninum*-associated abortion. All cows from this herd were bled in May 1989 for neosporosis study and also tested for *T. gondii* antibodies. Three cows had *T. gondii* antibodies by the MAT (Table 8.1). One cow had a MAT titer of 1:1,600. I purchased this 500-kg cow for isolation of *T. gondii*. The cow was killed and 100–500 g portions of most of its tissues were bioassayed in cats (500 g each tissue) and mice (100 g each tissue). None of the 12 cats fed approximately 6 kg of beef (liver to 3 cats, the heart to 2 cats, minced hamburger to 3 cats, kidneys, brain, tongue, and spinal cord to 1 cat each) shed oocysts. None of the 150 mice inoculated with homogenates of 15 tissues became infected. Strangely, *T. gondii* was isolated from a homogenate of intestine of the cow. Ten mice were inoculated s.c. with homogenate of the small intestine; five of the ten mice died and five mice remained seronegative. Of the five mice that died, four died on day 5 p.i. and were not examined because of autolysis. Virulent *T. gondii* was found in the fifth mouse that had died on day 8 p.i. This bovine isolate was designated CT1, and it is highly pathogenic for mice; one oocyst or one tachyzoite was lethal to mice.[320]

In a recent study of beef from retail grocers, antibodies to *T. gondii* were not found by ELISA in meat juice from any of the 2,049 samples of beef bioassayed during the National Retail Meat Survey for *T. gondii*, nor were viable organisms detected.[396] In this survey, six 100-g samples of beef were pooled, and fed to cats; none of the 350 cats fed beef from 2,049 samples shed *T. gondii* oocysts. Freyre et al.[549] also did not isolate *T. gondii* from 20-g samples of beef from Uruguay by feeding to cats. Wyss et al.[1377] isolated *T. gondii* DNA from tissues of 3% of 100 adult cows, 6% of 50 heifers, 2% of 100 bulls, and 1% of 100 calves in Switzerland.

Table 8.1 Seroprevalence of *T. gondii* in Cattle

Location	Test	No. Exam	% Pos.	Cutoff Titer	No. with Terminal Titers of:						Ref
					<10	16–20	25–40	50–64	128	≥256	
Argentina											
Chaco	IHA	249	39	64				97			887
Bangladesh	LAT	205	16.1	64				33			1140
Brazil	IFA	400	25.8	64							568
Paraná	IFA	400	25.8	64							568
Bahia	LAT	194	1	64				2			597
	LAT	63	4.8	64				3			596
Rio de Janeiro	IFA	589	14.8	64				79		8	22
São Paulo	IFA	2000	71.0	64							1142a
Costa Rica	ELISA	209	12.4	16		16		7		3	1109
	IFA	601	34.4	NS							42
Czech Republic											
South Bohemian Region	DT	1,926	4.1	4							640
	CFT	1,238	1.6	10							
	IFA	12	33	40			4				1161
Ethiopia	IHA	785	6.6	64				52			80
France											
La Réunion Island	ELISA	780	12.7	NS							1111
Haute-Vienne	MAT	198	27.5	20							1120
Champagne-Ardenne	MAT	1,329	7.8	24							593

India	IHA	50	52	18		20		6			930
Punjab	ELISA	83	2.4	NS			2				1175
Iran	LAT	2000	0	8							633
	ELISA	200	9	100					18		585
Mazadaran	IFA	290	0	128							1174
Tabriz	IFA	490	15.9	20							975
Iraq											
Mosul	CFT	204	48	5	98						1136
Israel	IFA	172	15	16		26					1178
Italy											
Lombardia	MAT	255	91.8	32				26	58	150	58
Sicily	MAT	253	20.6	16		52					1195
Malaysia	IHA	132	0	64							1082
	IFA	126	6.3	200							162a
Selangor	IFA	73	4.1	64							987
Mexico	ELISA	397	11.8	NS							567
Netherlands	ELISA	7132	15.8	NS							1307
Pakistan	LAT	100	25	64				14	7	4	1387
People's Republic of China											
Guangdong	IHA	90	4.4	64				4			833
Yunnan	IHA	208	1.4	64				3			686
	ELISA	262	2.3	NS							1383
Philippines	ELISA	96	5.2	NS							780
Portugal											
Porto	MAT	60	43	10		26					518
Saudi Arabia	IHA	60	1.7	128						1	460
Serbia	MAT	611	76.3	25			155	233	61	17	775

(*continued*)

Table 8.1 Seroprevalence of *T. gondii* in Cattle (Continued)

					No. with Terminal Titers of:						
Location	**Test**	**No. Exam**	**% Pos.**	**Cutoff Titer**	**<10**	**16–20**	**25–40**	**50–64**	**128**	**≥256**	**Ref**
Spain											
Cordoba	IFA	304	40.1	40			88		27	7	947
	MAT	304	41.1	8	89			30		6	
Galicia	MAT	5,196	1.6	40						6	598
Switzerland	ELISA	350	20.8	NS							1377
Thailand											
Northeastern	LAT	445	22.3	64			99				729
Turkey											
Elazig	IHA	272	8.45	32			19	3	1		787
Adana	IHA	370	0	64							1019
	DT	2,037	27.3	16		366		121		69	26
Amasya	DT	100	58	16		32		19		7	753
	DT	96	54.1	16							4
Uruguay	IHA	233	23.6	16		18	21	16			549
	DT	129	3.1	16							
United States	MAT	59	5	100				2		1	320
Vietnam	MAT	200	10.5	50				19		2	693

The ingestion of beef or dairy products is not considered important in the epidemiology of *T. gondii* because cattle are not a good host for this parasite. However, we cannot be sure that beef does not play a role in *T. gondii* transmission as only relatively small amounts of beef have been tested for viable *T. gondii* parasites. Epidemiologically, two small outbreaks of toxoplasmosis more than 30 years ago were linked to ingestion of infected beef. Five medical students developed symptoms of acute toxoplasmosis (see Chapter 2) during the second week after eating rare hamburgers at a university snack bar.[758] The restaurant had bought the meat from a local butcher who insisted that the meat was unadulterated beef; he never ground lamb in the same grinder, and when pork was ground it was at the end of the day and the grinder was washed thoroughly after use. In another instance, three persons developed symptomatic toxoplasmosis linked to eating Kibee Nayee (a meat dish made with raw beef) at a Syrian restaurant.[866]

8.1.3 Clinical Infections

Abortion is a major economic problem in the cattle industry worldwide. The diagnosis of abortion is difficult because of multiple etiologies and the advanced stage of autolysis of the many fetuses submitted for diagnosis. There are no confirmed reports of clinical toxoplasmosis in adult cattle.[290] Before the discovery of *Neospora caninum* as a cause of abortion in cattle,[1278] it is likely that this parasite in cattle was misdiagnosed as *T. gondii*; *T. gondii* and *N. caninum* are morphologically similar parasites.

Gottstein et al.[602] detected *T. gondii* DNA in 5% of the aborted bovine fetuses in Switzerland. They examined 83 bovine fetuses for the presence of *N. caninum* and *T. gondii* by several diagnostic procedures. They detected *N. caninum* DNA in 24 and *T. gondii* DNA in 4 of the fetuses; in 1 fetus DNA for both parasites was detected. In addition, they found antibodies to *T. gondii* in two dead calves suggesting transplacental infection. Neither *N. caninum* nor *T. gondii* was found in 54 of the cases. Neither parasite was cultivated in vitro from tissues of 27 of the 83 fetuses that were suitable for in vitro studies. Subsequently from the same laboratory, *N. caninum* DNA was found in 50 (21%) and *T. gondii* DNA in one (<1%) of 242 aborted fetuses tested.[1132] Ellis[467] from Australia also found *T. gondii* DNA in 2 and *N. caninum* DNA in 16 of 40 fetuses with histologically confirmed encephalitis. Therefore, there is a need for reassessing the prevalence of transplacental *T. gondii* infection in cattle.

Viable *T. gondii* has been isolated twice from aborted bovine fetuses, once in my laboratory from an aborted fetus from a cow in Washington state, and once from a fetal brain in Portugal.[148] Both aborted cows were Holstein. Whether these two cows had aborted due to toxoplasmosis could not be determined because a histological examination of the fetuses was not made. The cow from the United States aborted full-term stillborn twins in October 1992. Antibodies to *T. gondii* were not detected in 1:25 dilution of thoracic fluids of the two bovine fetuses. The dam of the fetuses had a MAT *T. gondii* titer of 1:100; it was culled and sent to slaughter before results of *T. gondii* testing were known. An avirulent *T. gondii* strain was isolated from fetal brain by bioassay in mice. Unfortunately, the strain was not cryopreserved.

A 5-month-old fetus, aborted in January 2002, was from Azores Island, Portugal.[148] *T. gondii* was isolated from the fetal brain by bioassay in mice, and the strain was cryopreserved for future studies.

Stalheim et al.[1233] mentioned in their paper that their colleagues had isolated *T. gondii* from the stomach contents of an aborted fetus in 1975, before the discovery of *N. caninum*. Their isolate of *T. gondii* was obtained by blind passages for 6 months in cell cultures inoculated with the stomach contents from 1 of 255 aborted bovine fetuses. Because this is the only isolation of a *T. gondii*-like parasite (including *Neospora*) ever made from stomach contents, the significance of the findings is unknown. Lack of isolation of *T. gondii* in any of the aborted fetuses from the United States[185] and Iran[633] also supports that *T. gondii* is not an important bovine abortifacient. It is clear from the above discussion that *T. gondii* can be transplacentally transmitted in cattle, but it is probably a rare occurrence.

8.2 EXPERIMENTAL INFECTIONS

Cattle are considered a poor host for *T. gondii*. Although cattle can be successfully infected with *T. gondii* oocysts, the parasite is eliminated or reduced to undetectable levels within a few weeks,[288] perhaps due to innate resistance. In one experiment, four steers weighing 100–150 kg were each fed 10,000 *T. gondii* oocysts. Except for mild anorexia and diarrhea between day 4 and 10 p.i., the inoculated steers remained healthy. The steers were killed 350, 539, 1,191, and 1,201 days p.i.[326] At necropsy, many tissues from each animal were bioassayed (100 g each for bioassay in mice and 500 g each for bioassay in cats) for viable *T. gondii*. Viable *T. gondii* were isolated from steers killed 350 to 1,191 days p.i. by bioassays in cats but not by bioassays in mice. *T. gondii* was recovered from cats fed the hearts of three steers, liver and tongue from two steers, intestine, roast, ribs, and brain from one steer each, and none of the kidneys and the tenderloin. The fourth steer became seronegative 15 months p.i. and viable *T. gondii* was not isolated from any tissue either in cats or mice. Results of these experiments highlight the difficulties in detecting *T. gondii* infection in cattle.

The four inoculated steers developed high titers of agglutinating antibodies within 2 weeks after inoculation. Antibody titers were higher when formalin-fixed tachyzoites were used in the MAT (test A) than those obtained when acetone-fixed (test B) was used. Antibody titers determined by test A remained high, whereas titers determined by test B decreased between 2 and 7 months after inoculation. Agglutination antibody titers determined by test B in steer 4 decreased to ≤1:20 between 15 and 19 months after infection. The dye test titers were inconsistent in all four steers.[326]

Oliveira et al.[237,238,1004] from Brazil compared experimental infections in two species of cattle (*Bos taurus* and *Bos indicus*). These authors fed 200,000 *T. gondii* oocysts to six (three for each species) 4–6-month-old calves. Calves developed mild illness and transient parasitemia in all six animals. The animals were killed 70 days p.i. and most of their tissues were bioassayed in mice after pepsin digestion. Mice inoculated with tissues of all six calves developed some evidence for *T. gondii* infection.[237] *T. gondii* tachyzoites were found in the peritoneal exudate of mice inoculated with liver of one *Bos indicus* and lymph nodes of one *Bos taurus*. Tissue cysts were found in the brains of mice inoculated with brain of another *Bos taurus*. Thus, isolation of *T. gondii* in these three instances was proven by finding tachyzoites or tissue cysts. In the remaining three animals (one *Bos taurus* and two *Bos indicus*) the mice inoculated with cattle tissues became seropositive but *T. gondii* was not found; *T. gondii* was not seen in histological sections of tissues of any of the six calves.

A similar experiment in *Bos tauras* and *Bos indicus* was reported by Scarpelli et al.[1149a] from another laboratory in Brazil with similar results. Additionally, they detected *T. gondii* in semen of these animals inoculated orally with oocysts or subcutaneously with RH strain tachyzoites, suggesting venereal transmission. The significance of these findings in the natural epidemiology of bovine toxoplasmosis is uncertain.

CHAPTER 9

Toxoplasmosis in Water Buffalo (*Bubalus bubalis*)

There are only a few seroprevalence studies on water buffalo (Table 9.1). The seroprevalence was generally very low except that in one report from India, Selvaraj et al.[1167] reported 100% prevalence at a MAT cutoff titer of 1:200. In my opinion, this result appears to be because of faulty technique. To my knowledge, there is no report of isolation of viable *T. gondii* from tissues of water buffalo, although buffalo meat and cheeses made from their milk are consumed by humans.

Buffalo, like cattle, are considered resistant to toxoplasmosis.[302] A group of scientists from Brazil[237,238,1004] fed 200,000 *T. gondii* oocysts to three 4- to 6-month-old calves. All three calves developed mild illness. Transient parasitemia was demonstrable in all three animals (day 7 and 14 in one, day 35 in one, and day 70 in one). The animals were killed 70 days p.i. and most of their tissues were bioassayed in mice after pepsin digestion.[237] *T. gondii* was isolated from two tissues (liver and skeletal muscle) of one of the three calves by bioassay. However, evidence for isolation of the parasite was based on seroconversion in mice; *T. gondii* organisms were not demonstrable in mice. *T. gondii* was not seen in histological sections of tissues of any of the three calves.

Table 9.1 Seroprevalence of *T. gondii* in Buffalo

					No. with Terminal Titers of:						
Location	**Test**	**No. Exam**	**% Pos.**	**Cutoff Titer**	**<10**	**16–20**	**25–40**	**50–64**	**128**	**≥256**	**Ref**
Brazil											
Bahia	LAT	104	3.9	64				4			597
Egypt	MAT	75	0	100							370
India											
Tamil Nadu	MAT	99	100	200					100		1167
Punjab	ELISA	103	2.9	NS			1	1		1	1175
Iran											
Khoozestan	IFA	385	8.8	16		34					972
People's of Republic of China	IHA	40	0	100							1383
	IHA	83	0	64							833
Vietnam	MAT	200	3	50			4			2	693

NS = not stated.

CHAPTER 10

Toxoplasmosis in Horses (*Equus caballus*)

10.1 NATURAL INFECTIONS

10.1.1 Serologic Prevalence

Serum antibodies to *T. gondii* have been found in horses and donkeys in many surveys worldwide (Table 10.1). Tassi[1266] reviewed in detail the current status of toxoplasmosis in horses. Ghazy et al.[586] reported that of 420 sera from horses in Egypt tested by different techniques, 40.5% were positive by IFA (1:64), 48.1% were positive by MAT (1:25), and 37.1% were positive by ELISA.

10.1.2 Clinical Infections

There is no confirmed report of clinical toxoplasmosis in horses.[371] However, in the past 20 years two protozoa, *Sarcocystis neurona* and *Neospora* spp. morphologically similar to *T. gondii,* have been demonstrated to cause clinical disease in horses.[376,407] The reports of fatal cases of toxoplasmosis in horses from the United Kingdom based on the detection of *T. gondii* DNA[1293,1295,1296] need histologic confirmation.

10.2 EXPERIMENTAL INFECTIONS

Two horses inoculated i.v. with 20 and 32 million RH strain tachyzoites developed transient fever (4–8 days p.i.) and specific antibodies between 6 and 12 days p.i., but the parasite did not persist in equid tissues 3 months p.i.[1228] Marques et al.[887a] reported transplacental transmission of *T. gondii* in horses fed oocysts, but these results need confirmation.

Table 10.1 Seroprevalence of *T. gondii* Antibodies in Equids

					No. with Terminal Titers of:						
Country	**Test**	**No. Exam**	**% Pos.**	**Cutoff Titer**	**<16**	**16–20**	**25–40**	**50–64**	**128**	**≥256**	**Ref**
Argentina (Chaco)	MAT	76	13.1	25			2	5	3		372
Brazil											
Minas Gerais	IFA	117	12.8	16		8		3		4	971
Paraná	IFA	173	12.1	64							568
Rio de Janeiro	IFA	430	4.4	16		19					579
Various areas	MAT	101	16	25			8	2	6		371
Czech Republic	DT	2886	7.7	4	181	31	7	2	2		644
Egypt	ELISA	*121	65.6	NS							458
	MAT	420	48.1	25			202				586
South Korea, Jeju Island	IFA	191	2.6	100					5		616
Sweden	ELISA	219	<1.0	NS							1299
	MAT	414	1	40			5				718
Switzerland	ELISA	120	4	NS							1377
Turkey											
Gemlik	DT	103	2	64				2			702
	LAT	*100	11	64							1394
	DT	194	8	16							1394
Kars	DT	111	1.8	16		2					53
Ankara Province	DT	100	28	16		23		5			609
Van	IHA	172	1.7	64							21
Kars	DT	189	20.6	16		27		11		1	19
United States and Canada	MAT	1788	6.9	20		69	37	9	9		373
	DT	339	15.9	10	29	12	4	9			373
Wyoming	MAT	276	0.4	25			1				388

* Donkeys.

CHAPTER 11

Toxoplasmosis in Camels (*Camelus dromedarius*)

Only a few seroprevalence studies on camels exist (Table 11.1). Viable *T. gondii* was isolated from tissues of asymptomatic camels in Egypt by Hilali et al.[654] Four cats fed meat pooled from 38 camels *(C. dromedarius)* shed *T. gondii* oocysts. The identity of *T. gondii* oocysts shed by cats was proven by bioassay in mice. To my knowledge, this is the first isolation of *T. gondii* from camel. This report is important because camel meat is eaten by humans in certain regions of the world.

I am aware of only one report of clinical toxoplasmosis in a camel. A 6-year-old female camel was admitted to the Iowa State Veterinary Teaching Hospital for evaluation of mild dyspnea of recent duration.[623] The camel had become anorectic 1 month earlier and aborted a near-term fetus 4 weeks before admission; the fetus was not examined. Thoracic radiography revealed fluid in the ventral half of the pleural cavity. Approximately 24 L of pale yellow, slightly turbid fluid was drained from both sides of the pleural cavity. The protein content of the fluid was 4.2 g/dl. Smears were made of the fresh pleural fluid, using a cytospin, and were air dried, fixed in methanol, and stained with Wright-Giemsa. I saw numerous *T. gondii*-like tachyzoites in the cytoplasm. Results of indirect hemagglutination antibody test for *T. gondii*, performed at the teaching hospital, were positive at dilutions of 1:256 and 1:1024 for the pleural fluid and serum, respectively. Results of the MAT performed in my laboratory were positive at a dilution of 1:20,000 on pleural fluid, using formalin-fixed tachyzoites, and 1:320, using acetone-fixed tachyzoites. Results of the DT on pleural fluid were positive at a dilution of 1:8,000.The camel was discharged after 2 days of hospitalization; however, it died in spite of treatment with trimethoprim sulfadiazine for 7 days. A necropsy was not performed, but the finding of numerous tachyzoites and high serologic titers indicated acute toxoplasmosis in the camel.

Table 11.1 Seroprevalence of *T. gondii* in Camels

					No. with Terminal Titers of:						
Location	**Test**	**No. Exam**	**% Pos.**	**Cutoff Titer**	**<10**	**16–20**	**25–40**	**50–64**	**128**	**≥256**	**Ref**
Egypt	IHA	19	26.3	16		4				1	463
	MAT	166	17.4				3	18		8	655
Iran (Mashhad)	IFA	120	4.2	20		3	2				1131
Saudi Arabia	IHA	227	16	64				8	6	22	695
	IHA	94	0	128							460
Sudan (Butana Plains)	LAT	482	67	8	127	71	68	25	17	15	464

CHAPTER 12

Toxoplasmosis in Chickens and Other Avian Species

12.1 CHICKENS (*GALLUS DOMESTICUS*)

12.1.1 Natural Infections

12.1.1.1 Prevalence

Worldwide serologic prevalences in free-range or backyard chickens using the MAT performed in my laboratory are given in Table 12.1. Prevalence varied from 2 to 100% depending on the source of the chickens. Additionally, Deyab and Hassanein et al. [255a] found MAT antibodies in 18.7% of 150 chickens from slaughterhouses in Egypt, and Yan et al.[1379b] reported MAT *T. gondii* antobodies in 11.4% of 361 FR chickens and in 4.1% of caged chickens in People's Republic of China.

Serological prevalences using tests other than the MAT were summarized by Dubey[431] and are not repeated here. Recently, Bártová et al.[77] reported *T. gondii* antibodies (IFA, 1:40) in 1 of 293 chickens from the Czech Republic and Chumpolbanchorn et al.[180] found *T. gondii* antibodies (IFA 1:16) in 64% of 303 FR chickens from Thailand.

Little is known concerning the validity of the serologic tests for the detection of *T. gondii* antibodies in avian sera. Viable *T. gondii* was isolated from up to 100% of free-range chickens bioassayed in mice (Table 12.2). Reasons for this variability could be many, including the age of the chicken, number examined, and the tissues bioassayed.

Overall, prevalence of viable *T. gondii* in chickens raised indoors was low (Table 12.3).

12.1.1.2 Clinical Infections

Chickens rarely have clinical toxoplasmosis.[431] Confirmed severe toxoplasmosis was first reported in a flock of 40 White Leghorn hens from Norway by Erichsen and Harboe[476,477]; such an outbreak has not been reported in the literature in the last 50 years. There are two recent reports of clinical toxoplasmosis in chickens from the United States.[404,599] Goodwin et al.[599] reported peripheral neuritis in three chickens from Georgia and the diagnosis was made by IHC; however, herpes virus infection (Marek's disease) could not be ruled out. More recently, out of a group of 14 backyard chickens in Illinois, three birds died suddenly.[404] Torticollis and lateral recumbancy were the only clinical signs. One of these birds was necropsied. Marked lesions were limited to the brain, which had multiple areas of necrosis, perivascular lymphocytic cuffs, and gliosis. An unusual finding was the presence of numerous tissue cysts and tachyzoites in the lesions. The protozoa in the brain reacted positively with *T. gondii* antibodies. The remaining 11 chickens remained asymptomatic, though all contained viable *T. gondii*.[404]

Table 12.1 Serological Prevalence of *T. gondii* in Free-Range Chickens from Different Countries Using MAT[a]

Country	No. of Chickens Tested	No. Positive (%)	No. of Chickens with MAT Titers of:						Reference
			5	10	20	40	100	160	
Argentina									
La Plata	29	19 (65.5)	1	1	6	2	2	7	Dubey et al. (2003e)
Santiago	61	25 (40.9)	6	1	2	5	4	7	Dubey et al. (2005h)
Austria	830	302 (36.3)	ND[d]	50	69	53	40	90	Dubey et al. (2005b)
Brazil									
Amazon	50	33 (66)	3	2	1	1	2	24	Dubey et al. (2006a)
Pará	34	20 (58.8)	ND	1	1	2	2	14	Dubey et al. (2007b)
Paraná	40	16 (40)	3	1	3	1	2	6	Dubey et al. (2003d)
Rio de Janeiro	198	129 (65.1)	ND	21			108		Silva et al. (2003)
Rio Grande do Sul	50	19 (38)	ND	0	1	2	5	11	Dubey et al. (2007b)
São Paulo	82	33 (40.2)	ND	2	1	2	6	22	Dubey et al. (2002)
7 northern states[b]	152	81 (53.3)	26	9	4	1	6	35	de Oliveira et al. (2009)
Chile	85	47 (55.3)	6	4	4	3	9	21	Dubey et al. (2006b)
Colombia	77	32 (44.4)	4	3	1	1	8	15	Dubey et al. (2005c)
Costa Rica	144	60 (40.1)	16	5	2	3	5	29	Dubey et al. (2006c)
DRC [c]	50	25 (50)	7	7	6	1	0	4	Dubey et al. (2005e)
Egypt	121	49 (40.4)	11	4	4	8	10	12	Dubey et al. (2003b)
	108	51 (47.2)	ND	ND	25	4	ND	22	El-Massry et al. (2000)
Ghana	64	41 (64)	5	15	1	4	3	9	Dubey et al. (2008a)
Grenada	102	53 (52)	6	4	4	4	15	20	Dubey et al. (2005a)
Guatemala	50	37 (74)	11	7	11	1	1	6	Dubey et al. (2005g)
Guyana	76	50 (65.8)	4	1	5	7	6	27	Dubey et al. (2007a)
India									
Chennai (Madras)	185	73 (39.5)	ND	ND	15	20	12	26	Devada et al. (1998)
Mumbai	741	133 (17.9)	ND	22	18	2	3	0	Sreekumar et al. (2003)
Indonesia	90	24 (26.6)	11	5	3	1	2	2	Dubey et al. (2008a)
Israel	96	45 (46.8)	3	2	0	5	5	30	Dubey et al. (2004c)
Italy	80	11 (13.7)	4	3	1	0	2	1	Dubey et al. (2008a)
Kenya	30	4 (13.3)	0	0	0	0	1	3	Dubey et al. (2005e)

Mexico	208	13 (6.2)	ND	3	4	1	1	4	Dubey et al. (2004b)
Nicaragua	98	84 (85.7)	10	8	7	9	11	35	Dubey et al. (2006d)
Nigeria	79	5	ND	3	1	1	0	0	Velmurugan et al. (2008)
Peru	50	13 (26)	1	0	3	0	0	9	Dubey et al. (2004a)
Poland	20	6 (30)	2	3	0	0	0	1	Dubey et al. (2008a)
Portugal	225	61(27.1)	8	6	3	23	5	16	Dubey et al. (2006e)
Sri Lanka	100	39 (39)	8	8	4	5	5	9	Dubey et al. (2005i)
Uganda	85	40 (47)	9	10	5	12	1	2	Lindström et al. (2008)
United States									
Ohio	118	20 (16.9)	5	5	1	1	4	4	Dubey et al. (2003a)
Illinois	11	11 (100)	0	0	0	1	0	10	Dubey et al. (2007c)
Venezuela	46	16 (32)	1	2	0	2	2	9	Dubey et al. (2005f)
Vietnam	330	80 (24.2)	34	22	13	1	1	9	Dubey et al. (2008a)

[a] Modified from Dubey.[431]
[b] Pernambuco, Rio Grande do Norte, Maranhão, Bahia, Ceará, Sergipe, and Alagoas.
[c] Democratic Republic of Congo.
[d] ND = no data.

Table 12.2 Isolation of *T. gondii* by Bioassay in Mice from Free-Range Chickens from Different Countries[a]

Country	No. of Chickens	No. of Chickens Bioassay Positive (%)	Tissues Bioassayed	Reference[b]
Argentina				
La Plata	19	9 (47.3)	H+B	Dubey et al. (2003e, 2005h)
Santiago	22	17 (77.2)	H+B+M	Dubey et al. (2005h)
Austria	209	56 (26.7)	H+B	Dubey et al. (2005b)
Brazil				
Amazon	33	24 (72.7)	B+H	Dubey et al. (2006a)
Rio de Janeiro	96	67 (69.7)	B+H	Silva et al.(2003); Dubey et al. (2003c)
Paraná	16	13 (81.2)	H+B	Dubey et al. (2003d)
São Paulo	29	22 (75.8)	H+B	Dubey et al. (2002)
Pará	20	15 (75)	H+B	Dubey et al. (2007b)
Rio Grande do Sul	19	18 (94.7)	H+B	Dubey et al. (2007b)
Rio de Janeiro	20	6	H+B	Peixoto and Lopes (1990)
Minas Gerais	28	11	H	Brandão et al. (2006)
7 northeastern states	81	23 (28.3)	H+B	de Oliveira et al. (2009); Dubey et al. (2008b)
Burkina Faso	40	1 (2.5)	H+B	Dubey et al. (2005e)
Chile	47	9 (19.1)	H+B	Dubey et al. (2006b)
Colombia	31	23 (74.1)	H+B	Dubey et al. (2005c)
Costa Rica	68	32 (47)	H+B	Dubey et al. (2006c)
	50	27 (54)	B+M	Ruiz and Frenkel (1980)
Czech Republic	10	2 (20)	H+B+M	Literák and Hejlíček (1993)
DRC	25	10 (40)	H+B+M	Dubey et al. (2005e)
Egypt	49	19 (38.7)	H+B	Dubey et al. (2003b)
Ghana	32	2 (6.2)	H+B	Dubey et al. (2008a)
Grenada	53	35 (66)	H+B+M	Dubey et al. (2005a)
Guatemala	19	8 (42.1)	H+B+M	Dubey et al. 2005g)
Guyana	50	35 (70)	H+B	Dubey et al. (2007a)
India	186	0	H+B	Sreekumar et al. (2003)
Indonesia	24	1 (4.1)	H+B	Dubey et al. (2008a)
Iran	23	6 (26)	H+B	Zia-Ali et al. (2007)
	109	6 (5.4)	B	Ghorbani et al. (1990)
Israel	45	19 (42.2)	H+B	Dubey et al. (2004c)
Italy	5	2 (40)	H+B	Dubey et al. (2008a)
	176	7 (3.9)	B	Zardi et al. (1967a)
Kenya	4	1 (25)	H+B	Dubey et al. (2005e)
Mali	48	5 (10.4)	H+B	Dubey et al. (2005e)
Mexico	13	6 (46.1)	H+B	Dubey et al. (2004b)
Nicaragua	66	47 (71.2)	H+B	Dubey et al. (2006d)
Nigeria	5	1	H	Velmurugan et al. (2008)
Peru	13	10 (76.9)	H+B+M	Dubey et al. (2004a)
Poland	5	2 (40)	H+B	Dubey et al. (2008a)

(*continued*)

Table 12.2 Isolation of *T. gondii* by Bioassay in Mice from Free-Range Chickens from Different Countries[a] (Continued)

Country	No. of Chickens	No. of Chickens Bioassay Positive (%)	Tissues Bioassayed	Reference[b]
Portugal	61	16 (26.2)	H+B+M	Dubey et al. (2006e)
Sri Lanka	36	11 (30.5)	H+B	Dubey et al. (2005i)
Uganda	85	9 (10.6)	H+B	Lindström et al. (2008)
United States				
Montana	11	3 (27.2)	H+B	Dubey (1981)
Illinois	11	11 (100)	H+B+M	Dubey et al. (2007c)
Tennessee	7	3 (42.8)	B	Gibson and Eyles (1957)
	60	0	B	Eyles et al. (1959)
Texas	11	7 (71.4)	H+B	Foster et al. (1969)
Iowa	12	11 (91.6)	H+B+M	McCulloch (1968)
Ohio	20	11 (55)	H+B	Dubey et al. (2003a)
Massachusetts	11	11	Bioassay in cats	Dubey et al. (2003a)
Venezuela	13	12 (92.3)	H+B+M	Dubey et al. (2005f)

[a] From Dubey.[431] For references see Dubey.[431]

12.1.2 Experimental Infections

In general, chickens are resistant to clinical toxoplasmosis. Overall, attempts to reproduce the clinical syndrome described from Norway have been unsuccessful, including efforts by Erichsen and Harboe.[476] Experimental infections induced by parenteral routes were summarized.[431] Chickens inoculated orally with *T. gondii* oocysts also remained asymptomatic or developed a mild illness.[100,332,752]

Table 12.3 Isolation of *T. gondii* from Indoor Chickens or from Slaughter House[a]

Country	Source	No. Bioassayed	Tissue	No. Positive (%)	Reference
Brazil	C	50	Heart	0	Brandão et al. (2006)
Croatia	SH	716	Brain	3 (0.4)	Kutičić and Wikerhauser (2000)
Czech Republic	C	1097	Brain, Heart, Muscle	4	Literák and Hejlíček (1993)
Egypt	SH	235	Muscle	0	ElMassry et al. (1990)
Germany	Farms	1636	Brain/and or hearts	5 (0.3)	Boch et al. (1968)
Iran	SH	109	Brain	6	Ghorbani et al. (1990)
Italy	Farm	176	Brain	7	Zardi et al. (1967a,b)
Poland	SH	1200	?	0	Grzywiński (1967b)
United States					
Nationwide	Retail meat	2094	Pectoral muscle	0	Dubey et al. (2005d)
Maryland	SH	108	Ovary, brain, leg muscle	4 (ovaries 3, leg muscle 1)	Jacobs and Melton (1966)
Yugoslavia	C	786	Brain	2	Simitch et al. (1961a)

[a] From Dubey.[431]

Of 13 hens each inoculated with 5000 oocysts directly into the crop, none became sick. The 13 hens given 50,000 oocysts also remained asymptomatic with the exception of a drop in egg production and high mortality in embryonated eggs.[100] Dubey et al.[332] examined effects of infection induced by feeding oocysts of two (Me-49, GT1) strains of *T. gondii* in 1-month-old chickens. None of the 17 chickens fed 1,000, 10,000, or 100,000 oocysts of the Me 49 strain developed clinical signs, whereas all five chickens fed 100,000 oocysts of the GT1 strain were anorectic 4–7 days p.i. and one bird died on day 7 p.i.; focal necrotic lesions were seen in the intestines, liver, and spleen of this bird. Tissues of all chickens that were killed days 15–58 p.i. were examined histologically; a single tissue cyst was found in the brain of a chicken on day 15 p.i. Of five chickens whose tissues were bioassayed on day 68 p.i., *T. gondii* was isolated from the brains of all five, hearts of three, and leg muscles of two, but not from the pectoral muscle and liver of any chicken.[332] Similar results were obtained in chickens fed tissue cysts or oocysts in experiments recently performed by Galli et al.[560a]

One-month-old broilers fed 500, 5,000, or 50,000 oocysts of the P strain of *T. gondii* remained asymptomatic, except pyrexia in chickens fed 50,000 oocysts.[752] In another related experiment by the same research group, *T. gondii* infection induced by feeding oocysts to broiler chickens was aggravated when they were co-infected with *Cryptosporidium baileyi*.[911]

In the following section, I have attempted to evaluate the efficiencies of different serological tests to detect antibodies in chicken sera; the information is broadly applicable to other avian species.

Although the DT is one of the most sensitive and specific tests for the diagnosis of toxoplasmosis in humans, there is ample evidence that it does not work with chicken sera or gives erratic results. *T. gondii* has been isolated or seen histologically in tissues of naturally infected chickens that had no demonstrable antibodies in 1:2 dilution of chicken sera.[476,1122] The failure to detect antibodies by the DT is not related to antigenic differences between mammalian and avian strains of *T. gondii* and does not apply to all birds; in contrast pigeons develop high DT antibody titers.[543] The DT is a complement-mediated test, and erratic results with DT in chickens may be related to fixation of the first component of the complement.[543] Chickens experimentally infected with *T. gondii* either did not develop antibodies detectable by the DT or had only low titers.[332]

The CFT, like the DT, does not detect antibodies to *T. gondii* in chicken sera.[476] Harboe and Reenaas[627] developed a complement inhibition test to detect *T. gondii* antibodies in chicken sera.

The IHAT is an insensitive test. In 13 experimentally infected chickens, its sensitivity was 46% and specificity of a negative test was 25%.[543] Ghorbani et al.[587] isolated *T. gondii* from 6 of 109 (5.4%) chickens from Iran; 30% had IHAT titers of 1:20 or higher, and the parasite was isolated from 5 seropositive and 1 seronegative chicken.

There is limited information on the efficacy of IFAT test for the detection of *T. gondii* antibodies in chicken sera. Chickens seroconverted 14 days p.i. after feeding them 100 or more *T. gondii* oocysts.[1162a] Brandão et al.[119] isolated viable *T. gondii* from tissues of 9 of 15 (60%) chickens with IFAT titers of 1:16 or higher and from 2 of 13 (15.3%) seronegative free-range chickens from Brazil.

Limited information is available concerning the LAT. The LAT is simple to perform and kits are available commercially. In seven chickens fed *T. gondii* oocysts, sera were tested by the LAT at 15, 35, 49, and 68 days p.i.[332] Antibodies were detected by LAT in four of seven chickens at day 15, seven of seven chickens at day 35, six of six chickens at day 49, and one of four at day 68. The highest titer detected was 1:128. In conclusion, only low titers were detected and in at least three chickens antibody titers had declined to undetectable levels between day 50 and day 68 p.i.[332] Zia-Ali et al.[1396] isolated *T. gondii* from 6 of 23 seropositive chickens in Iran; one of these animals had a LAT titer of 1:8, considered negative in this test.

The ELISA test has the advantage that it can be automated and is convenient for large-scale surveys. In chickens fed oocysts, Biancifiori et al.[100] studied the kinetics of IgG-ELISA using the soluble fraction of tachyzoites. They reported IgG titers of 1:800 or higher starting at day 12 p.i. and titers peaked at 1:12,800 at day 41 when the experiment was terminated. Dubey et al.[332] reported on the ELISA values in 10 chickens fed the Me-49 strain of *T. gondii*. Seroconversion was observed by day

14 p.i. Chicken sera were diluted in buffered 7% NaCl (instead of 0.85%) aqueous solution (saline) because most chicken immunoglobulins precipate when diluted with phosphate buffered saline.

The sensitivity and specificity of the MAT in naturally exposed chickens is under investigation in my laboratory using isolation of the parasite as a standard. Preliminary data in Table 12.1 suggest that the MAT is efficient in detecting *T. gondii* antibodies in chickens. In chickens fed oocysts, MAT was positive in 1:100 dilution of serum by day 15 p.i.[332]

12.2 TOXOPLASMOSIS IN OTHER AVIAN SPECIES

12.2.1 Natural Infections

12.2.1.1 Prevalence

Many species of birds are a host for *T. gondii*. In the early 1900s, there were many reports of *T. gondii*-like parasites in birds but several of them were likely hemoprotozoan infections; I have summarized them.[280] True hosts of *T. gondii* are those birds from whom viable *T. gondii* was isolated and these worldwide reports are summarized in Table 12.4.

Serologic prevalence of *T. gondii* infections in different species of birds is summarized in Table 12.5, but not all surveys are listed. Caution should be used when interpreting the serologic prevalence data because not all serologic tests used in mammals work with avian sera.

12.2.1.2 Clinical Infections

Reports are summarized in Table 12.6. Among all avian species susceptible to *T. gondii* infection, most severe toxoplasmosis has been reported in canaries (*Serinus canarius*) from Uruguay, Australia, Italy, New Zealand, the United Kingdom, and the United States. An unusual clinical picture reported is ophthalmitis (Figure 12.1) including cataracts and blindness. In ocular cases, the eyes became dull, sightless, closed, and sunken into the head, but birds were otherwise alert and continued to feed.[588,842,1320,1363] Some birds had neurological signs.

Reports of fatal toxoplasmosis in some Hawaiian birds are also of interest. The Hawaiian crow (*Corvus hawaiiensis*) is an endangered species with fewer than 25 birds left in captivity or in the wild.[1374] Toxoplasmosis was diagnosed in five 321–791-day-old birds that were captured or found dead in the wild; all were wearing radio-collars. Two birds were found dead and partially scavanged. The main lesion in birds 1 and 2 was encephalitis associated with tissue cysts. Bird 3 was completely eaten by maggots except its head; the carcass was decomposed and not studied histologically; *T. gondii* was isolated from the brain of bird 3 by bioassay. Bird 4 had generalized acute toxoplasmosis involving the spleen, liver, brain, adrenal gland, and skeletal muscle. Numerous tachyzoites were present in the liver and spleen and *T. gondii* was isolated by bioassay from the brain of this crow. Bird 5 had serologic evidence of *T. gondii* infection with antibody titer of 1:1600 in the MAT. The bird was found to be depressed and losing weight. After administration of an anticoccidial diclazuril (10 mg/kg) for 18 days its physical condition improved and the bird was released in the wild.

Work et al.[1375] also reported acute toxoplasmosis in two endangered Hawaiian goose nene goslings (*Nesochen sandvicensis*) at a zoo in Maui, Hawaii. Both birds died suddenly and had edematous and consolidated lungs. Histologically, the female gosling had severe interstitial mononuclear cell pneumonia, and necrosis in the liver, brain, heart, and muscles. There was also full thickness necrosis of the intestinal wall extending through the lamina propria and the muscular layers. Tachyzoites were found in lesions and the diagnosis was confirmed by IHC staining. The intestinal and hepatic

Table 12.4 Isolation of *Toxoplasma gondii* from Tissues of Naturally Infected Wild Birds[a]

Order	Species		Country	N	% Infected	Reference
Anseriformes	Mallard	*Anas platyrhynchos*	Czech Republic	184	12	Literák et al. (1992)
			Egypt	19	5	Dubey et al. (2003)
	Common Pochard	*Aythya ferina*	Czech Republic	8	12.5	Literák et al. (1992)
	Tufted Duck	*Aythya fuligula*	Czech Republic	25	28	Literák et al. (1992)
	Northern Pintail	*Anas acuta*	Kazakhstan	57	1.8	Pak (1976)
	Gadwall	*Anas strepera*	Kazakhstan	93	1.1	Pak (1976)
	Canada Goose	*Branta canadensis*	United States	1	100	Dubey et al. (2004)
	Domestic Goose	*Anser anser*	United States	1	100	Dubey et al. (2007)
Falconiformes	Northern Goshawk	*Accipiter gentilis*	Czech Republic	10	10	Literák et al. (1992)
	Cooper´s Hawk	*Accipiter cooperii*	United States	4	25	Lindsay et al. (1993)
	Eurasian Buzzard	*Buteo buteo*	Czech Republic	123	8.1	Literák et al. (1992)
			Kazakhstan	12	8.3	Pak (1976)
	Eurasian Kestrel	*Falco tinnunculus*	Czech Republic	1	100	Literák et al. (1992)
	American Kestrel	*Falco sparverius*	United States	3	33.3	Lindsay et al. (1993)
	Pallid Harrier	*Circus macrourus*	Kazakhstan	3	33.3	Pak (1976)
	Cinereous Vulture	*Aegypius monachus*	Kazakhstan	4	25	Pak (1976)
	Red-Tailed Hawk	*Buteo jamaicensis*	United States	27	41.1	Lindsay et al. (1993)
	Red-Shouldered Hawk	*Buteo lineatus*	United States	12	66.7	Lindsay et al. (1993)
Galliformes	Gray Partridge	*Perdix perdix*	Czech Republic	16	18.7	Literák et al. (1992)
	Ring-Necked Pheasant	*Phasianus colchicus*	Czech Republic	590	2.4	Literák et al. (1992)
			Slovakia	1	100	Čatár (1974)
	Wild Turkey	*Meleagris gallopavo*	United States	16	50	Lindsay et al. (1994)
Gruiformes	Eurasian Coot	*Fulica atra*	Czech Republic	43	4.6	Literák et al. (1992)
			Kazakhstan	29	3.4	Pak (1976)
Charadriiformes	Black-Headed Gull	*Larus ridibundus*	Czech Republic	61	16.4	Literák et al. (1992)
			Kazakhstan	84	1.2	Pak (1976)
	Common Tern	*Sterna hirundo*	Kazakhstan	14	7.1	Pak (1976)
			USSR	3	33.3	Pak (1970)

Columbiformes	Eurasian Collared-Dove	*Streptopelia decaocto*	Czech Republic	60	5	Literák et al. (1992)
			Slovakia	12	50	Čatár (1974)
	Common Wood-Pigeon	*Columba palumbus*	Czech Republic	12	8.3	Literák et al. (1992)
	Rock Pigeon	*Columba livia*	Czech Republic	606	1	Literák et al. (1992)
			Denmark	3	100	Siim et al. (1963)
			Portugal	12[b]	75	1328a
			Slovakia	16	12.5	Čatár (1974)
			United States	1	100	Feldman and Sabin (1949)
				50	2	Manwell and Drobeck (1951)
				80	5	Jacobs et al. (1952)
				16	6	Gibson and Eyles (1957)
	Laughing Dove	*Streptopelia senegalensis*	Kazakhstan	20	5	Pak (1976)
	Ruddy Ground-Dove	*Columbina talpacoti*	Panama	79	3	Frenkel et al. (1995)
Psittaciformes	Budgerigar	*Melopsittacus undulatus*	Switzerland	2	100	Galli-Valerio (1939)
Piciformes	Keel-Billed Toucan	*Ramphastos sulfuratus*	Costa Rica	1	100	435a
Strigiformes	Ferruginous Pygmy-Owl	*Glaucidium brasilianum*	Costa Rica	1	Not given	Holst and Chinchilla (1990)
	Little Owl	*Athene noctua*	Kazakhstan	15	6.7	Pak (1976)
	Great Horned Owl	*Bubo virginianus*	United States	5	20	Lindsay et al. (1993)
	Barred Owl	*Strix varia*	United States	15	26.7	Lindsay et al. (1993)
	Tawny Owl	*Strix aluco*	France	1	100	Aubert et al. (2008)
Passeriformes	Northern Shrike	*Lanius excubitor*	Czech Republic	1	100	Literák et al. (1992)
	Yellowhammer	*Emberiza citrinella*	Czech Republic	5	20	Hejlíček et al. (1981)
			Czech Republic	185	0.5	Literák et al. (1992)
	Chaffinch	*Fringilla coelebs*	Czech Republic	133	0.7	Literák et al. (1992)
			Czech Republic	152	0.7	Literák et al. (1992)
	House Sparrow	*Passer domesticus*	Costa Rica	106	16	Ruiz and Frenkel (1980)
			Czech Republic	1907	0.5	Literák et al. (1992)
			Czech Republic	40	17.5	Hejlíček et al. (1981)
			Kazakhstan	177	1.7	Pak (1976)

(*continued*)

Table 12.4 Isolation of *Toxoplasma gondii* from Tissues of Naturally Infected Wild Birds (Continued)

Order	Species		Country	N	% Infected	Reference
			Slovakia	5	40	Čatár (1974)
			USSR	412	0.5	Pak (1972)
	Eurasian Tree Sparrow	*Passer montanus*	Czech Republic	4	25	Hejlíček et al. (1981)
			Czech Republic	316	0.6	Literák et al. (1992)
			Kazakhstan	178	0.6	Pak (1976)
	Eurasian Jay	*Garrulus glandarius*	Czech Republic	43	2.3	Literák et al. (1992)
	European Starling	*Sturnus vulgaris*	Czech Republic	69	1.4	Literák et al. (1992)
			Kazakhstan	430	0.5	Pak (1976)
	Palm Tanager	*Thraupis palmarum*	Panama	3	33.3	Frenkel et al. (1995)
	Eurasian Blackbird	*Turdus merula*	Czech Republic	54	1.9	Literák et al. (1992)
			Slovakia	4	25	Čatár (1974)
	Mistle Thrush	*Turdus viscivorus*	Slovakia	1	100	Čatár (1974)
	Song Thrush	*Turdus philomelos*	Slovakia	7	71.4	Čatár (1974)
	European Robin	*Erithacus rubecula*	Slovakia	8	37.5	Čatár (1974)
	Great Tit	*Parus major*	Czech Republic	215	1.4	Literák et al. (1992)
			Slovakia	5	40	Čatár (1974)
	Eurasian Nuthatch	*Sitta europaea*	Slovakia	6	33	Čatár (1974)
	Eurasian Treecreeper	*Certhia familiaris*	Slovakia	1	100	Čatár (1974)
	European Greenfinch	*Carduelis chloris*	Slovakia	1	100	Čatár (1974)
	American Crow	*Corvus brachyrhynchos*	United States	82	1.2	Finlay and Manwell (1956)
	Carrion Crow	*Corvus corone*	Kazakhstan	58	1.7	Pak (1976)
			Slovakia	4	50	Čatár (1974)
	Eurasian Jackdaw	*Corvus monedula*	Czech Republic	5	20	Literák et al. (1992)
	Rook	*Corvus frugilegus*	Czech Republic	495	18	Literák et al. (1992)

[a] Modified from Dubey.[380,421]

[b] From seropositive pigeons. Mice inoculated with pigeon brains developed *T.gondii* antibodies but it is uncertain if viable *T.gondii* was isolated.

Table 12.5 Serologic Prevalence of Antibodies to *T. gondii* in Wild Birds

Order	Species	Scientific Name	Country	N	% Positive	Test	Cutoff	Reference
Struthioniformes	Ostrich	*Struthio camelus*	Canada	973	2.9	MAT	1:25	Dubey et al. (2000)
Ciconiiformes	Cattle Egret	*Bubulcus ibis*	United States	40	2.5	IHAT	1:64	Burridge et al. (1979)
Accipitriformes	Goshawk	*Accipter gentilis*	Poland	28	100	MAT	6 I.U.	1047
Anseriformes	Duck	*Anas* spp.	Czech Republic	360	14	IFA	40	77
	Duck	*Anas* spp.	Japan	221	21.17	ELISA	100	961
	Wood Duck	*Aix sponsa*	United States	16	6	IHAT	1:64	Burridge et al. (1979)
	Goose	*Anser* spp.	Czech Republic	178	43	IFA	40	77
	Goose	*Anser* spp.	Japan	123	14.63	ELISA	100	961
	Goose	*Anser* spp.	Russia	74	18.92	ELISA	100	961
	Magpie Goose	*Anseranas semipalmata*	United States	11	10.8	MAT	1:25	Dubey et al. (2001)
	Barnacle goose	*Branta leucopsis*	Norway	149	7	MAT	1:40	Prestrud et al. (2007)
Falconiformes	White-backed Vulture	*Gyps africanus*	Nigeria	240	64.8	MAT	1:25	Arene 1999
	Turkey Vulture	*Cathartes aura*	United States	2	50	IHAT	1:64	Franti et al. (1975)
	Eurasian Buzzard	*Buteo buteo*	France	14	79	MAT	1:25	Aubert et al. (2008)
Galliformes	Wild Turkey	*Meleagris gallopavo*	United States	130	10	MAT	1:25	Quist et al. (1995)
			United States	16	71	MAT	1:25	Lindsay et al. (1994)
Gruiformes	American Coot	*Fulica americana*	United States	38	3	IHAT	1:64	Franti et al. (1976)
Charadriiformes	Ring-Billed Gull	*Larus delawarensis*	United States	13	15.3	IHAT	1:64	Burridge et al. (1979)
	Laughing Gull	*Larus atricilla*	United States	33	6	IHAT	1:64	Burridge et al. (1979)
Columbiformes	Rock Pigeon	*Columba livia*	Belgium	220	3.18	MAT	1:64	Cotteleer and Famerée (1978)

(*continued*)

Table 12.5 Serologic Prevalence of Antibodies to *T. gondii* in Wild Birds (Continued)

Order	Species	Scientific Name	Country	N	% Positive	Test	Cutoff	Reference
			Germany	49	2	DT	1:16	Niederehe (1964)
			Israel	495	4	MAT	1:5	1134a
			Italy	108	3	DT	1:50	Mandelli and Persiani (1966)
			Poland	230	74.8	MAT	6 I.U.	1047
			Portugal	695	4.6	MAT	1:20	1328a
			South Africa	16	100	IHAT	1:64	Mushi et al. (2001)
			Taiwan	665	4.7	LAT	1:32	Tsai et al. (2006)
			United States	20	10	DT	1:16	Feldman and Sabin (1949)
			United States	15	6	DT	1:16	Gibson and Eyles (1957)
			United States	80	8.7	DT	1:16	Jacobs et al. (1952)
			United States	34	5.9	MAT	1:40	Kirkpatrick et al. (1990)
			United States	322	8.6	IHAT	1:16	Pendergraph (1972)
	Spotted Dove	*Streptopelia chinensis*	United States	134	8.2	DT	1:16	Wallace (1973)
	Ruddy Ground-Dove	*Columbina talpacoti*	Panama	79	12.6	MAT	1:05	Frenkel et al. (1995)
Strigiformes	Barn Owl	*Tyto alba*	United States	38	27.3	MAT	1:40	Kirkpatrick et al. (1990)
			United States	80	22.5	MAT	1:40	Kirkpatrick et al. (1990)
			United States	28	10.7	MAT	1:40	Kirkpatrick et al. (1990)
			France	18	11	MAT	1:25	Aubert et al. (2008)
	Tawny Owl	*Strix aluco*	France	12	50	MAT	1:25	Aubert et al. (2008)

Passeriformes	Plain Wren	*Thryothorus modestus*	Panama	1	100	MAT	1:05	Frenkel et al. (1995)
	Northern Mockingbird	*Mimus polyglottos*	United States	133	0.75	IHAT	1:64	Burridge et al. (1979)
	American Robin	*Turdus migratorius*	United States	23	8.6	IHAT	1:64	Franti et al. (1975)
			United States	20	5	IHAT	1:64	Franti et al. (1976)
	Clay-colored Robin	*Turdus grayi*	Panama	12	16.6	MAT	1:05	Frenkel et al. (1995)
	Crimson-backed Tanager	*Ramphocelus dimidiatus*	Panama	8	12.5	MAT	1:05	Frenkel et al. (1995)
	Blue-gray Tanager	*Thraupis episcopus*	Panama	15	33	MAT	1:05	Frenkel et al. (1995)
	Palm Tanager	*Thraupis palmarum*	Panama	3	33	MAT	1:05	Frenkel et al. (1995)
	Common Grackle	*Quiscalus quiscula*	United States	27	37	IHAT	1:64	Burridge et al. (1979)
	Great-tailed Grackle	*Quiscalus mexicanus*	Panama	33	33	MAT	1:05	Frenkel et al. (1995)
	Red-winged Blackbird	*Agelaius phoeniceus*	United States	31	6.4	IHAT	1:64	Franti et al. (1975)
	Brewer's Blackbird	*Euphagus cyanocephalus*	United States	4	25	IHAT	1:64	Franti et al. (1975)
	House Sparrow	*Passer domesticus*	Czech Republic	227	12.3	IFAT	1:10	Literák et al. (1997)
	Eurasian Tree Sparrow	*Passer montanus*	Czech Republic	41	4.9	IFAT	1:10	Literák et al. (1997)
	European Starling	*Sturnus vulgaris*	United States	563	4.8	IHAT	1:64	Haslett and Schneider (1978)
	American crow	*Corvus brachyrhynchos*	United States	74	14	IHAT	1:64	Franti et al. (1976)

Modified from Dubey.[380,421]
DT = dye test, IFAT = indirect fluorescent antibody test, IHAT = indirect hemagglutination test, LAT = latex agglutination test, MAT = modified agglutination test.

Table 12.6 Reports of Toxoplasmosis at Necropsy from Birds that Died in Captivity

Ordem	Common Name	Scientific Name	Country	No.	Captive or Wild	Diagnostic Method Reference	Reference
Sphenisciformes	Humboldt Penguin	*Spheniscus humboldti*	United States	4	C	H	Ratcliffe and Worth (1951)
	Magellanic Penguin	*Spheniscus magellanicus*	United States	2	C		Ratcliffe and Worth (1951)
	Jackass Penguin	*Spheniscus demersus*	United States	1	C		Ratcliffe and Worth (1951)
	Little Penguin	*Eudyptula minor*	Australia	1	C	H, IHC	Mason et al. (1991)
Pelecaniformes	Red-footed Booby	*Sula sula*	United States	1	C	H, IHC	Work et al. (2002)
Anseriformes	Magpie Goose	*Anseranus semipalmata*		2	C	H, IHC	Dubey et al. (2001)
	Hawaiian Goose	*Branta sandvicensis*	United States	2	C	H, IHC	Work et al. (2002)
	Mallard	*Anas platyrhynchos*	Argentina	Many	C	H	Boehringer et al. (1962)
Galliformes	Wild Turkey	*Meleagris gallopavo*	United States	1	W	H, TEM	Howerth and Rodenroth (1985)
			United States	1	W	H, IHC	Quist et al. (1995)
	Gray Partridge	*Perdix perdix*	Czech Republic	3	C	H	Pokorný (1955)
	Erckel's Francolin	*Francolinus erckelii*	United States	1	W	H, IHC	Work et al. (2002)
Columbiformes	Western Crowned-Pigeon	*Goura cristata*	Belgium	1	C	H, I	Kageruka and Willaert (1971)
			Belgium	1	C	H	Tackaert-Henry and Kageruka (1977)
			The Netherlands	3	C	H, I	Poelma and Zwart (1972)
	Victoria Crowned-Pigeon	*Goura victoria*	United States	1	C	H	Ratcliffe and Worth (1951)
			Belgium	4	C	H	Tackaert-Henry and Kageruka (1977)
			The Netherlands	2	C	H, I	Poelma and Zwart (1972)

	Pied Imperial-Pigeon	*Ducula bicolor*	The Netherlands	1	C	H, I	Poelma and Zwart (1972)
	Southern Crowned-Pigeon	*Goura scheepmakeri*	The Netherlands	4	C	H, I	Poelma and Zwart (1972)
	Torresian Imperial-Pigeon	*Ducula spilorrhoa*	Australia	3	C	H, IHC	Hartley and Dubey (1991)
	Wonga Pigeon	*Leucosarcia melanoleuca*	Australia	1	C	H, IHC	Hartley and Dubey (1991)
	Luzon Bleeding-Heart	*Gallicolumba luzonica*	The Netherlands	1		H, I	Poelma and Zwart (1972)
Strigiformes	Barred Owl	*Strix varia*	Canada	1	C	H, IHC	Mikaelian et al. (1997)
Psittaciformes	Regent Parrot	*Polytelis anthopeplus*	Australia	1	C	H, IHC	Hartley and Dubey (1991)
	Superb Parrot	*Polytelis swainsonii*	Australia	1	C	H, IHC	Hartley and Dubey (1991)
	Red Lorry	*Eos bornea*	United States	1	W	H, IHC, TEM	Howerth et al. (1991)
			United States	5	C	H, I	Howerth et al. (1991)
	Black-Winged Lorry	*Eos cyanogenia*					Dubey et al. (2004)
	Rainbow Lorikeet	*Trichoglossus haematodus moluccanus*	The Netherlands	1	C	H, I	Poelma and Zwart (1972)
	Crimson Rosella	*Platycercus elegans*	Australia	1	C	H, IHC	Hartley and Dubey (1991)
	Kakarikis	*Cyanoramphus* spp.	Australia	6	C	H, IHC	632
Piciformes	Red-Bellied Woodpecker	*Melanerpes carolinus*	United States	1	C	H, IHC	Gerhold and Yabsley (2007)
Passeriformes	Hawaiian Crow	*Corvus hawaiiensis*	United States	5		A, H, I, IHC	Work et al. (2000)
	Satin Bowerbird	*Ptilonorhynchus violaceus*	Australia	1	C	H, IHC	Hartley and Dubey (1991)
	Regent Bowerbird	*Sericulus chrysocephalus*	Australia	1	C	H, IHC	Hartley and Dubey (1991)

(*continued*)

Table 12.6 Reports of Toxoplasmosis at Necropsy from Birds that Died in Captivity (Continued)

Order	Common Name	Scientific Name	Country	No.	Captive or Wild	Diagnostic Method	Reference
	Red-Whiskered Bulbul	*Pycnonotus jocosus*	Australia	1	C	H, IHC	Hartley and Dubey (1991)
	Island Canary	*Serinus canaria*	Australia	12/24^	C	H, I, IHC	Vickers et al. (1992)
			Australia	2/40^	C	H	Lindsay et al. (1995)
			United Kingdom	2/44^	C	H	Gibbens et al. (1997)
			United States	2/9^	C	H	Williams et al. (2001)

Modified from Dubey.[421] For references see Dubey[421] unless stated otherwise.

Captive (C) or in the wild (W). Methods of diagnosis include antibodies to *Toxoplasma gondii* in serum (A), histology (H), immunohistochemical staining with anti-*T. gondii* antibodies (IHC), isolation in mice (I), and transmission electron microscopy (TEM). In most cases, birds died from pneumonia, hepatitis, splenitis, encephalitis, and/or ophthalmitis. In addition to data compiled in this table, clinical toxoplasmosis has been reported in unspecified species of pigeons as well as Rock Pigeons (*Columba livia*) from Brazil (Carini 1911; Pires and Santos 1934; Reis and Nobrega 1936; Springer 1942), Democratic Republic of Congo (Wiktor 1950), Ecuador (Rodriguez 1954), Italy (de Mello 1915; Alosi and Iannanuzzi 1966), Mexico (Paasch 1983), Panama (Johnson 1943), Scandinavia (Siim et al. 1963), Venezuela (Vogelsang and Gallo 1954), Uruguay (Cassamagnaghi et al. 1952, 1977), and from an unspecified source (Hubbard et al. 1986). Toxoplasmosis has also been reported from canaries and finches from Uruguay (Cassamagnaghi et al. 1952, 1977) and Italy (Parenti et al. 1986) and from budgerigars (*Melopsittacus undulatus*) from Switzerland (Galli-Valerio 1939). The number of birds affected and the methods of diagnosis were not detailed in several of these reports.

^ Fraction of total that were examined by histopathology.

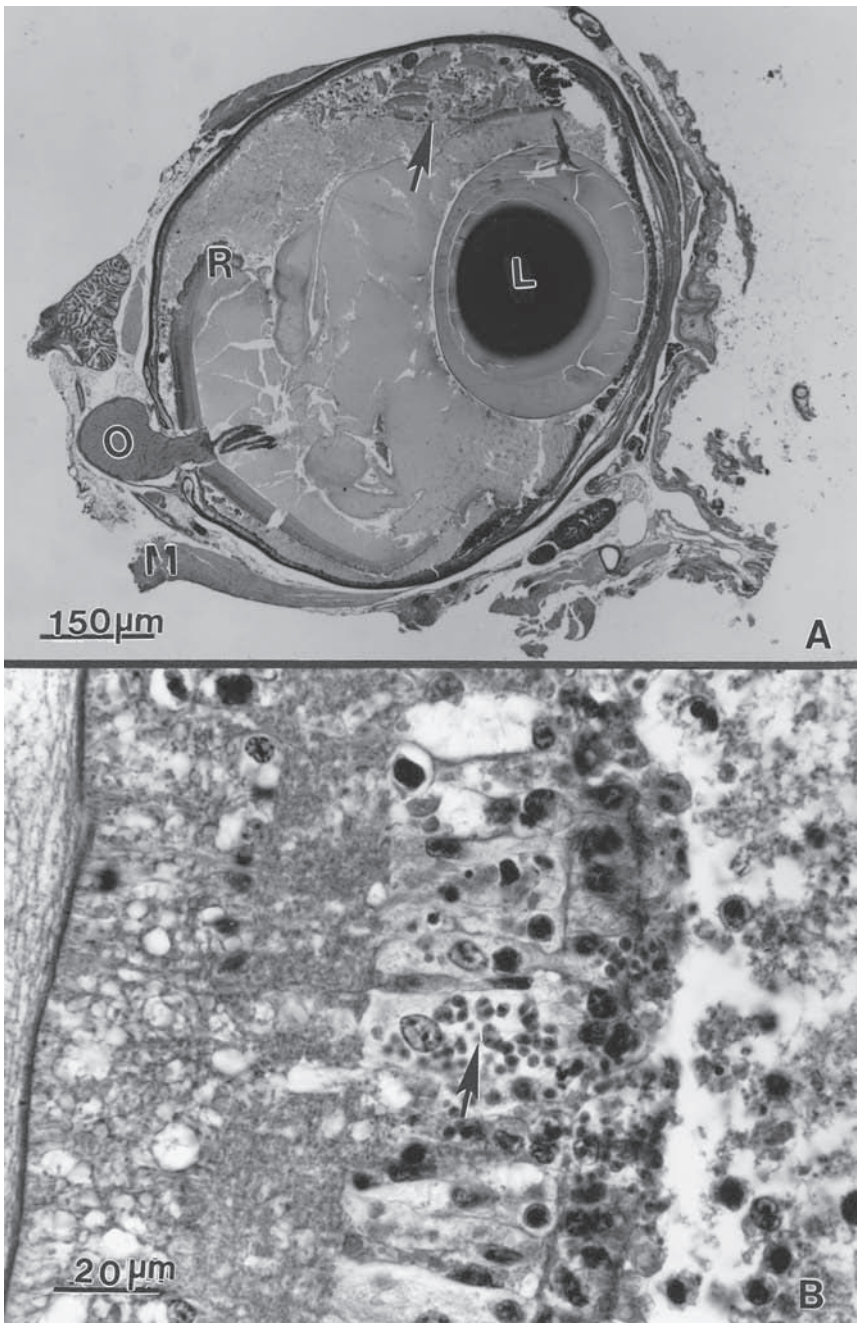

Figure 12.1 Ophthalmitis in a canary naturally infected with *T. gondii*. H and E. **(A)** Section of eye through the optic nerve. Note destruction of retina (R) with portions floating (arrow) in the vitreous. O, optic nerve; L, lens; M, ocular muscles. **(B)** Numerous tachyzoites in retina causing necrosis. Arrow points to a dividing tachyzoite. From Vickers et al.[1320]

Table 12.7 Experimental Toxoplasmosis in Gallinaceous Birds (Except Chickens) Fed *T. gondii* Oocysts

Species	No. of Oocysts Fed	*T. gondii* Strain	No. Died/ No. Fed	Clinical Disease	Day of Death	Reference
Japanese quail (*Coturnix japonica*)	10^5	GT-1	6/6	Acute	6/8	Dubey et al. (1994a)
	10^3	GT-1	5/6	Acute	8/23	
	10^5	Me-49	1/6	Mild disease	9	
	10^3	Me-49	1/6	Mild disease	16	
Bobwhites (*Colinus virginianus)*	10^5	GT-1	2/6	Acute, chronic	6, 7	Dubey et al. (1993b)
	10^4	GT-1	1/6	Acute, chronic	8	
	10^5	Me-49	1/6	Acute, chronic	18	
	10^4	Me-49	0/6	None		
Pheasants *(Phasianus colchicus)*	10^4	GT-1	1/5	Depressed	19	Dubey et al. (1994b)
	10^5	Me-49	0/6	None		
	10^4	Me-49	0/6	None		
Turkeys (*Meleagris gallopavo*)	10^5	Me-49	3*/8		12/14	Dubey et al. (1993a)
	10^4	Me-49	0/6	None		
	10^5	K-7	0/5	None		Sedlák et al. (2000)
Rock partridges (*Alectoris graeca*)	10^4	GT-1	6/6	Acute	4/6	Dubey et al. (1995)
	10^4	Me-49	5/6	Acute	7/11	
	10^3	Me-49	4/6	Acute	10, 11	
	10^2	Me-49	4/6	Acute	10/14	
	10	Me-49	2/6	Acute	12, 13	
Chukars (*Alectoris chukar*)	10^5	K-7	0/5	None		Sedlák et al. (2000)
	10^3	K-7	0/5	None		
Red-legged partridge (*Alectoris rufa*)	10^4	OV-51/95	1/4	Acute in 1, none in others	7 in 1	821
				None		
	10^3		0/4	None		
	10^2		0/4	Acute in 1, none in others		
	$5x10^1$		1/14	None	7 in 1	
	10		0/4			
Partridges (*Perdix perdix*)	10^5	K-7	6/8	Acute	6/10	Sedlák et al. (2000)
	10^3	K-7	2/5	Acute, chronic	16, 16	
Helmeted guineafowl (*Numida meleagris*)	10^5	K-7	0/5	None		Sedlák et al. (2000)
	10^3	K-7	0/5	None		

* Died of concurrent aspergillosis. Modified from Dubey.[380]

lesions were suggestive of recently acquired toxoplasmosis, probably by ingesting food contaminated with oocysts.

There are several old reports of clinical toxoplasmosis in the rock dove (common pigeon, *Columba livia)*, sometimes in epizootic form, but these epizootics have not been reported in the last 50 years.[421,380] Apparently, some species or breeds of pigeons appear to be more susceptible to clinical toxoplasmosis than others. For example, severe toxoplasmosis has occurred in crown pigeons, ornamental pigeons, and kakarikis from Australia and New Zealand (Table 12.6).

12.2.2 Experimental Infections

Among the avian species fed *T. gondii* oocysts, the rock partridge (*Alectoris graeca*) was found to be highly susceptible to toxoplasmosis (Table 12.7). Rock partridges fed 10 *T. gondii* oocysts died, whereas pheasants fed 100,000 oocysts of the same isolate survived (Table 12.7). Birds that died acutely succumbed from enteritis. Grossly, the intestines were hyperemic and had fibrino-necrotic enteritis, sometimes with desquamation of the entire mucosa. Microscopically, the enteric lesions resulted from the multiplication of *T. gondii* tachyzoites in the lamina propria. The parasites multiplied in virtually all cells of the lamina propria including endothelial cells. Subsequently, there was oozing out of the contents of the lamina propria into the intestinal lumen. Desquamation of surface epithelium led to ulceration; sometimes, ulcers extended to the serosal layer. Birds that recovered from enteritis usually became clinically normal. Encephalitis was found in a few birds that survived 15 days p.i.[334,335,347] Swarms of *T. gondii* tachyzoites invaded neuropil. Tissue cysts were seen in birds that survived 3 weeks p.i. Lesions were not seen in the eyes of any of the birds fed oocysts.

Limited experimental data indicate that owls and other predatory birds are resistant to clinical *T. gondii* infection. Seven owls (one great horned owl, *Bubo virginianus*, three barred owls, *Strix varia*, and three screech owls, *Asio otus*) and three red-tailed hawks (*Buteo jamaicensis*) fed *T. gondii* tissue cysts became infected but did not develop clinical signs.[321] *T. gondii* was isolated from all ten birds and all birds developed antibodies to *T. gondii*.

CHAPTER 13

Toxoplasmosis in Non-Human Primates

13.1 NATURAL INFECTIONS

There is only limited information on the prevalence of *T. gondii* in monkeys in the wild. Garcia et al.[571] found MAT (1:16) antibodies in 30.2% of *Cebus* spp. (capuchin monkeys), and 17.6% of 17 *Alouatta* spp. (howler monkeys); these animals were caught wild in remote areas of the Paraná River basin, Paraná state, Brazil. Yabsley et al.[1378] reported MAT (1:50) antibodies in 3 of 52 ring-tailed lemurs (*Lemur catta*) but not in 4 black and white ruffed lemurs (*Varecia variegata*), and 6 blue-eyed black lemurs (*Eulemur macaco flavifrons*); these animals were caught in the wild on St. Catherines Island, Georgia. File and Kessler[510] found *T. gondii* (1:16 titer, ELISA) in 10.2% of 243 *Macaca mulatta* from Cayo Santiago, Puerto Rico; these sera were from animals bled between 1956 and 1988. Ekanayake et al.[457] found *T. gondii* antibodies in 12% of 170 toque macaques (*Macaca sinica*) from Sri Lanka. They did not find any clinical signs or evidence of maternal transmission of *T. gondii* in these animals. *T. gondii* antibodies were found in 17.5% by MAT and 20% by IFA in sera of 40 captive *M. mulatta* in Brazil.[1255]

As concluded previously,[302] New World monkeys are highly susceptible to toxoplasmosis, particularly squirrel monkeys. Some of these animals can die suddenly, without obvious symptoms. Reports of fatal toxoplasmosis in New World monkeys after 1987 are summarized here (Table 13.1). Toxoplasmosis was diagnosed in squirrel monkeys and lemurs in zoos. Salant et al.[1134] detected low levels of *T. gondii* antibodies (MAT titer 1:16 in 19 and 1:64 in 1 of the 24) in monkeys sampled at a zoo in Israel where an outbreak of toxoplasmosis had occurred (Table 13.1). The diagnosis in monkeys was supported by PCR. Based on further characterization, the *T. gondii* DNA was SAG2 genotype. Pertz et al.[1045] described an episode of fatal toxoplasmosis in five golden lion tamarins circumstantially linked to eating a wild caught mouse.

Spencer et al.[1225] described acute toxoplasmosis in a lemur from the Alabama Gulf Coast Zoo. They isolated viable *T. gondii* from the lung of this animal by bioassay in cell culture and named the isolate AU Tg1. To my knowledge, this is the second isolate of *T. gondii* from a monkey; we have cryopreserved this isolate for further studies. The first isolate (BWM) was obtained fortuitously from the skin of a monkey.[84] Dr. Benirschke made a culture of skin fibroblasts of this monkey; *T. gondii* coexisted in the cell culture and was not discovered for several months. We used this slow-growing BWM isolate to study cystogenesis of *T. gondii* in cell culture.[665]

Gyimesi et al.[621] found that two woolly monkeys that had died of acute toxoplasmosis were seropositive by using MAT, IHA, and LAT, and their tissues were positive for *T. gondii* DNA tested by PCR (Table 13.1); samples from 13 other woolly monkeys that were living or that died of unrelated causes were negative by all four tests.

Table 13.1 Summary of Reports of Clinical Toxoplasmosis in Non-Human Primates

			T. gondii Demonstrated[a]					
Species	**Source**	**Remarks**	**Histo**	**Iso/ PCR**	**Imm**	**Tach**	**Cyst**	**Ref**
Squirrel monkey (*Saimiri sciureus*)	2, captive, zoo, Japan	Visceral toxoplasmosis, TEM	Yes	ND	Yes	Yes	Yes	708
	21, captive, zoo, Hungary	Disseminated toxoplasmosis	Yes	ND	Yes	Yes	No	475
	16 of 25 captive, zoo, Mexico	Visceral toxoplasmosis diagnosed in 8 animals necropsied	Yes	ND	Yes	Yes	Unknown	478
	6, captive, zoo, Israel	Disseminated toxoplasmosis	Yes	PCR	ND	Yes	Yes	1134
	6, captive, zoo, UK	6 of 17 monkeys died, disseminated toxoplasmosis	Yes	No	Yes	Yes	ND	201
	47, captive, laboratory, French Guiana	Sudden death	Yes	Yes	ND	Yes	ND	189, 242
Ring-tailed lemur (*Lemur catta*)	1, captive, zoo, United States	Pneumonia, visceral toxoplasmosis	Yes	Yes	Yes	Yes	No	1225
New World primates	33*, captive, zoos, Brazil	Disseminared toxoplasmosis	Yes	ND	Yes	Yes	ND	472, 473, 474
	32**, captive, zoo, Germany	Myocarditis, pneumonia	Yes	PCR	Yes	Yes	ND	118,1367
Golden lion tamarin (*Leontopithecus rosalia rosalia*)	5, captive, zoo, United States	Visceral toxoplasmosis, TEM	Yes	ND	Yes	Yes	ND	1045
Woolly monkey (*Lagothrix lagotricha*)	2, captive, zoo, United States	Disseminated toxoplasmosis	Yes	ND	Yes	Yes	No	621
New World monkeys[a]	7, captive, zoo, Denmark	Confirmed disseminated toxoplasmosis	Yes	ND	ND	Yes	?	261a

* 3 squirrel monkeys (*Saimiri sciureus*), 7 golden-headed lion tamarin (*Leontopithecus chrysomelas*), 3 emperor marmoset (*Saguinus imperator*), 1 yellow handed marmoset (*Saguinus midus*), 1 black marmoset (*Saguinus niger*), 5 woolly monkey (*Lagothrix lagotricha*), 1 black ear-rufted marmoset (*Callithrix penicillata*), 1 night monkey (*Aotus triviragatus*),1 black lion tamarin (*Leontopithecus chrysopygus*), 2 golden lion tamarin (*Leontopithecus rosalia*), 6 howler monkey (*Alouatta fusca*), and 2 white ear-tufted marmoset (*Callithrix jacchus*).

** 17 *Lemur catta*, 15 S*aimiri sciureus*.

ND = No dta.

[a] 1 common marmoset *(Callithrix jacchus),* 2 cotton-top tamarin (*Saguinus oedipus*), 1 red-bellied white-lipped tamarin (*Saguinus labiatus*), 2 golden lion tamarin (*Leontopithecus rosal*), 1 pale-headed saki (*Pithecia pithecia*).

13.2 EXPERIMENTAL INFECTIONS

Old World monkeys are resistant to *T. gondii*, but New World monkeys are highly susceptible to clinical toxoplasmosis.[302] For example, squirrel monkeys are highly susceptible to toxoplasmosis and can die of severe visceral toxoplasmosis before lesions develop in the brain. Oral inoculation with a few *T. gondii* is lethal to them.[928] To investigate if the parasite can be transmitted to cage mates, Furata et al.[557] fed Me49 tissue cysts to three squirrel monkeys. As expected, all three developed acute toxoplasmosis and died or were euthanized 6 or 7 days p.i. Two control squirrel monkeys not inoculated with *T. gondii* remained clinically normal, but seroconverted, and *T. gondii* was demonstrated by PCR in their tissues when the monkeys were killed day 21 p.i. Furuta et al.[557] suggest that the un-inoculated monkeys became infected by ingesting or inhaling *T. gondii* tachyzoites excreted by inoculated monkeys. Carme et al.[189] reported an acute pulmonary toxoplasmosis in squirrel monkeys in contact with sick monkeys. Therefore, precautions should be taken to prevent transmission of *T. gondii* to humans while handling sick monkeys.

Although Old World monkeys are resistant to *T. gondii*, experiments with these animals have been useful to answer specific questions relating to toxoplasmosis in humans. Schoondermark et al.[1156,1157] used experimental infections in Rhesus monkeys (*Macaca mulatta*) to study pathogenesis of congenital toxoplasmosis because these animals have the same type of hemochorial placenta as humans. They inoculated intravenously 5×10^6 RH strain tachyzoites at 90 or 130 days of gestation and followed the kinetics of infection in the dam and fetuses. The dams developed asymptomatic infection, but 11 of 18 transmitted *T. gondii* to their fetuses. Transmission to fetuses occurred during maternal parasitemia. These authors provide details of congenital toxoplasmosis including detection of *T. gondii* in the fetus, antibodies, and clinical disease in the offspring. This is an excellent study, unfortunately performed using a highly lab-adapted strain of *T. gondii* inoculated by an unnatural route.

Holland et al.[668] used the cynomologus monkey (*Macaca fascicularis*) to study pathogenesis of ocular toxoplasmosis. Seven *M. fascicularis* were inoculated with 30,000 tachyzoites of the Beverley strain directly in the macula of one eye in each monkey. All developed retinochoroidal lesions at the site of inoculation. These lesions healed within a month of inoculation. Three months from the time of inoculation of tachyzoites six of these monkeys were given total lymphoid irradiation to reduce cellular immunity. The animals did not develop recurrent retinochoroiditis in spite of successful lymphoid depletion. However, five of six animals developed retinochoroiditis when their eyes were re-inoculated with *T. gondii* 4 months after irradiation. Results of this study indicated that recurrence of eye disease may be a complex immunologic phenomenon.

CHAPTER 14

Toxoplasmosis in Australasian Marsupials

14.1 INTRODUCTION

Australasian marsupials are in general highly susceptible to *T. gondii* infection. There are numerous reports of deaths in zoos, and these animals have also died from toxoplasmosis in the wild.[149,731] Additionally, infected kangaroo meat may serve as a source of infection for humans.[1105]

14.2 KANGAROOS AND WALLABIES

14.2.1 Natural Infections

14.2.1.1 Serologic Prevalence

Serologic surveys of wild animals caught in Australia indicate that certain macropods can develop chronic infection (Table 14.1).

14.2.1.2 Isolation of Viable T. gondii *from Macropods*

Johnson et al.[730] isolated viable *T. gondii* by mouse bioassay from the brains of 6 of 17 *Macropus rufogriseus* and 4 of 17 Tasmanian pademelons (*Thylogale billardierii*) from Tasmania, Australia. These animals were killed as part of a crop protection program and their clinical status was not stated.[730] To my knowledge, this is the only isolation made from macropods in the wild.

There is only scant information concerning isolation of *T. gondii* from wallabies from other countries, and these were from captive animals. Mandelli et al.[884] isolated viable *T. gondii* in mice inoculated from tissues of three Bennett's kangaroos (*Macropus bennetti*) that died in a zoo in Milan, Italy. Dobos-Kovács et al.[263] fed tissues of two derby kangaroos (*Thylogale eugenii*) to a cat and the cat shed *T. gondii* oocysts; the kangaroos had died in a zoo in Budapest, Hungary. Basso et al.[74] isolated *T. gondii* from two captive *M. rufogriseus* in Argentina. Dubey and Crutchley[424] isolated *T. gondii* from four *M. rufogriseus* and one *M. eugenii* in the United States. Three of the four isolates were designated TgWyUs 1-3; the fourth isolate was lost and not cryopreserved. Preliminary genotyping of these three *T. gondii* isolates using PCR-RFLP marker SAG2 revealed that three of the four isolates were genotype III.

Recently, Paramerswaran et al.[1024] detected *T. gondii* DNA in tissues (brain or tongue) of nine seropositive free-ranging kangaroos (*M. fuliginosus*) from Australia.

14.2.1.3 Clinical Infections

14.2.1.3.1 Free-Range

A Registry for Comparative Pathology was established at the Taranga Zoo, Sydney, Australia through the efforts of Dr. Bill Hartley. Canfield et al.[149] reviewed cases of toxoplasmosis in marsupials submitted to this registry which included histologic sections from 43 macropods, as well as 2 common wombats (*Vombatus ursinus*), 2 koalas (*Phascolarctos cinereus*), 6 possums (*Trichosurus* spp.), 15 dasyurids (species not given), 2 numbats (*Myrmecobius fasciatus*), 8 bandicoots (species not given), and 1 bilby (*Macrotis lagotis*). The lesions were detailed in various tissues collectively, but not for each animal individually. The diagnosis was confirmed immunohistochemically in tissue sections from four macropods, two koalas, two dasyurids, one wombat, and one possum.[149]

Subsequently, fatal toxoplasmosis was reported in free-ranging eastern barred bandicoots (*Perameles gunnii*) in Tasmania, Australia.[993] These animals had signs of incoordination, apparent blindness, and unstable gait. Histologically, myocarditis and encephalitis were the predominant lesions. Seven animals serologically examined had antibodies by the MAT.[995]

14.2.1.3.2 Zoo Animals

Initially, wallabies and other kangaroos in zoos around the world were imported from New Zealand and Australia. Many of these died in the zoos because of stress of transportation and relocation; these animals are stressed easily. Some zoos now have breeding programs; however, wallabies in zoos continue to die of toxoplasmosis. Recent reports are summarized in Table 14.1. Earlier literature was summarized by Dubey and Odening.[378]

Table 14.1 Serological Prevalence of *T. gondii* in Free-Range Macropods from Australia

Species	Location	No. Tested	No. Pos.	% Pos.	Test	Cutoff	Remarks	Ref
Tasmanian pademelons (*Thylogale billardierii*)	5 locations, Tasmania	85	15	17.7	ELISA	NS	Test verification	730
Bennett's wallabies (*Macropus rufogriseus*)	5 locations, Tasmania	151	5	3.3	ELISA	NS	Age-related	730
Eastern barred bandicoots (*Perameles gunnii*)	2 locations, Tasmania	150	10	6.7	MAT	64	DAT, MAT comparison	995
Eastern barred bandicoots (*Perameles gunnii*)	Zoos, Victoria	57	5	9	MAT	25	DAT, MAT comparison	918
Western grey kangaroo (*Macropus fuliginosus*)	7 locations, Perth area	219	34	15.5	ELISA	NS	ELISA, verified with MAT	1024
Wombats (*Vombatus ursinus*)	1 location, New South Wales	23	6	26.1	MAT	1:64	Test comp. LAT, DAT	630

NS = not stated.

Table 14.2 Reports of Clinical Toxoplasmosis in Captive Macropods

Location	Species	Remarks	Ref
Argentina, zoo, La Plata	Bennett's wallabies *(M. rufogriseus)*	2 adults died suddenly with generalized toxoplasmosis. A 3 mo-old joey was not infected. Viable *T. gondii* was isolated from brains of both adult females by bioassay in cell culture.	74
Spain, zoo in Lugo	Bennett's wallabies *(M. rufogriseus)*	Three of 5 wallabies died after relocation. 1 animal was necropsied; it died of visceral toxoplasmosis, including ulcerative gastritis. Disease was thought to be reactivation of chronic infection. *T. gondii*-like parasites were found in biopsy of ulcers in buccal mucosa of the second wallaby.	89
UK, animal farm	Western grey kangaroo (*Macropus fuliginosus*)	2 of 12 kangaroos died, 1 necropsied. Pulmonary toxoplasmosis was the main finding.	1298
United States			
Brookfield Zoo, Illinois	Black-faced kangaroos (*M. fuliginosus)*	8 of 25 adults and 2 joeys died of acute toxoplasmosis. Serologic data on 6 animals were compared by different tests. Skeletal myositis was associated with tissue cysts found in 1 joey.	299
Exotic animal farm, Missouri	Bennett's wallabies (*M. rufogriseus)*	6 of 18 adult and 1 joey died within 2 mo. Two adult wallabies were necropsied. Toxoplasmic myocarditis was the main lesion.	921
Zoo, Illinois	Bennett's wallabies (*M. rufogriseus)*	Acute toxoplasmosis was diagnosed ante-mortem, tachyzoites were seen in peripheral leukocytes. The animal died of generalized toxoplasmosis in spite of therapy.	5
Australia	Common wombat (*Vombatus ursinis*)	Histologically confirmed toxoplasmosis in 3 wombats.	157a, 1198a

14.2.1.3.3 Pet Wallabies

Recently, we reported on various aspects of toxoplasmosis in pet wallabies.[424] The unusual aspect of this report is that the animals belonged to one of us, Dr. Carolyn Crutchley. Carolyn is a human physician, devoted to saving marsupials. These animals were part of her household and treated as pets. This allowed her to make close observations of these animals, treat them, obtain blood samples frequently for serology (Table 14.3), and perform post-mortem examinations.[424]

Ten adult wallabies (five Tammar, *Macropus eugenii*, and five Bennett's, *Macropus rufogriseus*) were imported from New Zealand by Crutchley; five of them (two Tammar and three Bennett's) died suddenly of toxoplasmosis, within two months of their arrival. One of the three remaining wallabies (no. 1, Tammar) eventually died in spite of various treatments and we isolated viable *T. gondii* from this animal. Wallabies no. 2 and 3 were adult female *Macropus rufogriseus.* They developed toxoplasmosis, and attempts were made to treat them initially with pyrimethamine, sulfadimethoxine, and later with atovaquone with apparent success. Wallaby no. 4, a male *Macropus rufogriseus*, was purchased from a breeder in Pennsylvania by Carolyn when approximately 7 months old. This wallaby developed toxoplasmosis (Table 14.3), and was treated with atovaquone. The wallaby was killed when 5 years old and *T. gondii* was isolated from its tissues. Wallaby no. 5, male *Macropus rufogriseus*, was born in Pennsylvania. The joey was hand raised after 6 months of age. At 9 months of age, the joey developed toxoplasmosis and was treated with atovaquone. The wallaby was killed when 4 years old and *T. gondii* was isolated from its tissues.[424]

Table 14.3 Serologic Responses of Wallabies (*Macropus rufogriseus*) Naturally Infected with *T. gondii

	MAT Titers of Bennett's Wallaby no.:		
Date Bled (mo/day/yr)	**2**	**3**	**4**
8/2/1996	<25	<25	<25
9/18/1996	≥500	≥3200	<25
9/29/1996	≥3200	≥3200	ND
10/27/1996	24,800	49,600	ND
12/16/1996	24,800	24,800	ND
2/16/1997	25,600	51,200	6400
4/5/1997	12,800	25,600	≥102,400
5/4/1997	12,800	102,400	ND
6/27/1997	6400	102,400	ND
7/27/1997	ND	102,400	ND
12/7/1997	204,800	409,600	409,600
4/30/1998	25,600	≥500	ND
11/8/1998	25,600	≥500	≥500
3/1/1999	≥3200	ND	ND
7/23/2002			≥12,800

≥ = end titration not done.
ND = no data.
* Modified from Dubey and Crutchley.[424]

14.2.1.4 Serologic Diagnosis

Ante-mortem diagnosis of toxoplasmosis in marsupials is problematic because they can die suddenly and before developing detectable antibodies. Among the agglutination tests (MAT, IHA, LAT, DAT) that do not require species-specific reagents, the MAT appears to be most sensitive. Hartley and English[630] found *T. gondii* antibodies (cutoff 1:64) in 6 of 23 free-range asymptomatic wombats by the MAT and DAT but only in 1 by the LAT. Antibodies (cutoff 1: 64) were found in all 5 adult black-faced kangaroos by the use of DT, MAT, IHA, and LAT; these animals had clinical toxoplasmosis.[299] Skerratt et al.[1198a] reported histologically confirmed toxoplasmosis in a wombat that was seronegative by MAT but had high titer by DAT.

Whole formalin-preserved tachyzoites are used as antigen in both DAT and MAT with the difference that mercaptoethanol is added in the MAT. The MAT detects only IgG antibodies because the mercaptoethanol used in the test destroys IgM and IgM-like substances that interfere with the test.[297] Without the addition of mercaptoethanol, this test gives false reactions[253] (see Chapter 1). Some authors have used the difference in titers in this test, with or without mercaptoethanol, as indicative of IgM antibodies.[731] I do not recommend DAT without mercaptoethanol, based on studies in humans and other animals.

Infected mammals generally develop IgM prior to the development of IgG. Therefore, a single high titer in MAT does not mean recent infection because the time of onset of IgG development in wallabies is largely unknown. Under controlled conditions, two *Macropus giganteus* fed 50 or 500 *T. gondii* oocysts and one inoculated i.m. with 250 tissue cysts developed IgG antibodies by 3 weeks p.i. using the MAT.[731] Four Tasmanian pademelons (*Thylogale billardierii*) with histologically confirmed acute toxoplasmosis had IgM but not the IgG antibodies to *T. gondii*.[731] Lynch et al.[878] found antibodies to *T. gondii* in Tammar wallabies by DAT, but not by MAT, 7 and 10 days p.i. However, not all macropods are equally susceptible to toxoplasmosis. The smaller species, traditionally referred to as wallabies, demonstrate a vulnerability to toxoplasmosis that is not apparent in the larger species,

commonly referred to as kangaroos. Many species of animals that I have fed oocysts seroconverted according to the MAT, indicating the quick onset of IgG antibodies. I would like to emphasize that the magnitude of titer obtained by the MAT is not indicative of recency of infection or clinical status (Table 14.3). Additionally, young marsupials may have antibodies passively transferred via milk.[919]

14.2.1.5 Epidemiology

The ingestion of oocysts from the environment is the most likely source of infection for most wallabies. Ingestion of earthworms (transport hosts) was thought to be a source of infection for bandicoots.[94] Although wallabies are herbivores, under captive conditions feeding uncooked meat can also be a source of infection.[993]

Little is known of neonatal toxoplasmosis in marsupials. The gestational period for wallabies is short (30 days for *M. rufogriseus).* At birth, the joey has rudimentary development, primarily of the head and forelimbs and is usually less than 10 mm long, but the size is species dependent. Using the head and forelimbs, the joey crawls from the opening of the cloaca up to the pouch and in. The joey is too undeveloped to be able to suck at the time of entry into the pouch. If the joey is successful in getting a nipple into its mouth, the nipple swells and prevents detachment for approximately 2 months, while milk is pumped into the joey's mouth. Throughout pouch life, development can be followed visually, by gently pulling open the mouth of the pouch and looking in. After about 7 months in the pouch, a joey is fully furred and can start going in and out of the pouch, but is nourished primarily by the mother's milk. After 8 months, a joey can maintain body heat and live outside the pouch but it is not fully weaned until nearly 12 months; this is a general concept, the time of acquisition and weaning are species dependent.

There is no confirmed report of *T. gondii* infection in suckling marsupials or the excretion of *T. gondii* in dam's milk. Dubey et al.[299] reported fatal toxoplasmosis in two young *M. fuliginosus* captive joeys. Joey no. 1 was hairless, approximately 3 months old, and weighed 0.6 kg; it was found dead. Joey no. 2 was approximately 7 months old and weighed 1.1 kg. This animal was found prematurely out of the pouch, and attempts to return the animal to the dam pouch were unsuccessful; it was hand raised on milk formula. Toxoplasmosis has been reported from many species of Australian marsupials but little is known of the biological character of *T. gondii* from these hosts. Why wallabies are highly susceptible to clinical toxoplasmosis is not known. The isolates of *T. gondii* from three wallabies that had clinical toxoplasmosis were genotype III.[424] Type III isolates are relatively avirulent for mice (see Chapter 1). Wallabies inoculated with a non-pathogenic, non-persistent vaccine strain of *T. gondii* (S48) died of acute toxoplasmosis.[878] Nothing is known of the genetic diversity of *T. gondii* isolates circulating in various species of marsupials in Australia.

14.2.1.6 Treatment

Treatment of acute toxoplasmosis with sulfonamides and pyrimethamine has been generally unsuccessful, but there are no well-documented reports of success or failure. Treatment of wallabies with atovaquone appears to be effective therapy for toxoplasmosis and the drug was well tolerated in wallabies.[424] It is worth noting that the dose that was received by the wallabies was approximately 10 times the amount customarily used to treat humans, when compared on the basis of mg/kg, and this drug is expensive.

14.2.2 Experimental Infections

Two eastern barred bandicoots (endangered species) fed 100 oocysts of the P89 strain died of acute toxoplasmosis 15 and 17 days p.i. The predominant lesions were necrosis of lymphoreticular

tissues[93]; these animals had titers of 1:64, and 1:256 by DAT and not by MAT. This study emphasized that these animals can die of primary toxoplasmosis.

Reddacliff et al.[1085,1086] and Lynch et al.[878] attempted to immunize Tammar wallabies using *Hammondia hammondi* or S48 strain of *T. gondii*. *H. hammondi* is related to *T. gondii,* but is nonpathogenic; mice fed *H. hammondi* oocysts develop protective immunity to *T. gondii*.[540] The S48 is a modified *T. gondii* strain used as vaccine for sheep (see Chapter 4). Four Tammar wallabies inoculated with S48 tachyzoites died of acute toxoplasmosis within 12 days p.i.[878] Reddacliff et al.[1085,1086] fed *H. hammondi* oocysts to five Tammar wallabies and challenged them with 500 Me49 (type II strain) *T. gondii* oocysts. These animals remained clinically normal after *H. hammondi* infection but three of five died of acute toxoplasmosis 11–14 days p.i. after challenge with *T. gondii*.[1086] Another group of ten wallabies, fed 1,000 or 10,000 *T. gondii* oocysts, and not given *H. hammondi*, died of acute toxoplasmosis 9–12 days p.i. Lesions in wallabies that died of acute toxoplasmosis included enteritis, necrosis of lymphoid organs, myositis, and encephalitis.[1085,1086]

14.3 OTHER MARSUPIALS

In addition to the report of Canfield et al.,[149] referred to above, fatal toxoplasmosis has been reported in two captive koalas (*Phascolarctos cinereus*) in San Francisco.[317] McOrist and Smales[904] reported fatal toxoplasmosis in 2 of 55 free-living and 2 of 10 captive echidnas (*Tachyglossus aculeatus*) from Victoria, Australia. McColl[900] reported chronic myocarditis associated with *T. gondii* tissue cysts in 5 of 20 captive platypus (*Ornithorhynchus anatinus*) in Victoria, Australia.

CHAPTER 15

Toxoplasmosis in Marine Mammals

15.1 INTRODUCTION

T. gondii infection in marine mammals is intriguing and deserves special discussion for the following reasons: (1) Most of them feed on aquatic cold-blooded animals, and *T. gondii* does not multiply in cold-blooded animals; (2) *T. gondii* was reported to be lethal for some species; and (3) these animals could serve as sentinels of environmental contamination by *T. gondii* oocysts.

15.2 SEROLOGIC PREVALENCE

Antibodies to *T. gondii* have been found in a variety of marine mammals including sea otters, dolphins, seals, and walruses (Table 15.1). Prevalence of *T. gondii* in sea otters was high but varied between 47 and 100% depending on the category (live versus dead), source (California versus Washington), and the serological test used. Prevalence in sea otters from California was higher than in Washington otters.

A very high seroprevalence of *T. gondii* was found in the bottlenose dolphin from California, Florida, North Carolina, and South Carolina in the United States, as well as in Japan and Spain, indicating that these marine mammals in both the Atlantic and Pacific oceans have been exposed. However, *T. gondii* antibodies were not found in sera from 316 harp seals (*Phoca groenlandica*), 48 ringed seals (*Phoca hispida*), 78 hooded seals (*Cystophora cristata*), and 202 minkle whales (*Balaenoptera acutorostrata*) from the North Atlantic.[1002]

How marine mammals become infected with *T. gondii* is enigmatic. Freshwater runoff has been suggested as a risk factor for *T. gondii* infection in California sea otters (see Chapter 1). *T. gondii* infection of other marine mammals that mainly eat fish is even more intriguing.

15.3 ISOLATION OF VIABLE *T. GONDII* FROM TISSUES OF MARINE MAMMALS

Viable *T. gondii* was isolated from sea otters, dolphins, a Pacific harbor seal,[922] and a California sea lion.[186] Most of the isolations were made from the sea otter. Cole et al.[183] isolated *T. gondii* from 15 of 67 selected sea otters. Lindsay et al.[847] isolated this parasite from another sea otter. Fifty isolates of *T. gondii* were obtained from sea otters in California in the laboratory of Dr. Pat Conrad.[186,924,925] All of these isolates were made by bioassay in cell cultures. Recently, Sundar et al.[1247] obtained 15 new isolates by bioassay in mice and cats. Sundar et al.[1247] reported that none of the isolates from sea otters were virulent for mice. Dubey et al.[419] isolated *T. gondii* from 3 of 32 bottlenose dolphins

15.1 Prevalence of *T. gondii* Antibodies in Marine Mammals

Species	Source	No. Tested	% Positive	Test	Titer	Ref
	United States					
Sea otter *(Enhydra lutris)*	California—Live	80	36	IFAT	1:320	924
	Dead	77	61	IFAT	1:320	924
	Dead	100	82	MAT	1:25	386
	Dead	25	52	MAT	1:25	1247
	Washington—Live	21	38	IFAT	1:320	924
	Dead	10	100	MAT	1:25	1247
Walrus (*Odobenus rosmarus)*	Alaska	53	5.6	MAT	1:25	386
Monk seal (*Monachus schauinslandi*)	Hawaii	117	1.7	MAT	1:25	11
Sea lion (*Zalophus californianus*)	Alaska	27	29.6	MAT	1:25	386
	California	18	61.1	MAT	1:25	386
Harbor seal (*Phoca vitulina*)	Washington	380	7.6	MAT	1:25	795
	Alaska	311	16.4	MAT	1:25	386
	Canada	34	9	MAT	1:25	906
Grey seal (*Halichoerus grypus*)	Canada	122	9	MAT	1:25	906
Hooded seal (*Cystophora cristata*)	Canada	60	1.6	MAT	1:25	906
Ringed seal (*Phoca hispida*)	Alaska	32	15.6	MAT	1:25	386
Bearded seal (*Erignathus barbatus*)	Alaska	8	50	MAT	1:25	386
Spotted seal (*Phoca largha*)	Alaska	9	11.1	MAT	1:25	386
Bottlenose dolphin (*Tursiops truncatus*)	California	94	96.8	MAT	1:25	386
	Florida	47	100	MAT	1:25	386
	Florida, South Carolina	146	100	MAT	1:25	395
	South Carolina	49	53	MAT	1:25	419
	Canada	8	100	MAT	1:25	435
	Japan (Solomon Island)	58[a]	13.7	LAT	1:64	1012
	Japan	59	10.1	IHA	1:64	962
	Spain	7	57.1	MAT	1:25	146
Common dolphin (*Delphinus delphis*)	UK	21	28.6	DT	NS	517
	Spain	4	50	MAT	1:25	146
Striped dolphin (*Stenella coeruleoalba*)	Spain	36	11.1	MAT	1:25	146
Humpback whale (*Megaptera novaengliae*)	UK	1	100	DT	NS	517
Harbor porpoise (*Phocoena phocoena*)	UK	70	1.4	DT	NS	517
	Spain	1	100	MAT	1:25	146
Steller eared sea lion (*Eumetopias jubatus)*	Russia	189	13.8	ELISA	NS	23

[a]Pacific bottlenose dolphin (*Tursiops aduncus*)

that had beached on the east coast of the United States. These isolates were designated TgDoUs1–3. More recently, a *T. gondii* isolate was obtained from a free-range striped dolphin (*Stenella coeruleoalba*) from Costa Rica[415] and a captive bottlenose dolphin from Canada.[435]

Recent studies on the mortality associated with toxoplasmosis in sea otters contributed new information on the host-parasite relationship linking clinical outcome with *T. gondii* genotype and also resulted in the description of a new genotype X.[186,925] Of the 50 isolates of *T. gondii* from sea otters genotyped using four polymorhic genes (B1, SAG1, SAG3, and GRA6), 70% of sea otter isolates were identified as type X, 28% type II, and no type I or III.[186] Sundar et al.[1247] applied 10 genetic markers against the 20 isolates previously isolated by Cole et al.,[183] and 17 new isolates. PCR-RFLP analysis at nine of the genetic markers and DNA sequencing at the other marker (GRA6) defined six genotypes, four related to type II, type X, and a new genotype they called type A. Although a suggestion was made by Conrad et al.[186] that type X might be associated with mortality in sea otters, no such association was found in the study reported by Sundar et al.[1247] The isolates from the Pacific harbor seal and the California sea lion were also type X.[186] The isolates from dolphins were type II.[415,419,435] Thus, no type I or type III isolate has been conclusively found in marine mammals (though Honnold et al.[673] reported a possible type III strain in a Hawaiian monk seal, see below). Sources of infection for marine mammals are discussed in Chapter 1.

15.4 CLINICAL TOXOPLASMOSIS

Case reports in animals, except sea otters, are summarized in 15.2. In some of these reports, *T. gondii* infection might have been coincidental. Among these cases, toxoplasmosis in the Atlantic bottlenose dolphin,[709] Indo-Pacific bottlenose,[721] Risso's dolphin,[1098] Indo-Pacific humpbacked dolphin,[115] and the Hawaiian monk seal[673] were well documented. I have seen tissue sections from all these cases except the report by Bowater et al.[115] Both the Atlantic bottlenose dolphin and the Indo-Pacific bottlenose adults had been caught wild; the former was accompanied by a calf, and the latter was pregnant; toxoplasmosis was found in all four. The fetal Risso's dolphin from Spain had overwhelming toxoplasmosis. The Hawaiian monk seal is an endangered pinniped, and this animal had extensive visceral toxoplasmosis. All four Indo-Pacific humpbacked dolphins that stranded off the Queensland, Australia, coast died of toxoplasmosis; fresh water runoff after heavy rains was suspected to be associated with source of infection.[115]

Southern sea otters of California are listed as a threatened species under the Endangered Species Act in the United States. There are at least three geographically separated, established populations off the coasts of California, Washington, and Alaska with minimal, if any, intermixing among groups.[925] Post-mortems and histopathologic evaluation are made on most sea otters that die in the United States, thus, data are available on causes of death.[786,1280] Another protozoa, *Sarcocystis neurona* morphologically related to *T. gondii*, also causes encephalitis in sea otters, and some sea otters have concurrent infections with both parasites. Two studies summarized causes of death in sea otters examined between 1985 and 2004. Thomas et al.[1280] reported on 344 sea otters from California and Washington state necropsied from 1985 to 2004; the same animals were used for isolation studies.[183,847,1247] Kreuder et al.[786] studied animals necropsied from 1998 to 2001; the same animals were used for isolation studies.[183,922,925,926] The prevalence of protozoal meningoencephalitis and the proportions attributed to *S. neurona* and *T. gondii* in the study reported by Thomas et al.[1280] differed from the study by Kreuder et al.[786] Thomas et al.[1280] found protozoal encephalitis in 39 (11.3%) of the 344 sea otters, 22 with *S. neurona*, 5 with *T. gondii*, and 12 with both organisms. Overall, more severe encephalitis had a greater association with active *S. neurona* than with toxoplasmosis.[1280] In contrast, *S. neurona* meningoencephalitis was diagnosed as a cause of death in 6.7% of otters examined and predominantly in otters dying in 2001.[786] *T. gondii* was diagnosed as a primary factor in the deaths of 16.2% and a contributor in the deaths of another 11.4% of otters

15.2 Summary of Reports of Clinical Toxoplasmosis in Marine Mammals*

Species	Source	Remarks	*T. gondii* Demonstrated[a]					References
			Histo	TEM	Imm	Tach	Cyst	
Seals								
Elephant seal (*Mirounga angustirostris*)	Wild, California	Encephalitis	Yes	ND	Yes	No	Yes	392
Northern fur seal (*Callorhinus ursinus*)	Wild, California	Encephalitis	Yes	ND	Yes	Yes	Yes	Holshuh et al. (1985)
Pacific harbor seal (*Phoca vitulina richardsi*)	Wild, Cold Bay, Alaska	1 day old, 11.5 kg, hepatitis	Yes	ND	ND	Yes	Yes	Van Pelt and Dieterich (1973)
Monk seal (*Monachus schauinslandi*)	Wild, Hawaii	1 adult male, good nutritional condition, lymphadenitis	Yes	ND	Yes	Yes	Yes	673
Sea lion (*Zalophus californianus*)	Captive, zoo, Pennsylvania	10 day old, disseminated	Yes	ND	ND	Yes	No	Ratcliffe and Worth (1951);
	Captive, California	Adult, myocarditis	Yes	ND	ND	Yes	Yes	Migaki et al. (1977);
	Captive, Florida	9-yr-old male, disseminated, myocarditis	Yes	ND	Yes	Yes	Yes	386
Dolphins								
Atlantic bottlenose (*Tursiops truncatus*)	Wild, Florida	One adult and her calf, disseminated	Yes	Yes	Yes	Yes	Yes	Inskeep et al. (1990)
	Wild, Florida	Young male, hepatitis, adrenalitis	Yes	Yes	Yes	Yes	Yes	Cruickshank et al. (1990)
	Tuscany, Italy	2 wild, adult	Yes	ND	ND	Yes	No	DiGuardo et al. (1995a,b);
	Wild, United States	1 of 97 stranded adults	Yes	ND	ND	Yes[b]	Yes[b]	Schulman et al. (1997), 386
	Captive, Canada	2 with encephalitis	Yes	No	Yes	No	Yes	435

Striped (*Stenella coeruleoalba*)	Wild, Spain	Lymphadenitis, encephalitis, 4 of 110 stranded animals	Yes	ND	ND	Yes	No	Domingo et al. (1992)
	Wild, Tuscany, Italy	4 wild adults, encephalitis with co-infection with morbillivirus	Yes	ND	ND	Yes	No	Di Guardo et al. (1995a,b)
Indo-Pacific bottlenose (*Tursiops truncatus aduncus*)	Wild, Australia	Late-term fetus, myocarditis, encephalitis	Yes	ND	Yes	Yes	Yes	Jardine and Dubey (2002)
Spinner (*Stenella longirostris*)	Wild, Hawaii	Adrenalitis	Yes	Yes	ND	Yes	Yes	Migaki et al. (1990)
Risso's (*Grampus griseus*)	Wild, Spain	Adult and her fetus, disseminated	Yes	ND	Yes	Yes	Yes	Resendes et al. (2002b)
	Wild, Italy	1 adult	Yes	ND	ND	Yes	No	Di Guardo et al. (1995a)
Tucuxi (*Sotalia fluviatilis guinensis*)	Wild, Rio de Janeiro, Brazil	1 adult, lymphadenitis	Yes	ND	ND	Yes	Yes	Bandoli and de Oliveira (1977)
Indo-Pacific humpbacked *(Sousa chinensis)*	Wild, Queensland, Australia	4 of 4 stranded adults	Yes	Yes	Yes	Yes	Yes	115
		Other Marine Mammals						
Walrus (*Odobenus rosmarus*)	Canada	Seizure[c]	No	No	No	No	No	435
West Indian manatee (*Trichechus manatus*)	Wild, Florida	Encephalitis	Yes	Yes	ND	No	Yes	Buergelt and Bonde (1983)
	Georgetown, Guyana	Myocarditis	Yes	Yes	Yes	Yes	Yes	386
Beluga whale (*Delphinapterus leucas*)	Wild, Quebec, Canada	6 mo old, encephalitis	Yes	ND	Yes	Yes	Yes	Mikaelian et al. (2000)
	Wild, Spain	31 yr old, disseminated	Yes	ND	Yes	Yes	Yes	De Guise et al. (1995); Mikaelian et al. (2000)

* Modified from Dubey et al.[386] For earlier references, see Dubey et al.[386]

[a] Histo = histology, TEM = Transmission electron microscopy, Imm = immunohistochemistry, Tach = tachyzoites, Cyst = tissue cysts, ND = no data.

[b] The stage of the parasite was not specified.

[c] *T. gondii* found by PCR.

examined. They also found that sea otters that died from shark attack were more likely to have *T. gondii* meningoencephalitis and proposed that this infection produced clinical effects that made otters more vulnerable to sharks. Encephalitis and myocarditis were the main lesions associated with toxoplasmosis.[786]

Recently, Miller et al.[920] reported an interesting case of neonatal toxoplasmosis in a sea otter with a finding of generalized lymphadenopathy and malformation of the left cerebral temporal lobe. Both of these lesions, well recognized in human toxoplasmosis (see Chapter 2), have not been confirmed for toxoplasmosis in other hosts. This animal also had lesions in other organs. *T. gondii* tachyzoites were demonstrated histologically and the parasite was isolated by bioassay; it was genetically type X.[920]

15.5 EXPERIMENTAL INFECTIONS

Grey seals (*Halichoerus grypus*) fed oocysts became infected but did not become sick.[560]

CHAPTER 16

Toxoplasmosis in Wild Canids

16.1 CANIDS (RACCOONS, FOXES, SKUNKS, MINK, AND OTHERS)

16.1.1 Introduction

Toxoplasmosis in wild carnivores is clinically and epidemiologically important because clinical toxoplasmosis in some of these hosts simulates rabies. Wild canids are also susceptible to the canine distemper virus (CDV) infection, which is immunosuppressive in these hosts, as it is in the domestic dog (see Chapter 7). *T. gondii* infection in some of these hosts (e.g., raccoons) is ecologically important because raccoons can act as sentinel hosts for *T. gondii* infection. Raccoons eat almost anything including garbage, soil, and plants, and thus they are good indicators of *T. gondii* infection in the environment. Therefore, I have attempted to summarize seroprevalence, isolation of *T. gondii* from their tissues, and available clinical reports.

16.1.2 Natural Infections

16.1.2.1 Serological Prevalence

A high prevalence of *T. gondii* antibodies was found in canids in North America (Table 16.1).

16.1.2.2 Isolation of Viable T. gondii from Canine Tissues and Genetic Characterization of Isolates

Most isolates were obtained from raccoons (Table 16.2). In addition to the data in Table 16.2, Prestrud et al.[1072] directly amplified *T. gondii* DNA from 55 of 167 seropositive arctic foxes from Norway; 46 samples were type II, 7 were type III, and 2 had atypical *T. gondii* genotypes. Smith et al.[1205a] detected *T. gondii* DNA in 5.6% of 61 fox tongues from U.K.

16.1.2.3 Clinical Infections

Isolated reports are summarized in Table 16.3. Previous reports were summarized by Dubey and Odening.[378] A major outbreak of toxoplasmosis in mink was reported by Frank[526] on a mink farm in Wisconsin. Approximately 26% of 7,800 females aborted or their kits died neonatally. The CDV infection was not found but had been diagnosed the previous year. Fulminating toxoplasmosis was diagnosed histologically with lesions in the brain, heart, liver, and lungs.[526] Tachyzoites were found in lesions and the diagnosis was confirmed by IHC staining.

Table 16.1 Seroprevalence of *T.gondii* in Canids and Other Carnivores

Species	Country	Test	No. Exam.	% Pos	Cutoff Titer	Ref
Badger (*Meles meles*)	Spain	MAT	29	75.9	25	1213
Coyote (*Canis latrans*)	United States					
		MAT	222	59	25	374
	Georgia	LAT	17	18	16	671
		MAT	1	100	25	393
	Kansas	DT	13	62	8	1205
	Texas	MAT	52	62	25	844
	Wisconsin	MAT	35	18	20	416
Dingoes (*Canis familiaris dingo*)	Australia	MAT	62	10	32	731a
Foxes						
Arctic (*Vulpes lagopus*)	Norway	MAT	594	43	64	1070
Gray (*Urocyon cinereoargenteus*)	United States	DT	4	25	8	1205
		MAT	97	75.3	25	374
	Georgia	MAT	2	100	25	393
Island (*Urocyon littoralis*)	United States—California					
	Channel Islands	IHA	194	29.9	64	566
Kit (*Vulpes macrotis*)	United States					
	California	IHA	35	6	64	901
		NS	47	14.9	NS	1234
Red (*Vulpes vulpes*)						
	Belgium	IFA	123	100	64	140
	Spain	MAT	89	60.8	25	1213
	UK	IHA	549	20	128	624
	United States	MAT	283	85.9	25	374
	Georgia	MAT	1	100	25	393
	Kansas	MAT	2	50	25	123
		DT	10	90	8	1205
Sand (*Vulpes ferrilata*)	United Arab Emirates					
	Sharjah	MAT	8	100	25	1029
Skunk (*Mephitis mephitis*)						
	Canada	MAT	64	15.6	25	699
	United States					
	Connecticut	MAT	24	42	50	932
	Illinois	MAT	18	38.9	25	344
	Iowa	MAT	81	47	32	661
	Kansas	MAT	1	0	25	123
		DT	4	50	8	1205
	Wisconsin	MAT	7	5	20	416
Raccoon (*Procyon lotor*)	Canada	MAT	91	27.5	25	699
	United States					
	Connecticut	MAT	12	100	50	932
	Georgia	MAT	75	52	25	393
	Kansas	MAT	20	70	25	123
		DT	52	13	8	1205
	Illinois	MAT	188	67	25	344

(*continued*)

Table 16.1 Seroprevalence of *T.gondii* in Canids and Other Carnivores

Species	Country	Test	No. Exam.	% Pos	Cutoff Titer	Ref
		MAT	379	48.5	25	931
	Iowa	MAT	14	29	25	1208
		MAT	885	15	32	661
	Virginia	MAT	256	84	50	626
	Wisconsin	MAT	54	59.2	20	416
	Various areas	MAT	427	50.3	25	325
		MAT	99	46.5	50	846
Wolves	Spain	MAT	27	48.1	25	1213
Canadian (*Canis lupus*)						
	United States—Alaska	MAT	125	9	25	1391
Maned (*Chrysocyon brachyurus*)	Brazil	IFA	59	74.6	25	1322
Genet (*Genetta genetta*)	Spain	MAT	18	66.6	25	1213
Marten						
Pine (*Martes martes*)	Spain	MAT	3	100	25	1213
	Czech Republic	DT	6	17	4	647
Stone (*Martes foina*)	Spain	MAT	14	92.8	25	1213
Mink (*Mustela vison*)	Bulgaria	ELISA	156	12.2	NS	46
	Poland	LAT	961	13.9	32	1204
	Denmark	LAT	195	3	64	650a
	United States	DT	24	54.2	16	1205
Mongoose Egyptian (*Herpestes ichneumon*)	Spain	MAT	22	59.1	25	1213
Otter (*Lutra lutra*)	Spain	MAT	5	100	25	1213
Wolverine (*Gulo gulo*)	Canada	MAT	41	41.5	25	1087

NS = not stated.

Table 16.2 Isolation of *T. gondii* from Tissues of Wild Canids

Species	Source	No. Tested	Tissues[b]	No. Pos.	Isolate Designation	Genetic Data, Type	Ref
Raccoon	Georgia	33[a]	H	7	No	Yes, I,II,III	393
	Wisconsin	30	B,H,T	5	TgRaW1-5	Yes	416
	Saskatoon, Canada	3			TgRaCa1-2	Atypical	429
Coyote	Georgia	1	H	1	No	Yes, I	393
Gray fox	Georgia	2	H	1	No	Yes, I	393
Red fox	Georgia	1	H	1	No	Yes, I	393
	Kansas	4[a]	B	1	No	No	1205
Arctic fox	Svalbard, Norway	1	B,H	1	No	Yes, II	1071
Skunk	Wisconsin	1	H,M	1	TgSkW1	Yes	416
	Saskatoon, Canada				TgRaCa1-2	Atypical	416
Mink	Kansas	8[a]	B	2	No	No	1205

[a] Seropositive
[b] B = brain, H = heart, T = tongue

Table 16.3 Summary of Reports of Clinical Toxoplasmosis in Selected Species of Carnivores*

Species	Source	Remarks	*T. gondii* Demonstrated[a]					
			Histo	TEM	Imm	Tach	Cyst	Ref
Raccoon (*Procyon lotor*)	1, Wild, Pennsylvania	Encephalitis, hepatitis, MAT 1:160	Yes	ND	Yes	Yes	Yes	325
Gray fox (*Urocyon cinereoargenteus*)	1,Wild, Mississippi	Encephalitis, cerebral hematoma	Yes	ND	Yes	Yes	Yes	338
	6 of 157, Wild, many states, United States	Pneumonia, concurrent CDV	Yes	ND	ND	Yes	No	226
	1 of 35, Wild, Virginia	Pneumonia, concurrent CDV	Yes	ND	ND	Yes	No	759
Sand fox (*Vulpes rueppelli*)	Captive, Sharjah, UAE	Enteritis, hepatitis, myocarditis	Yes	ND	Yes	Yes	No	1029
Arctic fox (*Alopex lagopus*)	Wild, Svalbard, Norway	Hepatitis, pneumonia, encephalitis	Yes	ND	Yes	Yes	No	1218
Red fox *(Vulpes vulpes)*	1, Wild, Pennsylvania	Neurologic, disseminated toxoplasmosis, no CDV	Yes	ND	Yes	Yes	No	313
	3 of 48, Wild, Virginia	Pneumonia, concurrent CDV	Yes	ND	ND	Yes	No	759
Fennec fox *(Fennecus zerda)*	1, Captive, Missouri	Disseminated toxoplasmosis	Yes	ND	Yes	Yes	No	782
Mink *(Mustela vison)*	Wisconsin	Abortion and neonatal mortality	Yes	ND	Yes	Yes	No	526
Blanford's fox (*Vulpes vulpes*)	1, Captive, Sharjah, UAE	10 wk-old, visceral toxoplasmosis	Yes	ND	Yes	Yes	No	425
Ferret (*Mustela putorius)*	New Zealand	Neonatal mortality	Yes	ND	Yes	Yes	Yes	1283; 1282
Black-footed ferrets (*Mustela nigripes*)	25, Captive, Kentucky United States	Disseminated toxoplasmosis	Yes	Yes	Yes	Yes	Yes	131
Slender-tailed meerkat (*Suricata suricatta*)	7, Captive, Spain	Disseminated toxoplasmosis, pulmonary lesions predominant	Yes	ND	Yes	Yes	Yes	742
	3, Captive, Argentina	Disseminated toxoplasmosis, pulmonary lesions predominant	Yes	ND	Yes	Yes[d]	Yes	74a

[a] Histo = histology, TEM = Transmission electron microscopy, Imm = immunohistochemistry, Tach = tachyzoites, Cyst = tissue cysts, ND = no data.
[b] The stage of the parasite was not specified.
[c] *T. gondii* found by PCR.
[d] *T. gondii* isolated (TG-Suricata-1) and characterized genetically.

Table 16.4 Experimental Toxoplasmosis in Wild Carnivores

Species	*T. gondii* Strain, Stage, Dose	Days	Serology	Isolation	Remarks	Ref.
Raccoon	Me 49,2 fed tissue cysts, 2 oocysts	59–61	Weekly serology, MAT, IHA, DT,LAT compared	Recovered from muscle but not brain	Subclinical	327
Red fox	TC-1, tissue cysts fed to 2, GT1 oocysts fed to 2	36–55	Weekly serology, MAT, IHA, DT,LAT compared	Recovered from tissues	Subclinical	374
Blue fox (*Alopex lagopus*)	RH 10^4–10^7, tachyzoites, s.c.	6–8	Histopathology, TEM	Described lesions	Severe toxoplasmosis	104
Mink *(Mustela vison*)	119 strain, 100,000 tissue cysts	56	Serology by DT at necropsy	Described lesions, serology	Subclinical	261
Skunk (*Mephitis mephitis*)	VEG, tissue cysts, fed to 10 skunks TgCKCa4 oocysts, fed to 9 skunks	25 days	MAT antibodies 20–23 days p.i.	Described clinical signs, lesions, serology	Clinical	1075

An outbreak similar to that in the mink was reported in ferrets (*Mustela putorius*) from New Zealand.[1282,1283] During several weeks 270 of 750 (36%) mink died suddenly without apparent clinical signs. Myocarditis and hepatitis were the predominant lesions.

An epizootic of toxoplasmosis occurred in 22 adults and 30 kit black-footed captive ferrets (*Mustela nigripes*) in Kentucky.[131] Clinical signs were observed in 19 adults and 6 kits and included anorexia, corneal edema, and ataxia. High MAT and LAT *T. gondii* antibody titers were found in ten animals tested. The diagnosis was confirmed by IHC staining and by ultrastructural examination. Lesions are detailed for most animals.

16.1.3 Experimental Infections

Red foxes and coyotes fed tissue cysts or oocysts remained asymptomatic, irrespective of the strain of *T. gondii*. The GT1 strain of *T. gondii*, a type I strain, is highly mouse virulent. It is noteworthy that in raccoons *T. gondii* was re-isolated from muscle tissue but not the brain (Table 16.4). In experimentally infected foxes and raccoons, the MAT and DT are more sensitive tests than the IHA and LAT.[374]

CHAPTER 17

Toxoplasmosis in Deer and Bears

17.1 INTRODUCTION

T. gondii infection in wild game is of epidemiological significance. Deer are popular game animals in several countries. In some countries red deer and reindeer are domesticated and raised for human consumption. In the United States the deer population is estimated in the millions. During the 2006 deer hunting seasons 150,552 and 270,778 deer were harvested in Iowa and Minnesota alone.[417] The white-tailed deer (WTD) is the most abundant species in the United States. Deer and bears are the two most common game and more information is available on *T. gondii* infection in these animals than other game. Therefore I have separated these from other game animals.

Deer are strictly herbivores and the high prevalence of *T. gondii* in deer suggests widespread contamination of the environment with oocysts. Bears are omnivores and infections in them indicate cumulative contamination with oocysts and intermediate hosts in the environment. Thousand of these animals are killed on roads, hunted, or preyed upon by carnivores. Carnivores scavenge the tissues of these animals, and if felids eat infected tissues, they can shed many oocysts in the environment. Cases of clinical toxoplasmosis,[1130] including ocular manifestations,[1117] have been documented in humans who had consumed undercooked venison. An outbreak of toxoplasmosis in pregnant women in Canada was linked to the consumption of caribou meat.[902] Jordan et al.[741] reported both *T. gondii* and *Trichinella spiralis* infections in a patient after eating wild bear meat.

17.2 DEER

17.2.1 White-Tailed Deer (WTD)

17.2.1.1 Serologic Prevalence

Among all surveys in deer, up to 60% of WTD had antibodies to *T. gondii* (Table 17.1).

17.2.1.2 Isolation of Viable T. gondii

Viable *T. gondii* was isolated from hunted WTD in the United States (Table 17.2). It was isolated from fetuses of 6 of 61 deer in early pregnancy (45–85 days of gestation) from Iowa and fetuses of 9 of 27 deer from Minnesota in mid-gestation (130–150 days) of a gestational period of 7 months. The objective of that study was to determine the possibility of transplacental transmission of *T. gondii* in

Table 17.1 Seroprevalence of *T. gondii* in Deer

Species	Country	Test	No. Exam.	%Pos	Cutoff Titer	Ref
Deer						
Black-tailed *(Odocoileus hemionus columbianus)*	United States					
	Washington	MAT	43	32.5	25	427
Marsh *(Blastocerus dichotomus)*	Brazil	IHA	66	27.2	16	503
Mule *(Odocoileus hemionus hemionus)*	United States					
	California	LAT	276[a]	15	32	175
	Nebraska	MAT	89	34.8	25	853
Fallow *(Dama dama)*	Czech Republic	IFA	143	17	40	76
	Spain	MAT	79	22.8	25	578
Red *(Cervus elaphus)*	Norway	MAT	571	7.7	40	1321
	Spain	MAT	441	15.6	25	578
	Czech Republic	IFA	377	45	40	76
Pampas *(Ozotoceros bezoarticus)*	Brazil	IHA	41	12.1	16	503
Roe *(Capreolus capreolus)*	Austria	IHA	40	12	32	450
	Czech Republic	DT	95	14	40	647
	Czech Republic	IFA	79	24	40	76
	Norway	MAT	760	33.9	40	1321
	Spain	MAT	278	39.2	25	562
	Spain	MAT	33	21.2	25	578
Sika *(Cervus nippon)*	Czech Republic	IFA	14	50	4	76
White-tailed *(Odocoileus virginianus)*	United States					
	Alabama	MAT	19	21	50	839
	Iowa	MAT	84	64.2	25	433
	Iowa	MAT	62	32.2	25	433
	Kansas	MAT	106	44	25	123
	Minnesota	MAT	1367	30	25	1309
	Minnesota	MAT	170	53.5	25	433
	Mississippi	MAT	73	46.5	25	393
	Ohio	MAT	147	44	25	198
	Pennsylvania	MAT	593	60	25	691
Reindeer or Caribou						
Fennoscandian *(Rangifer tarandus* spp.*)*	Norway and Finland	MAT	2,577	0.9	40	1001
	Czech Republic	IFA	2	50	40	76
Svalbard *(Rangifer tarandus)*	Norway	MAT	866	1	40	1321
		MAT	390	0	64	1070
Caribou *(Rangifer tarandus)*	United States Alaska	MAT	241	6	25	1391
Barren-Ground *(Rangifer tarandus groelandicus)*	Canada	MAT	147	29.9	25	791

[a] Samples were from various species of deer, including mule deer.

Table 17.2 Isolation of *T. gondii* from White-Tailed Deer (WTD) and Black Bears in the United States

Species	Source	No. Bioassayed	% Positive	Ref
WTD	Alabama	19	21	839
	Mississippi	73	28.7	393
	Iowa, Minnesota	88	17	417
Black bear	Pennsylvania	28	35.7	343
	Pennsylvania	10	70	393

game, because this information was not available for any large wild animal species. In total *T. gondii* was isolated from 15 deer, and these isolates were designated TgWtdUs1–15.

17.2.1.3 Genetic Characteristics of T. gondii from WTD

Genotyping of the 15 deer isolates revealed that nine isolates were *T. gondii* clonal type II strains, two isolates were clonal type III strains, and four isolates belong to three nonclonal genotypes. DNA sequencing analysis of representative isolates (TgWtdUs2, 4, 5, 6, 8, 10, 13, and 15) at loci SAG2, c22-8, L358, and PK1 revealed that the sequence data of the deer isolates match perfectly with PCR-RFLP genotyping data at all four loci. The data indicate that the three nonclonal genotypes of deer isolates are closely related to the clonal type I, II, and III lineages, and they were likely derived from natural recombination among the three clonal lineages. Howe and Sibley[681] genotyped two WTD *T. gondii* isolates (WTD-1, WTD-3) as SAG2 genotype II; these isolates were from Alabama deer.[839]

Like the deer, common game animals in the United States have high seropositive rates of *T. gondii* infection, and they may be important in the transmission of the parasite to humans. The high prevalence of clonal type II strains in both humans and deer in the United States suggests the possible link of transmission from game animals to humans. This is important in public health and would suggest that more attention should be paid in preparing meat from game animals for human consumption.

17.2.1.4 Clinical Toxoplasmosis in WTD

There is no report of clinical toxoplasmosis in WTD.

17.2.2 Reindeer

Fatal toxoplasmosis was diagnosed in a full-term stillborn reindeer (*Rangifer tarandus*) fetus from the Houston Zoo, Texas.[382] The fetus had encephalitis and placentitis associated with *T. gondii*. Tissue cysts were identified histologically in sections of brain and tachyzoites were present in placenta and the myocardium. Protozoa in the brain, heart, and placenta stained positively with *T. gondii* antibodies in an IHC test. The dam of the fetus had a high (MAT, 1:12,800) *T. gondii* antibody titer.

Experimentally, reindeer (*R. tarandus*) were susceptible to toxoplasmosis. Two yearling reindeer were inoculated with 5,000 or 50,000 oocysts of the ME 49 strain directly in the rumen to avoid spillage.[1000] Both animals became ill 4 days p.i. and the reindeer given the higher dose died of acute visceral toxoplasmosis 9 days p.i. The deer given 5,000 oocysts was treated successfully with sulfatrimethoprim from days 2–9, it recovered by day 16 p.i. and remained asymptomatic 707 days p.i. (last day of the experiment).

Table 17.3 Seroprevalence of *T. gondii* in Bears

Species	Country	Test	No. Exam.	% Pos.	Cutoff Titer	Ref
Black (*Ursus americanus*)	Canada	ELISA	38	34.2	NS[a]	1058
	United States					
	Alaska	LAT	40	15	64	176
		MAT	143	43	25	1391
	Florida	LAT	66	56.1	64	443
	North Carolina	MAT	143	84	25	988
	Pennsylvania	MAT	665	80	25	124
		MAT	322	79.8	25	333
		MAT	28	78.6	40	343
		MAT	80	82.5	25	393
Grizzly (*Ursus arctos horribilis*)	Canada	ELISA	36	0	NS	1058
	United States					
	Alaska	LAT	480	18	64	176
		MAT	892	25	25	1390
Polar (*Ursus maritimus*)	Canada	ELISA	60	0	NS	1058
	Norway[b]	MAT	cubs 83	3.6	25	1003
		MAT	adults 444	21.4	25	
	United States					
	Alaska	LAT	500	6	64	1079

[a] NS = not stated.
[b] Including Svalbard, Greenland, and Barents sea.

17.3 BEARS

17.3.1 Serologic Prevalence

A very high prevalence of *T. gondii* antibodies was found in the black bear and the prevalences have remained stable around 80% (Table 17.3).

17.3.2 Isolation of Viable *T. gondii* from Black Bears

Viable *T. gondii* was isolated from 35% and 70% of animals (Table 17.2). *T. gondii* was isolated from the hearts of 10 of the 28 bears killed in 1993. Seven of the isolates were obtained by bioassay in cats, two isolates were obtained by bioassay in mice, and one isolate was obtained by bioassay in both cat and mice.[343] Howe and Sibley[681] used oocysts of seven of these isolates for genotyping. They identified four of these as type III, two as type II, and one as a recombinant of type II and III. The genotypes of the two isolates obtained by bioassays in mice were type III in one and type II in one.[393] Thus, of the 16 *T. gondii* isolates from Pennsylvania bears studied, type III lineage was found in 7, type II in 7, type I in 1, and a recombinant of type II and III in 1.

17.3.3 Clinical Toxoplasmosis in Bears

There is no confirmed report of clinical toxoplasmosis in bears.[378]

CHAPTER 18

Toxoplasmosis in Rodents and Small Mammals

18.1 RODENTS

18.1.1 Rats, Mice, and Shrews

Dubey and Frenkel,[362] Afonso et al.,[9] and Dabritz et al.[212] tabulated worldwide serologic *T. gondii* prevalence in rodents; I have not repeated them here.

The method used for *T. gondii* examination of rodents is critical. Serological surveys are the easiest, but specificity and sensitivity depends on the test, and the dilution of serum tested. As an example, Webster[1343] reported a high (35%) seroprevalence in 235 rats from farms in England, but the study was based on 1:10 titer in the LAT, which is a very insensitive test and titers below 1:32 are regarded nonspecific. Confirmation of positive samples was sought by IgG-ELISA and by direct microscopic examination of the rat brains for *T. gondii* tissue cysts. The percentage of sera and brains that were so checked was not indicated.[362] She proposed that *T. gondii* infection can be maintained in the absence of cats on some farms.[1343] It is unfortunate that such a profound statement was based on a 1:10 titer in LAT.[362]

Bioassays for viable *T. gondii* have revealed that only a small proportion of rodents remain persistently infected (Table 18.1). The largest survey was reported by Hejlíček and Literák[648] from the Czech Republic. They tested by bioassay tissues of 17 species of 5,166 small mammals from various locations; 0.9% had viable *T. gondii*. The highest prevalence (7.4% of 94) was in the striped field mouse (*Apodemus agrarius*). Six of 884 (0.7%) of the house mouse (*Mus musculus*) were also infected (Table 18.1). It is noteworthy that seven of eight mice from whom we isolated viable *T. gondii* were seronegative both in the DT and MAT.[344] Surveys based on small samples can be misleading. As an example, attempts were made to isolate *T. gondii* from animals trapped in and around a stable in Atlanta where an outbreak of toxoplasmosis had occurred in people.[285,1274] Viable *T. gondii* were isolated from tissues of 4 mice trapped in the stable in November 1977 (sample 1) but not from 12 mice, 3 rats, and 4 cotton rats trapped in the stable in December 1977 (sample 2). All four mice from whose tissues viable *T. gondii* were isolated had no detectable antibodies to *T. gondii* at a 1:2 serum dilution (prozone was excluded) of serum tested using a dye test. Afonso et al.[8] surveyed 218 small mammals from France. Antibodies (MAT, 1:25) were found in 4 of 92 *Apodemus* spp., 3 of 64 *Clethrionomys glareolus*, 3 of 5 *Sorex* spp., 2 of 9 *Arvicola terrestris*, and 7 of 18 *Talpa europaea*, but *T. gondii* was not isolated from the hearts of any of the seropositive animals.

Bioassay in mice, although expensive, is the definitive proof of *T. gondii* infection. All currently available evidence indicates that a mouse can detect as few as one viable *T. gondii* by the parenteral routes (see Chapter 1 Table 1.18). PCR is also sensitive but it cannot differentiate between live or dead organisms, and contamination is a major issue. A very high prevalence of *T. gondii* infection

Table 18.1 Isolation of *T. gondii* from Rodents and Other Small Mammals

Species	Country	Remarks	No. Total	No. Positive (%)	Ref
Mice					
House mouse (*Mus musculus*)	Czech Republic		884	6 (0.7%)	648
	United States	6/7 isolates were from seronegative mice	1,502	7 (0.5%)	344
Striped field (*Apodemus agrarius*)	Czech Republic		94	7 (7.4%)	648
White-footed deer mouse	United States	1/2 isolates from seronegative mice	67	2 (3%)	344
(*Peromyscus* sp.)		2/33 seropositive	33	2 (6%)	817
Field mouse (*Apodemus* spp.)	Czech Republic		1,897	16 (0.8%)	648
Rats					
(*Rattus novergicus, Rattus rattus*)	Czech Republic		41	0	648
	Mexico	2/249 rats seropositive	249	0	434
	Panama		23[a]	1 (4.3%)	546
	Grenada — West Indies	The rat had a MAT titer of 1:320	238	1 (0.4%)	403
	United States	2 seropositive	7	0	817
(*Rattus novergicus*)	United States	The rat had a MAT titer of 1:500	107	1 (0.9%)	344
Muskrat (*Ondatra zibethicus*)	Czech Republic	69/146 isolates from rats on site A	256	69 (26.9)%	976
		10/110 isolates from sites A, B, C, D			
Shrews					
Eurasian (*Sorex araneus*)	Czech Republic		465	2 (0.4%)	648
Lesser white-toothed (*Crocidura suaveolens*)	Czech Republic		45	0	648
Pygmy (*Sorex minutus*)	Czech Republic		33	0	648
Squirrels					
(*Spermophilus variegatus*)	Mexico	All seronegative	69	0	434
(Sciurus carolinensis)	United States	2/11 were seropositive	2[a]	1 (50%)	1205
Voles					
Bank (*Clethrionomys glareolus*)	Czech Republic		299	3 (1%)	648
Common (*Microtus arvalis*)	Czech Republic		1,337	12 (0.9%)	
Field (*Microtus agrestis*)	Czech Republic		39	1 (2.6%)	
Meadow (*Microtus pennsylvanicus*)	United States	1 seropositive	8	0	817
Capybara	Brazil	49/64 capybaras were seropositives	40[a]	36 (90%)	1379
(*Hydrochaerus hydrochaeris*)					

[a] Only seropositive animals were bioassayed.

was reported in rodents by Hughes et al.[689] They found *T. gondii* DNA in the brains of 42.2% *Rattus norvegicus* from urban areas of Manchester, England. Kijlstra et al.[769] found *T. gondii* in 12 (hearts of 8, brains of 4, but not both from the same animal) of 101 rodents from 3 pig farms in the Netherlands. On one poorly managed farm, *T. gondii* DNA was detected in 1 of 7 wood mice (*Apodemus sylvaticus*), 2 of 8 house mice (*Mus musculus*), 3 of 19 white-toothed shrews (*Crocidura russula*), 3 of 35 rats (*Rattus norvegicus*), 1 of 1 bank vole (*Clethrionomys glareolus*), and 1 of 1 common vole (*Microtus arvalis*). On farm 2, *T. gondii* DNA was found in 1 (*Rattus norvegicus*) of 5 rodents, but all 25 rodents on farm 3 were negative.

How rodents acquire *T. gondii* infection in nature is unknown, and the host species or strains are also important. It is 50 years since congenital infection was demonstrated in the outbred laboratory albino mouse (*Mus musculus*). Repeated congenital infection can occur in progeny and in the same dam without re-infection from outside.[97] Theoretically, *T. gondii* may be maintained in the mouse by the congenital route. However, it is unknown if this occurs in nature. Actually, Beverley terminated his experiments after nine generations in mice because of the high mortality in the progeny, and because the progeny became seronegative, although infected by the parasite. This fact was unknown at that time, and is now considered due to immunological tolerance. Viable *T. gondii* has now been isolated from the tissues of rodents without demonstrable antibodies.[344,355]

I would like to emphasize that this repeated congenital transmission does not occur in all strains of mice. For example, inbred BALB/C mice and rats transmit the parasite only once.[355,1103] Owen and Trees showed repeat congenital transmission in field mice (*Apodemus sylvaticus*) fed 50 *T. gondii* oocysts.[1015] They found that 19 of 26 pups in the first litter, 23 of 27 pups in the second litter, and 24 of 30 pups in subsequent litters were infected with *T. gondii*, based on the detection of *T. gondii* DNA in fetal tissues by PCR. They also reported that the PCR was as sensitive as the mouse inoculation, but serology (MAT, cut-off 1:40) did not reflect *T. gondii* infection status. They compared MAT, PCR, and mouse inoculation in 12 pups (5 MAT-positive and 7 MAT-negative); of the MAT-positive pups, all 5 were positive by PCR, and 4 were positive by mouse test. In contrast, of the MAT-negative pups, 5 were positive by both PCR and mouse test. Thus, of the 12 pups tested, 5 were positive by MAT, 9 by mouse bioassay, and 10 were positive by PCR.[1015]

An alarmingly high rate of *T. gondii* infection has been reported[689,888,963] (all three papers discuss the same mice) in house mice (*Mus musculus*). In a well-planned study, they live trapped 200 mice on 27 domestic properties in Cheetham Hill, an inner city area in Manchester, England. The mice were dissected and brains were collected for PCR and sera were obtained for serology. By PCR 59% of 200 mice contained *T. gondii* DNA, but antibodies to *T. gondii* were detected in only one mouse. They found 61.3% prevalence in mice weighing 10 g, 55.3% in mice weighing 10–15 g, and 52.9% in mice weighing 15 g, suggesting congenital transmission of *T. gondii*. Sixteen of these mice (12 of them were PCR-positive) were pregnant at the time of capture. *T. gondii* DNA was detected in 63 (74.6%) of 78 fetuses from these 12 dams; at least 1 fetus was infected in each litter.[888,963] I am not aware of a previous study of *T. gondii* infection in mice from human dwellings.

Dabritz et al.[212] performed an excellent epidemiologic survey of rodents for *T. gondii* infection in 18 locations in Morro Bay, California. Rodents were trapped on 13 locations away (>200 m) and 5 locations close (<200 m) to human dwellings. Sera were tested by a conservative cutoff of 1:80 in IFA test (mice and rats) and 1:32 in LAT (other species) to decrease the possibilities of nonspecificity. *T. gondii* antibodies were not found in any of the 15 house mice, 10 rats, 25 *Microtus californicus*, 50 *Neotoma fuscipes*, 14 *Perognathus californicus*, and 44 *Reithrodontomys megalotis*. The increasing

frequency of infected hosts were 8% of 37 *Spermophilus beecheyi,* 9% of 11 *Peromyscus truei*, 23% of 214 *Peromyscus californicus*, 31% of 29 *Peromyscus boylii*, and 34% of 74 *Peromyscus maniculatus*. They identified two main risk factors: location of trapping and age of the animal. Infections were higher in rodents trapped near dwellings than away from the dwellings. The odds of *T. gondii* seropositivity was three times greater in older animals than in juveniles.[212] The results indicate postnatal transmission of *T. gondii* in *Peromyscus* spp.

I am not aware of any reports of clinical toxoplasmosis in naturally infected rodents, including rats and mice.

18.1.2 Capybaras (*Hydrochaeris hydrochaeris*)

The capybara is a herbivorous rodent throughout tropical South America. Capybara meat is consumed by humans in many countries. Antibodies (MAT 1:25) were found in 42.3% of 149 capybaras killed for food.[151] In a subsequent study, *T. gondii* antibodies were found in 75% of 64 capybaras and viable *T. gondii* were isolated from tissue homogenates of 36 capybaras and the isolates were designated TgCyBr1–36.[1379] Most isolates were lethal to mice; 17 of the 36 isolates killed 100% of infected mice, 11 isolates caused mortality in 25–90% of infected mice, and 8 isolates were nonpathogenic to mice. All capybaras were clinically normal. Results indicated that asymptomatic capybaras can harbor mouse-virulent *T. gondii,* and hence they can serve as a source of infection for humans. Genetic characterization of these isolates was recently reported.[1379a]

18.2 HARES AND RABBITS

18.2.1 Serologic Prevalence

Demand for rabbit meat for human consumption is increasing, therefore toxoplasmosis in rabbits is of epidemiologic significance. Almería et al.[25] surveyed wild rabbits from five regions of Spain. The overall prevalence was 14.2% of 456 by the MAT (cutoff 1:25). Seroprevalence was the highest (53.8% of 26) for rabbits from Catolina and the lowest (6.1% of 148) for rabbits from Cadiz. Prevalence in young (<7 months, 16.4% of 67) was similar to older (>7 months, 11% of 363) rabbits, indicating congenital infection or early postnatal infection. Stutzin et al.[1241] found *T. gondii* antibodies (IHA, 1:16) in 8% of 50 wild rabbits from Chile.

Figueroa-Castillo et al.[509] detected *T. gondii* antibodies in 26.9% of 286 rabbits from three rabbit farms in Mexico; seroprevalence was higher in two poorly managed farms (39.7 and 33.3%), and low (18.7%) on a better managed farm. Hejlíček et al.[647] found DT antibodies (cutoff 1:4) in 8% of rabbits from the Czech Republic.

There are several reports of *T. gondii* infection in hares from Europe. Frölich et al.[554] examined tissues and sera from 321 hunter killed European brown hares (*Lepus europaeus*) from Germany for *T. gondii* antibodies by the DT (cutoff 1:16) and *T. gondii* antigen by IHC staining. Antibodies to *T. gondii* were found in 46% of 318 hares and *T. gondii* antigen was demonstrated in 57% of 201 hares. Apparently, there were no cases of clinical toxoplasmosis. Results of this study are in marked contrast to the report of Gustafsson and Uggla[618] who did not find antibodies to *T. gondii* in any of 176 brown hares (*Lepus europaeus*) from Sweden, suggesting that this animal is highly susceptible to *T. gondii* and perhaps the disease is fatal in most of them. By using the Ouchterlony agar diffusion test Arnaudov et al.[46] found *T. gondii* antibodies in 9 of 58 wild hares from Bulgaria. Edelhoffer et al.[450] reported a 2% seroprevalence in 3,124 hares from

Austria by the IHA test (cutoff 1:32). Hejlíček et al.[647] found DT antibodies (cutoff 1:4) in 4% of 164 hares from the Czech Republic.

Smith and Frenkel[1205] found *T. gondii* antibodies by DT in 3 of 12 cottontail rabbits (*Sylvilagus floridanus*) from Kansas.

18.2.2 Isolation of Viable *T. gondii*

Hejlíček and Literák[643] examined 366 rabbits from 48 properties in Strakonice, Czech Republic for antibodies and viable *T. gondii*. Antibodies to *T. gondii* were found in 54 (17.8) of 366 sera at a 1:4 dilution by the DT. Homogenates from portions of diaphragm, brain, and liver (pooled for each rabbit) from 304 rabbits were inoculated i.p. into two mice. Viable *T. gondii* was isolated from 54%. It is worth noting that of the 54 rabbits with demonstrable *T. gondii*, 18 rabbits were seronegative by the DT (cutoff 1:4).

Sroka et al.[1230] found *T. gondii* antibodies (MAT, 1:8,000) in two of nine rabbits from a farm in Poland where cases of human toxoplasmosis were detected. They isolated viable *T. gondii* from the brain of one of these two seropositive rabbits. *T. gondii* tachyzoites were detected in tissues of the mice inoculated with rabbit tissues. The strain was mildly pathogenic to mice. *T. gondii* tissue cysts were found in histological sections of the rabbit's brain.

18.2.3 Detection of *T. gondii* DNA in Tissues

Hughes et al.[690] detected *T. gondii* DNA in brains of 68.4% of 57 wild rabbits from the UK.

18.2.4 Clinical Infections

Epizootics of toxoplasmosis have been reported from rabbits and hares mainly from Scandinavia and these epizootics have been recognized since the early 1950s.[178,617] To my knowledge, these epizootics have not been seen in other countries. We have described two reports of acute toxoplasmosis involving a few rabbits from the United States (Table 18.2). The predominant lesions were in the spleen (Table 18.2).

18.2.5 Experimental Infections

Experimentally, rabbits are susceptible to clinical toxoplasmosis, particularly by the oral inoculation of oocysts, and hares are even more susceptible to toxoplasmosis than the rabbits.[620] Seven hares and nine rabbits fed 50 *T. gondii* oocysts of the Swedish isolate TgSwef were killed 7 or 8 days p.i., before they become severely ill. All hares had histologic evidence of acute visceral toxoplasmosis, characterized by necrosis whereas lesions in rabbits were mainly inflammatory. Sedlák et al.[1162] conducted a similar experiment in the Czech Republic. Twelve rabbits and 12 hares were fed 10, 1,000, or 100,000 *T. gondii* oocysts (4 for each dose) and observed for 33 days p.i. All hares died of acute visceral toxoplasmosis between 8 and 19 days p.i.; hares fed 10 oocysts died 12–19 days p.i. Three rabbits (one in each dose) died of concurrent bacterial infection, but the remainder survived and were found to have tissue cysts. Both hares and rabbits seroconverted between 7 and 12 days p.i.

Rabbits have been used in the laboratory to study the pathogenesis of toxoplasmic chorioretinitis, because they are highly susceptible to *T. gondii* infection and have big enough eyes for manipulation compared with rodents.[576]

Table 18.2 Summary of Reports of Clinical Toxoplasmosis in Hares, Rabbits, and Squirrels*

Species	Source	Remarks	*T. gondii* Demonstrated[a]					
			Histo	TEM	Imm	Tach	Cyst	Ref
Squirrels								
American red squirrel (*Tamiasciurus hudsonicus)*	2, FR, Indiana	Toxoplasmic pneumonia, PCR-positive	Yes	Yes	Yes	Yes	Yes	68
Gray squirrel (*Sciurus carolinensis)*	2, captive, Louisiana, 1 FR, Philadelphia	Neurologic signs, disseminated toxoplasmosis	Yes	ND	Yes	Yes	Yes	400
Korean squirrel *(Tanias sibericus)*	4, transported from People's Republic of China, died in Spain	Transport stress, disseminated toxoplasmosis	Yes	Yes	Yes	Yes	Yes	156
Red Squirrel (*Sciurus vulgaris*)	1, captive 1, feral	Acute toxoplasmosis	Yes	ND	Yes	ND	ND	438a
Rabbits and hares								
Rabbit (*Oryctolagus cuniculus*)	2, domestic, Georgia 1, rabbitry, Massachusetts	Visceral toxoplasmosis with severe splenitis Disease outbreak in a 50 animal Rhinelanders	Yes	ND	Yes	Yes	No	322
	3, Texas	3 of 5 French lop rabbits from a private owner died, 1 rabbit necropsied Visceral toxoplasmosis with severe lesions in spleen	Yes	ND	Yes	Yes	No	819
Brown hare (*Lepus europaeus)*	39, Sweden	Retrospective study of 388 wild brown hares and 202 wild mountain hares that died 1980–1985	Yes	ND	Yes	Yes	Yes	617
Mountain hare *(Lepus timidus)*	8, Sweden							

[a] Histo = histology, TEM = transmission electron microscopy, Imm = immunohistochemistry, Tach = tachyzoites, Cyst = tissue cysts, ND = no data.

18.3 SQUIRRELS

Serologic surveys in squirrels indicate low prevalence of *T. gondii*. We were unable to isolate *T. gondii* from 69 squirrels (*Spermophilus variegatus*) from Durango, Mexico[434] (Table 18.1). Smith and Frenkel isolated viable *T. gondii* from one of two seropositive gray squirrels from Kansas.[1205]

Sporadic cases of clinical toxoplasmosis occur in squirrels (Table 18.2). Toxoplasmosis in squirrels can simulate signs of rabies. Reported clinical signs in squirrels were anorexia, diarrhea, lethargy, viciousness, and labored breathing, and in two cases squirrels had bitten children.

18.4 VOLES

Voles appear to be an important reservoir of *T. gondii* infection in Europe (Table 18.1).

18.5 SMALL MAMMALS AS SOURCE OF *T. GONDII* INFECTION FOR CATS

As said earlier, how cats become infected in nature is epidemiologically important. There are no studies that examined the comparative role of different animal species as source of infection for *T. gondii* in cats. This would vary in each location. Such studies would be difficult in locations where *T. gondii* infections are endemic in small mammals, birds, and in cats. For example, in Costa Rica, viable *T. gondii* was isolated from 16% of sparrows, 54% of chickens, 12.5% of rats, 3.5% of mice, and 23% of cats.[1121,1122] In contrast, we found a very low prevalence of *T. gondii* in rodents and birds on pig farms in the United States[344,817] (Table 18.1). *T. gondii* was isolated from 7 of 1,502 house mice on 5 farms, 2 of 67 white-footed mice on 2 farms, 1 of 107 rats but not from 8 voles, 3 ground squirrels, 2 moles, 2 shrews, and 21 sparrows that were trapped.[344] On another pig farm in Massachusetts, *T. gondii* was isolated from 2 of 33 white-footed mice, 1 of 29 European starling, but not from 23 other small mammals, and 134 other avian species.[817] These studies were very labor intensive and all trapped animals were bioassayed in mice, irrespective of serologic status. Studies in Costa Rica and the United States were based on isolation of viable *T. gondii.* As said earlier, caution should be used in interpreting the serologic data in rodents and avian species because seronegative animals can harbor viable *T. gondii*.

Afonso et al.[9] proposed an interesting concept of correlating *T. gondii* infection in cats and the body mass of intermediate hosts; a positive correlation was found between feline infection and the body mass of intermediate hosts. Thus, rabbits were more likely a source of infection than other small mammals. More information is needed concerning diets of free-ranging cats.[512] *T. gondii* infection in rats was much lower than in white-footed mice[212] (see Table 18.1). Recently, *T. gondii* was isolated from the tissues of only 1 of 238 rats from Grenada, West Indies, and none of 249 rats from Mexico.[434] Childs had proposed that predation was size dependent, and cats were unlikely to catch adult rats or rabbits.[172]

CHAPTER 19

Toxoplasmosis in Miscellaneous Animals

19.1 INTRODUCTION

T. gondii is thought to infect virtually all warm-blooded animals. In this chapter I have lumped all hosts not discussed in Chapters 2–18.

19.2 MISCELLANEOUS ANIMALS

Serologic prevalence is summarized in Table 19.1. da Silva et al.[207b] found *T. gondii* antibodies in 1 of 3 six-banded armadillo (*Euphractus sexcinctus*) and 2 of 9 nine-banded armadillo (*Dasypus novemcinctus*) from Brazil; *T. gondii* was isolated from tissues of 2 of 3 *E. sexcnctus*. Cases of fatal toxoplasmosis are summarized in Table 19.2.

Carme et al.[154] discussed *T. gondii* infections in free-ranging neotropical mammals in French Guiana.

19.3 ANTELOPES

19.3.1 Saiga Antelope (*Saiga tatarica*)

The *Saiga tatarica* is an antelope that is found only in a few areas in Russia, Kazakhstan, and western Mongolia. They have an unusual, oversized, and flexible nose structure. Visceral toxoplasmosis was diagnosed in a 1.5-year-old captive that died in the Czech Republic.[1163] *T. gondii* DNA was detected in frozen samples of liver, lungs, kidneys, spleen, and intestine. Sections of liver were studied histologically. There was marked hepatitis characterized by necrosis, hemorrhage, and perivascular inflammation. There were numerous tachyzoites and tissue cysts associated with hepatitis; the identity of tissue cysts was confirmed by PAS staining and reactivity to bradyzoite-specific BAG 5 antibodies.[1163]

19.3.2 Dik-Dik (*Madoqua guentheri smithii*)

The dik-dik is a small antelope that lives in the bush of some parts of Africa. Two captive dik-diks at the Houston Zoo, Texas, died suddenly of visceral toxoplasmosis and toxoplasmic pneumonia.[379] An unusual finding was gastroenteritis with tachyzoites and tissue cysts demonstrated in the intestines, rumen, and reticulum. Although the animals died 2 years apart, similar lesions were found in both. Both animals had a MAT titer of 1:800.[379]

Table 19.1 Seroprevalence of *T. gondii* in Miscellaneous Animals

Species	Country	Test	No. Exam	% Pos	Cutoff Titer	Ref
Red acouchy (*Myoprocta acouchy*)	French Guiana	MAT	26	4	40	242
Agouti (*Dasyprocta aguti*)	French Guiana	MAT	43	23.3	40	154
	French Guiana	MAT	45	18	40	242
Alpacas (*Lama pacos*)	Peru	Immunoblot	356	0.8	2 IDA	1370
	Germany	Immunoblot	12	33	2 IDA	1370
	Peru	IFA	278	34.5	NS	1268
Nine-banded armadillos (*Dasypus novemcinctus*)	Brazil (São Paulo)	MAT	31	12.9	256	208
African buffalo (*Syncerus caffer*)	Zimbabwe	MAT	18	5.6	25	677
Sable (*Hippotragus niger*)	Zimbabwe	MAT	67	11.9	25	677
Pronghorn (*Antilocapra americana*)	United States (Kansas)	MAT	63	4.7	25	123
Bushbuck (*Tragelaphus scriptus*)	Zimbabwe	MAT	14	57.1	25	680
Common brushtail possums (*Trichosurus vulpecula*)	Australia (Sydney)	MAT MAT	126 142	4.8 6.3	25 25	660 485
Barbary sheep (*Ammotragus lervia*)	Spain	MAT	10	10	25	578
American beaver (*Castor canadensis*)	United States (Kansas)	DT	14	*7	8	1205
Bighorn sheep (*Ovis canadensis*)	United States	MAT	697	3.6	25	375
	(California)	NS	178	12.3	NS	181
		NS	998	21.6–25.0	NS	466
American bison (*Bison bison*)	United States (Alaska)	MAT	241	0.8	25	1391
Pyrenean chamois (*Rupicapra pyrenaica*)	Spain	MAT	10	20	25	578
Dall sheep (*Ovis dalli*)	United States (Alaska)	MAT	319	6.9	25	1391
Giraffe (*Giraffa camelopardalis*)	Zimbabwe	MAT	10	10	25	680
Eland (*Taurotragus oryx*)	Zimbabwe	MAT	19	36.8	25	677
Elephants						
Indian (*Elephas maximus indicus*)	Thailand	MAT	156	45.5	50	1292
		LAT	156	25.6	64	
Asian (*Elephas maximus maximus*)	Sri Lanka	MAT	53	26.4	25	216
African (*Loxodonta africana*)	Zimbabwe	MAT	19	10.5	25	677
		MAT	20	10	25	680
Greater kudu (*Tragelaphus strepsiceros*)	Zimbabwe	MAT	10	20	25	680
Llamas (*Lama glama*)	Argentina	IFA	308	30	50	949
	Peru	IFA	43	55.8	200	169
		Immunoblot	81	8.6	2 IDA	1370

(*continued*)

Table 19.1 Seroprevalence of *T. gondii* in Miscellaneous Animals (Continued)

Species	Country	Test	No. Exam	% Pos	Cutoff Titer	Ref
	United States	MAT	283	33.5	25	323
Moose (*Alces alces*)	Canada	IHA	125	15	64	1181
	Norway	MAT	2142	12.6	40	1321
	United States (Alaska)	MAT	240	1.2	25	1391
Mouflon (*Ovis musimon*)	Bulgaria	Ouchterlony test	60	0	NS	46
	Czech Republic	IFA	105	8.5	4	76
(*Ovis ammon*)	Spain	MAT	27	14.8	25	578
Muskox (*Ovibos moschatus*)	Canada	MAT	203	6.4	25	790
Nyala (*Tragelaphus angasii*)	Zimbabwe	MAT	10	90	25	680
Opossum (*Didelphis virginiana*)	Mexico	CFT	29	10.4	NS	1248
		MAT	66	16.6[a]	25	434
	United States	DT	38	15.8[b]	4	1205
Red panda (*Ailurus fulgens*)	People's Republic of China	IHA	73	15.1	64	1073
Black rhinoceros (*Diceros bicomis*)	Zimbabwe	MAT	11	27.3	25	680
White rhinoceros (*Ceratotherium simus*)		MAT	2	50	25	677
Spanish ibex (*Capra pyrenaica*)	Spain	MAT	3	33	25	578
Vicuna (*Vicugna vicugna*)	Peru	IFA	200	5.5	50	169
	Peru	Immunoblot	103	2.9	2 IDA	1370
Yak (*Bos grunniens*)	Bulgaria	Ouchterlony test	267	6	NS	46
Wildebeest (*Connochaetes taurinus*)	Zimbabwe	MAT	69	14.5	25	677
Woodchuck (*Marmota monax*)	United States (Pennsylvania)	MAT	545	9.4	25	1235a

* *T. gondii* isolated from one of one seropositive beaver.
[a] *T. gondii* not isolated by biossay.
[b] *T. gondii* isolated from from one of three seropositive opossums.
NS = not stated.
2 IDA = two or more immunodominant antigens recognized.

19.3.3 Nilgais (*Boselaphus tragocamelus*)

The Nilgai is also an antelope, but looks like an ox, as the name implies (nil = blue, gai = cow in Hindi). It is one of the most commonly seen wild animals of northern India. Three captive nilgai had stillbirth or their newborns died within 2 days of birth.[1163] *T. gondii* DNA was found in the brain and liver of each of two fetuses, and in the brain of one of the two neonates. Histologically, the fetuses had pneumonitis and hepatitis. Antibodies (>1:640, IFA) were found in all three adult females and one male nilgai.

19.4 ROCKY MOUNTAIN BIGHORN SHEEP (*OVIS CANADENSIS CANADENSIS*)

Baszler et al. diagnosed toxoplasmic encephalitis in a 4-month-old sheep from Washington.[75] The brain had multifocal necrotizing and nonsuppurative encephalitis with *T. gondii* tissue cysts in the lesions.

Table 19.2 Summary of Reports of Clinical Toxoplasmosis in Miscellaneous Animals

Species	Source	Remarks	*T. gondii* Demonstrated[a]					
			Histo	TEM	Imm	Tach	Cyst	Ref
Beaver (*Castor canadensis*)	1, FR, Connecticut	Disseminated toxoplasmosis, no evidence for paramyxoal virus	Yes	ND	Yes	Yes	Yes	519
Woodchuck (*Marmota monax*)	1, FR, New York	Encephalitis, PCR -positive	Yes	ND	No	No	Yes	68
Porcupine (*Coendou mexicanus*)	1, captive, zoo, Costa Rica	Disseminated toxoplasmosis	Yes	ND	Yes	Yes	Yes	944
North American porcupine (*Erethizon dorsatum*)	2, captive, New Jersey	Neurologic, concurrent infection with *Baylisascaris* spp.	Yes	ND	ND	Yes	No	907
Slender-tailed meerkat (*Suricata suricatta*)	7, captive, Spain	Disseminated toxoplasmosis, pulmonary lesions predominant	Yes	ND	Yes	Yes	Yes	742
Dama gazelle (*Gazella dama*)	1, captive, Florida	Disseminated toxoplasmosis	Yes	ND	ND	Yes	Yes	1237
Cuvier's gazelle (*Gazella cuvieri*)	1, captive, Missouri	Visceral toxoplasmosis, hepatitis	Yes	ND	Yes	Yes	Yes	746
Slender-horned gazelle (*Gazella leptoceros*)	1, captive, Florida	Disseminated toxoplasmosis	Yes	ND	ND	Yes	Yes	1237
Gerenuk (*Litocranius walleri*)	2, captive, Florida	Disseminated toxoplasmosis	Yes	ND	ND	Yes	Yes	1237
Three-toed sloth (*Bradypus tridactylus*)	1, captive, Pará, Brazil	Sudden death, visceral toxoplasmosis	Yes	ND	Yes	Yes	Yes	1297
Guinea pig (*Cavia porcellus*)	1, captive, United Kingdom	Abortion, Cesarean section, tachyzoites placenta, and fetal liver, dam IFA titer 1:8198, IHA 1:2048	Yes	ND	ND	Yes	No	604

[a] Histo = histology, TEM = transmission electron microscopy, Imm = immunohistochemistry, Tach = tachyzoites, Cyst = tissue cysts, ND = no data.
[b] The stage of the parasite was not specified.
[c] *T. gondii* found by PCR.

19.5 MUSKOX (*OVIBOS MOSCHATUS*)

The muskox is an Arctic mammal of the Bovidae family, noted for its thick coat and for the strong odor emitted by males. Toxoplasmosis was diagnosed in the herd at the San Francisco Zoo.[196] A 7-year-old muskox born at the zoo aborted a full-term calf (gestational period 8.5 months). The cow passed the placenta 4 days after birth. *T. gondii* tachyzoites were identified histologically and the diagnosis was confirmed by IHC staining. Fetal tissues were autolysed. The cow had aborted also in the previous gestation. The most unusual aspect of this case was that sera from all muskox had been frozen; we could retroactively test all sera by MAT. The aborted cow was initially

seronegative, and became seropositive (MAT 1:3200-1:12,800); the MAT titer 4 days after abortion (the last tested) was 1:6400. Five other muskox at the zoo had MAT titers of 1:800–1:12,800.

19.6 WILD PIGS

See Chapter 6.

19.7 BATS

Because bats can be a host for rabies, the question is asked if they are also a host for *T. gondii*. I am not aware of any recent studies on these animals, but there are two old French studies. Akinchina and Doby[20] saw *T. gondii* in the brain of one dye test negative *Myotis bechsteinii* and isolated viable *T. gondii* by mouse bioassay. Doby et al.[264] examined 35 bats belonging to 15 species and found *T. gondii* antibodies (dye test between 1:10 and 1:40) in one.

19.8 HIBERNATING AND POIKILOTHERMIC ANIMALS

An interesting phenomenon is the ability of *T. gondii* to remain dormant in hibernating animals and to resume activity when they waken. Rodhain and Hendrix[1107] infected a hibernating marmot with *T. gondii*. The animal remained well as long as it hibernated, but when it wakened, it developed acute toxoplasmosis and died. It would appear that *T. gondii* hibernates with its host and resumes activity when its host wakens. This is paralleled by its behavior in tissue culture in which it has been found to persist for long periods at lower temperatures without damaging the cells, and to become fully active when the temperature is raised to 37°C.

T. gondii can survive, but does not multiply in poikilothermic animals.[538,885] A recent study affirmed this hypothesis and showed that the goldfish can be infected with *T. gondii* but the parasite does not multiply or persist in fish tissues.[1011] This subject is of renewed interest because of the potential for contamination of our seas with *T. gondii* oocysts.[186] There are many intriguing issues including the uptake and concentration of *T. gondii* oocysts by molluscs. Whether *T. gondii* sporozoites can excyst in the gut of these molluscs is unknown. Before the discovery of the oocyst in 1970, who would have thought that we would be living in a universe of *Toxoplasma*.

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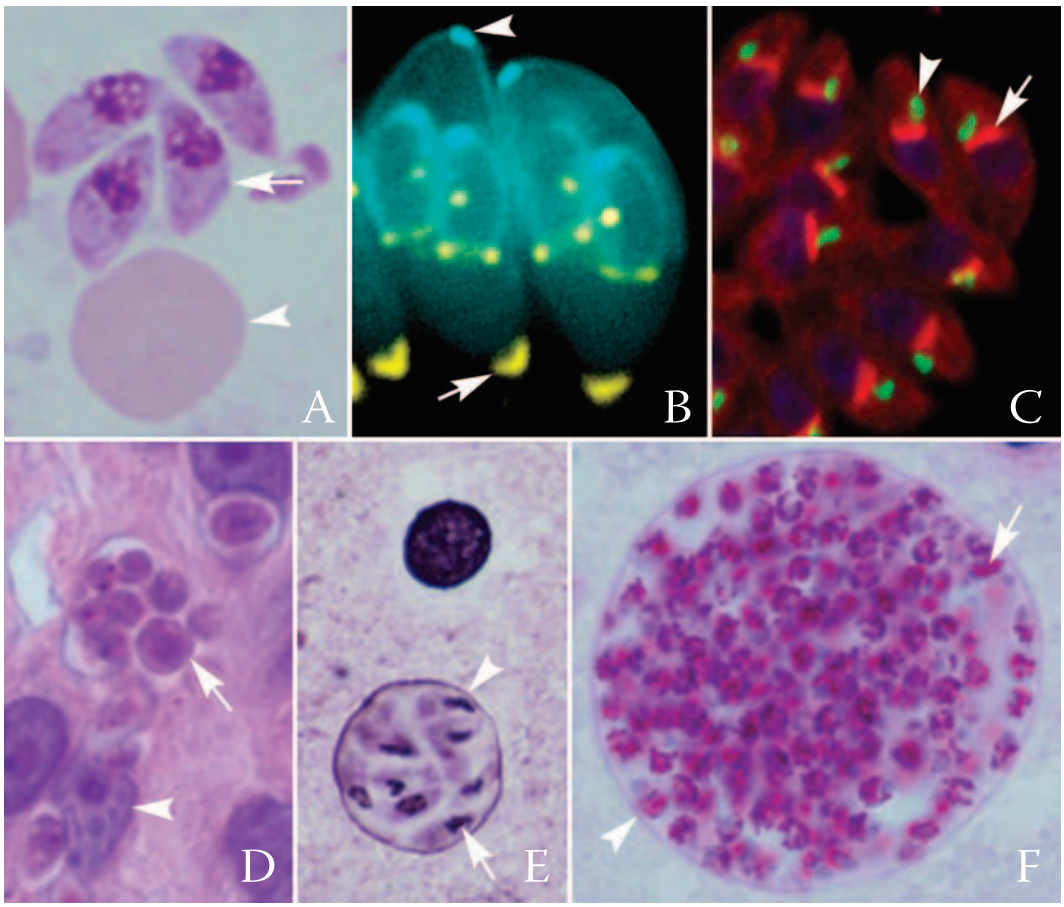

Figure 1.2 Tachyzoites (A–D) and tissue cysts (E,F) of *T. gondii.* **A.** Tachyzoites (arrow) are crescent shaped and as long as the red blood cell (arrowhead), impression smear, mouse lung. Giemsa. **B.** Fluorescent image of two dividing tachyzoites, expressing YFP-±-tubulin (cyan) and RFP-TgMORN1 (yellow). The apical ends (arrowhead) of the parasites are oriented toward the top of the image. The two dome-like structures in each parasite are daughter cortical cytoskeletons highlighted by YFP-±-tubulin (cyan) and RFP-TgMORN1 (yellow). Arrow points to nonoconidal end. Courtesy of Drs. K. Hu and John Murray. **C**. Group of tachyzoites showing Golgi (Grasp-mRFP; red, arrow) and apicoplast (anti-acyl carrier protein with Alexa 488; green, arrowhead) with DAPI staining. Courtesy of Dr. Boris Striepen. **D.** Group of tachyzoites (arrow) in a parasitophorous vacuole in the lamina propria of small intestine of cat. Dividing tachyzoites (arrow) are bigger in size and plump. Arrowhead points to a host cell nucleus. Histologic section. H and E. **E.** Small tissue cyst. Note thin silver-positive cyst wall (arrowhead) and bradyzoites with terminal nuclei (arrow). Impression smear, mouse brain. Wilder's silver stain plus Giemsa. **F.** Tissue cyst with a thin PAS-negative (or faintly stained) cyst wall (arrowhead) and intensely PAS-positive bradyzoites (arrow). Histologic section of brain. PAS reaction counter stained with hematoxylin.

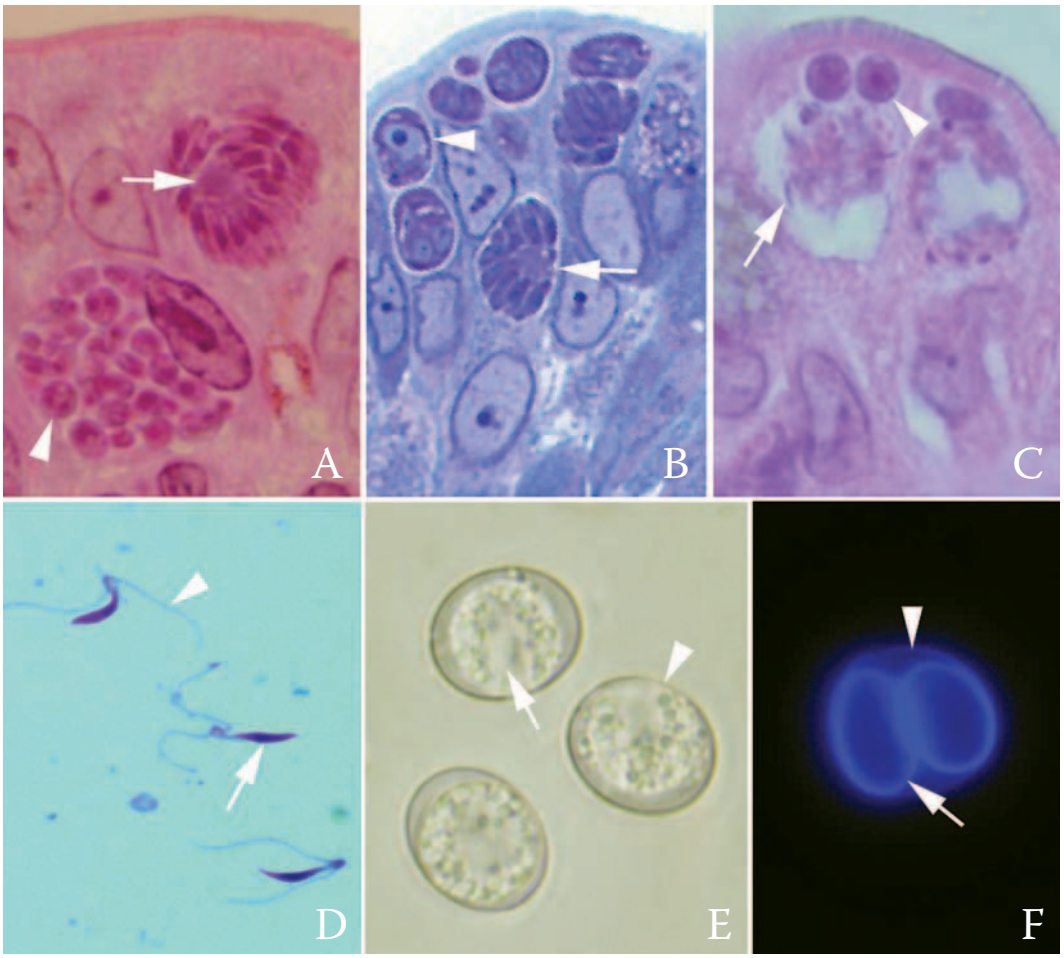

Figure 1.17 Enteroepithelial stages of *T. gondii.* **A.** Type C (arrow) schizont with a residual body and a type B schizont with a hypertrophied host cell nucleus (arrowhead) in sections of small intestine of cat fed tissue cysts 52 hr p.i. **B**. A mature schizont (arrow) and developing stages (arrowhead) in section of the epithelium of small intestine. 7 days p.i. All stages are located above the nuclei of enterocytes. Toluidine Blue. Courtesy of Dr. David Ferguson. **C.** Two male gamonts with peripherally located microgametes (arrow) and uninucleate organisms (arrowhead) in section of the epithelium of small intestine. 5 days p.i. Hand E stain.**D.** Three biflagellated microgametes. Note long slender flagella (arrowhead) and the small microgamete nucleus (arrow). Impression smear of small intestine, 5 days p.i. Giemsa stain **E.** Three unsporulated oocysts in cat feces. Note thin oocyst wall (arrowhead) and an unsporulated mass called sporont (arrow). Unstained. **F.** An autofluorescing sporulated oocyst, ultraviolet. Note thin oocyst wall (arrowhead) and two sporocysts (arrow). Courtesy of Dr. Bill Everson.

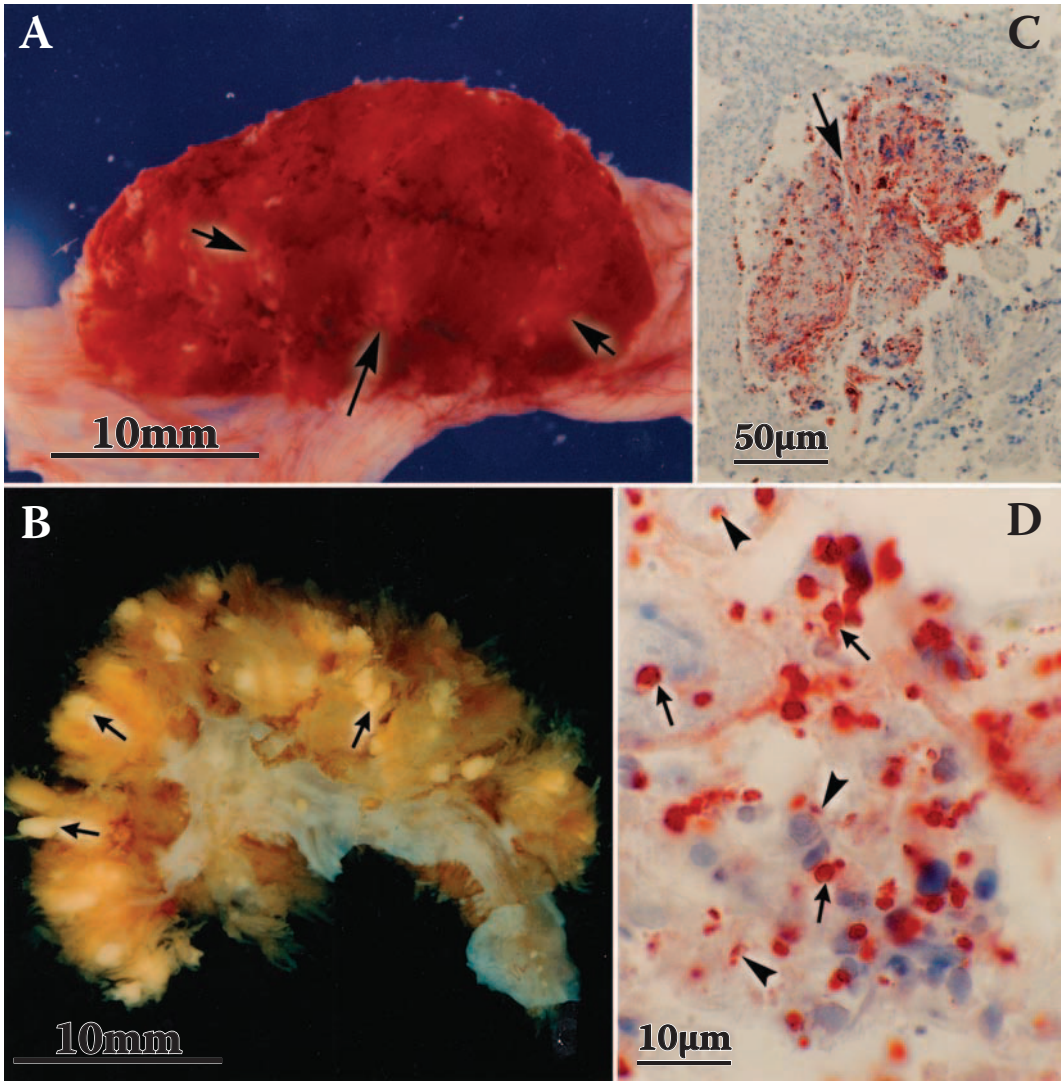

Figure 1.31 *T. gondii*-induced placentitis in naturally infected goat (A, C, D), and sheep (B). Both of these animals aborted dead fetuses. Placenta in goats and sheep consists of numerous (>300) cotyledons, the site of fetal attachment. *T. gondii* causes necrosis of the cotyledons that can be macroscopic. **A.** Grossly visible foci of necrosis (arrows) in fetal cotyledon. Unstained. **B.** Section of fetal cotyledon. Note foci of necrosis (arrows). Unstained. **C.** Histologic section of fetal cotyledon. Note a focus of necrosis and mineralization. IHC staining with *T. gondii*-specific antibodies. **D.** Higher magnification of the lesion in **(C)**. Note numerous intact tachyzoites and particulate antigen from degenerating tachyzoites. From Dubey and Stewart.[391a]

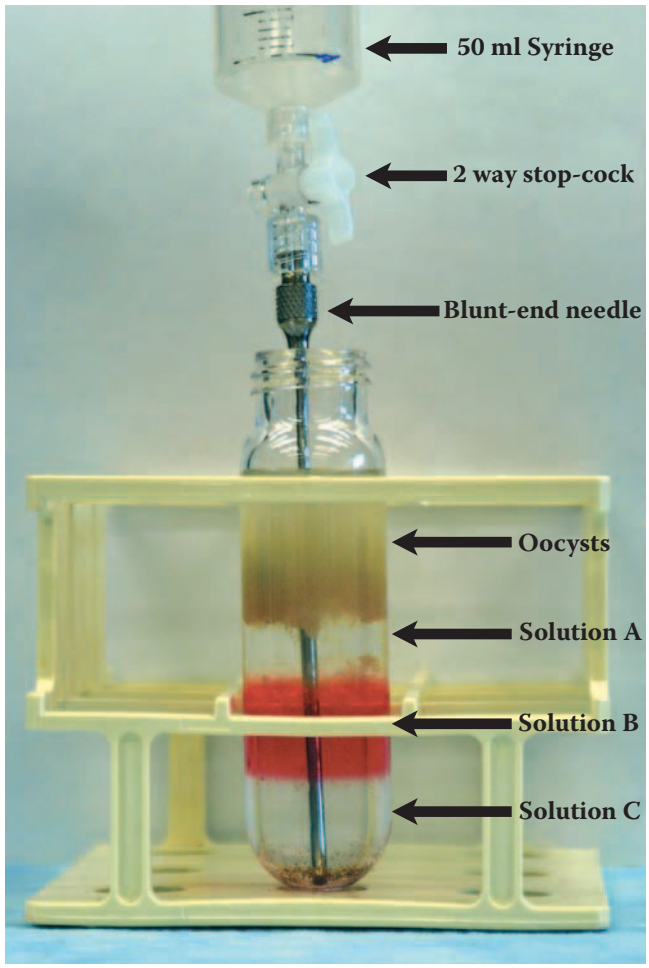

Figure 1.32 Cesium chloride gradient for separating *T. gondii* oocysts from cat feces. Courtesy of Dr. Eric Villegas.

Life Sciences

Found worldwide from Alaska to Australasia, *Toxoplasma gondii* knows no geographic boundaries. The protozoan is the source of one of the most common parasitic infections in humans, livestock, companion animals, and wildlife, and has gained notoriety with its inclusion on the list of potential bioterrorism microbes. In the two decades since the publication of the first edition of **Toxoplasmosis of Animals and Humans** there has been an explosion of knowledge concerning *T. gondii* and toxoplasmosis. Still used extensively as a cell model, its genome has recently been published, making it a subject of even greater scientific interest. Keeping the organizational style that made the previous edition so popular, this second edition has been completely revised and updated.

New in the Second Edition:

- Expanded information on the cultivation, maintenance, and preservation of *T. gondii*
- Expanded information on the cell biology and molecular biology of the parasite
- Coverage of food safety concerns and methods to kill Toxoplasma in meat
- Reviews all literature from the past 20 years for each domestic animal
- Summarizes information on the worldwide prevalence of toxoplasmosis in pregnant women and the devastating disease it can cause in newborns

Written by one of the pioneers of the field, the book provides unique information on all known host types for this parasite. It distills the voluminous and potentially confusing scientific literature that has grown geometrically in the 20 years since the publication of the first edition into a comprehensive resource. The single-author approach ensures a strong foundation in biology and a seamless integration of topics.

The new edition of this groundbreaking work is the only volume to cover toxoplasmosis of animals and humans thoroughly in one source. It supplies an entry point to further research by cutting through the morass of literature to identify the most relevant references.

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ISBN: 978-1-4200-9236-3

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