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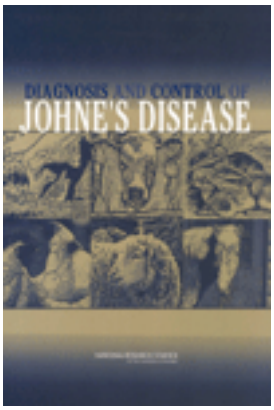

Diagnosis and Control of Johne's Disease

Committee on Diagnosis and Control of Johne's Disease, National Research Council

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DIAGNOSIS AND CONTROL OF JOHNE'S DISEASE

Committee on Diagnosis and Control of Johne's Disease
Board on Agriculture and Natural Resources
Division on Earth and Life Studies

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This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

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Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by Dr. Norman Cheville. Appointed by the National Research Council, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

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Preface

Control or eradication of important livestock diseases has been a top priority for the United States Department of Agriculture (USDA) throughout much of the previous century. Most efforts have involved mandatory national eradication programs. But mandatory programs bring a host of difficulties: they require a large program infrastructure, administration of indemnity programs, and constant scrutiny to uncover and eliminate loopholes. As most eradication programs near completion, attention is turning to other diseases that previously were not of sufficiently high priority to warrant mandatory control. These other diseases pose new challenges and may require novel approaches for control.

Johne's disease (JD) is one such disease. Although its significance as an animal health and economic problem has been recognized for years, it is only now beginning to receive attention and prioritization for control. The United States Animal Health Association's National Johne's Disease Working Group has been leading the effort to provide national coordination and minimum standards for voluntary control, and USDA recently stepped in with the appointment of a national JD coordinator. Some of this recent attention is being driven by concern over the potential role of *Mycobacterium avium* subsp. *paratuberculosis (Map),* the causative agent of JD, in Crohn's disease in humans. This issue warrants concern because it could affect the diagnosis and

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treatment of Crohn's disease and because it could change strategies for control of JD in livestock.

The USDA saw an opportunity to address this important issue and to facilitate progress in the control of JD in livestock. In August 2001, the Board on Agriculture and Natural Resources was asked to convene the Committee on the Diagnosis and Control of Johne's Disease. The committee's task was to review and synthesize the state of knowledge of JD in livestock, to evaluate current control plans, to critically assess the evidence for and against a causal relationship between *Map* and Crohn's disease, and to provide recommendations for control and research.

The committee had a relatively short period in which to accomplish these ambitious goals, but I am pleased that its members approached the task with great enthusiasm and determination. The committee met twice in Washington D.C., and once in Hershey, Pennsylvania in conjunction with the annual meeting of the United States Animal Health Association. The latter meeting included a public workshop, where committee members heard from experts on JD and from a leading Crohn's disease patient advocacy group. At its final meeting, the committee received an update on research into the link between *Map* and Crohn's disease and a progress report on the *Map* genome project at the National Animal Disease Laboratory.

This report represents the outcome of many hours of hard work and spirited debate by committee members. I am grateful for their talent, dedication, intellectual integrity, and perseverance in the face of many challenges posed by our schedule and task. I am also very grateful to the Board on Agriculture and Natural Resources for the great care they took in assembling such a distinguished panel and for the opportunity to serve with them on this important committee. Finally, on behalf of the committee I would like to thank and acknowledge study director Tina Rouse and project assistant Cindy Lochhead for their outstanding dedication and persistence. Without their expertise, organizational skills, resilience, and resourcefulness, we could not have completed the task.

BRUCE A.RIDEOUT, *Chair*
Committee on Diagnosis and Control of Johne's Disease

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Contents

EXECUTIVE SUMMARY 1
The Task 2
Conclusions 3
Disease Control 4
Education and Training 7
Research 7

1 INTRODUCTION 13
The Committee's Task 15

2 JOHNE'S DISEASE IN DOMESTICATED AND WILD ANIMALS 16
Spectrum of Disease in Domesticated Animals 18
Spectrum of Disease in Other Animals 20
Pathology 21
Epidemiology 24
Modes of Transmission 27
Pathogenesis in Cattle 37

3 DIAGNOSTICS 45
Testing of Domesticated Animals 45

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Molecular Methods 59
Epidemiologic Tools 62

4 CONTROL PRINCIPLES AND PROGRAMS 66
Control Principles 66
Current Control Programs 78
Principles of Control Implementation 97

5 ECONOMIC IMPLICATIONS OF JOHNE'S DISEASE 99
Trade 100

6 JOHNE'S DISEASE AND CROHN'S DISEASE 104
Crohn's Disease 105
Implications of the Link 107
Conclusions 119

7 CONCLUSIONS AND RECOMMENDATIONS 121
Control Programs 122
Control Recommendations 124
Education and Training Recommendations 126
Research Recommendations 127

REFERENCES 133

APPENDIXES

A Interpretation of Diagnostic Results 175

B USAHA Voluntary Johne's Disease Herd Status Program for Cattle 179

C USDA/APHIS Draft Johne's Disease Control Program 194

ABOUT THE AUTHORS 221

BOARD ON AGRICULTURE AND NATURAL RESOURCES PUBLICATIONS 226

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Tables, Boxes, and Figures

TABLES

2–1	Species Infected with *Mycobacterium avium* subsp. *paratuberculosis*	16
2–2	Clinical Stages of Johne's Disease in Cattle	20
2–3	Global Seroprevalence of Johne's Disease in Dairy Cattle	25
2–4	Seroprevalence of Johne's Disease in U.S. Dairy Cattle in 1996	26
2–5	Environmental Factors Associated with *Mycobacterium avium* subsp. *paratuberculosis*	32
2–6	*Mycobacterium avium* subsp. *paratuberculosis* Response to Heat Treatment, Pasteurization, and Other Processes	35
3–1	Detectability of Johne's Disease at Varying Clinical Stages	46
3–2	Comparison of Diagnostic Tests for Johne's Disease	47
3–3	Utility of Diagnostic Tests in Clinical Stages of Johne's Disease	47
4–1	Standard-Track Certification from the U.S. Voluntary Johne's Disease Herd Status Program for Cattle	81
4–2	Fast-Track Certification from the U.S. Voluntary Johne's Disease Herd Status Program for Cattle	81
4–3	Test-Positive Requirements from the Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program	84

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Diagnosis and Control of Johne's Disease
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4–4 Standard-Track Test-Negative Components from the Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program 84
4–5 Fast-Track Test-Negative Components from the Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program 85
4–6 National Veterinary Services Laboratory Approved Johne's Disease Tests 87
4–7 Canadian and U.S. National Veterinary Services Laboratory Approved Laboratories for Johne's Disease 87
4–8 Components of State Johne's Disease Herd Status/Control Programs 96
4–9 Components of Nationwide Johne's Disease Herd Status/Control Programs 97
6–1 Clinical Features of Crohn's Disease and Johne's Disease 111
6–2 Pathologic Features of Crohn's Disease and Johne's Disease 112
6–3 Antimycobacterial Therapy Studies for Crohn's Disease 113

BOXES

ES-1 Components of a Coordinated Industry/Government JD Control Program 5
4–1 Approval Process for Laboratories Performing Official Johne's Disease Tests 86
4–2 Approval Process for Laboratories Performing Johne's Disease Screening Tests (Serology Tests) 86
7–1 Components of a Coordinated Industry/Government JD Control Program 125

FIGURES

4–1 Johne's Disease State Control Programs, January 2002 92
4–2 Johne's Disease State Certification and Status Programs, January 2002 93
4–3 Johne's Disease State Advisory Committees, January 2002 93

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Executive Summary

Johne's disease (JD) is a chronic, progressive intestinal disease caused by infection with *Mycobacterium avium* subspecies *paratuberculosis (Map)*. The agent was first identified in European cattle a century ago, and it was discovered in the United States in the early 1900s. Control of JD has been problematic because it has a long incubation period, it is clinically similar to many other common diseases of cattle, available diagnostic tests are expensive and relatively low in sensitivity, and there are no accepted standards for diagnosis and control. These problems are compounded by a lack of awareness of the disease and the fact that its slow progression makes financial losses not easily perceptible to the individual producer.

In recent decades, concern has been growing about the apparent increase in the global prevalence of JD and the attendant animal health, economic, and trade implications. The recognition that the *Map* host range includes ruminant and nonruminant wildlife also has raised concerns. The spread of JD from domesticated animals to wildlife could alter wildlife populations, and, if wildlife reservoirs become established, it could limit the ability to control or eradicate JD in domesticated livestock.

Finally, there is increasing concern over the human health implications of JD. The possibility that *Map* infection could be a cause of some cases of

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Crohn's disease in humans, combined with concern that *Map* is becoming widespread in the environment and the food chain, could transform JD into a serious public health problem.

Several approaches have been taken to begin addressing growing concern over JD. The National Johne's Disease Working Group (NJWG) of the United States Animal Health Association has implemented an educational program to increase awareness among livestock producers, developed a voluntary herd status program to encourage producers to rear JD-free herds, and has developed minimum standards for state JD control programs. All of these efforts are yielding positive results, but the lack of a nationally coordinated control effort has limited progress and the sense of urgency continues to grow.

THE TASK

Participants at national and international meetings have reviewed the status of JD research and developed strategies to control the spread of *Map*. In July 2000, the U.S. Department of Agriculture (USDA) requested that the Board on Agriculture and Natural Resources of the National Academies convene a committee on the diagnosis and control of JD. Specifically, the committee was instructed to conduct a thorough review, evaluation, and compilation of all scientific research related to JD in domesticated and wild ruminants. The committee's task was to: (1) review and synthesize current information regarding diagnostic techniques, mode of transmission, clinical expression, global prevalence, and potential animal and human health implications associated with JD in domesticated and wild ruminants; (2) evaluate current programs for controlling and preventing JD in ruminants; (3) provide policy recommendations for identification, monitoring, and management strategies applicable to U.S. livestock herds; (4) conduct an objective, critical assessment and summary of the state of knowledge regarding the relationship of JD in ruminants and Crohn's disease in humans; and (5) provide recommendations on future research priorities and potential mechanisms to facilitate prevention and control of the disease.

The committee reviewed the literature on diagnosis, modes of transmission, clinical expression, global prevalence, and potential animal and human health implications of JD with the following goals in mind:

- Determine whether JD presents a problem sufficient to warrant control efforts.
- Objectively review the data that provide the basis for control plans.
- Identify significant gaps in knowledge that affect diagnosis and control.
- Differentiate well-established facts from dogma or prevailing opinion.
- Determine whether available diagnostic tests are adequate for control programs.

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- Make recommendations on the research needed to fill in knowledge gaps.

The committee also evaluated existing state, national, and international control programs with the goal of identifying sound control principles to guide program development and to make recommendations for a national control program.

Finally, the committee evaluated the evidence for and against a role for *Map* in some cases of Crohn's disease in humans. Considerable controversy has surrounded this issue and there are diverse viewpoints about how some of the evidence should be weighed against other evidence. This problem is not unique to Crohn's disease. Determining the cause of chronic human diseases is a difficult and often controversial process. One outcome of the debate has been the proposal by Hill and Evans (Evans, 1976) for a set of objective criteria, which, if met, could be considered sufficient evidence to determine that a causal relationship exists between an agent and a chronic disease. To approach this important task most objectively, the committee used the Hill-Evans criteria to develop a list of the research results that would be necessary to establish with reasonable certainty that infection by *Map* causes some cases of Crohn's disease. The list of necessary research results was developed before the literature review and it was used to evaluate the strength of the evidence.

CONCLUSIONS

Based on its review and synthesis of the literature, the evaluation of existing control and herd status programs, and information presented at the public workshop, the committee reached the following conclusions.

CONCLUSION 1. JD is a significant animal health problem that warrants implementation of control programs tailored to specific animal species and specific segments of the agriculture industry. Furthermore, JD control deserves high priority from USDA, individual states, and industry. The significance of the disease derives primarily from its consequences for animal and herd health, for the agriculture industry, and for national and international trade. The current concern about JD in industry and government agencies and the potential link with Crohn's disease in humans provide additional support for making JD control a high priority.

CONCLUSION 2. There remains insufficient evidence to prove or disprove that *Mycobacterium avium* subsp. *paratuberculosis* is a cause of some or all cases of Crohn's disease in humans; a new approach is needed to resolve the issue—one that is based on a research agenda that will provide answers to specific criteria set forward in Hill-Evans postulates. A causal link between *Map* and Crohn's disease remains a plausible hypothesis that warrants a new research approach and steps by industry and government agencies to identify and mitigate avenues of exposure.

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CONCLUSION 3. Available diagnostic tests and information about the biology of JD and methods to control it are adequate for immediate implementation of control programs.

CONCLUSION 4. There are significant gaps in knowledge about some areas relevant to control that are discussed in the recommendations section. The committee emphasizes that closing knowledge gaps will improve control programs, although the need for more information should not delay implementation.

CONCLUSION 5. Control will require a long-term commitment and iterative program implementation to maximize the chance of success. This commitment must come from all constituencies, including USDA, state agencies, and industry.

CONCLUSION 6. Because control of JD is of greatest concern to the dairy industry, much of the emphasis in control recommendations is directed there. Other industries, however, should consider this an opportune time to deal aggressively with the disease, before infection prevalence increases and the disease becomes more widespread.

CONCLUSION 7. The USDA National Animal Health Monitoring System (NAHMS) prevalence surveys have been a critical element in laying the groundwork for control programs.

CONCLUSION 8. The Voluntary Bovine Johne's Disease Control Program proposed by the NJWG has most of the elements necessary for a successful control program, but prospects for success are and will be limited by a lack of uniform implementation among individual states.

CONCLUSION 9. The committee endorses the NJWG's efforts in educating producers and veterinarians, and advocates the expansion of these efforts.

DISEASE CONTROL

The occurrence of a particular infectious agent in a herd is a consequence of one or more environmental or management risk factors being out of control. If the focus is placed on controlling the risk factor(s), which may be common to a number of infectious agents, rather than on the agent itself, a producer will be more likely to adopt a control practice because the incidental control of other agents will result in a greater return on the investment. While the committee strongly endorses a Best Management Practices approach to control, it felt that control programs should initially focus on JD to take advantage of growing support for control of the disease. In addition, it is

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anticipated that funding for various aspects of control will be more readily available through government-industry partnerships if control has an easily identifiable target, such as JD, rather than a broader concept of Best Management Practices. As control efforts progress, an incremental transition to a Best Management Practices approach should be more feasible.

RECOMMENDATION 1. *An integrated, bottom-up approach to on-farm disease control is required that meets the needs of the livestock producer and motivates behavioral change, with support at broader industry, state, and federal levels. Components of such a control program are described in Box ES-1.*

BOX ES-1 COMPONENTS OF A COORDINATED INDUSTRY/ GOVERNMENT JOHNE'S DISEASE CONTROL PROGRAM

The following elements are important for a successful control program. At the farm level:

1. On-farm risk assessment and development of a farm plan
2. Manure management that minimizes potential for transmission of pathogens by the fecal-oral route
3. Protection of young stock
4. Acquisition of replacement animals free from infection by *Map* and other significant pathogens that are shed in the feces
5. Removal of infected animals from the farm
6. Reduction of environmental contamination by *Map*

At the state-federal program level:

1. Minimum national standards for program implementation
2. Performance-based criteria for diagnostic testing and laboratory accreditation
3. Rapid identification and protection of JD-free herds that can be used to provide *Map*-free replacement animals
4. Incremental implementation, progressing from a voluntary herd status program to a system of strong (preferably market-based) incentives for participation and disincentives for nonparticipation, culminating in a mandated herd control program, if JD eradication is the ultimate goal
5. A gradual transition from an exclusive focus on Map to a broader health and market assurance program that emphasizes Best Management Practices to prevent the spread of all pathogens by the fecal-oral route

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6. A mechanism for periodic program review and self-correction
7. A program to prevent the reemergence of disease after low prevalence or eradication is achieved

Recommendations for a stepwise expansion of the federal role in JD prevention and control:

1. The government should promulgate uniform methods and rules (UM&R) for voluntary JD status and control programs. The federal standards would provide consistent definitions and program guidelines for a baseline across all states. Individual states could mandate additional requirements.
2. The federal government should provide control infrastructure, including support and incentives to upgrade diagnostic laboratories across the United States, to promote large-scale testing.
3. All states should be required to implement a control program that is voluntary for producers in accordance with the UM&R.
4. Producers should be encouraged to test all herds and register them either in status programs or in control programs, based on test results. Federal subsidies may be needed to cover the cost of initial testing, in order to encourage participation.
5. Federal restrictions should continue on interstate and international transport of cattle from *Map*-positive herds.
6. A federal plan should be established to monitor the success of the control program. The plan should provide for periodic program review and self-correction.

General control program outlines for a given category of management and husbandry situation will be the same, but must be sufficiently flexible to be easily adaptable to the specific circumstances of each farm. Finally, the information must be packaged and delivered in a manner that is in harmony with the style with which the producer manages information and can motivate them to change their behavior. This motivation may require feedback signals in the form of market price differentials established through testing of the farm product by the downstream purchaser.

While control programs for dairy herds may be of highest priority, control programs for beef cattle, sheep, goats, and captive cervids should be developed and implemented. Control programs for zoo animals and wildlife should also be monitored to ensure that a non-domesticated animal reservoir does not compromise control efforts for any species.

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EDUCATION AND TRAINING

The National Johne's Working Group has made education of producers and veterinarians a high priority. The committee endorses this effort and offers the following additional recommendations for action by federal and state authorities:

RECOMMENDATION 2. *Commodity-oriented (dairy, beef, sheep, goat, llama) materials should be developed that are standardized nationally, and a rationale and guidelines for development of control and certification plans should be provided. These should not be considered the same as national program standards, but they should serve as the information base for participation in national programs.*

RECOMMENDATION 3. *Informational resources should be developed for practicing veterinarians that includes guidance on diagnostic test selection, sample size and selection of animals for testing, interpretation of test results, development of risk assessment methods, writing of herd plans, and monitoring of compliance and progress.*

RECOMMENDATION 4. *Educational resources that emphasize control of risk factors (Best-Management Practices) should be developed instead of materials that emphasize control of a single etiologic agent.*

RECOMMENDATION 5. *Training programs are needed for state Johne's coordinators, USDA personnel, practicing veterinarians, and laboratory personnel to ensure a uniform base of knowledge and practice.*

RESEARCH

The committee identified significant gaps in the current state of knowledge of the pathophysiology, immunology, diagnosis, and control of JD in domesticated livestock and in wildlife. Choosing the research projects needed to fill those gaps will be important to the success of any JD herd status or control program. The issues also are complex enough to warrant the convening of a USDA expert panel to formulate consensus methods to address the research questions. The committee considered ongoing research to be important for the success of any control program and therefore felt that a research element should be integral to future program development. The committee developed recommendations in several areas.

Epidemiology of Map Infection and JD

The committee found significant gaps in the understanding of the epidemiology of JD that could affect the success of proposed control programs. In particular, the committee recommends five additional areas of research.

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RECOMMENDATION 6. *Age-dependent dose-response curves are needed to clarify the magnitude and significance of age-related susceptibility or resistance to infection and the degree to which horizontal transmission occurs in different age groups. Use of epidemiologic modeling would help to determine the best measure of infectivity.*

With the increase in concentrated calf-rearing operations, there is an urgent need to investigate the possibility of horizontal transmission in young animals. Current control strategies assume that horizontal transmission among adult cattle is insignificant, but this should be confirmed because the success of control strategies could be at stake.

RECOMMENDATION 7. *The effects of chronic, low-level exposure on infectivity and on the outcome of infection should be studied.*

Much of the data on infectivity and age susceptibility has been derived from decades-old studies in which one or a few large infective doses typically were administered. Although this provides valuable information, because it does not mimic natural exposure, the conclusions that can be drawn are limited. It would be helpful to have a better understanding of the outcome of chronic, low-level, or intermittent exposure to *Map* in the environment.

RECOMMENDATION 8. *Experimental studies and field investigations of natural infection in nonruminant and ruminant wildlife in the United States should focus initially on native lagomorphs and other small mammals prevalent on or around livestock operations with endemic JD.*

Recent investigations on the role of wildlife in the epidemiology of JD in livestock in Scotland, Australia, and the Czech Republic have yielded interesting results. The identification of endemic *Map* infections in European rabbits in Scotland has important ramifications for control programs there. Little work has been done in the United States on the susceptibility of nonruminant wildlife to *Map* infection. Determining the prevalence of *Map* infection in wildlife on or around livestock operations will be important to understanding the success or failure of any livestock JD control programs.

RECOMMENDATION 9. *Results of diagnostic testing, control practices, and other epidemiologic data should be evaluated and used to answer remaining research questions and to refine and optimize control programs.*

RECOMMENDATION 10. *The USDA NAHMS prevalence surveys should continue, with attention paid to maximizing the data obtained from the samples collected through "add-on" projects and investigations.*

As control programs are implemented, they present opportunities to take advantage of resulting "natural experiments." The committee recommends that these opportunities not be lost. Results of the two investigations proposed

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above will help regulators to establish a scientific basis for several control measures, including how long to wait before restocking contaminated land, whether environmental decontamination can expedite restocking, and whether removal of calves from dams at birth is the best means of breaking the transmission cycle.

Diagnostics and Immunology

Although the committee acknowledges that available diagnostic tools are sufficient to implement control programs, significant deficiencies still exist. To address these important gaps, the committee recommends the following:

RECOMMENDATION 11. *Epidemiologically sound sampling and sample-pooling protocols should be developed and validated to facilitate screening and monitoring of large cattle herds and sheep flocks.*

Recent research in Australia suggests that pooling of sheep fecal samples could enable more cost-effective flock screening for control programs. This work should be repeated in the United States and expanded to include cattle. Another important need is for a rapid, sensitive test to detect the presence of *Map* in bulk-milk samples. This would promote more efficient and cost-effective collection of herd prevalence data, which will be important for control. Some promising studies have been conducted, but additional research is needed in this area.

RECOMMENDATION 12. *Sensitive and specific serologic and fecal culture methods should be developed and validated for use in sheep and goats.*

The development of diagnostic tests for JD in sheep, goats, and other species has trailed that for cattle. Although much of the current control emphasis is on dairy cattle, development of more sensitive and specific tests is needed for these other species. Recent reports suggest that the difficulty in isolating sheep strains of *Map* by fecal culture has largely been overcome, but methods are still slow and not ideal for use in control programs.

RECOMMENDATION 13. *Methods for detecting an early, specific immune response to* Map *should be developed.*

There are no reliable tests to identify animals in the early stages of infection, before fecal shedding. Early identification of infected animals would be helpful for control programs, especially for prepurchase testing of replacement animals. Exposure to other mycobacteria, such as *M. avium* subsp. *avium,* is likely to be common in cattle, so it is essential that any test to identify animals in the early stages of infection be highly specific for *Map*. This might require identification of unique antigenic epitopes in *Map,* against which an early immune response is generated.

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Map Genome Studies

As the committee evaluated knowledge gaps and research needs, the importance of complete sequencing of the *Map* genome became evident. Sequencing would yield many benefits, including identification of unique *Map* antigens for development of diagnostic tests and vaccines, improvement of diagnostic methods based on PCR, and identification of potential virulence factors. The *Map* genome sequencing project at USDA's National Animal Disease Center is nearing completion, and the committee strongly recommends the following:

> **RECOMMENDATION 14.** *USDA and other agencies should seize the opportunity presented by the completion of the* Map *genome project to accelerate progress in JD research, diagnostic test improvement, and vaccine development.*
>
> Information about the completion of this project needs to be disseminated to the international research communities for JD and Crohn's disease, and the sequence data should be made available as soon as possible. Research funds should be directed to research and development that use the results of the *Map* genome project. The paucity of funds available for JD research has limited progress in several important areas. The completion of the *Map* genome project provides a unique opportunity to correct this oversight, and it should not be neglected.

Vaccine Development

Current vaccines for *Map* are highly problematic. There are conflicting data on their ability to reduce shedding of *Map,* and the fact that they generate cross-reactions to intradermal tests for *M. bovis* makes them unsuitable for widespread use in control programs. Because vaccines can expedite the reduction of disease prevalence, the committee recommends the following:

> **RECOMMENDATION 15.** *Research should be done on the nature and evolution of the immune response to* Map, *and ways to modulate the immune response to elicit protection should be studied.*
>
> **RECOMMENDATION 16.** *Research is needed on the feasibility of using recombinant-vaccine technology to create a vaccine that generates a specific, protective immune response in domesticated livestock without interfering with diagnostic tests for JD, bovine tuberculosis, or other diseases.*

Development of an efficacious vaccine will require identification of unique *Map* antigens that will elicit a protective immune response without

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generating cross-reactions to other mycobacteria. The *Map* genome project has the greatest potential for providing the basis for these advances.

Human and Animal Health Issues

After evaluating all of the available evidence for and against a causal role for *Map* in Crohn's disease, the committee was of the unanimous opinion that the evidence was insufficient either to establish or to refute a causal connection. The committee considered the following research to be important to the resolution of this question:

RECOMMENDATION 17. *A blinded study should be done for the detection of* Map *and* Map *RNA-DNA in identical coded intestinal tissue samples sent to various laboratories using standardized methods for* Map *culture and detection. This will help clarify the degree to which conflicting research results have been the result of variations in methods versus operators.*

RECOMMENDATION 18. *A large-scale, double-blind, multicenter study should be done to detect the presence of* Map *and* Map *RNA-DNA in tissue specimens from patients with Crohn's disease, using the same standardized methods as above. The specimens should be stratified by type of disease, duration of disease, presence or absence of known Crohn's susceptibility genes, and treatment. Control subjects without Crohn's disease should be included in the study.*

RECOMMENDATION 19. *A large-scale, multicenter, double-blind study of the treatment of Crohn's disease patients with anti-Map combination antimicrobial therapy should be undertaken. The patients should be stratified by type of disease, duration of disease, presence or absence of known susceptibility genes, treatment, and presence or absence of* Map *by culture or PCR methods. There also should be an appropriate control group of patients with Crohn's disease who do not receive anti-*Map *therapy.*

RECOMMENDATION 20. *A multicenter, double-blind study is needed on the presence of* Map, Map *antigens, and* Map *RNA-DNA in breast milk of lactating women with Crohn's disease, compared to controls. Finding such in lactating women with Crohn's disease would provide strong support for the proposed connection between* Map *and Crohn's disease.*

If a subset of CD patients responds to *anti-Map* therapy, or *Map* is otherwise implicated as a cause of CD in a subset of patients, research on methods to better identify this subset will be needed. Other research considered important by the committee included on-going studies of Crohn's susceptibility genes and familial tendencies, gene microarray studies to determine which genes

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are up- and down-regulated in Crohn's disease and animal models, especially in genetically altered animals.

RECOMMENDATION 21. *The National Institutes of Health or a similar body should convene a panel with experts in gastroenterology, Crohn's disease, infectious disease, mycobacteriology, biostatistics, epidemiology, etc., to define the precise study designs and to rank order the various studies to be done.*

Although the committee did not find sufficient evidence to implicate *Map* as a cause of Crohn's disease, there was consensus that efforts to identify and mitigate avenues of exposure to *Map* would be prudent while awaiting definitive resolution of this important question.

Identifying environmental sources of *Map* also is an important element of JD control in livestock, so there is additional justification for such investigations. The committee therefore recommends researching the following projects:

RECOMMENDATION 22. *Research should be conducted to determine the prevalence of viable Map in potable-water supplies, streams, ponds, and other bodies of water with potential for* Map *contamination. This may require development of better methods for identifying and quantifying* Map *in environmental samples.*

RECOMMENDATION 23. *Additional studies are needed to determine whether* Map *is present in retail milk or other dairy products, as well as in pasteurized colostrum or commercial colostrum replacers that are fed to calves.*

RECOMMENDATION 24. *Research should be done to determine the prevalence of viable* Map *in peripheral lymph nodes, muscle, and other tissues that are processed for human consumption.*

RECOMMENDATION 25. *Research should be done to determine the prevalence and concentration of* Map *in other environmental materials likely to be contaminated with ruminant manure and associated with exposure to humans or susceptible animals. Those materials could include composted manure, fruits and vegetables, pastures, and crops fed to livestock.*

If a causal relationship is established between human *Map* infection and even a subset of Crohn's disease cases, the above research recommendations will be essential for implementation of new control programs aimed at protecting public health by minimizing exposure to *Map*. Additional research would then be needed to develop methods for routine screening of dairy products, meat, and meat products for *Map*.

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1

Introduction

Johne's disease (JD), sometimes called paratuberculosis, is a chronic, progressive intestinal disease caused by infection with *Mycobacterium avium* subsp. *paratuberculosis*. A variety of animals can be infected, but the primary hosts are ruminants—cattle, sheep, goats, bison, deer, and other non-domesticated hoofstock. The clinical syndrome was recognized in the early 1800s, but it was not until 1895 that Johne and Frothingham demonstrated the presence of mycobacteria in the intestines of affected cattle in Germany. By the early 1900s, the disease had been found in several European countries and in the United States. Since then, it has spread throughout the world and is now found in virtually every country where there is a significant livestock industry.

The causative agent has undergone taxonomic reclassification and name changes over the years. There is still some debate, but in 1990 a proposal was made to change the name of the agent from *Mycobacterium paratuberculosis* to *Mycobacterium avium* subsp. *paratuberculosis* (which will be abbreviated as *Map* in this report) in recognition of its close genetic relationship to *M. avium.* Throughout this report, the Committee on the Diagnosis and Control of Johne's Disease uses the taxonomy name, although *M. paratuberculosis* is still in common usage. The proposed taxonomic reorganization placed *Map* in the group of mycobacterial agents known as the *Mycobacterium avium* Complex, or MAC. As a result, there are several places

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in the body of the report in which *Map* as a disease agent is discussed in the larger context of its position in the MAC.

Control of JD on farms has been difficult for several reasons. The disease has a long subclinical phase, during which animals can spread the infection without themselves exhibiting signs of illness. Current diagnostic tests are relatively insensitive at detecting this phase of the disease. It thus is a simple matter for a producer to unknowingly purchase a *Map*-infected animal, which can then spread the infection throughout a herd. Subsequent detection of JD in a herd can be delayed because of a lack of producer awareness, poor sensitivity of diagnostic tests when applied to individual animals, and the fact that JD is clinically similar to other common ruminant diseases. Finally, the lack of an efficacious vaccine means that control is undertaken primarily through management practices that are designed to interrupt transmission of the agent. Producers often are slow to adopt those new methods, however, because they often fail to recognize JD as a problem of sufficient economic importance to warrant dramatic management changes. This reluctance could limit the success of control programs that focus exclusively on *Map* as the disease agent.

Historically, control of JD in the United States has been left to the discretion of individual states. Some states have no program at all, others have chosen from a variety of voluntary options. The United States Animal Health Association has attempted to provide more coordination through the creation of its National Johne's Disease Working Group (NJWG), which promulgated a series of recommendations, including minimum standards for administering voluntary state herd JD status programs. NJWG also has developed educational programs to increase awareness among producers and an accreditation program for laboratories that test for JD. Despite significant strides, the lack of perceived priority or cost by the industry and veterinary profession, lack of market incentives, and lack of a nationally coordinated effort and supporting infrastructure have resulted in haphazard implementation of some of NJWG's important recommendations.

In recent decades, there has been growing concern over the lack of effective JD control; the apparent increase in global prevalence; and the implications for accompanying animal health, economies, and trade. The recognition that the *Map* host range includes ruminant and nonruminant wildlife also is alarming. The spread of JD from domesticated animals to wildlife could significantly alter some wildlife populations, and the development of wildlife reservoirs could hamper the ability to control or eradicate JD in domesticated livestock. Finally, there is increasing concern over the human health implications of JD in livestock. Although considerable controversy surrounds this issue, there is some evidence that *Map* plays a role in some cases of Crohn's disease in humans. There also is concern that *Map* is becoming more widespread in the environment and in the food chain (possibly through milk and meat), resulting in greater risk of human exposure. If *Map* were determined to be a human pathogen, JD would be transformed from an animal health and economic issue into a serious public health concern.

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THE COMMITTEE'S TASK

The current status of JD research has been reviewed at national and international meetings and strategies to control the spread of *Map* have been presented. As part of these endeavors, in July 2000 the U.S. Department of Agriculture requested that the Board on Agriculture and Natural Resources of the National Academies convene a committee on the diagnosis and control of JD. Specifically, the committee was instructed to conduct a thorough review, evaluation, and compilation of all scientific research related to JD in domesticated and wild ruminants. The committee's task was to: (1) review and synthesize current information regarding diagnostic techniques, mode of transmission, clinical expression, global prevalence, and potential animal and human health implications associated with JD in domesticated and wild ruminants; (2) evaluate current programs for controlling and preventing JD in ruminants; (3) provide policy recommendations for identification, monitoring, and management strategies applicable to U.S. livestock herds; (4) conduct an objective, critical assessment and summary of the state of knowledge regarding the relationship of JD in ruminants and Crohn's disease in humans; and (5) provide recommendations on future research priorities and potential mechanisms to facilitate prevention and control of the disease.

Much of the emphasis in this report is on knowledge and control of JD in dairy cattle. This emphasis is difficult to avoid, because most of the published research, diagnostic test development, epidemiology, and control efforts have focused on dairy cattle. This could be the result of the higher prevalence of JD in dairy cattle, the potentially greater economic consequences of JD for the dairy industry, or the fact that some dairy management practices facilitate the introduction and spread of the agent within a herd. This report mirrors the bias toward dairy cattle, but the committee strove to address significant gaps in knowledge, research needs, and control measures for other species of domesticated livestock and wildlife. The committee anticipates that progress with other species will accelerate as JD control in dairy cattle advances.

In addition to discussions and recommendations on specific topics, the committee presents a broad outline of the steps that should be taken to control the disease, reduce the spread of *Map,* and minimize effects of the disease in animals. The committee also makes recommendations on research that should be undertaken to enhance control programs, to develop improved diagnostic tests and an efficacious vaccine, and to clarify the role of *Map* in the pathogenesis of Crohn's disease.

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2

Johne's Disease in Domesticated and Wild Animals

Most information about Johne's disease (JD) comes from dairy cattle. However, it is important to consider how the disease also is exhibited in other domesticated and wild animals. The significance of species other than dairy cattle as potential sources of exposure could increase as control programs are implemented and as within-herd transmission declines on dairy farms. Table 2– 1 summarizes the reported clinical indications of JD in domesticated and wild animal species. The following summary begins with dairy cattle, which reflect closely the condition in beef cattle, and concludes with the current state of knowledge about other species.

Table 2–1. Species Infected with Mycobacterium avium subsp. paratuberculosis

Species	Location	Reference
Domesticated ruminants		
Cattle	Global	Buergelt et al., 1978b
Sheep	Global	Rajya and Singh, 1961
Goats	Global	Nakamatsu et al., 1968
Camels	Unspecified	Ganke et al., 1964

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Species	Location	Reference
Reindeer	Unspecified	Katic, 1961
Llamas	Unspecified	Rankin, 1958
Zebu Cattle	Unspecified	Katic, 1961
Wildlife and exotic ruminants		
White-tailed deer *(Odocoileus virginianus)*	Unspecified	Chiodini and Van Kruiningen, 1983
Reindeer *(Rangifer tarandus)*	Unspecified	Katic, 1961
Sika deer *(Cervus nippon)*	Unspecified	Chiodini et al., 1984
Red deer *(Cervus elaphus)*	Unspecified	Hillermark, 1966
Axis deer *(Axis axis)*	Unspecified	Chiodini et al., 1984
Roe deer *(Capreoleus capreoleus)*	Unspecified	Katic, 1961
Fallow deer *(Dama dama)*	Unspecified	Chiodini et al., 1984
Moose *(Alces alces)*	Unspecified	Chiodini et al., 1984
Rocky Mountain bighorn sheep *(Ovis canadensis)*	Colorado	Quist, 1998; Williams et al., 1979
Aoudads *(Ammotragus lervia)*	Unspecified	Boever and Peters, 1974
Mouflons *(Ovis musimon)*	Unspecified	Boever and Peters, 1974
Rocky Mountain bighorn goats *(Oreamnos americanus)*	Colorado	Quist, 1998; Williams et al., 1979
Dwarf goats	Unspecified	Katic, 1960
American bison *(Bison bison)*	Unspecified	Chiodini et al., 1984
American buffalo *(Syncerus caffer)*	Unspecified	Katic, 1961
Water buffalo *(Bubalus bubalis)*	Unspecified	Katic, 1961
Bactrian camels *(Camelus bactrianus)*	Unspecified	Katic, 1961
Dromedary camels *(Camelus dromedarius)*	Unspecified	Amand, 1974
Antelopes	Unspecified	Katic, 1961
Stonebuck *(Capra aegagraus ibex)*	Unspecified	Williams and Spraker, 1979
Tule elk *(Cervus elaphus nannodes)*	California	Quist, 1998; Jessup et al., 1981
Llamas *(Llama glama)*	Unspecified	Appleby and Head, 1954
Yaks *(Bos grunniens)*	Unspecified	Almejan, 1958

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Species	Location	Reference
Gnus *(Connochaetes albojubatus)*	Unspecified	Rankin, 1958b
Zebu cattle *(Bos indicus)*	Unspecified	Katic, 1961
Wild rabbits	Scotland	Grieg et al., 1999; Beard et al., 2001b
Monogastric animals		
Mules	Global	Eveleth and Gifford, 1943
Hogs	Global	Larsen et al., 1971
Chickens	Global	Larsen et al, 1981
Monkeys	Global	Chiodini et al., 1984
Pygmy asses	Global	Van Ulsen, 1970
Mandrills *(Papio sphinx)*	Unspecified	Zwick et al., 2002
Horses	Global	Larsen et al., 1972

SOURCE: Adapted from Chiodini et al., 1984.

SPECTRUM OF DISEASE IN DOMESTICATED ANIMALS

Cattle

JD is characterized by vague and often variable clinical signs, and the clinical signs and the severity of gross and histological lesions do not always correspond (Allen et al., 1968; Downham, 1950; Hallman and Witter, 1933; Macindoe, 1950; Smyth, 1935; Smyth and Christie, 1950). Whitlock and Buergelt (1996) have described the following progression of disease in cattle (summarized in Table 2–2).

Stage I: Silent Infection

In Stage I, animals typically exhibit no overt evidence of infection with *Mycobacterium avium* subsp. *paratuberculosis (Map)*. Stage I JD is typically found in calves and heifers, most immature young stock, and many adult cattle. No routine or special clinicopathologic tests or serology will detect disease in these animals. Only postmortum tissue culture or, less often, histopathology can detect infection at this early stage of disease.

Stage II: Subclinical Disease

Most animals in Stage II JD are adults that are carriers of *Map*. The animals do not exhibit clinical signs typical of JD, but they sometimes have detectable antibodies or exhibit altered cellular immune responses. Many are fecal-culture negative, although they intermittently shed low numbers of organisms in feces. In a small percentage (15–25 percent), disease can be detected by fecal culture, by altered cellular immune response, by serum antibodies, or by histopathology. An unknown proportion of Stage II animals progress slowly to Stage III (clinical disease), but because so many are culled

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from herds for other reasons and before clinical signs typical of JD are recognized, the magnitude of the *Map* infection within a herd can be obscured.

Stage III: Clinical Disease

The clinical signs characteristic of Stage III JD typically develop only after several years of *Map* incubation. The initial signs are subtle; they include a drop in milk production, roughening of the hair coat, and gradual weight loss despite an apparently normal appetite. Over a period of several weeks, diarrhea (often intermittent at first) develops. In the absence of a history of herd infection, clinical diagnosis is difficult because other conditions (gastrointestinal parasitism, peritonitis, renal amyloidosis, lymphosarcoma, copper deficiency, chronic salmonellosis, chronic infectious diseases, starvation, kidney failure) often result in similar signs. Because JD diagnosis based on the signs is challenging, the first cases in a herd often are misdiagnosed (Whittington and Sergeant, 2001).

Histopathologic lesions can occasionally be found in the intestinal tract, with the most common site being the terminal ileum. Serum and plasma biochemical changes are predictable and characteristic of the clinical signs, but they are not specific enough to be of use in diagnosis of JD. Most animals test positive on fecal culture for *Map* and have detectable concentrations of antibodies on commercial serum enzyme-linked immunosorbent assay (ELISA) and agar gel immunodiffusion tests, although these diagnostic tests, discussed in Chapter 3, may misidentify animals. A few unusual cases will regress to Stage II and remain there for an indeterminate period.

Stage IV: Advanced Clinical Disease

Animals can progress from Stage III to Stage IV JD in a few weeks, and their health deteriorates rapidly. They become increasingly lethargic, weak, and emaciated as the disease progresses to Stage IV. Intermandibular edema (bottle jaw) due to hypoproteinemia, cachexia, and profuse diarrhea characterize Stage IV. Dissemination of *Map* throughout the tissues can occur. Although the organism can sometimes be cultured from sites distant from the gastrointestinal tract, extraintestinal lesions are rarely detected. When extraintestinal lesions are present, the liver, other parts of the GI tract, and the lymph nodes are the most common sites. At this stage, most animals are culled from the herd because of decreased milk production or severe weight loss. Those animals often are sent for salvage to slaughter, where their carcasses may not pass tests for human consumption. Death from JD is often the result of the severe dehydration and cachexia.

At any given time in an infected herd, the majority of infected animals will be in Stages I and II, with relatively few animals exhibiting clinical signs of disease (Stages III and IV). Because the total number of animals with clinical signs grossly underestimates the prevalence of infection in the herd, the animals in Stages III and IV have been referred to as the “tip of the iceberg” (Whitlock and Buergelt, 1996).

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Table 2–2. Clinical Stages of Johne's Disease in Cattle

Stage	Sign	Presence of Histopathologic Lesion
I Silent infection	No overt evidence	Absent or undetectable
II Subclinical	No clinical signs exhibited	Sometimes
III Clinical	Production loss, roughening of hair coat, gradual weight loss despite normal appetite, diarrhea	Can be found in intestinal tract; most common site: terminal ileum
IV Advanced clinical	Lethargy, weakness, emaciation, intermandibular edema due to hypoproteinemia, cachexia, profuse diarrhea	Can be found in organs other than intestinal tract; most common secondary site: liver and lymph nodes

Sheep and Goats

Clinical disease in sheep and goats is reported to be similar to that in cattle, with the exception that diarrhea is less frequent and onset occurs in younger animals. When diarrhea does occur, it typically attends end-stage disease (Smith and Sherman, 1994; Stehman, 1996; Williams et al., 1983).

In a study of 50 clinically affected sheep—45 of which were emaciated and 5 of which were in fair condition—50 percent had hard feces; 30 percent had soft, non-pelleted feces; and 20 percent had diarrhea (Carrigan and Seaman, 1990). Wool fragility, gaps in wool, weak fibers (metabolic alopecia subsequent to malnutrition or severe disease), and poor fleece condition have also been reported in sheep with JD (Cranwell, 1993). Diseases with similar clinical signs in small ruminants include chronic intestinal parasitism, internal abscesses such as those caused by *Corynebacterium pseudotuberculosis,* chronic hepatic disease, and chronic malnutrition (Beeman et al., 1989). Clinical signs thus are not a reliable indicator of the presence or absence of *Map* infection in sheep or goats (Whittington and Sergeant, 2001). Moreover, *Map* until recently was difficult to isolate from feces of infected sheep (Juste et al., 1991); advances in culture technique, however, appear to have largely solved that problem (Whittington et al., 1999).

SPECTRUM OF DISEASE IN OTHER ANIMALS

Clinical manifestations of JD in non-domesticated ruminants are highly variable in prevalence and timing, but otherwise are similar to those of their domesticated counterparts (Manning, 1998; Power et al., 1993; Ridge, 1991; Williams et al., 1979, 1983). Little has been published specifically on the clinical signs of JD in non-ruminant wildlife, but *Map*-infected European rabbits rarely exhibit signs (Beard et al., 2001b; Greig, 1997).

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PATHOLOGY

Cattle

Lesions of Subclinical Infection

Gross lesions in Stage I and Stage II JD cattle are usually absent or undetectable. The earliest histologic lesions observed in experimentally infected calves are focal aggregates of epithelioid macrophages or Langerhans giant cells in Peyer's patches and in the villous tips of the intestine (Buergelt et al., 1978b; Gilmour et al., 1965; Payne and Rankin, 1961a, b). Similar histologic changes and involvement of mesenteric lymph nodes also characterize naturally infected animals in subclinical stages of disease. Acid-fast staining of lesions may reveal a few mycobacteria, but staining often is negative.

Lesions of Clinical Infection

Primary gross lesions in cattle are confined to the intestinal tract, where they can extend from the duodenum to the rectum. The terminal ileum is the most common site. Gross lesions can be subtle, but the affected intestine is usually thickened, corrugated, and highly folded. In animals infected with strains of *Map* that produce pigment, the intestinal mucosa can take on a yellow-orange cast (Stuart, 1965a, b; Taylor, 1951, 1953; Watt, 1954). The regional lymph nodes also can be involved, sometimes with enlarged and tortuous mesenteric lymphatics. Common secondary changes include effusion into body cavities, atrophy of fat, general wasting, and dependent subcutaneous edema.

The histologic lesion generally is described as a diffuse granulomatous or histiocytic enteritis, without necrosis, hyperemia, or reactionary fibrosis (Buergelt et al., 1978b; Hallman and Witter, 1933; Taylor, 1953). Early granulomatous infiltrates in the intestine are often nodular (tuberculoid), and, in later stages of disease, they can coalesce to form diffuse (lepromatous) infiltrates (Chiodini et al., 1984). The villous tips of the mucosa often fuse, reducing the surface area and the absorptive capacity of the intestine (Whitlock and Buergelt, 1996). The lamina propria and submucosa of the intestine are variably infiltrated with macrophages. Giant cells, primarily of the Langerhans type, often are observed. Epitheloid macrophages and giant cells can contain variable numbers of typical acid-fast bacilli (Buergelt et al., 1978b; Hallman and Witter, 1933; Harding, 1957; Nguyen and Buergelt, 1983; Rajya and Singh, 1961; Stamp and Watt, 1954). Affected lymph nodes exhibit a granulomatous lymphadenitis. Granulomatous lymphangitis also can occur, although less so in cattle than in sheep and goats. In the terminal stages of disease, lesions can occur in other organs; the liver is the most common secondary site (Buergelt et al., 1978b; Hallman and Witter, 1933; Mathews, 1930; Whitlock and Buergelt, 1996).

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Sheep

Several descriptions of the lesions of JD in sheep have been published (Carrigan and Seaman, 1990; Clarke, 1997; Clarke and Little, 1996; Perez et al., 1996; Rajya and Singh, 1961; Stamp and Watt, 1954). The pathology of naturally occurring and experimental cases in sheep has been extensively reviewed by Clarke (1997). Gross lesions are generally similar to those described for cattle, with thickening and corrugation of intestinal mucosa, thickening of lymphatic vessels, and lymph node enlargement. Lesions are more prevalent in the terminal ileum, but they often extend to other parts of the small intestine and colon. Carcass emaciation, intermandibular edema, ascites, hydropericardium, and serous atrophy of fat are common secondary findings. Several ovine strains of *Map* produce large amounts of an orange-yellow pigment that can result in brown-yellow pigmented intestinal lesions (Clarke, 1997).

Lesion classification systems have been developed for JD in sheep. One of the more comprehensive systems designed to represent stages in the pathogenesis of the disease was described by Perez and colleagues (1996) and reviewed by Clarke (1997). The following summary of this system is adapted from Clarke (1997) and Perez and colleagues (1996).

Category 1 This category has mild focal aggregates of foamy macrophages, forming small granulomas in the ileal Peyer's patches with no visible organisms or visible gross lesions. The researchers indicated that this stage is exhibited in animals that were infected as lambs but in which disease has been arrested by effective cell-mediated immunity.

Categories 2 and 3 represent disease progression into adulthood, with failure of effective immune mechanisms.

Category 2 Animals in this category exhibit more extensive lesions in the ileal Peyer's patches, with granulomas extending into the submucosa, and with obvious presence of organisms histologically but no visible gross lesions.

Category 3a. Grossly visible thickening of the intestinal mucosa is present in this category. Multifocal large granulomas are in the lamina propria, submucosa, and serosa of the ileum and in draining lymph nodes, with extension of lesions into the jejunum. Organisms are obvious histologically.

Category 3b. In this category numerous macrophages and a few multinucleate giant cells spread in mosaic-like sheets through the submucosa and lamina propria to create villous fusion and marked thickening of the intestine with abundant organisms.

Category 3c. A diffuse granulomatous enteritis is present in this category, with marked lymphocytic infiltrates within the mucosa and small, well-defined granulomas and giant cells scattered throughout the lesions.

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Organisms are either sparse or undetectable. Granulomas and focal areas of giant-cell necrosis are present within mesenteric lymph nodes.

Goats

Various breeds of domesticated and exotic goats are susceptible to *Map* infection. Subclinical paratuberculosis has been described in goats after experimental infection (Storset et al., 2001). Gross lesions in goats can be variable in type and location (Clarke, 1997; Stehman, 1996). The most common gross lesions are thickening of the terminal small intestine, mesenteric lymph node enlargement, and corrugation of the ileal mucosa. The earliest histologic lesions in experimental cases consisted of clusters of epithelioid macrophages and giant cells in the basal regions of ileal and jejunal Peyer's patches at three months after inoculation. Lesions tended to coalesce and had extended into the large intestine by 10 months, when mucosal ulceration without caseous necrosis was evident (Clarke, 1997). In some cases, nodular foci of caseous necrosis with mineralization have been described in the mucosa, submucosa, serosa, lymphatics, and particularly in the mesenteric lymph nodes, which could be easily confused with signs typical of *M. bovis* or *M. tuberculosis* infection (Clarke, 1997). There is some evidence that subspecies other than *Map,* such as *M. avium* subsp. *silvaticum,* could be the etiologic agent in some caprine cases, possibly accounting for some of the prominent nodular caseonecrotic lesions that resemble tuberculosis (Collins et al., 1984; Thorel et al., 1990a). In advanced cases, the lesions of granulomatous enteritis are similar to those seen in other ruminants, but goats also exhibit lesions in the sciatic and brachial plexus nerves that resemble those in human leprosy. Granulomatous lesions also are present in the liver and lungs of some goats with advanced clinical disease (Stehman, 1996). A classification system for the lesions of JD in goats has been proposed (Corpa et al., 2000) that closely follows the system described above for sheep (Perez et al., 1996).

Exotic Ruminants

JD in small ruminants, deer, and South American camelids (llamas, alpacas) has been reviewed by Stehman (1996), Clarke (1997), and Collins et al. (1994). As in cattle, transmission of *Map* in sheep, goats, South American camelids, and deer is presumed to occur primarily through the fecal-oral route. Gross and microscopic lesions in South American camelids are similar to those reported in cattle, but lymph node necrosis and mineralization, along with multiorgan dissemination, have also been reported (Stehman, 1996). Gross and microscopic lesions in deer are similar to those reported in sheep and goats (Stehman, 1996). *M. bovis* and *M. avium* subsp. *avium* infections, which produce gross and histologic lesions indistinguishable from JD, have been reported in deer (Stehman, 1996).

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Non-ruminant Species

Primates

Map infection produced lesions confined to the intestine and abdominal lymph nodes in a colony of stump-tailed macaques at Yerkes Primate Center, resulting in a condition very similar to JD in cattle and Crohn's disease in humans (McClure et al., 1987). Thirty-eight animals in the colony were infected and were shedding *Map* in feces. Using a serum ELISA, antibodies to *Map* could be detected in 79–84 percent of the animals in the colony; antibodies could not be detected in six animals with clinical disease (McClure et al., 1987). *Map* also was recently reported in a mandrill *(Papio sphinx)* (Zwick et al., 2002).

Rabbits

European rabbits *(Oryctolagus cuniculi)* infected with *Map* develop intestinal disease that is similar to ruminant JD (Angus, 1990). Laboratory rabbits, which are derived from the European rabbit, are occasionally used as an experimental model (Mokresh et al., 1989). Severe lesions in rabbits consist of extensive granulomas and numerous giant cells, with many intracellular acid-fast bacilli in the small intestine (Beard et al., 2001a).

Other Species

Monogastric species—including pigs, horses, dogs, and other laboratory animals—have demonstrated susceptibility to infection with *Map* under natural or experimental conditions (Chiodini et al., 1984; Clarke, 1997; Hines et al., 1995; Thoen et al., 1977, 1981), although reports of such cases are rare. Lesions in these species generally consisted of granulomatous enteritis and mesenteric lymphadenitis with the presence of organisms, but often without clinical signs. Occasional disseminated disease also occurs (Clarke, 1997).

EPIDEMIOLOGY

Global Prevalence in Domesticated Animals

No systematic global survey has been completed for JD or for the presence of *Map* in domesticated animals, but JD has been reported on every continent of the world except Antarctica ([International Office of Epizootics] OIE, 2001b). Some countries, principally island nations, have reported no cases of JD, and others have reported JD limited to specific geographic zones. This self-reporting, however, requires no documentation and has not been independently verified. OIE, which consists of the veterinary administrations of 158 countries, has provided international standards and guidance for the JD diagnostic techniques, vaccines, and biologics (OIE, 2000). However, determining worldwide prevalence with any degree of certainty is complicated by the lack of international consensus on population-testing protocols.

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Furthermore, no international scientific consensus exists concerning the definition and verification of JD-free zones or regions (Table 2–3).

Numerous JD prevalence studies have been completed in domesticated-animal populations of various countries (Kennedy and Benedictus, 2001). Differences in sampling design and diagnostic strategies make direct comparison of the studies difficult, but critical evaluation of published surveys suggests considerable variation in the prevalence of infected herds in different countries and within specific geographic areas. The prevalence of *Map*-infected animals and clinical JD also varies greatly within affected herds. Several estimates for JD herd prevalence exist (Tables 2–3 and 2–4).

The most extensive surveys for JD and *Map* across multiple states in the United States have been completed by the United States Department of Agriculture (USDA). Merkal and colleagues (1987) cultured ileocecal lymph node specimens from clinically normal cull cattle at slaughterhouses in 32 states during 1983–1984. They isolated *Map* from 119 of 7540 cattle; isolates from dairy cattle culls were more than 250 percent higher than were those from beef culls (2.9 percent and 0.8 percent, respectively). USDA's National Animal Health Monitoring System (NAHMS) serologically tested a sample of dairy and beef cattle using ELISA to obtain estimates of the prevalence of JD in herds and in individual animals. In the U.S., 32,622 cows were tested from 1004 dairy operations. Herd prevalence for JD was estimated at 21.6 percent (NAHMS, 1997a). Testing of 10,371 beef cows from 380 herds in 1997 generated an estimate of 7.9 percent infected herds, although the results are not directly comparable to those from dairy cattle because of differences in the investigators' definitions of a positive test in a herd (Dargatz et al., 2001). Nevertheless, the surveys confirm the beliefs that JD is widespread in the United States and that dairy herds are more likely to be infected than are beef herds.

Prevalence data for sheep in the United States will be available when the NAHMS Sheep 2001 report is released. No prevalence studies have been conducted on goats in the United States.

Table 2–3. Global Seroprevalence of Johne's Disease in Dairy Cattle

Country	**Herd Prevalence (%)**
Australia (Victoria)	14–17
New Zealand	60
Netherlands	55
Belgium	22
Austria	7
England, Wales	17
Germany (Arnesberg)	10–30

SOURCE: Collins and Manning, 2002b.

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Table 2–4. Seroprevalence of Mine's Disease in U.S. Dairy Cattle in 1996

Region	Herd Prevalence (%)
West[a]	23.5
Midwest[b]	24.2
Northeast[c]	16.1
Southeast[d]	17.2

[a]Oregon, Washington, Idaho, California, Texas, New Mexico.
[b]Minnesota, Iowa, Missouri, Wisconsin, Illinois, Indiana, Ohio, Michigan.
[c]Vermont, New York, Pennsylvania.
[d]Florida, Tennessee, Kentucky.
SOURCE: Adapted from Garry et al., 1999.

Prevalence in Wildlife and Zoo Animals

Although *Map* is primarily known as a pathogen of cattle and other domesticated ruminants, natural and experimental *Map* infection of ruminant and non-ruminant wildlife has been well documented (Angus, 1990; Beard et al., 2001a, b; Buergelt et al., 2000; Chiodini and van Kruiningen, 1983; Cook et al., 1997; Dukes et al., 1992; Hillermark, 1966; Jessup et al., 1981; Libke and Walton, 1975; McClure et al., 1987; Nebbia et al., 2000; Riemann et al., 1979; Shulaw et al., 1986; Stehman, 1996; Williams et al., 1983). However, the prevalence of JD in wildlife in the United States has not been investigated. Quist (1998) summarized the reports of JD in wildlife in the United States and identified only two endemic foci: Rocky Mountain bighorn sheep *(Ovis canadensis)* and mountain goats *(Oreamnos americanus)* in Colorado (Williams et al., 1979), and tule elk *(Cervus elaphus nannodes)* in California (Cook et al., 1997; Jessup et al., 1981). This could be interpreted as evidence of low JD prevalence in U.S. wildlife, but the lack of active surveillance suggests that data are insufficient to warrant any conclusions.

Sporadic cases or outbreaks of JD have been reported in exotic hoofstock in U.S. zoos (Boever and Peters, 1974; Dukes et al., 1992; Weber et al., 1992) and game farms (de Lisle et al., 1993; Fawcett et al., 1995; Manning et al., 1998; Power et al., 1993). However, the prevalence of JD in zoos could be higher than is suggested by the paucity of reports. A recent survey of zoos accredited by the American Zoo and Aquarium Association revealed that 31 percent of responding institutions had experienced at least one case of JD, but up to 67 percent lacked effective surveillance for JD (Manning and Ziccardi, 2000). This suggests that true institutional prevalence is much higher. No prevalence studies have been conducted on game-ranched or farmed non-domesticated ruminants.

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MODES OF TRANSMISSION

A number of routes of transmission of *Map* infection have been proposed. The relative effectiveness of each route can vary with age and management practices.

Fecal-Oral Transmission

The primary route of *Map* transmission in all species is thought to be fecal-oral (Clarke, 1997; Stehman, 1996). Excretion (shedding) of variable numbers of *Map* in the feces of most affected animal species has been documented (Clarke, 1997; Chiodini et al., 1984; Stehman, 1996; Sweeney, 1996), as has the relative ease of experimental oral transmission in young cattle and sheep (Clarke, 1997). Age-related resistance has been clearly demonstrated only in cattle, but young animals are assumed to be most susceptible in all species (Stehman, 1996). Fecal contamination of the udder or calving environment is therefore thought to be the primary risk factor for neonatal infection. This conclusion is supported by epidemiologic evidence that removal of newborn calves from dams at birth (before suckling) was associated with lower within-herd prevalence of *Map* infection (Collins, 1994).

Congenital Transmission

The possibility of prenatal transmission in cattle has long been suspected: *Map* has been recovered from bovine testes (Tunkl and Aleraj, 1965), semen (Larsen and Kopecky, 1970; Larsen et al., 1981; Lukashaw et al., 1962; Tunkl and Aleraj, 1965), bulbourethral gland (Larsen and Kopecky, 1970), prostate and seminal vesicles (Larsen and Kopecky, 1970; Larsen et al., 1981), uterus or uterine flushes (Goudswaard, 1970; Kopecky et al., 1967; Lawrence, 1956; Pearson and McCelland, 1955; Rohde and Shulaw, 1990), and fetal tissue (Doyle, 1958; Lawrence, 1956; Muhammed and Eliasson, 1979; Pearson and McCelland, 1955; Seitz et al., 1989; Sweeney et al., 1992c). The estimated risk of fetal infection from culture-positive dams is reported to be 26.4 percent with a 95 percent confidence interval (CI) of 11.3–40.7 percent (Seitz et al., 1989). In another study, only 8.6 percent of fetuses from asymptomatic infected cows were *Map*-culture positive (compared with 20–40 percent reported for symptomatic cows), suggesting that risk of fetal infection in “light shedders” (less than 3000 colony-forming-units per gram [cfu/g] of feces) is low (Sweeney et al., 1992a). Despite evidence of fetal infections, the prevalence of prenatal modes of transmission is unknown.

Lactation Transmission

In dairy cows, *Map* has been isolated from mammary gland (Doyle, 1954; Goudswaard, 1970; Taylor et al., 1981), and up to 35 percent of infected cows with clinical signs shed the organism in milk (Hole, 1958; Goudswaard, 1970; Taylor et al., 1981). In more recent studies, 19 percent of asymptomatic

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"heavy shedders" (fecal colony counts greater than 70 per culture tube) and only 3 percent of "light shedders" (fecal colony counts less than 10 per culture tube) had the organism present in milk (Sweeney et al., 1992b). The organism also can be detected in colostrum from asymptomatic cows at a rate of 36 percent in "heavy shedders" (>3000 cfu/g of feces) and 9 percent in "light shedders" (<3000 cfu/g of feces) (Streeter et al., 1995). Isolation of *Map* from milk and colostrum from infected cattle raises the possibility that this route of transmission from dam to offspring could be more important than was previously recognized. The shedding of *Map* in milk of other species has not been documented.

Cross-Species Transmission

It is important to understand the role of cross-species transmission in causing JD outbreaks and in maintaining infection cycles. Molecular strain typing has greatly enhanced knowledge about cross-species transmission of *Map*. But even before molecular strain typing was available, epidemiologic evidence suggested that natural transmission of JD between cattle and sheep was uncommon (e.g., Allworth and Kennedy, 1999; Fridriksdottir et al., 2000; Kennedy and Allworth, 1999). This suggested that cattle and sheep harbor different strains of the organism, and that those different strains were at least somewhat host adapted. The ease of isolating *Map* from cattle and the difficulty of isolating it from sheep also suggested the existence of different strains in the two species.

It is now well documented that there are at least two main strain types of *Map,* designated C (cattle) and S (sheep), which can be distinguished by restriction fragment length polymorphism patterns (Bauerfeind et al., 1996; Collins et al., 1990a; Cousins et al., 2000; Pavlik et al., 1995; Whittington et al., 1998a, 2001c). There also is evidence of a goat-specific strain in Norway (Collins et al., 1990a) that does not appear to be pathogenic for calves (Saxegaard, 1990). A unique bison *(Bison bison)* strain has been found in Montana (Whittington et al., 2001b), but whether it can be transmitted to other species is not yet known.

Despite strain differences, cross-species transmission of *Map* between sheep and cattle has been demonstrated both naturally and experimentally (Feizabadi et al., 1997; Muskens et al., 2001; Whittington et al., 2001c). Nevertheless, molecular epidemiology has confirmed that C strains predominate in cattle populations and S strains predominate in sheep (Bauerfeind et al., 1996; Collins et al., 1990a; Cousins et al., 2000; Whittington et al., 1998a, 2000). C strains also predominate in goats, camelids, and ruminant and non-ruminant wildlife (Bauerfeind et al., 1996; Cousins et al., 2000; Greig et al., 1999; Nebbia et al., 2000; Pavlik et al., 2000; Thoresen and Olsaker, 1994; Whittington et al., 2000). This evidence suggests that sheep strains are less likely to be transmitted to other species than cattle strains are, but it is not clear how much of this is attributable to differences in susceptibility or to differences in exposure. There also is evidence that milder disease results when sheep strains are transmitted to cattle (Fridriksdottir et al., 2000).

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The role of wildlife in maintaining infection cycles in domesticated animals is unknown. Experimental exposure and inoculation studies have shown that domesticated animals can be infected with *Map* from wildlife (Beard et al., 2001c; Dukes et al., 1992; Williams et al., 1983), but there are no published reports of natural transmission of *Map* from wildlife to domesticated livestock. There is, however, epidemiologic evidence that having infected farmed red deer on dairy farms increases the risk of JD in cattle (Cetinkaya et al., 1997), suggesting that, under the right circumstances, natural transmission can occur.

In addition to the risks posed to livestock by endemic infection in wild ruminants, there is potential for non-ruminant wildlife to serve as reservoirs of *Map*. JD is endemic in wild European rabbits *(Oryctolagus cuniculi)* in Scotland (Angus, 1990; Beard et al., 2001b, c; Greig et al., 1997, 1999). The high prevalence of *Map* infection in rabbits is attributed to sharing pasture with domesticated livestock herds in which JD is prevalent (Greig et al., 1997, 1999). The infected rabbits shed very large numbers of *Map* in their feces (Beard et al., 2001c), making them potentially important amplifiers of *Map* in the environment. Because domesticated cattle do not avoid grazing on pasture heavily contaminated by rabbit feces, even when cleaner pasture is available (Daniels et al., 2001), the potential for wild rabbits to be important reservoir hosts exists in Scotland. It remains to be determined whether North American lagomorphs, such as the cottontail (*Sylvilagus* spp.) and jackrabbit (*Lepus* spp.), can be infected with *Map*. Several studies have shown, however, that *Map* can be isolated from other non-ruminant mammals and from birds (Beard et al., 2001a; McClure et al., 1987; Ol'iakova and Antoniuk, 1989). The role of these species as transport or reservoir hosts has not been established. The isolation of *Map* from foxes *(Vulpes vulpes)* and stoats *(Mustela erminea)* in Scotland raises the possibility of sylvatic predator-prey cycles (Beard et al., 2001a).

Natural transmission of JD from wildlife to domesticated animals has not been documented, but domesticated animals have been implicated as the source of several naturally occurring outbreaks in wildlife (Beard et al., 2001a, b; Jessup et al., 1981; Pavlik et al., 2000; Riemann et al., 1979). Furthermore, captive and free-ranging wild animals can harbor the same strains of *Map* as domesticated cattle and goats (Greig et al., 1999; Moreira et al., 1999; Nebbia et al., 2000; Pavlik et al., 2000). Because *Map* can persist for long periods in the environment (Jorgensen, 1977; Larsen et al., 1956), there is potential for transmission from domesticated animals to wildlife even when there is not concurrent occupation of a contaminated area. Infection may also occur from exposure to effluent run-off from a contaminated area.

Once *Map* is established in a wildlife population, a self-perpetuating cycle of infection can become established (Cook et al., 1997). Endemic JD in wildlife can then increase the risk of transmission back to domesticated livestock, and it can reduce the overall productivity and well-being of wildlife populations, making them more vulnerable to local extinction. This is at least as great a concern to some constituencies as the economic losses to the livestock industry are to others. Because it is much more difficult to control JD in free-

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ranging wildlife, the success of JD control and eradication programs depends to a large degree on preventing the spread from livestock to wildlife. The success of some wildlife conservation programs also could depend on preventing establishment of *Map* in wildlife.

The data on cross-species transmission have important ramifications for control programs. The relatively restricted host range of S strains suggests that the greatest risk for JD in sheep comes from exposure to other infected sheep, rather than from exposure to other species. The relatively broad host range of C strains suggests that there is potential for greater epidemiologic complexity in the control of JD in cattle. Nevertheless, absent on-farm wildlife reservoirs, the greatest risk for JD in cattle is other cattle, rather than exposure to other species. With the limited information available, it is impossible to accurately determine the relative risk of transmission of *Map* from wild ruminants to domesticated livestock in the United States (Sweeney, 1996). However, the role of non-ruminant wildlife in the epidemiology of JD in the United States has not been investigated, and it therefore warrants attention before large-scale control programs begin.

Sanitation

- Little direct evidence is available for critically assessing the importance of farm manure management and disposal. However, it is generally considered an important risk factor for JD because large numbers of *Map* can be present in the manure (Chiodini et al., 1984). Two recent epidemiologic studies have implicated manure management as a major risk factor. Specific high-risk management practices include: (Johnson-Ifearulundu and Kaneene, 1998; Obasanjo et al., 1997)
- The use of an exercise lot for lactating cows
- Failure to clean maternity pens after each use
- Failure to apply lime to contaminated pastures Spreading of feces on fields from which forage is later harvested
- Infrequent cleaning of cow barns
- Use of common equipment to clean facilities of both young stock and older animals
- Exposure of calves under 6 weeks old to feces of adult cows

Several methods of disposal of *Map*-infected feces have been suggested, including landfilling, lime stabilization, and distribution on permanent cropland. It has been suggested that treatment of liquid feces (slurry) with 20 percent hydrated lime (v/v pH 11.0) might be an effective method of reducing environmental contamination (Chiodini et al., 1984).

Many dairy operations use a water flush system to remove manure from common feeding and loafing areas around milking parlors. Some of these farms recycle untreated water—which is contaminated with feces that can contain significant numbers of *Map*—for use in the flush system. Repeated cycles of

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flushing likely would tend to concentrate *Map* in the recycled water, thus increasing the potential risk of JD exposure. Apparently, no studies have evaluated this as a risk factor for the spread of JD. Nothing has been reported regarding the risks associated with the use of recycled, uncomposted manure solids for feed, bedding, or fertilizer.

Two recent studies have addressed the possibility that endoparasites could serve as mechanical vectors in the transmission of *Map* and other mycobacteria to cattle and sheep. In one study, *Map* was recovered from washing water, third-stage larvae, and larval sheaths of trichostrongylid nematodes obtained from feces of sheep with JD (Whittington et al., 2001a). *Map* was not recovered from exsheathed larvae. The authors concluded that nematode parasites from sheep could act as mechanical vectors for *Map* infection. In another study, *Map* was recovered from various adult flies (Diptera) collected from pastures grazed by a *Map*-infected cattle herd and from a waste bin at a slaughterhouse where *Map*-infected cattle were slaughtered. The authors suggested that *Map* and other mycobacteria were spread by various species of adult flies that had been in contact with contaminated material from *Map*-infected cattle (Fischer et al., 2001).

Environmental Factors

Most studies on the environmental survival of mycobacteria have focused on *M. avium* subsp. *avium,* rather than on *Map*. However, if *Map* is taxonomically classified as a member of the *Mycobacterium avium* complex (MAC), some of those data could be relevant. MAC organisms are ubiquitous in the environment and have been isolated from soil, water, food, dust, and domesticated and wild animals (Ashford et al., 2001). It appears that the natural habitat for MAC is the soil rather than water (Dailloux et al., 1999).

Specific data for *Map* indicate that the organism is extremely resistant to marked variation in environmental conditions. In 1954, Lovell reported that *Map* could survive approximately 270 days when stored in pond water, tap water, or distilled water, and approximately 246 days in bovine feces (Lovell et al., 1944). A Russian study reported that *Map* could survive in bovine urine for 7 days and in feces for 11 months (Larsen et al., 1956). In 1956, Larsen and colleagues reported that *Map* survival time in a mixture of bovine feces and urine was less than 30 days; in tap water it was between 17 and 19 months. In tap water containing 1 percent gelatin at pH 5.0 or pH 8.5, *Map* survived 14–17 months. *Map* maintained in a sulfuric acid dessicator survived 47 months; and *Map* similarly treated for 7 days then placed in the dark at 38°C were still viable after 17 months. *Map* stored at `14° C for 5 months, then at 4°C for 5 months, and then at 38°C for 8 months also were still viable. *Map* stored at `14°C for 12 months and then at 4°C for 5 months remained viable (Larsen et al., 1956). Jorgensen reported that experimentally inoculated *Map* could survive in cattle and swine fecal slurries for 252 days at 5°C. At 15°C, *Map* survived for 98 days in cattle fecal slurry, 182 days in swine fecal slurry, and 168 days in mixed cattle and swine fecal slurries (Jorgensen, 1977) (Table 2–5).

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Table 2–5. Environmental Factors Associated with Mycobacterium avium subsp. paratuberculosis

Medium	Survival Time	pH	Temp (°C)	Reference
Pond water	270 Days	—	—	Lovell et al., 1944
Saline	17–19 Months	—	38	Larsen et al., 1956
Tap water	17–19 Months	7	38	Larsen et al., 1956
	14–17 Months	5	38	Larsen et al., 1956
	14–17 Months	8.5	38	Larsen et al., 1956
Bovine urine	No growth observed	—	38	Larsen et al., 1956
90% Bovine urine, 10% feces	No growth observed	—	38	Larsen et al., 1956
Cattle fecal slurry	252 Days	—	5	Jorgensen, 1977
	98 Days	—	15	Jorgensen, 1977
Swine fecal slurry	252 Days	—	5	Jorgensen, 1977
	182 Days	—	15	Jorgensen, 1977
50% Swine, 50% cattle fecal slurry	252 Days	—	5	Jorgensen, 1977
	168 Days	—	15	Jorgensen, 1977

Water

Some information is available about water-related factors important for the growth of MAC and *Map*. Mycobacterial contamination of water depends on chemical factors and temperature. Chlorine at concentrations used to treat drinking water (0.2 mg/L) is not effective in eliminating *Map* (Dailloux et al., 1999; Whan et al., 2001). The presence of organic material and, in particular, concentrations of humic and fulvic acids favor the multiplication of MAC. MAC can tolerate low pH and high temperature (40–50°C), which likely explains their concentration in hot-water supply systems (Dailloux et al., 1999). High numbers of MAC are usually associated with warm temperatures, low pH, low dissolved oxygen, high soluble zinc, high humic acid, and high fulvic acid (Kirschner et al., 1992). Fewer MAC were isolated from marine waters (20 percent) than in fresh water (37 percent). The highest numbers of MAC were isolated in waters that ranged from 0.1 percent to 1.9 percent sodium chloride (Falkinham et al., 1980). A study to evaluate the numbers of mycobacteria in drinking-water distribution systems reported a correlation with water turbidity; water treatment substantially reduced the number of MAC by 2 to 4 log units. However, the MAC counts were substantially higher in the downstream distribution system samples than in those taken immediately downstream from water treatment facilities, indicating mycobacterial growth in the distribution system (pipes). Falkinham and colleagues (2001) also reported that the increase in mycobacteria was correlated with assimilable carbon and biodegradable

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organic carbon concentrations. A multinational study (von Reyn et al., 1993) reported MAC isolation rates of 35 percent (U.S. and Finland) and 10 percent (Zaire and Kenya) in environmental water samples; with 32 percent (U.S. and Finland) and 5 percent (Zaire and Kenya) in drinking-water supply system samples. The authors concluded that the likelihood of human exposure to MAC through water is higher in countries within the Northern hemisphere than in sub-Saharan Africa (von Reyn et al., 1993).

Another study (Iivanainen et al., 1993) in Finland reported mycobacterial counts correlated positively ($P<0.001$) with the presence of peatlands; rainfall; chemical oxygen demand; water color; and concentrations of iron, aluminum, copper, cobalt, and chromium. That study also reported a negative correlation ($P<0.001$) with increasing pH; other non-mycobacterial counts were unaffected. Using a linear-regression model, eighty-three percent of the variation in mycobacterial counts from brook waters could be accounted for by peatlands in the drainage area, chemical oxygen demand, concentration of potassium, and pH. The authors concluded that acidification might enhance the growth of environmental mycobacteria (Iivanainen et al., 1993).

Much of the above information relates to MAC in general as opposed to *Map* specifically. However, there is reason to expect that water management will be important in controlling risk for JD in cattle and in reducing human exposure.

Soil Characteristics

Kopecky (1977) performed a study of the distribution of clinical JD in relation to soil types in Wisconsin. He concluded that the disease persisted in regions with acid soils, but was self-limiting in regions with alkaline, calcareous soils. He also concluded that the differences were not attributable to relative differences in cattle populations within the evaluated regions (Kopecky, 1977). Kopecky also described a region on the southwest coast of England where the calcium carbonate content of the soil was as much as 25 percent. In that area, clinical JD did not occur, but lesions could be detected at necropsy. In an adjoining granite area, where the soil was acid and deficient in lime, animals with clinical JD were present. Kopecky (1977) also described situations in the Netherlands where clinical JD was common in areas of low calcium content and low pH: as the pH and calcium content of the soils increased, the incidence of clinical JD decreased. He reported similar findings from France, where JD is found in areas where animals are kept on acid soil with lime and phosphorus deficiencies (Kopecky, 1977). In another report, lime was applied to the pastures to elevate the soil pH in excess of recommended pasture guidelines, and cases of JD abated and had not returned for several years (Richards, 1989). Another study demonstrated a highly significant correlation between high numbers of MAC and high acidity (low pH) of the corresponding soils. No correlations or weak correlations were found with other soil characteristics, such as high concentrations of organic matter, high conductivity, or high moisture content (Brooks et al., 1984). The findings of those reports are biologically

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plausible, as it is general knowledge that *Map* requires an acid medium for effective growth in culture.

Pasteurization

With concerns focused on transmission within and across species (especially cattle to humans), reports of *Map* in raw and processed milk (Grant et al., 1996, 1999, 2002a, 2002b; Millar et al., 1996; Streeter et al., 1995; Sweeney et al., 1992b; Taylor et al., 1981) have raised questions about the capability of heat treatment, pasteurization, and other processes to eliminate live organisms in colostrum and milk. Several researchers have examined this method of breaking the potential cycle of transmission through milk and dairy products (Table 2–6).

Data indicate that *Map* is remarkably tolerant of a wide range of environmental conditions. Consistent with these data, early studies indicated that *Map* were more thermally resistant than were other milkborne pathogens (Chiodini and Hermon-Taylor, 1993; Sung and Collins, 1998). At relatively low inoculation concentrations and at a range of temperature and time treatments, live *Map* organisms were still detectable after incubation (Chiodini and Hermon-Taylor, 1993; Grant et al., 1996; Meylan et al., 1996; Rowe et al., 2000) (Table 2–6). They are known to form colonial clumps, and the clumped colonies demonstrate higher thermal resistance than do declumped samples, which has been attributed to the protective function of the outer layer of clumped cells allowing the inner layers to survive for longer heating times (Rowe et al., 2000). However, *Map* generally is more susceptible to even low-temperature treatments, when those treatments extend over long periods (5–30 min vs. 15 s) (Stabel, 2001). *Map* also can be inactivated by ionizing irradiation (Stabel et al., 2001).

Should *Map* be identified as a significant zoonotic pathogen, pasteurization of milk will be a potential control action that can address a major point of human exposure. Studies currently leave the question of the effectiveness of heat treatment unresolved because of methodologic differences and the difficulty of quantifying viable *Map* organisms, and further work is warranted (Collins, 1997).

A more pressing question is whether to implement changes in pasteurization and sterilization procedures before the zoonotic potential of *Map* has been clarified. This question was recently addressed in deliberations of the United Kingdom Food Standards Agency. Their report reached the conclusion that a link between *Map* and Crohn's disease can neither be proved nor disproved with available evidence. However, they felt it important to take the possibility of such a link seriously. They included a recommendation that minimum pasteurization times be extended from 15 to 25 minutes. It was acknowledged that the evidence supporting this recommendation remains incomplete.

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Table 2–6. Mycobacterium avium subsp. paratuberculosis Response to Heat Treatment, Pasteurization, and Other Processes

Inoculation	Treatment	Treatment Type	Duration	Incubation Period	Result	Reference
Waste milk						
n/a	150°F	Heat	30 min	28 weeks	Complete inactivation	Stabel, 2001
Milk						
10^4 cfu/mL	0 kGy	Ionizing radiation	1.2 kGy/h	Unspecified	Complete inactivation	Stabel et al., 2001
10^4 cfu/mL	5 kGy	Ionizing radiation	1.2 kGy/h	Unspecified	Complete inactivation	Stabel et al., 2001
10^4 cfu/mL	10 kGy	Ionizing radiation	1.2 kGy/h	Unspecified	Complete inactivation	Stabel et al., 2001
10^8 cfu/mL	0 kGy	Ionizing radiation	1.2 kGy/h	Unspecified	Complete inactivation	Stabel et al., 2001
10^8 cfu/mL	5 kGy	Ionizing radiation	1.2 kGy/h	Unspecified	Complete inactivation	Stabel et al., 2001
10^8 cfu/mL	10 kGy	Ionizing radiation	1.2 kGy/h	Unspecified	Complete inactivation	Stabel et al., 2001
Colostrum						
10^4 cfu/mL	63°C	Heat	30 min	16 weeks	Reduction	Meylan et al., 1996
10^4 cfu/mL	20–23°C	n/a	Unspecified	16 weeks	Reduction	Meylan et al., 1996
10^3 cfu/mL	63°C	Heat	30 min	16 weeks	Reduction	Meylan et al., 1996
10^3 cfu/mL	20–23°C	n/a	Unspecified	16 weeks	Reduction	Meylan et al., 1996
10^2 cfu/mL	63°C	Heat	30 min	16 weeks	Reduction	Meylan et al., 1996
10^2 cfu/mL	20–23°C	n/a	Unspecified	16 weeks	Reduction	Meylan et al., 1996
Raw cow's milk						
106 cfu/mL	72°C	Heat	15 sec	Unspecified	Reduction	Grant et al., 1999
106 cfu/mL	75°C	Heat	15 sec	Unspecified	Reduction	Grant et al., 1999
106 cfu/mL	78°C	Heat	15 sec	Unspecified	Reduction	Grant et al., 1999

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Inoculation	Treatment	Treatment Type	Duration	Incubation Period	Result	Reference
106 cfu/mL	80°C	Heat	15 sec	Unspecified	Reduction	Grant et al., 1999
106 cfu/mL	85°C	Heat	15 sec	Unspecified	Reduction	Grant et al., 1999
106 cfu/mL	90°C	Heat	15 sec	Unspecified	Reduction	Grant et al., 1999
106 cfu/mL	72°C	Heat	20 sec	Unspecified	Incomplete inactivation	Grant et al., 1999
106 cfu/mL	72°C	Heat	25 sec	Unspecified	Complete inactivation	Grant et al., 1999
107 cfu/mL	63.5°C	Heat	30 min	Unspecified	Reduction	Grant et al., 1996
102 cfu/mL	Unspecified	Unspecified	Unspecified	Unspecified	Reduction	Grant et al., 1996
104 cfu/mL	71.7°C	Heat	15 sec	Unspecified	3–100% Inactivation	Chiodini and Hermon-Taylor, 1993
>103 cfu/mL	71.7°C	Heat	15 sec	Unspecified	Reduction	Grant et al., 1996
Raw cow's milk, w/ clumped *Map*						
Unspecified	63°C	Heat	0 min	18 weeks at 37°C	Reduction	Rowe et al., 2000
Unspecified	63°C	Heat	2 min	18 weeks at 37°C	Reduction	Rowe et al., 2000
Unspecified	63°C	Heat	4 min	18 weeks at 37°C	Reduction	Rowe et al., 2000
Unspecified	63°C	Heat	6 min	18 weeks at 37°C	Reduction	Rowe et al., 2000
Unspecified	63°C	Heat	8 min	18 weeks at 37°C	Reduction	Rowe et al., 2000
Unspecified	63°C	Heat	10 min	18 weeks at 37°C	Complete inactivation	Rowe et al., 2000
Raw cow's milk, w/ declumped *Map*						
Unspecified	63°C	Heat	0 min	18 weeks at 37°C	Reduction	Rowe et al., 2000
Unspecified	63°C	Heat	1 min	18 weeks at 37°C	Reduction	Rowe et al., 2000
Unspecified	63°C	Heat	2 min	18 weeks at 37°C	Reduction	Rowe et al., 2000

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Inoculation	Treatment	Treatment Type	Duration	Incubation Period	Result	Reference
Unspecified	63°C	Heat	3 min	18 weeks at 37°C	Reduction	Rowe et al., 2000
Unspecified	63°C	Heat	4 min	18 weeks at 37°C	Reduction	Rowe et al., 2000
Unspecified	63°C	Heat	5 min	18 weeks at 37°C	Complete inactivation	Rowe et al., 2000

Notes: cfu/mL, colony forming units/per milliliter; kGy, kiloGray.

PATHOGENESIS IN CATTLE

The severity of disease and the rate of its progression depend on several variables, among the most important of which are the dose of *Map* and the age of the animal at exposure. Only a small dose of the organism might be required to establish infection in a newborn calf, even though large doses might be necessary to infect older animals (reviewed in Sweeney, 1996). The incubation period for JD also is variable but often protracted, ranging from 4 months to 15 years (Macindoe, 1950; Smyth and Christie, 1950). In oral transmission, after ingestion, the organism is taken up by specialized M cells lining the intestine (Gilmour et al., 1965; Momotani et al., 1988; Payne and Rankin, 1961a, b). The mechanism by which attachment and uptake of *Map* occurs is not known, but recent in-vitro studies suggest that soluble fibronectin binds to fibronectin attachment proteins on both *Map* and epithelial cells to mediate uptake (Secott et al., 2001, 2002). After uptake by the M cells, *Map* bacilli are transferred to underlying lymphoid tissue. Dissemination via the bloodstream can then occur, with subsequent localization to secondary sites—the liver, spleen, and peripheral lymph nodes. The organism is ultimately taken up by macrophages, where it survives and is isolated from normal humoral and cellular defense mechanisms (Momotani et al., 1988; Zurbrick and Czuprynski, 1987). The mechanism by which *Map* survives within macrophages has not been elucidated, but it has been shown with other mycobacteria that the normal phagosome-lysosome fusion with acidification and the release of hydrolytic enzymes does not occur (Armstrong and Hart, 1971, 1975; de Chastellier and Thilo, 1999; Frehel et al., 1986a; Goren et al., 1987a, b; Hackam et al., 1998; Hart and Young, 1988; Pieters, 2001a; Sibley and Krahenbuhl, 1987; Sibley et al., 1987). The mechanism by which this inhibition occurs is unclear, but recent work suggests that two host cell components, the steroid cholesterol and a phagosomal coat protein called TACO (tryptophane aspartate-containing), promote the establishment of an intracellular infection by mycobacteria (Ferrari et al., 1999; Pieters, 2001a). Cheville and colleagues (2001) suggested that pathogenic and wild strains of *Map* block phagosomal acidification, so that the phagosome fails to obtain markers of the late phagosome and phagolysosome. This then leads to

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replication within bacteriophorous vacuoles (Cheville et al., 2001). Their study and a previous study by Draper and Rees (1973) show that, during growth within phagosomes of murine or bovine macrophages, the mycobacteria develop a capsule-like lamellar coat. This coat has an ultrastructural organization that is compatible with mycoside (a mycobacterial glycopeptidolipid), which could be an intraphagolysosomal defense mechanism (Cheville et al., 2001; Frehel et al., 1986b; Rastogi et al., 1994; Rulong et al., 1991).

Iron is a requirement for mycobacterial growth. However, unlike other mycobacteria, *Map* does not produce siderophores (mycobactins) that facilitate iron uptake. In-vitro growth of *Map* therefore requires supplementation with mycobactin. The mechanism by which *Map* organisms obtain iron in vivo is not known, but recent studies suggest that *Map* produces its own ferric reductase enzyme that mobilizes host iron to allow growth (Homuth et al., 1998).

In animals that fail to contain the infection, replication within tissues progresses, and more and more of the intestinal lining (mucosa) and regional lymph nodes are infiltrated by macrophages filled with mycobacteria. This phase of infection corresponds with the prolonged incubation period observed clinically. Eventually, the accumulating mycobacteria-laden macrophages interfere with intestinal absorption, resulting in weight loss and an initially intermittent diarrhea. It has been proposed that formation of mycobacterial antigen-antibody complexes in the infected intestine results in histamine release, thus exacerbating the diarrhea (Merkal et al., 1970). Failure of the immune system to contain the infection results in a continuously increasing mycobacterial burden and progressively more severe clinical disease. In the terminal stages of the infection, immune cells become functionally nonresponsive, resulting in uncontrolled replication and spread of *Map* in tissues (Chiodini et al., 1984). The mechanism by which this unresponsiveness occurs in the advanced stages of the disease is unknown and has not been thoroughly investigated. The release of soluble mycobacterial products, bacterial-cell components, or mediators from macrophages could be involved, as in *M. leprae* infections (Birdi et al., 1980; Salgame et al., 1980; Zurbrick et al., 1988).

Age-Related Susceptibility

Reports from experimental inoculations in cattle suggest that infection must occur early because of the development of age-related resistance (Bendixen, 1978; Doyle, 1953, 1956; Hagan, 1938; Larsen et al., 1975; Levi, 1948; Rankin, 1958a, 1961a, b, 1962; Taylor, 1953). The mechanism by which age-related resistance occurs is still not known. It has been proposed that the greater susceptibility in neonates is related to increased permeability of the intestine in the first 24 hours after birth, when colostral immunoglobulins are being absorbed. Alternatively, an inherent weakness of the intestinal mucosal barrier at this time could be involved (Sweeney, 1996). One study suggests that by one year of age, resistance appears equal to that of mature adult cattle (Buergelt et al., 1978b). Recent work has shown that there is a differential distribution of γδ-T-lymphocyte subpopulations between young and mature cattle (Wyatt et al., 1994). It has been suggested that cytotoxic inactivation of

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antigen specific CD4+ helper lymphocyte populations by γδ-T-cells could be involved in the proliferation of *Map* and in the development of clinical disease (Chiodini and Davis, 1993). Some of the mechanisms underlying age-related resistance have been explained, but there are still questions about the importance of horizontal transmission in older animals. Age-related susceptibility to *Map* infection has not been investigated thoroughly in species other than cattle.

Genetic Susceptibility

Genetic susceptibility to JD has been investigated only in mice, cattle, and humans. The susceptibility of mice to the establishment of mycobacterial infections has been shown to be controlled by a single, dominant, autosomal gene called the *Bcg* gene locus, which directly regulates the process of T-cell-dependent macrophage activation for antimycobacterial function and, indirectly, the quality and magnitude of the specific immune response to *M. bovis* (Skamene, 1989). It is thought that the *Bcg*-resistant allele confers to the macrophage the ability to inhibit the proliferation of mycobacteria (Frelier et al., 1990). Similar genetic resistance has been described for *M. intracellulare* (Goto et al., 1984). Inbred C57BL/6J mice are more susceptible to infection by *Map* than are outbred Swiss mice, and resistance to *Map* and *M. bovis* is regulated by the same locus or by linked loci (Frelier et al., 1990). The *Nramp gene* (a component of the *Bcg locus*) has been linked to resistance in mycobacterial infections, including murine models of JD (Blackwell et al., 1994; Chandler, 1961; Frelier et al., 1990; Hackam et al., 1998; Levin and Newport, 2000; Veazey et al., 1995). The bovine equivalent of that gene has been identified, and based on its homology to the murine *Nramp1* gene, it could have similar functions (Feng et al., 1996). However, Barthel and colleagues (2000) were unable to detect an association between resistance or susceptibility to infection with *M. bovis* and polymorphism in the *Nramp1* gene, suggesting that the *Nramp1* gene might not determine resistance or susceptibility to *M. bovis* infections in cattle. The involvement of the *Nramp1* gene in the elimination of *Map* in cattle is currently unknown (Valentin-Weigand and Goethe, 1999). Mutations in other genes critical for macrophage up-regulation, including interferon-γ (IFN-γ), IFN-γ receptor, tumor necrosis factor (TNF) receptor, or interleukin-12 (IL-12), or IL-12-receptor genes, are all associated with decreased resistance to mycobacterial infection in humans (Levin and Newport, 2000). Studies of genetic resistance could be important in the control of JD if they promote the identification or development of resistant cattle (Koets et al., 2000).

Immune Response

Efforts to dissect the immune system and identify the mechanisms that regulate the immune response to pathogens have involved the use of multiple laboratory species, especially the mouse. The use of inbred mouse strains with inherited differences in susceptibility to infectious agents, and knockout mice missing genes encoding molecules involved in development of an immune

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response, have greatly advanced our knowledge of the composition of the immune system and mechanisms of immune regulation. The use of other species has provided further information on factors regulating immune responses, and also revealed there are species differences in the composition of the immune system that need to be taken into consideration when attempting to understand the immune response to a pathogen in the target species (Davis and Hamilton, 1998). This is especially important for studies in ruminants (Davis et al., 1996; Goddeeris, 1998; MacHugh et al., 1993; Wijngaard et al., 1994).

Three major lineages of lymphocytes have been identified: αβ- and γδ-T-lymphocytes, and B lymphocytes. The subset composition of the αβ-T and B lymphocyte lineages is similar in most species (Goddeeris, 1998). However, the composition of the γδ-T-lymphocyte lineage differs. In most species, there is one lineage of γδ-T-lymphocytes that is present in low frequency (three to five percent) in peripheral blood, but widely distributed in mucosal tissue at sites of entry of pathogens. There are two lineages of γδ-T-lymphocytes in ruminants and other Artiodactyla (pigs and camelids) that differ in phenotype and tissue distribution (Davis et al., 1996, 1998, 2000; Goddeeris, 1998; MacHugh et al., 1998). One population with a phenotype similar to γδ-T-lymphocytes in humans and mice is present in blood (three to five percent) and tissues in comparable proportions, except in the spleen where they may comprise 30 percent or more of the lymphocytes present (Davis et al., 1996). The second population is distinguished by the expression of a unique molecule, workshop cluster 1 (WC1). It differs from the WC1` population in frequency in peripheral blood and in the pattern of trafficking, potentially associated with differences in function (Wilson et al., 1998, 1999). The WC1+ population may comprise 30 to 50 percent of lymphocytes in peripheral blood of young animals. Except for the spleen, the WC1+ and WC1` γδ-T-lymphocytes are present in similar proportions in secondary lymphoid tissue and in epithelial tissues at points of entry of pathogens (Wyatt et al., 1994, 1996). Additional smaller subsets of lymphocytes have also been identified, including natural killer cells (NK). Limited information is available on the role of these subsets and γδ-T-lymphocytes in host defense (Kaufmann, 1996). However, it is thought that γδ-T-lymphocytes and NK cells may play a role in first line of defense against infectious agents (Kaufmann, 1996).

The development of a protective immune response is complex (Seder and Hill, 2000; Van Parijs and Abbas, 1998). It involves the interaction of multiple cell types following exposure to a pathogen. In general, there are four phases to the response: (1) antigen recognition following encounter with a pathogen, (2) increase in the frequency of antigen-specific lymphocytes involved in cell-mediated immune (CMI) response and humoral immunity, (3) contraction of the responding populations through apoptosis following control of the infection, and (4) appearance of memory lymphocytes. Protective immunity is dependent on the development and maintenance of memory cells following encounter with pathogens. Immunity is lost if the concentration of antigen-specific memory cells drops below a threshold level needed for a rapid recall

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response on subsequent encounters with a pathogen (Seder and Hill, 2000; Sprent and Surh, 2001).

Cumulative studies have shown αβ-T- and B-lymphocytes are the primary mediators of immunity. Their function is under the control of dendritic cells (DC), the sentinel antigen presenting cells (APC) in peripheral lymphoid tissue that capture and process pathogen derived antigens for presentation to CD4+ and CD8+ subsets of αβ-T-lymphocytes (Banchereau and Steiman, 1998; Flores-Romo, 2001; Huang et al., 2000; Iwasaki and Kelsall, 2000; Kelsall and Strober, 1996). Following uptake of antigen at sites of infection, DC migrate to secondary lymphoid organs, where they present signatory peptide fragments of antigens to CD4+ and CD8+ T-lymphocytes in association with major histocompatibility complex (MHC) class II or I molecules, respectively. Second signals mediated through co-stimulatory molecules expressed on DC, and secreted chemical messengers (chemokines and cytokines) modulate differentiation and maturation of T-lymphocytes to cells with specific effector and/or memory activity (Banchereau and Steinman, 1998; Dubois et al., 1998; Flores-Romo, 2001; Moser and Murphy, 2000). In particular, secretion of IL-12 by DC promotes differentiation of CD4+ lymphocytes with a type 1 cytokine profile dominated by secretion of IFN-γ (Bloom, 1992; Dubois et al., 1998). Lymphocytes with this cytokine profile develop into effector cells that function in CMI and provide help in activation of B cells that produce complement-fixing antibodies. IFN-γ secreted by type 1 CD4+ lymphocytes activates macrophages and increases their capacity to kill ingested bacteria. Direct killing of bacteria is mediated by secretion of perforin and granulysin at the sites of infection (Canaday et al., 2001; Dieli et al., 2001; Smyth et al., 2001). Down-regulation of secretion of IL-12 promotes differentiation of CD4+ lymphocytes with a type 2 cytokine profile dominated by secretion of IL-4. Lymphocytes with this cytokine profile function as helper cells that stimulate activation of B cells that produce antibodies without complement fixing activity. DC also promote differentiation of CD4+ lymphocytes with a type 3 cytokine profile dominated by expression of IL-10 and TGF-*β*. Lymphocytes with this cytokine profile modulate the maturation of lymphocytes with type 1 and type 2 cytokine profiles by down regulating their effector activity. Stimulation of CD8+ T-lymphocytes by DC promotes differentiation of cytotoxic T-lymphocytes (CTL) with a type 1 cytokine profile and/or T-lymphocytes with a type 2 cytokine profile that regulate CD4+ and CD8+ T-lymphocyte effector activity. The role of DC in the response of γδ-T-lymphocytes is not clear, but may involve the interaction with another MHC related molecule, CD1 (Spada et al., 2000). A subset of γδ-T-lymphocytes in humans has been shown to be selectively activated through CD1c. This subset contains perforin and granulysin and, when activated, secrete IFN-γ and IL-2 (Spada et al., 2000). Granulysin-dependent killing has also been reported for γδ-T-lymphocytes expressing a specific arrangement of the γδ-T-cell receptor (Dieli et al., 2001).

The magnitude and type of immune response depends on the pathogen and in part on which receptors are used for internalization of the pathogen and which signaling pathways are used for antigen processing (Huang et al., 2001;

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Lutz et al., 1996; Pulendran et al., 2001; Thoma-Uszynski et al., 2000, 2001). Some pathogens have evolved ways to persist in APC and modulate the pathways of antigen processing and secretion of cytokines for a sufficient time to cause disease (Gewurz et al., 2001; Pieters, 2001a). In spite of these various strategies to evade immune elimination, either or both cellular and humoral responses develop against pathogens. The response elicited may lead to either resolution and sterile immunity, or to immune control of infection, with the pathogen persisting in a dormant state or replicating at a low rate.

Elucidation of how different pathogens influence the evolution of an immune response is crucial to understanding how to develop efficacious vaccines against known and emerging pathogens (Zinkernagel, 2000). The most informative data on the immune response to mycobacteria have been obtained from studies that used *M. tuberculosis, M. bovis* and *M. leprae* in humans, and *M. tuberculosis* and *M. bovis* in mice and cattle. The limited *Map* studies have shown the factors regulating the immune response could be similar to those that regulate other mycobacteria. Common features include tropism for macrophages and use of the same receptors for internalization and sequestration in a phagosomal compartment that does not fuse with lysosomes. Once the mycobacteria take up residence in macrophages, several immunologic mechanisms become involved in the control or containment of the infection. Studies with humans and mice with genetic defects in genes encoding IFN-γ, TNF-α, IL-1, IL-12, and their receptors have shown the mechanisms of those cytokines and receptors in the development and maintenance of an immune response that controls infection (Dorman and Holland, 2000; Jouanguy et al, 1999; Ottenhoff, 2000). Genetically susceptible mice and knockout mice with defects in the αβ and γδ-T-cell receptors, CD4, and CD8 have shown that CD4+ memory T-lymphocytes with a type 1 cytokine profile (Bloom, 1992) operate in the control of infection (Follett and Czuprynski, 1990; Hamilton et al., 1991; Koets et al., 2002; Mogues et al., 2001). The effector mechanisms used by CD4+ T-lymphocytes include secretion of IFN-γ, which activates bactericidal activity in macrophages; secretion of lymphotoxin, which promotes formation and maintenance of tuberculoid granulomas at sites of infection (Roach et al., 2001); and secretion of perforin and granulysin, which kill bacteria on contact (Canaday et al., 2001). The role of CD8+ memory and γδ-T-lymphocytes in protective immunity is less clear but there is evidence that CD8+ T-lymphocytes also kill *M. tuberculosis* using granulysin (Canaday et al., 2001). Their presence in lesions suggests they synergize with CD4+ T-lymphocytes.

Some studies suggest that the type-1 (Bloom, 1992) cell-mediated immune response described above contains the infection during the early, subclinical stages (Demangel and Britton, 2000; Koets et al., 2002). The type-1 immune response can be recognized histologically by the presence of microscopic granulomas and infiltrating lymphocytes with very few mycobacteria, and it is known as the tuberculoid form of the disease. The mechanisms by which mycobacteria ultimately evade host responses and replicate in macrophages are still being elucidated, but one important factor appears to be retention of the TACO protein in the macrophage phagosome,

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which in some way protects replicating bacteria from bactericidal oxygen and nitrous oxide intermediates produced by activated macrophages (Pieters, 2001b; Raupach and Kaufmann, 2001). Progression to clinical disease is thought to occur when the type-1 response declines, allowing proliferation and dissemination of mycobacteria. This stage of the immune response is characterized histologically by diffuse lesions (rather than discrete granulomas) and large numbers of *Map,* and it is called the lepromatous form of the disease (Burrells et al., 1999; Perez, 1996, 1999; Stabel, 2000). Additional work is needed to elucidate the factors that contribute to the decline in type-1 immune response and progression of disease, but a constellation of host factors and microbial virulence factors is likely involved.

No vaccine has been developed that elicits an immune response that completely eliminates viable *Map* from the host (sterile immunity). This is in part attributable to a lack of knowledge of the factors that regulate the immune response to *Map* or to pathogenic mycobacteria in general (Flynn and Chan, 2001). What is known is that not all animals exposed to *Map* progress to clinical disease. It is not clear whether infection is never established in some animals, or whether they develop an immune response that controls or eliminates the pathogen. The appearance of a strong CMI response and the secretion of IFN-γ by cultures of peripheral blood lymphocytes stimulated with *Map* antigens may prove to be the earliest indications that animals have been permanently or transiently infected with *Map* (Köhler et al., 2001; Gwozdz and Thompson, 2002). If infection has occurred and is under immune control, CMI and secretion of IFN-γ by activated T-lymphocytes could be the only indicators of latent, persistent infection (Koets et al., 2002; Köhler et al., 2001). It is not possible, however to use those indicators to distinguish between animals that have developed sterile immunity and those with latent infections. If immune control is lost in latently infected animals, disease can progress slowly through subclinical to clinical stages, with associated lesions. The rate of progression would depend on the factors that influence the immune response to *Map* antigens. The indicators of disease progression are a depression in the CMI response to *Map* antigens and the appearance of a strong humoral antibody response to *Map* antigens (Chiodini, 1996; Koets et al., 2002; Stabel, 1998).

Commercial and experimental live-attenuated and killed vaccines have elicited a CMI response that has not prevented infection or shedding of bacteria (Harris and Barletta, 2001; Kalis et al., 2001). Experimental trials with vaccinated animals challenged with virulent *Map* have, however, shown that immunization does have an effect. Vaccination leads to a reduction in the severity of lesions and bacterial load and to a reduction in clinical signs of disease (Harris and Barletta, 2001). The fact that some protective immunity is elicited suggests that further research could reveal which bacterial antigens to pursue for development of an efficacious vaccine.

Sequencing of the Map Genome

Complete sequencing of the *Map* genome is now at an advanced stage. This joint USDA National Animal Disease Center and University of Minnesota

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project has benefited from parallel efforts to sequence the genome of *M. avium*. Preliminary reports from the *Map* sequencing project demonstrate overall 99 percent sequence identity between *Map* and MAC. The *Map* genome size, at 5.5 million base-pairs, appears larger than that of *M. tuberculosis* and *M. avium*. Twenty-one unique genes had been identified as of January 2002 and it is projected that a total of about 50 will be identified when the project is complete. Among these is a new gene cluster without homology to any known genes at both the genome and possibly at the protein level. Preliminary expression studies have been started comparing expressed genes from culture vs. organisms cultivated in an immortalized macrophage cell line. Several repetitive elements have also been identified in addition to IS900 and IS1311. As might be expected, there are variations in sequence length between different copies of IS900 suggesting heterogeneity in this insertion element. This may be of value in more refined studies of strain relatedness.

Results of the gene-sequencing project have the promise to provide diagnostic reagents with improved sensitivity and specificity. Expression libraries may help to identify unique antigens that can be used in developing serological assays and tests of cell-mediated immunity. They may also provide the foundation for rational vaccine development. These diagnostic approaches may additionally provide adequate specificity and sensitivity to assess definitively the role of *Map* in human disease.

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3

Diagnostics

TESTING OF DOMESTICATED ANIMALS

Tests for Johne's disease (JD) can be divided into two categories: those that detect the organism and those that assess the host response to infection. The first category includes fecal smear and acid-fast stain, culture, and polymerase chain reaction (PCR) tests. There are no tests of metabolic products or unique antigens of *Map*. The second category, detection of host response, includes clinical signs in combination with gross and microscopic pathology and immunologic markers of infection, which include antibody response to *Map* (serology), delayed-type hypersensitivity (DTH) reaction, lymphocyte proliferation, and increased cytokine (IFN-γ) production. Most of the development and evaluation of diagnostic tests has occurred in domesticated cattle and sheep. Despite considerable research effort, all methods are fraught with difficulties that have impeded the control and eradication of JD.

Diagnostic test interpretation and evaluation are important subjects that are discussed in greater detail in Appendix A. The best measure of diagnostic-test performance is predictive value, which is the ability of a test to accurately predict whether disease is present in a particular population. Rates of sensitivity (the ability of a test to correctly identify a diseased individual in a population) and specificity (the ability of a test to correctly identify a healthy individual in a

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population) are established by comparing how well a test correlates with an established "gold standard" for the condition. The gold standard for JD is the identification of the etiologic agent, *Map,* in tissues that show characteristic histopathologic lesions. A test's sensitivity and specificity must be determined, along with the prevalence of the condition, in order to calculate the positive predictive value of a positive test and the negative predictive value of a negative test.

Diagnostic test performance depends on the stage of disease. JD in dairy cattle is clinically categorized into four stages (see Table 2–2). In Stage 1, animals are infected, asymptomatic, and no organisms are detected in feces. In Stage 2, animals are asymptomatic, but organisms can be detected in feces. Stage 3 animals are symptomatic with weight loss and diarrhea. Stage 4 is advanced clinical disease, animals are symptomatic with signs of lethargy, emaciation and profuse diarrhea. Diagnostic tests will generally tend to perform better in individual animals in the later stages of the disease. (This may not be true for immunological tests, where anergy of either cell-mediated or antibody responses to *Map* has been noted in animals with a heavy bacterial burden.) At the herd level, tests will tend to perform better as the proportion of individuals in more advanced stages of disease increases.

For control programs, it is important to make this distinction between test performance at the individual animal level and test performance at the herd level. One valuable screening test for control programs is the enzyme-linked immunosorbent assay (ELISA) for antibodies against *Map*. This test has relatively low sensitivity at the individual animal level, but fairly good sensitivity at the herd level. It also has significant advantages over fecal culture for screening, which is important in large-scale control programs. These advantages include relatively low cost, simplicity, and rapid results (Tables 3–1, 3–2, 3–3).

Table 3–1. Detectability of Johne's Disease at Varying Clinical Stages

	Stage I	**Stage II**	**Stages III, IV**
Signs of disease	No	No	Yes
Fecal culture	No	Maybe	Yes
PCR	No	Maybe	Yes
Acid-fast bacilli	No	Maybe	Yes
IFN-γ	Maybe	Yes	Maybe
Serology	No	Maybe	Yes

Notes: PCR: polymerase chain reaction

IFN-γ: gamma interferon

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Table 3–2. Comparison of Diagnostic Tests for Johne's Disease

Test	Cost	Turn-Around Time	Sensitivity	Specificity	Species[b]	Clinical Stage
Fecal culture	Moderate	Months	Moderate	High	All	II, III, IV
PCR	High	Hours	Low	High	All	II, III, IV
Acid-fast bacilli	Low	Hours	Low	Moderate	All	III, IV
IFN-γ	High	Days	Moderate	Moderate	Bovine, ovine, caprine	II, III
Serology						
ELISA	Low	Hours	Low-high	Moderate	Bovine, ovine, caprine, alpaca, deer	II, III, IV
AGID	Low	Days	Low-moderate	High	Bovine	III, IV

Notes: PCR: polymerase chain reaction

IFN-γ: gamma interferon

ELISA: enzyme-linked imunosorbent assay

AGID: agar gel immunodiffusion

[a] Sensitivity is highly variable with stage of disease.
[b] Payeur, 1998.

Table 3–3. Utility of Diagnostic Tests in Clinical Stages of Johne's Disease

Test	Stage I	Stage II	Stages III, IV
Pathologic lesions	Generally useful	Generally useful	Useful
Signs of disease	n/a	n/a	Very useful
Fecal culture	n/a	Very useful	Extremely useful
PCR	Limited utility	Useful, depending on clinical progression	Useful
Acid-fast bacilli fecal smear	n/a	n/a	Useful, depending on clinical progression
IFN-γ	Limited utility	Generally useful, depending on clinical progression	Useful
Serology	Generally n/a	Generally useful, depending on clinical progression	Useful

Notes: N/A: not applicable

PCR: polymerase chain reaction

IFN-γ: gamma interferon

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An ideal diagnostic test for a control program would identify most animals in Stage I. No antemortem test meets this standard. Surveillance sampling for histopathologic changes is both impractical and likely to be insensitive because visible mucosal changes are later manifestations of disease. A more practical goal for a control program would be to identify animals as they enter Stage II and to continue to improve the sensitivity of detection so that an increasing proportion of Stage I animals can be recategorized as Stage II.

Fecal culture is the accepted standard for identification of Stage II animals. Several other laboratory methods are available as alternatives to fecal culture, but none has proven superior in identifying individual infected animals. It has been erroneously claimed that because *Map* is an obligate pathogen there are no false-positive fecal cultures. In other words, whenever the organism is isolated from clinical specimens, the JD pathologic process will be present in the infected animal. This assertion is based on the fastidiousness of the organism and its inability to proliferate without externally provided cofactors, which would make it an obligate (as opposed to facultative) intracellular pathogen. However, that now seems unlikely, in view of the large number of species *Map* can infect, its widespread geographic distribution, and its capacity to persist under harsh environmental conditions. Little is known about whether there are microenvironments that permit proliferation outside the animal host. However, it is reasonable to expect that this could be the case. In addition, in cattle from contaminated environments, pass-through *Map* can be detected in uninfected animals, yielding a false-positive result (Sweeney et al., 1992a).

Bacteriologic Culture

The Johne's bacillus was first described in the inflamed intestine of a cow in 1895. The bacillus was eventually grown in 1912 on enriched culture media to which extracts of *M. tuberculosis* and *M. phlei* had been added (Twort and Ingram, 1912). Culture techniques were refined after the mycobactins, which are iron-chelating proteins, were subsequently identified as the essential ingredient from those extracts that enabled cultivation of the Johne's bacillus. Detection of *Map* in conjunction with histopathologic lesions of JD is regarded as the gold standard for determining whether an animal is infected. The most common cultivation method involved processing of a sample matrix—most commonly feces but also intestinal tissues, milk, or fetal tissues—and the detection of subsequent visible growth of the agent on a variety of artificial culture media. Occasionally, fecal culture by itself has been erroneously regarded as a gold-standard procedure for determining an animal's infection status. Although the specificity of fecal culture is high, it is not absolute because of the potential for pass-through of orally ingested organisms by uninfected cattle (Sweeney et al., 1992a) and because of laboratory errors, such as sample misidentification or cross-contamination. Because of the relatively low sensitivity of conventional fecal culture in subclinically infected animals, and because *Map* grows very slowly on artificial media, much has been done to improve performance of the procedure and, more recently, to develop hybrid test procedures (e.g., Collins et al., 1990b; Secott et al., 1999).

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All culture methods require long incubation (8–16 weeks or more, in some cases), decontamination of the specimen to selectively kill faster growing non-mycobacterial organisms that would otherwise overgrow the culture medium, and some way to concentrate the organisms from within the specimen before inoculation of the medium. A variety of methods and parameters have been evaluated over the years to improve the sensitivity of *Map* detection by culture. The following discussion briefly highlights the most common methods and techniques for isolating *Map* from clinical specimens.

Decontamination of Specimens

Various chemicals and antibiotics that are selectively toxic to organisms other than *Map* have been included in decontamination methods. Hexadecylpyridium chloride (HPC) is probably the most commonly used decontaminant; it is less toxic to *Map* than are other commonly used decontaminants (Merkal et al., 1982). HPC is commonly used in North America, but sodium hydroxide is more common in Europe (Whitlock et al., 1992). Other decontaminants are benzalkonium chloride and oxalic acid (Merkal et al., 1982); sodium hydroxide and oxalic acid followed with neomycin and amphotercin B (Jorgensen, 1982); cycloheximide with nystatin and tetracycline (Merkal and Richards, 1972); and PANTA—amphotercin B mixed with polymyxin B, nalidixic acid, trimethoprim, and azlocillin (Merkal and Richards, 1972). PANTA is a premixed antibiotic supplement (Becton Dickinson Laboratories, Sparks, Maryland, USA) that has been recommended by the manufacturer for use with the BACTEC radiometric culture system.

The double-incubation method (also called the Cornell method) is commonly used for decontamination (Shin, 1989; Whitlock et al., 1992). It includes a preincubation step with brain-heart infusion medium that initiates germination of bacterial and fungal spores, followed by centrifugation, and then a second step with the addition of antibiotics (amphotercin B, vancomycin, and nalidixic acid) to kill the spores that subsequently germinate (Shin, 1989; Whitlock et al., 1992). Singh and co-workers (1992) reported that the double-incubation culture method demonstrated a higher sensitivity of detection and that it reduced contamination more effectively than did the conventional sedimentation method.

Concentration of Map from Specimens

After the culture specimen is decontaminated, the *Map* organisms must be concentrated to increase the sensitivity of the technique (i.e., to enable detection of lower numbers of organisms). Several methods, including centrifugation, sedimentation, filtration, and immunomagnetic separation have been used to accomplish this task.

Centrifugation of the supernatant increases recovery of *Map* (Whipple et al., 1991; Whitlock and Rosenberger, 1990). Various combinations of speed and duration of centrifugation have been suggested, including 900g (gravity units) for 30 min (Whitlock and Rosenberger, 1990), 1000g for 30 min

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(Whitlock et al., 1989), and 1700g for 20 min (Stabel, 1997). High-speed centrifugation resulting in compaction of the pellet can result in increased contamination rates, and it can cause difficulty in resuspending the pellet (Whipple et al., 1991). Kalis and co-workers (1999b) reported that high-speed centrifugation (3000g for 15 minutes) did not increase the efficiency of identification of animals that shed *Map*. Allowing the supernatant to settle overnight or longer improves recovery of *Map* organisms by sedimentation. Stabel (1997) compared three methods of *Map* concentration and reported that centrifugation yielded more organisms than did sedimentation, but fewer than a modified version of double incubation: centrifugation had higher rates of contamination than did sedimentation or double incubation. Filtration of the supernatant through polycarbonate filters with a 3 micron pore size (to take advantage the organisms' tendency to form large clumps) has been used with the BACTEC system to concentrate *Map* while letting contaminants pass through (Collins et al., 1990b). Paramagnetic beads coated with specific antibodies to *Map* have been used to quickly extract *Map* organisms from feces and milk by immunomagnetic separation. The beads and the attached *Map* organisms are easily separated from the specimen with the use of a magnetic field and thorough washing. This method has been highly effective for milk specimens in which the quantity of *Map* per unit volume is low (Grant et al., 1998), but it is much less effective in isolating *Map* from feces.

Conventional Culture for Map

Conventional *Map* culture consists of decontaminating the specimen, concentrating the organisms, and inoculating a growth medium. Solid media are most commonly used; Herrold's egg yolk medium (HEYM) is popular in many regions, but a modified Lowenstein-Jensen medium is preferred in some areas of Europe. Each medium is supplemented with mycobactin J (Jorgensen, 1982; Whitlock et al., 1992). A thorough discussion of medium constituents and techniques for these and other less commonly used solid media was published by Whitlock and colleagues (1992). Multiple tubes of medium with and without mycobactin J supplementation are used for each specimen to assess the mycobactin dependence commonly associated with *Map* (Whitlock et al., 1992). The tubes are monitored for at least 16 weeks, after which the presence and number of colonies and the demonstration of mycobactin dependence consistent with *Map* are recorded (Whitlock et al., 1992). A molecular based confirmatory test, such as PCR to detect the *Map* marker sequence IS900, can be used to confirm positive specimens.

Automated Culture

Radiometric Systems

The radiometric system in greatest current use is the BACTEC system (Becton Dickinson Laboratories, Sparks, Maryland, USA). It was originally designed for human clinical laboratories for the diagnosis of tuberculosis and other human mycobacterial infections. Collins and co-workers (1990b)

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demonstrated that the system could be modified to culture *Map*. BACTEC is highly automated, faster, and apparently has a slightly higher sensitivity than conventional culture systems do, but it is quite expensive and requires the use of radioisotopes (^{14}C-labeled palmitic acid). Specimens are decontaminated and then mixed with PANTA and specific liquid medium that contains ^{14}C-labeled palmitic acid in sealed tubes. The instrumentation detects the ^{14}C-labeled CO_2 that is produced by metabolism of the labeled palmitic acid. Although many microorganisms can metabolize labeled palmitic acid, the decontamination step and the antibiotic brew (PANTA) select for mycobacterial species (Collins et al., 1990b). A confirmatory test, such as IS900 PCR, is required on positive specimens. When the BACTEC system is combined with IS900 PCR, rapid and specific identification of *Map* is possible—often within only a few weeks (Whittington et al., 1998b). This system is used only in a few laboratories throughout the world, primarily because the equipment is expensive, but also because it requires the use and disposal of radioactive materials.

Nonradiometric Systems

Several new non-radiometric automated culture systems have become available. They are highly automated, incubating and evaluating the culture vials simultaneously and downloading results directly to a computer. The systems require special defined media and incorporate a detector system that reacts to alterations in oxygen, CO_2, or pressure within a sealed tube (Nielsen et al., 2001). Studies that evaluate their utility for the detection of *Map* in clinical specimens have not been published, but several studies of their ability to detect mycobacteria in human clinical specimens have been published: MB/Bact system by Organon-Teknika (Brunello et al., 1999), BACTEC MGIT 960 and BACTEC 9000 MB systems by Becton Dickinson (Tortoli et al., 1998), MB Redox by Bioquest (Piersimoni et al., 1999), and ESP system by Trek Diagnostics (Tortoli et al., 1998).

Pooled Fecal Culture

The use of pooled fecal specimens from several animals within a herd has been evaluated as a means of determining a herd's infection status. Pooling samples reduces the number of fecal cultures necessary to determine infection, thereby reducing the cost of a large-scale JD control or eradication program. One study (Kalis et al., 2001) compared the results of strategically pooled culture specimens (five animals of the same age per pool) and individual fecal specimens. Using individual fecal culture as the gold standard, the authors reported a sensitivity of 86 percent and specificity of 96 percent—higher than expected. The authors concluded that pooling of the samples and using fecal culture influenced diagnostic sensitivity and that strategic pooling of samples would considerably reduce testing costs when a herd is not suspected of being infected. If a herd were infected, however, individual cultures or other organism-based tests would be necessary to identify infected individuals (Kalis et al., 2001). The same research group also evaluated strategically pooled fecal

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cultures as a method for certifying *Map*-free dairy herds. They concluded that the absence of clinical signs of JD in a herd was not a good indicator of the infection status of the herd, that culture of pooled fecal specimens could be used to detect *Map* infections in herd that was believed to be uninfected, that repeated cultures of strategically pooled fecal specimens in combination with closed herd management were necessary to effectively determine the *Map* infection status, and that the optimal number of herd cultures necessary to provide a sufficient level of confidence that a herd is indeed free is still an open question (Kalis et al., 1999a). At the Seventh International Colloquium on Paratuberculosis, Garner and co-workers (2002) presented a preliminary report of a continuing study on the diagnostic sensitivity of pooled fecal culture for *Map* in dairy herds. The results indicated a sensitivity of 30–100 percent on pools of 5 and 10 animals and that sensitivity depends on pool size and the number of heavy versus light shedders (Garner et al., 2002).

Sensitivity of Fecal Culture

Merkal et al (1970) reported that fecal culture has a diagnostic sensitivity of roughly 50 percent and that it detects animals shedding more than 100 colony-forming units per gram (cfu/g) of feces. Several other studies have evaluated the sensitivity of various fecal-culture methods and report sensitivities ranging from 38 to 55 percent and specificities of near 100 percent (Eamens et al., 2000; Sockett et al., 1992a; Stabel, 1997; Whitlock et al., 2000b). Sockett and colleagues (1992a) reported on one study in which radiometric culture (BACTEC) produced a slightly higher sensitivity than did conventional fecal culture (54.4 percent vs. 45.1 percent). Another study (Eamens et al., 2000) evaluated five conventional and radiometric culture methods. The authors reported that the Whitlock decontamination to BACTEC medium was most sensitive for detecting shedder cattle, followed in order by Whitlock decontamination to HEYM, modified Whitlock decontamination to BACTEC medium, conventional decontamination with sedimentation to HEYM, and conventional decontamination and filtration to BACTEC medium (Eamens et al., 2000).

Immunologic Tests

Detecting the Cell-Mediated Response

Various tests can detect cell-mediated immune response to *Map,* but the most common are assays for IFN-γ production (Rothel et al., 1990; Wood et al., 1989, 1992). In the past, intradermal skin tests (Johnson et al., 1977; Wentink et al., 1992) and lymphocyte blastogenesis, also called lymphocyte transformation (Buergelt et al., 1977, 1978a; Johnson et al., 1977), were used more commonly.

Skin Testing

The skin test takes advantage of the development of a delayed-type hypersensitivity (DTH) reaction to the intradermal injection of a mycobacterial

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extract, purified protein derivative (PPD). Intradermal skin testing has been and continues to be commonly used for the diagnosis of both bovine and human tuberculosis. The PPD used in JD skin testing in the United States has been named Johnin, and the PPD extract was isolated from *M. avium* serovar 2 (formerly, *M. paratuberculosis* strain 18), which, before the development of more sensitive genetic methods of identification (Chiodini, 1993), was presumed to be a laboratory-adapted strain of *Map*. A positive test results in an increase in skin thickness (greater than 3 mm) at the site of injection within 72 hours of intradermal injection of Johnin PPD. Significant cross-reactions occur with exposure to other environmental mycobacteria, such as *M. avium* species and *M. bovis,* or with vaccination for JD, resulting in a lack of specificity and a poor correlation with the infection status of the animal (Cocito et al., 1994; Collins, 1996; Wentink et al., 1992, 1994). A nonspecific response can be clarified in cattle by the use of the comparative cervical skin test, because a stronger reaction will be given to the *M. avium* (PPD-A) injection site than to *M. bovis* (PPD-B) (Manning and Collins, 2001).

Lymphocyte Blastogenesis and Transformation

Lymphocyte blastogenesis transformation is a relatively complex in vitro bioassay that uses the antigen Johnin PPD to stimulate lymphocytes present in fresh bovine whole blood co-incubated with ^{125}I-5-iodo-2'-deoxyuridine (Buergelt et al., 1977, 1978a) or, in some cases, ^{3}H thymidine (Kreeger et al., 1991). The incorporation of labeled deoxyuridine is measured using a gamma counter to determine the degree of lymphocyte blastogenesis in response to Johnin stimulation (Buergelt et al., 1977, 1978a). Lymphocyte blastogenesis also has been used as a diagnostic test in North American wild ruminants and domesticated sheep (Williams et al., 1985). The method requires the use and disposal of radioisotopes, relatively expensive instrumentation, and a fairly large volume of fresh whole blood. It must be done immediately after specimen collection, and it suffers from specificity problems similar to those for skin testing related to exposure to other mycobacteria.

Gamma Interferon

The basis of the IFN-γ test is the production and release of IFN-γ by sensitized bovine lymphocytes in response to in vitro stimulation with a series of mycobacterial antigens, including Johnin PPD and *M. bovis* PPD (Wood et al., 1992). The method was developed for the diagnosis of bovine tuberculosis (*M. bovis* infection), as an in vitro correlate of skin testing (Rothel et al., 1990). Two methods have been developed to detect bovine IFN-γ: a bioassay (Wood et al., 1989), and a sandwich enzyme immunoassay (EIA) (Wood et al., 1992). An EIA test kit is commercially available (BOVIGAM, CSL Ltd., Parkville, Australia). The sandwich EIA is more sensitive and specific than the bioassay is for detecting *Map*-infected cattle at all stages of the disease: it offers sensitivity of 71.8 percent for subclinical cases with no fecal shedding, 93.3 percent for subclinical cases with fecal shedding, and 100 percent for clinical cases. The

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bioassay, by comparison, had sensitivity of 16.7 percent for subclinical cases with no fecal shedding, 33.3 percent for subclinical cases with fecal shedding, and 80 percent for clinical cases (Billman-Jacobe et al., 1992; Nielsen et al., 2001; Wood et al., 1992). Collins and Zhao (1995) showed that it is possible to differentiate experimentally infected animals from noninfected animals at 17 months post-challenge. Jungersen and co-workers (2002) reported that cross-reactivity with *Map* could be documented in cattle with specificities of 95–99 percent after Johnin stimulation, irrespective of interpretation relative to *M. bovis* PPD or no antigen stimulation. However, false-positive test results were found in animals under the age of 15 months, and false negatives were reported for infected animals when the test was performed on day-old specimens (Jungersen et al., 2002). EIA evaluated on young cattle has produced mixed results that were related to specificity and uncertain test interpretation. The cause could have been related to exposure to other mycobacterial antigens, as described with other cell-mediated tests (Collins and Zhao, 1995; McDonald et al., 1999). A modification of this test is reported to be available soon for use in Cervidae (Manning and Collins, 2001).

Serologic Tests to Detect the Humoral Response

Serologic tests for *Map* are most useful in establishing the herd prevalence of infection, for presumptive identification of infected animals, and for confirming the diagnosis of JD in animals that demonstrate compatible clinical signs (Nielsen et al., 2001). A variety of tests can detect humoral antibodies to *Map* in bovine serum, but because only the agar gel immunodiffusion (AGID) test, the complement fixation test (CFT), and absorbed indirect ELISA have been used widely to diagnose JD, the discussion below is limited to those tests. In a recent review of *Map* in veterinary medicine, Harris and Barletta (2001) briefly describe the results of studies conducted over the past decade on assays using serum or blood to detect humoral and cell-mediated responses to *Map*.

In general, because CMI develops early in *Map* infection and humoral immunity develops 10–17 months after infection, serologic tests are not recommended for animals younger than 15 months (Lepper et al., 1989; Nielsen et al., 2001). Because the humoral response tends to occur relatively late in infection, those tests are better used for detection of clinical than subclinical disease (Nielsen et al., 2001). All of them depend heavily on the use of *Map*-specific antigens to prevent cross-reaction of antibodies that develop after exposure to other mycobacteria, such as *M. bovis* or other *M. avium* species (Nielsen et al., 2001). This has been a particular problem because the original antigen used for some of the tests (particularly ELISA) was derived from a lysate of *M. avium* serovar 2, formerly known as *M. paratuberculosis* strain 18 (Chiodini, 1993).

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Complement Fixation Test

The complement fixation test (CFT) was one of the earliest serologic tests for JD, and it is still required by some countries for export or import of livestock (Colgrove et al., 1989; Larsen et al., 1963). CFT's major advantage is its ability to detect heavily infected animals (de Lisle et al., 1980). Most animals with a CFT titer of 1:32 or above are likely to be fecal-culture positive, but animals with lower titers also can be positive on fecal culture, indicating a lack of sensitivity (Whitlock, 1994). CFT's usefulness is limited by its tendency to produce false-positive results and its lack of sensitivity (Colgrove et al., 1989; Larsen, 1973; Merkal, 1984). CFT can detect serum antibodies 1–5 months later than does ELISA (Ridge et al., 1991), and its sensitivity and specificity have been reported as 38.4 and 99 percent respectively (Sockett et al., 1992b).

Agar Gel Immunodiffusion

AGAR gel immunodifussion (AGID) was developed after CFT as a quick test for animals that were showing clinical signs of JD (Sherman et al., 1984, 1989). Positive test results correlate well with clinical signs of JD, but failure to detect subclinically infected animals is a major drawback. Whitlock (1994) estimated that when AGID results are positive, there is a 95 percent chance of actual *Map* infection. Sherman and colleagues (1990) reported that AGID has slightly better sensitivity (18.9 percent) and specificity (99.4 percent) for detecting subclinically infected animals than does CFT (sensitivity, 10.8 percent; specificity, 94.7 percent). However, other results have not been conclusive. Sockett and colleagues (1992b) reported sensitivities of 26.6 percent (AGID) and 38.4 percent (CFT) and specificities of 100 percent (AGID) and 99 percent (CFT) (a titer of ≥1:8 was considered positive). Colgrove and co-workers (1989) reported that, when the results of AGID testing on 192 cattle were compared with fecal culture results, there was poor agreement, although there was fair to good agreement among fecal-culture, CFT, and ELISA results. Nielsen and colleagues (2001) reported that AGID was generally less sensitive than ELISA or CFT, particularly for subclinical infection. Because the antigen used for AGID differs from one country to the next, results for the United States and European countries, for example, are not always directly comparable (Goudswaard et al., 1976).

Enzyme-Linked Immunosorbent Assay

Most ELISA tests in current use are modifications of the method developed by Yokomizo and colleagues (1983). For many years, this test used a protoplasmic antigen derived from *M. avium* serovar 2, formerly known as *M. paratuberculosis* strain 18 (Chiodini, 1993; Whitlock, 1994; Yokimizo et al., 1983), although by the mid-1990s this antigen was finally replaced with a *Map*-derived antigen. Current versions employ a step to reduce the cross-reaction to nonspecific mycobacterial antigens by absorbing the bovine test serum with *M. phlei* before performing the ELISA (Bech-Nielsen et al., 1992). Other antigens have been used in some ELISA studies, including various extracts of *Map,*

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purified lipoarabinomannan, and lipid-free arabinomamman (Harris and Barletta, 2001; Sugden et al., 1987, 1989). Care must be taken in comparing the results of the various ELISA studies to be sure similar antigens were used. ELISA test kits or services are commercially available from a number of sources (IDEXX, Portland, Maine, USA; Allied Monitor, Fayette, Missouri, USA; Synbiotics, San Diego, California, USA; Biocor Animal Health, Inc., Omaha, Nebraska, USA; CSL, Parkville, Victoria, Australia). Depending on the specific ELISA, sensitivities range from 43.4 to 58.8 percent and specificities range from 95.4 to 99.8 percent (Collins et al., 1991; Nielsen et al., 2001; Ridge et al., 1991; Sockett et al., 1992b). Apparently the best currently available serologic test for JD is the absorbed ELISA (Collins, 1996; Socket et al., 1992b).

ELISA for bulk-tank milk also has been developed for estimating the prevalence of JD in dairy cattle (Nielsen et al, 2001). Those authors describe a sensitivity of 97 percent and specificity of 83 percent. However, the number of infected animals that must be present in the herd to result in a positive bulk-tank sample is apparently unknown, and it is likely that a herd with only a few infected animals would be missed (Nielsen et al., 2001). An earlier study (Hardin and Thorne, 1996) compared the results of individual animal milk ELISA versus serum ELISA in 821 dairy cattle from 12 Missouri herds and reported a low correlation (k=0.08 and R^2=0.02) between the two tests. More study will be necessary to determine the usefulness of ELISA on individual milk and bulk-tank milk samples for use in JD status or control programs.

Interpretation of ELISA results generally has been made on a single, arbitrary cutoff value with a resultant positive-negative test result. Although this makes results easier for veterinary practitioners and herd owners to understand, valuable information is lost. Ranking of the values obtained can be used to determine the likelihood ratio that cattle are infected with *Map* or are fecal shedders (Collins, 1996; Nielsen et al., 2001; Spangler et al., 1992).

Test Result Measures

Fixed-Decision Thresholds or Cutoffs

For serologic tests with a continuous result, such as conventional ELISA, a positive test is one that yields a signal of sufficient intensity (optical density) that it is above or below an arbitrary decision threshold or cutoff value (Greiner et al., 2000). Such a cutoff is selected to optimize the tradeoff between false-negative and false-positive results, the occurrence of which incur different costs. Because of inherent procedure variability and a surprising degree of individual animal response variability across repeated samples, recent findings suggest that such cutoffs are particularly problematic for *Map*-antibody ELISA (Barrington et al., 2003, Sockett, 2000). For example, using a commercial kit licensed by the U.S. Department of Agriculture, Sockett (2000) found that 6 of 22 ELISA-positive cows were ELISA-negative 30 days later. On testing a subset of samples in 72 replicates, Sockett found that the coefficient of variation for the procedure was 19 percent; a procedure is considered robust if its

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coefficient of variation is below 10 percent. As a consequence of this inherent lack of precision, samples from cows with true values near the cutoff are likely to yield false results on any given test and therefore are likely to produce inconsistent results on repeated testing. Because of the high prevalence of subclinical infection, a large proportion of *Map*-infected cattle in a herd could be close to the cutoff. Furthermore, for unknown reasons, serum antibody concentrations are more variable in infected animals than our understanding of antibody kinetics would predict (Barrington et al., 2003). These findings support the anecdotal reports of difficulty with replicating *Map*-antibody ELISA in the field. Variation in results, particularly near a threshold value for test interpretation, seriously undermines the confidence of producers and veterinary practitioners in such tests. One approach to reducing the effect of procedure variability is to run replicates of each specimen or, to minimize total testing costs, to repeat those tests that yield results within a given range of the cutoff value.

Information Cost and Degree of Result Categorization

When a result from a laboratory procedure that is inherently continuous, such as a colony count or an analyte concentration, is classified into one of a few categories (positive, suspect, negative), potentially useful information about the disease or infection state in the individual is often lost (Shapiro, 1999). For example, reporting the result of fecal bacterial culture only as positive indicates that organisms were shed in feces, but provides no information about the level of shedding. Many laboratories increase the utility of the reported information by further dividing positive culture results into several categories (light, moderate, heavy, too numerous to count [TNTC]). Because shedding is likely related both to an animal's disease state and to the risk it poses to other animals in its environment (Whitlock et al., 2000b), information on shedding is useful for management decisions, such as whether to cull an animal immediately or to wait for a later stage in the production cycle.

Increasing the information yield by further categorization, such as providing a more precise estimate of fecal shedding, increases the cost of testing. The necessity of improving the yield of information from procedures depends on the balance between the increased utility of the information to its users—producers and veterinary practitioners—and increased costs. Producers generally are willing to pay more for information that is more accurate or more useful. As the analytic sensitivity of a test procedure improves and additional result categories become available, additional research to validate the relationship between these categories and biologically or economically important outcomes also is needed. Some work has been done to determine the relationship between minimally categorized fecal shedding and such factors as the likelihood of in utero transmission (Sweeney et al., 1992c), the presence of the agent in colostrum or milk (Sweeney et al., 1992b), the degree of milk and other production losses (Abbas et al., 1983; Benedictus et al., 1987, Buergelt and Duncan, 1978), and the time to development of clinical disease. The

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analytic sensitivity of fecal-culture procedures has improved considerably since much of this work was done, and more research is needed to improve test credibility and to provide a better basis for management decisions. The relationships between the test result categories and outcomes beyond the presence or absence of infection should be studied. Little work has been published that relates serologic test results to infection states and to the associated production and health; however, because of their rapid turnaround, the use of such tests is increasing.

Likelihood Ratios and ROC Curves

Likelihood ratios that correspond to specific test results are a useful approach for reporting test procedures that yield a continuous quantitative result, such as optical density from conventional-format ELISA (Radack et al., 1986). The power of the likelihood ratio is that it allows clinicians to revise estimates of infection or disease probability (Giocoli, 2000). Using a conversion algorithm, the test value—optical density, for example—for each animal is converted to a likelihood ratio that is then reported as a result. A major advantage of the algorithmic approach is that the effects of covariates on test performance (age, breed, vaccination status, gestational status) can be incorporated to increase accuracy—and thus the usefulness—of the test information (Greiner and Gardner, 2000). The failure to formally incorporate this readily available covariate information into the calculation of test likelihood ratios has been named as a major oversight in this field (Brenner et al., 2002; Feinstein, 2002).

Using a likelihood ratio for each tested animal and a known or assumed prior probability of infection in the herd, the producer or veterinarian can obtain positive and negative predictive values for each animal tested. These indices can be calculated from formulas (Fletcher et al., 1996; Sacket et al., 1991) or obtained directly from the graph nomogram of the relationship between likelihood ratios and pre- and post-test disease prevalence or probability (Pagan, 1975; Giocoli, 2000; Moller-Petersen, 1985). Utilizing farm-specific estimates of prevalence with false-positive and false-negative costs, a farm-specific cutoff can be calculated to satisfy an optimality criterion (Greiner et al., 2000).

The use of likelihood ratios avoids the problem of information loss associated with the too-broad categorization of results, as well as the confusion that arises from repeated testing and the use of a fixed cutoff in the presence of procedure and animal variability. Producers and their veterinarians need educational materials explaining the interpretation of likelihood ratios as test results and the use of calculations or other indices, because those measures are not yet in wide use. Recent evidence from the practice of human medicine shows that very few physicians are familiar with or use these measures correctly, despite their clinical usefulness (Hoffrage et al., 2000; Reid et al., 1998; Steurer et al., 2002). Veterinary practitioners are likely to have a similar lack of familiarity and understanding.

Establishing the algorithm for converting a particular test value (optical density, percentage inhibition) to the corresponding likelihood ratio, whether by equation or empiric table, requires determining the test performance over a full

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range of typical results in groups of infected and uninfected animals representative of those in which the test will be used. The results of such evaluations are expressed as receiver operating characteristic (ROC) curves, which are essentially expressions of all true-positive (sensitivity) versus false-positive (1`specificity) pairs over the range of the test (Shapiro, 1999; Zweig and Campbell, 1993). Because of these common scales, the use of ROC curves also promotes direct accuracy comparisons among test formats. The application of ROC curves to medical laboratory test evaluation is relatively recent, and ROC data analysis methodology is an active area of research in biostatistics. ROC curves are not yet widely accepted, particularly for tests that provide continuous results (Pepe, 2000b). Recent examples of advances are the ROC analysis approaches that use specialized regression techniques, as published by Lloyd (2002) and Pepe (2000a). The application of ROC curves to veterinary diagnostic test evaluation has been reviewed by Greiner and colleagues (2000).

As with the evaluation of any laboratory test, proper design and execution of a study used to establish a ROC curve is critical, and the fundamental principles are no different. Despite well-established methodologic test evaluation, those studies often have serious flaws (Reid et al., 1995), with the design flaws most often resulting in an overestimation of test performance (Lijmer et al., 1999). Greiner and Gardner (2000) provided a design checklist and discussed sample selection methods and strategies for avoiding bias in veterinary laboratory test evaluation studies. Because test sensitivity and specificity generally vary across herds (Brenner and Gefeller, 1997; Greiner and Gardner, 2000), the results from studies involving multiple herds could best be analyzed as clustered data. At least one paper has been published on the analysis of clustered ordinal, rather than continuous, data for ROC curves (Beam, 1998). Although a sufficient number of test subjects must be carefully selected for sampling, clear guidelines for estimating the sample size required for developing ROC curves for a test with a continuous result are not yet available. Without regard to cluster effects, Sintchenko and Gilbert (2001) suggest that a minimum of 500 animals is needed on each side (infected and noninfected) of a study. Although not directly applicable to establishing ROC curves for laboratory tests that yield continuous results, Obuchowski (2000) provides tables containing some smaller sample sizes as preliminary estimates.

MOLECULAR METHODS

The first molecular studies of *Map* were connected with efforts to identify a small number of mycobacterial isolates obtained from long-term cultures of human intestine from patients with Crohn's disease (Chiodini et al., 1984; McFadden et al., 1987a). The organisms were first evaluated by DNA-DNA hybridization with known strains of mycobacteria and were found to be indistinguishable from organisms of the *M. avium* complex (McFadden, et al., 1987b). Genetic fingerprinting techniques using restriction fragment length polymorphism (RFLP) analysis of DNA subsequently identified these isolates as

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identical to known isolates of *Map*. Application of these and other techniques to standard laboratory strains of *Map* led to the proposed taxonomic reclassification of *Map* as a subspecies of *M. avium* (Rogall, et al., 1990; Thorel et al., 1990a). Those analyses showed that, with more than 98 percent genetic identity, *Map* was more closely related to *M. avium* subsp. *avium* than it is to *M. intracellulare* or *M. scrofulaceum.*

IS900

In 1989, a novel DNA insertion sequence in *Map* was reported independently from two laboratories (Collins, et al., 1989; Green, 1989). This insertion sequence, IS900, was the first described in mycobacteria and has attributes that, when combined with modern molecular methods, make it a powerful tool for studying *Map* infection. Studies of mycobacterial strain collections showed the presence of multiple copies of IS900 in all isolates of *Map* and in no other mycobacterial species analyzed. However, recent studies have shown that insertion sequences similar to IS900 exist and can yield false-positive test results if stringent procedures are not followed (Cousins et al., 1999).

The apparent specificity of IS900 for *Map* was quickly exploited by using PCR to develop a rapid assay for identification of *Map* from clinical specimens (Vary, 1990). A commercially available assay kit (IDEXX Laboratories, Inc. Westbrook, Maine, USA) has been evaluated in several host species. In cows, IS900 PCR testing of feces is highly specific, as well as sensitive, when fecal shedding is frequent (Sockett et al., 1992a; Thoresen and Saxegaard, 1991). Although PCR testing is not as sensitive as fecal culture, results can be obtained within a few days rather than after many weeks—an important attribute for JD control programs. Other genetic sequences (hspX and F57), purportedly specific for *Map,* also have been reported, but additional work is needed to clarify their utility (Bannantine and Stabel, 2000; Coetsier et al., 2000; Ellingson et al., 1998, 2000; Poupart, 1993).

At least two studies have raised doubts about reliance on IS900 PCR typing alone for identification of *Map* from clinical specimens. Critical review reveals some of the uncertainties in interpretation that can arise when new findings are presented. Cousins and colleagues (1999) reported that mycobacteria isolated from feces of four healthy ruminants were positive by IS900 PCR typing. Those findings are relevant to JD control programs because the isolates were obtained during monitoring of herds that had been established as JD-free. A careful analysis of the amplified product by sequencing the DNA showed that the IS900 primers used had amplified DNA elements that were only moderately similar to IS900 from *Map* (71–83 percent DNA-DNA homology). Closer sequence relatedness existed between three of these isolates and strains of *M. paraffinicum,* and with *M. scrofulaceum* for the fourth. In addition, DNA typing by RFLP analysis showed patterns distinct from IS900. Thus, isolates that were presumptive *Map* by PCR were found, on closer examination, to be distinct mycobacterial species.

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Another study (Naser et al., 1999) evaluated IS900 PCR on *M. avium* isolates derived from human clinical sources and reported positive amplification reactions in 15 of 28 isolates. The results were confirmed by hybridizing the PCR reaction products with a labeled plasmid probe derived from *Map.* However, hybridization of this probe with *M. avium* subsp. *avium* has been accomplished by Hampson and colleagues (1989) using low stringency conditions, permitting interaction with chromosomal flanking sequences for IS900 insertion or with non-IS900 sequences of lower specificity. Naser et al. (1999) did not identify the stringency conditions, so it is difficult to interpret the results of their method of validation. Sequencing of the amplified products would have provided a more convincing demonstration of the presence of IS900 in these *M. avium* clinical isolates. When sequencing of PCR products was done by Cousins and colleagues (1999), it became clear that their mycobacteria were distinct from *Map* and that IS900 was not present.

These results highlight several concerns about the use of molecular methods as a surrogate for isolation of the whole organism. It is essential that the methods' specificity be tested in well-designed collaborative studies and measured against clear and accepted standards. Any departures from established methods should be validated by a similar comparison with the primary standard, or with a widely accepted surrogate, although consensus standards should be used whenever possible. Care in interpretation must be exercised whenever an established test is applied to a new clinical entity or to a new clinical context (such as when prevalence differs markedly from the conditions for which the assay was developed). Molecular diagnostic methods, such as PCR for IS900, are often erroneously claimed to have 100 percent specificity, despite occurrence of clearly demonstrated false-positive results, even in the absence of contamination. Limitations of PCR in routine diagnostic applications include the high cost; the demand for technical sophistication; and the need for well-defined, stringent quality control (Collins, 1996).

Molecular Strain Typing

The capacity to differentiate individual strains of *Map* is essential for evaluating routes of transmission and characteristics of pathogenesis. It is important for producers to be able to identify the source of a new infection because that information often will dictate corrective action. Different control strategies are warranted depending on whether a new infection is the result of introducing livestock from another herd or is attributable to animal contact with something in the farm environment, such as contaminated pasture.

Isolates of *Map* from different clinical sources have few distinguishing phenotypic characteristics. The only features that differentiate strains of *Map* in culture are the rate at which they grow and, sometimes, variations in pigment (Stevenson et al., 2002). However, several methods have been developed to discriminate closely related strains. Nonmolecular methods are usually based on serology, differences in biochemical properties, antimicrobial susceptibility, and phage typing. Multilocus enzyme electophoresis (MEE), which compares strain differences in the size of common metabolic enzymes, could be useful, but few

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of these techniques are practically useful for differentiating strains of *Map*. MEE is also technically ponderous and its use is limited to a few research laboratories (Feizabadi, 1997; Thorel et al., 1990b; Wasem et al., 1991). Molecular-strain typing has had a great influence on studies of *Map*. Among the techniques used have been RFLP analysis of DNA, pulsed-field gel electrophoresis of DNA, and multiplex PCR typing. RFLP has been used most extensively.

In the most common application of RFLP analysis, DNA is cut into small, nonrandom lengths that are separated by size. A probe that can recognize regions that occur in multiple copies in the genome reveals fragment sizes that contain those regions. In the correct circumstances, the resulting pattern will be characteristic of closely related strains but will change as relatedness between strains becomes more distant. There are many variations on RFLP typing, and the results can vary depending on the type of probe used, the manner in which the DNA is cut, and the choice of reaction conditions. Sequences from IS900 are the most widely used probe in RFLP analysis of *Map*. IS900 has been particularly useful because 15–20 copies typically are present in the genome, and its insertion remains stable over many generations of growth of the organism (Cousins et al., 2000; Green et al., 1989; Pavlik et al., 1995; Whipple, 1990).

IS900 RFLP typing has been widely applied to isolates of *Map* from animal and human sources (Thoresen and Olsaker, 1994). It is now well documented that there are at least two main strain types of *Map,* designated C (cattle) and S (sheep), which can be distinguished by RFLP patterns. These strain types are discussed in greater detail in Chapter 2.

EPIDEMIOLOGIC TOOLS

Test Protocol Standardization

Because of the relatively poor epidemiologic sensitivity and specificity of current laboratory tests for subclinical infections, considerable research is focused on improving test performance. Some have proposed methods for optimizing conventional *Map* culture of bovine-origin specimens (Whipple et al., 1991; Whitlock and Rosenberger, 1990), but specific standard protocols have not been established for any JD tests. Even the International Office of Epizootics (OIE) *Manual of Standards,* the principal source of standard methodologies prescribed for international trade (OIE, 2000), implies a large degree of flexibility in diagnostic methods. OIE does not prescribe a JD test for international trade or detail an international primary reference standard. For example, it acknowledges that conventional culture is technically difficult and time-consuming, and it cites seven variations in the method (OIE, 2000). The USDA National Veterinary Services Laboratory (NVSL) recommends three protocols (Cornell, National Animal Disease Center, University of Pennsylvania) for culture of bovine fecal specimens (Payeur and Kinker, 1999). However, the version of the Pennsylvania protocol in the reference cited by Payeur and Kinker (Stabel, 1997) as a source of these protocols is different from

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that protocol as published by its originator (Whitlock and Rosenberger, 1990). Protocols for conventional culture are therefore not readily available in the refereed primary scientific literature and are in a continuous state of improvement (Shin, 2000). In light of relatively poor test standardization and the considerable research to improve it, the absence of specific standard protocols is probably justified. Beyond reagent and reference standard quality control, the incorporation of rigid procedural standards into control program requirements is likely to retard the development and incorporation of improved tests into infection control plans.

Although the use of ELISA serologic testing for bovine JD is increasing, the large variation in test procedures compounds the problem of relatively poor diagnostic performance, even with standardized protocols in the form of commercial test kits. After repeated testing of six serum samples across multiple plates and days with the IDEXX ELISA, Sockett (2000) reported an average coefficient of variation of 19 percent, with variation greatest at the lower sample-to-positive control (S/P) ratios. Because the arbitrary positive-negative threshold is 0.25, positive samples with a mean S/P of 0.27 would test negative 35 percent of the time, and negative samples with a mean S/P of 0.21 would test positive 25 percent of the time. Sockett (2000) noted that discrepant results from repeated testing—a consequence of this relatively large variation— seriously undermine confidence in the reliability of testing as part of control and eradication programs. One response to the problem of variability is to move away from using a fixed S/P cutoff for positive or negative classification to using S/P ranges as an indication of likelihood that an animal is infected (Coffins, 2002).

Although OIE provides international standard sera for many diseases, which is crucial for international standardization of testing procedures, it is not yet providing such standards for JD in cattle or other species. The international availability of standard sera for all ruminant livestock species is important because they would make it possible to establish a baseline for diagnostic test performance in any laboratory, worldwide. This also would provide a comparison standard within and between laboratories for developing and improving tests and for establishing international trade criteria (Wright, 1998).

Laboratory Proficiency Evaluation

The variability in ELISA test performance makes laboratory proficiency testing and quality control all the more important for JD control programs, but commercial test design can run counter to this goal. In a study involving eight laboratories using an earlier version of this ELISA kit, the within-plate coefficient of variation averaged 7 percent, but it ranged from 5 percent to 29 percent across laboratories. The across-day coefficient of variation averaged 14 percent, but it ranged from 6 to 29 percent (Collins et al., 1993). The authors indicated that, because of economic pressure to keep the cost of the test low, the kit was being marketed as a single-well assay and concluded that this was justified based on the results. Running ELISA as a single-well test is contrary to what is considered best laboratory practice. The lack of data on

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within-sample variability also markedly reduces a laboratory's ability to monitor coefficients of variation as a component of laboratory quality control.

Because the effects of well position within a 96-well plate on ELISA variability are not random—owing to edge effects—a duplicate placement scheme was developed several decades ago that takes advantage of this systematic pattern (Stemshorn et al., 1983; Wright, 1987). Placing duplicates in a systematic pattern (rather than to next to one another) reduces the coefficient of variation by 30 percent (Stemshorn et al., 1983), yet, for convenience, ELISA kit instructions from two companies suggest placing duplicate positive and negative controls in sequential wells at the beginning of each plate (CSL Laboratories, 2000; IDEXX, 2000). The number and types of controls in these kits are inadequate: the OIE guidelines specify that four replicates of an antibody-negative control, a weak-positive control, and a strong-positive control be run on each plate (Wright et al., 1993). Within-sample variability data would enable continuous monitoring of quality control using computerized procedures such as Shewart cumulative sum (Shewart—CUSUM) program (Blacksell et al., 1994; 1996), which allows continuous evaluation of many factors that affect performance within the laboratory. Such procedures also enable comparison of performance between laboratories on a near real-time basis. Sharing quality control data via the Internet would allow rapid identification of differences between lots of reagents or kits (Rebeski et al., 2001).

A strong, unbiased, external laboratory proficiency evaluation or quality assessment program is critical to establishing and maintaining confidence in the testing aspects of disease control programs. Indeed, proficiency evaluations are required under ISO 9000 guidelines implemented by OIE for international trade harmonization (OIE, 2000). Proficiency programs should be designed to encourage laboratories to adopt better-performing tests and to refine existing protocols rather than restrict them to performing a set of standard test protocols (Salkin et al., 1997). Given the range of protocols— whether for culture or serology—comparing performance across laboratories in a continuous proficiency evaluation program provides the basis for determining what factors or procedures are critical to improved performance (Somoskovi et al., 2001). Publication of proficiency testing results in media read by producers and veterinary practitioners will encourage underperforming laboratories to adopt better methods. It also will encourage improvement in quality control and will provide consumers of laboratory services (producers and veterinarians) important information for selecting laboratories. The result will be an increase in confidence in JD test results.

At the request of the Laboratory Certification Subcommittee of the Johne's Disease Committee of the United States Animal Health Association, in 1996 NVSL began an annual check testing program for veterinary diagnostic laboratories to become approved for JD fecal culture or serology of bovine-origin specimens (Whitlock et al., 2000a). The procedure is based on a set of serum and fecal-sample unknowns that NVSL provides from known, naturally infected, culture-positive cattle and from known culture-negative cattle herds. In 1999, testing involved 30 samples; in 2000, 25 samples were provided.

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Laboratories must correctly identify all of the culture-negative fecal samples, all of the culture-positive TNT (too numerous to count) samples, and 70 percent of the remaining samples identified as positive by 70 percent of the participating laboratories. Laboratories must correctly identify 90 percent of the serology samples. In the first year, the performance of only five of 35 laboratories met fecal-culture-approval standards, and none of 16 met those for serology. This increased to 35 of 41 for fecal culture, to 2 of 6 for fecal PCR, and to 61 of 63 for serology in the 2000 round of testing. Veterinary diagnostic laboratories have an incentive to participate in this program: the current U.S. voluntary cattle herd status program recommends that all tests be performed in an approved laboratory (USAHA, 1998). No such check testing program exists for veterinary diagnostic laboratories that test JD specimens originating from other ruminant livestock species.

In contrast to the proficiency assessment procedures currently in use, and those recommended by OIE (OIE, 2000), considerable research has shown that assessment programs should use a process that is as blind as possible: the participating laboratory should not be able to distinguish check samples from routine samples. Open schemes, such as those currently in use, overestimate day-to-day laboratory proficiency, presumably because more care is taken with the check samples than with regular diagnostic samples. Rather than estimating routine laboratory proficiency, open schemes indicate the optimal proficiency of participating laboratories (Black and Dorse, 1976; Reilly et al., 1999). Libeer (2001) reported that assurance plans that use identifiable samples overestimated clinical chemistry laboratory performance by 25 percent, compared with blind samples. In a Centers for Disease Control and Prevention study (Hansen et al., 1985) of samples submitted for drug testing, the average error rate across six drug classes was 49 percent higher for blind positive samples than for mailed positive samples. In a study of mycology samples, the frequency of errors on covert samples was as much as 25 percent higher than those for overt samples (Reilly et al., 1999). This bias occurs even when both the laboratory director and the individuals who perform the testing are required by law to sign statements that the proficiency program samples are handled in the same manner as regular submissions. In a study of 42 laboratories in proficiency testing programs requiring such a statement, 18 percent of results from blind proficiency testing samples were unacceptable, but only 4 percent of samples from open tests were unacceptable. In this study, 60 percent of the laboratories exhibited significantly better performance on the open samples than on the blind samples (Parsons et al., 2001). Use of the appropriate sample matrix, in addition to blinding, is important: Only 47 percent of laboratories properly identified *E. coli* in a blind urine specimen; 94 percent did so in a lyophilized specimen (Black and Dorse, 1976). The conclusion is that the current USDA laboratory certification system considerably overestimates the actual performance of veterinary diagnostic laboratories on routine submissions, particularly for difficult, labor-intensive procedures, such as fecal culture for *Map,* or for procedures with many sources of variability, such as bovine JD ELISA.

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4

Control Principles and Programs

CONTROL PRINCIPLES

Although there are large gaps in the understanding of *Mycobacterium avium* subsp. *paratuberculosis (Map)* transmission, enough detail is known that the essential control program components for dairy herds were proposed almost a half-century ago (Organisation for European Economic Co-operation, 1956) and reiterated more recently (Moyle, 1975). They differ surprisingly little from current proposals for the control of Johne's disease (JD) (Collins, 1994; Rossiter and Burhans, 1996).

For dairy herds, the recommendations include:

- Taking precautions against introducing the disease through purchased animals
- Isolating and slaughtering clinically infected animals
- Culling recent offspring of clinical cases as soon as possible
- Removing calves from dams immediately upon birth (before suckling)
- Isolating calves in separate calf-rearing area
- Harvesting colostrum from cows with cleaned and sanitized udders

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- Feeding colostrum to calves by bucket, and thereafter feeding only milk replacer or pasteurized milk
- Preventing contamination of calf feedstuffs, water, or bedding by effluent from the adult herd
- Applying manure from the adult herd only to cropland or to pastures grazed by adult stock

Few empirical studies of those control program components have been done, and their justification is based on biologic plausibility, limited observation, and anecdotal evidence.

Implementation of herd or flock level control programs, establishment of test-negative or low-risk herds and flocks, and reduction of environment and food contamination with *Map* are attainable current goals. The possibility of eradication of JD in the United States should be evaluated after significant progress in control programs is attained.

Environmental Factors in Infection Control Practices

In most, if not all, affected species, *Map* is believed to be transmitted primarily in a fecal-oral cycle shed in the feces of infected animals and then ingested by susceptible animals. Such a cycle is also the primary means by which most other communicable enteric infectious agents are transmitted. For *Map* transmission by indirect contact, factors include the number of organisms shed in the feces and the organism's survival characteristics in the environment. The relationship between *Map* and the environment is complex, involving factors such as the physical characteristics of the substrate material (feces, water, milk, manure slurry, dust, environmental surface, dirt), temperature, pH, water activity or content, and competing microorganisms. The relationships are not well defined for the many combinations encountered in the farm environment, but decisions must still be made for control programs. More importantly, current information relates to the duration of environmental survival of a large inoculation of laboratory-origin *Map,* which could respond differently from *Map* originating directly from an infected host (Mitscherlich and Marth, 1984). More information on the environmental survival characteristics of *Map* is needed to determine how long the organism remains infectious once the area or material (water, grass, forage, or other feedstuff) becomes contaminated, leading to an estimate of dose and response over time. All of this is critical information for determining how to manage livestock flow through housing facilities or paddocks and for how to otherwise minimize disease transmission. Some recommendations do not provide risk estimates of cost relative to benefit. For example, there is a recommendation that livestock producers keep young animals from grazing pasture that has been fertilized with *Map*-contaminated manure for at least one year after birth. But because producers could regard that practice as infeasible and the risk relatively insignificant, they choose to ignore it. In fact, these recommendations may well

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conflict with recommendations being made or required of the operation for other reasons, such as comprehensive nutrient management plans.

There is not enough information available about the environmental survival of *Map* to support adequate risk assessments of many farm practices. *Map* survival information also is needed to address emerging off-farm issues, such as concerns about the persistence of *Map* in waterways that receive farm effluent or the survival of *Map* in other farm products (such as forages) potentially contaminated with *Map* through normal irrigation or manure spreading.

Producer Awareness and Adoption of Biosecurity Practices and Control Measures

Infectious disease biosecurity (preventing the introduction of disease to a farm) has two major components: The first is to reduce the likelihood of introduction of an infectious agent into a group (external biosecurity). The second is to reduce the likelihood of transmission once a disease is present (internal biosecurity or biocontainment). Unfortunately, many dairy producers have not adopted long-advocated practices for either, and many are largely unaware of JD and its associated preventive measures (National Animal Health Monitoring System [NAHMS], 1996b, 1997a). According to NAHMS, an astounding 10 percent of dairy producers admitted never having heard of JD, 35 percent knew of it by name only, 37 percent knew some basics, and only 18 percent considered themselves knowledgeable about the disease. Many of those operations were at significant risk: 64 percent had milk cows born off the operation, 44 percent had purchased cattle during the previous year, and only 9 percent of those who had purchased cattle during the previous year normally required testing of additions to the herd. The most important prepurchase information—the infection status of the herd of origin—was probably unidentified in most of the cases, although the survey did not address this issue (NAHMS, 1996b, 1997a).

In addition to low awareness, there has been poor adoption of general biosecurity practices by producers. Of the dairy operations in the NAHMS (1996b) survey that brought mature cattle onto the operation, 38 percent had required no previous vaccinations, and fewer than half required vaccinations for bovine virus diarrhea (BVD), infectious bovine rhinotracheitis (IBR), or leptospirosis. Sixty-six percent of the producers required no testing of purchased cattle for JD. Only 26 percent of dairy producers who purchased cows required an individual-cow somatic-cell count for mastitis, a disease Wells and colleagues (1998) ranked of highest concern because it causes the largest direct losses to the producer. Purchasing such animals is most likely the way many infectious agents are introduced into a herd. MacNaughton (2001) stated that “the trigger that pushes many herds from elevated-level test results to penalty-level test results is the introduction of new animals into the milking herd without taking biosecurity precautions.”

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Low awareness and poor adoption of biosecurity practices are not unique to the dairy industry. A NAHMS study (1997b) of 2713 randomly selected producers of U.S. beef cow-calves reported that understanding of bovine JD was even lower among that group than it was among dairy producers: seventy percent had never heard of the disease, 22 percent recognized its name only, 5 percent knew some basics, and 2 percent classified themselves as "fairly knowledgeable" (Dargatz et al., 1999). The frequency of possible JD exposure via purchased animals in the beef cow-calf herds surveyed was similar to that in the dairy herd survey (NAHMS, 1996a), with 39 percent of operations purchasing cattle during the survey year and 22 percent of the calved-cow inventory consisting of purchased animals.

As with external biosecurity practices, producers are slow to adopt well-established internal biosecurity measures. The NAHMS study (1996b, 1997b) of 1219 randomly selected dairy herds across the United States and reported that 55 percent used a common hospital and calving area, 47 percent left newborn calves with their dams more than six hours, 32 percent did not clean udders before harvesting colostrum, 23 percent used the same equipment to handle both young stock feed and herd manure, and 12 percent allowed contact between calves younger than six months and adult cows. This lack of prevention is not unique to U.S. dairy producers. A recent study of 534 Australian dairy producers (Wraight et al., 2000) reported that 48 percent had adopted none of six long-recommended control measures. This failure is even more surprising in light of the facts that these producers ranked JD second only to scours as the calfhood infection of greatest concern in Australia, and the proportion adopting no control measures was not significantly different between herds with and without *Map* infection recognized on their premises.

Failure to adopt internal biosecurity measures by dairy producers is not limited to *Map* control. Mastitis control practices are also not adopted by a significant proportion of producers, which is even more surprising given that mastitis is more prevalent and it causes the largest farm losses attributable to disease (Wells et al., 1998). The efficacy of these control program components has been reported in an extensive literature from experimental studies and field trials beginning in the mid-1960s (Neave et al., 1969).

There are likely any number of reasons for low producer awareness of JD and poor adoption of biosecurity practices, but one factor could be a general failure on the part of livestock veterinarians to educate their clients. Dairy producers reported that the most important source of health information was a veterinarian—at 78 percent, far outranking any other source (trade journals, university extension services, other professionals). A lack of contact with veterinarians also does not appear to be the problem: veterinarians made between 13 and 24 visits to the surveyed farms during the year prior to the survey (NAHMS, 1996b, 1997a). Beef producers also ranked veterinarians as their most important source of health information, outranking any other source almost threefold (NAHMS, 1996a).

Education to increase producer awareness is an essential component of JD control, but it will be insufficient by itself to generate widespread

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compliance. Additional incentives could be required. In a Canadian mastitis program initiated in 1989 based on bulk-tank somatic-cell counts in milk, penalties for violating standards and premiums for exceeding standards resulted in adoption of control practices that reduced somatic cell counts. Premiums appeared to generate the greatest response: premiums of approximately one-tenth the magnitude of the penalties resulted in similar compliance (Geyer, 1990). This response to market price signals is interesting in light of the fact that information on the production losses associated with subclinical mastitis was readily available from studies carried out in the mid-1970s (Hortet and Seegers, 1998). It appears that many dairy producers who do not respond to information about unobservable losses will respond if there is direct market feedback. Thus, providing additional information to dairy producers that includes the economics of subclinical *Map* infection is likely to have far less influence on their adoption of *Map* control practices than will a market price signal—even when the unobserved losses are greater than the drop in market price.

At least in the cattle industries, a major weakness of current biosecurity programs is the failure of producers to adopt well-established control practices, not a lack of scientific support for such programs. Clearly, research into factors that affect producer adoption of control practices, particularly in association with their veterinarians, is a major need. In fairness to the producers, rapid changes in the industry structure—the growth in average herd size, the emergence of off-site rearing, and the intensification of management and housing systems over recent decades—has contributed to disease risk. But those same structural changes also increase the producers' need for specialized knowledge. Little published work addresses the veterinary profession's role in this area (Brown et al., 1988). To its credit, the National Johne's Working Group (NJWG) recognized the lack of producer understanding as an impediment to *Map* control and proposed an educational plan that includes marketing and follow-up effectiveness evaluation (Hansen, 1997). In a subsequent five-year review (Whitlock et al., 2000a) in which "educational issues for producers" was ranked as the highest current concern, it was not clear whether the proposed plan was fully funded or fully adopted. The USDA NAHMS 2002 Dairy Survey, which is currently under way, could provide some evidence about the effectiveness of the education program. Furthermore, given both the lack of understanding about the disease and the lack of control program adoption among cattle producers, similar studies of owners of the other ruminant livestock species are probably needed. No evidence on the adoption of control practices or on the knowledge of producers of the other ruminant species affected by *Map* disease is currently available.

Control Programs with a Single-Agent Focus

With the possible exception of the New York State Cattle Health Assurance Program (NYSCHAP, 2002), all current on-farm *Map* control programs, as well as those for most other agents, are focused on controlling a single infectious agent without considering other agents transmitted by the same

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mechanisms. Those programs also ignore the associated broader fundamental interests of the stakeholders. This rather myopic focus on a single agent reflects two outdated paradigms: first, that an infectious agent by itself is the necessary and sufficient cause of disease, and second, that infectious disease is a linear process that starts with the entry of the agent into a susceptible host, continues with a series of pathologic events, and culminates in clinical disease and sometimes death (Morris, 1995; Schwabe, 1982, 1993). The modern paradigm places infectious disease in a causal web (Thrusfield, 1995), so infection is not simply a matter of the agents being present or absent (the focal point of historical control programs [Morris, 1995; Schwabe, 1982, 1993]). The occurrence of disease is the result of many factors that act as a set of sufficient causes (Rothman, 1976). The infectious agent, the host, and the environment interact in complex, dynamic ways over time. An infectious agent most often acts as an opportunist, rather than as a primary pathogen. As a consequence, individual animals in a herd manifest the effects of this causal web in different ways. Suboptimal production of milk is the most common manifestation, and thus the most important economically. Subclinical infection is the next-most-prevalent and economically important manifestation, followed by clinical disease, which is least common and economically least important.

In this modern paradigm, the excessive incidence or prevalence of a controllable infectious disease is more correctly viewed as a sign that one or several major risk factors are at work, and that the risk factors work in concert to present an opportunity for other infectious agents to flourish, as well. The fact that a significant number, if not a majority, of similar herds are not infected by a particular agent, or that they do not exhibit an excessive amount of a very common infectious agent, is primary evidence that control interventions are possible. The sets of risk factors against which interventions can be made differ in the strength of their influence on infection and disease risk for different farms and management systems.

Some have suggested that, with this shift in the way infectious diseases are viewed, livestock veterinarians need to shift focus from traditional hands-on diagnosis and treatment of individual animals (after disease has already occurred), to a more comprehensive approach that includes disease prevention, herd health, and client education (Leman, 1988; Radostits, 2001; Schwabe, 1982, 1993). Although not an explicit component of its current program, the NJWG has adopted this concept for cattle herds and is promoting it through its education materials that were distributed to states throughout the country, as well as to all members of the American Association of Bovine Practioners. A glance at the tables of contents of the recent editions of major texts used in agricultural animal disease courses, however, reveals content that is still mostly organized by specific disease, suggesting that the veterinary curriculum retains its traditional focus on the individual animal. Morris (1995) suggested that teaching veterinary students in this new paradigm will be difficult because their lack of experience with complex problems limits their ability to learn the methods necessary to solve them.

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From the producer perspective, the most important and fundamental goals are, first, the short-term, and then the long-term economic survival of their own farming enterprises. Many chronic, difficult-to-detect infectious agents (including *Map*) reduce herd profitability through decreased production, increased morbidity and mortality, and decreased product value, which in turn jeopardize a herd's economic viability. Many agents are difficult to control once they become established and, for most of those agents, the most important risk factor for acquiring the infection is the purchase of infected animals. Both the ease of acquiring the infection and the subsequent difficulty with control are consequences of the silent—and thus difficult to detect—carrier state of infected animals.

From the consumer perspective, the fundamental objectives are wholesome, high-quality foods produced without unwarranted environmental impact. Wholesomeness includes minimal risk from any zoonotic agent in livestock-origin foodstuffs, not just the mitigation of risk attributable to a specific infectious agent in a specific product. Expending stakeholders' resources on the control of one agent, such as in an indemnity program targeted at a single agent, while ignoring the other agents of concern to the stakeholders is less than optimal. For example, some of the individual-agent control measures typically recommended can either directly or indirectly increase the risk of a herd's acquiring other infectious agents. Those in turn can have direct and indirect farm-level economic effects or adverse consequences for human health. For example, application of a test-and-cull program increases the need for replacement animals, which often are purchased rather than reared on the farm. Although it is commonly recommended that purchased replacements be tested for *Map,* little consideration appears to have been given to preventing the acquisition of other economically and zoonotically important infectious agents. Because the largest risk factor for acquiring most communicable infectious agents is the purchase of a subclinically infected animal, and given the demonstrated lack of knowledge on the part of producers about disease biosecurity, such recommendations must be placed within an integrated disease biosecurity program that considers other infectious agents of concern.

Another *Map* control recommendation that can increase the risk of acquiring other infectious agents is contract rearing of heifers off-site (Groenendaal and Galligan, 1999)—18 percent of dairy producers with more than 200 lactating cows in a herd follow this practice (NAHMS, 1996b). In many contract rearing operations, neonatal calves originating from different farms initially are housed in close quarters and commingled after weaning. This creates an opportunity for the exchange of infectious agents between calves from different herds. Although many disease agents can establish long-term carrier states (*Map,* bovine leukemia virus, IBR virus, *Mycoplasma bovis, Staphlococcus aureus, Salmonella enterica* var Dublin, *Leptospira borgpetersenii* serovar hardjo-type Bovis), the individual health consequences and subsequent biosecurity risks associated with return of such calves to the herd of origin are currently unknown. If pregnant heifers are commingled at midgestation, BVD virus also must be added to the list because of the potential

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for fetal infections that result in persistent infections after birth. Both the prevalence of contract rearing and the plausibility of its attendant disease risk are sufficient to warrant further assessment and risk analysis, for *Map* and other infectious agents.

Single-agent control programs also commonly overlook the on- and off-farm economies of integrating multiple control programs. For example, the fecal and serum samples taken during a *Map* control program also could be used for surveillance for other infectious agents. To do so reduces the on-farm expense associated with the sampling for *Map,* because the costs are apportioned across other agents as well, reducing the unit cost of this aspect of controlling *Map*. Similarly, diagnostic laboratory testing based on integrated technology—microarrays, multiplex primers in PCR (polymerase chain reaction), and different antibody-signal conjugates in ELISA (enzyme-linked immunosorbent assay)—could be used to reduce the supply and labor costs per agent because they would be apportioned across the agents.

Finally, the approach of starting with the infectious agent and then packaging information on how to control associated risk factors is a convenient focus and a convenient way to organize information for researchers, administrators, and veterinary practitioners, but it does not meet the immediate needs of the producer. On a dairy farm, the producer is focused on managing an intensive, complex animal husbandry system, which requires the day-to-day balancing of many competing risks and expenditures of scarce resources. Time is the farm manager's most precious resource, and acquiring new information takes time. Information is needed on everything from current commodity prices to where to find new milkers. As a result, information on disease control must be delivered concisely and efficiently. Most researchers and practitioners consider themselves information seekers (looking for any and all information on a topic with little regard to its current applicability), but producers are information satisfiers, who seek only the information they need to make a specific decision. Information relevant to a current problem—where to purchase heifers to fill the new free-stall barn—is of considerably higher value than information related to a potential future problem, such as the possibility of JD developing in five years in one of those purchased heifers. A control practice could be completely feasible and biologically correct in the long term, but if implementing it results in cash-flow problems that lead to bankruptcy in the short-term, it is a failure.

Producers are best served by “pull” information systems, such as the Johne's Information Center at http://johnes.org/ (Collins and Manning, 2002a), which allow them to find and use information as the need arises, rather than “push” information systems, such as newsletters or journal articles. The occurrence of a particular infectious agent in a herd is a consequence of one or more risk factors being out of control. If the focus is on controlling the risk factors, which could be common to several infectious agents, rather than on the agent itself, the producer will be more likely to adopt the control practice because there is a greater return on the investment. In contrast, if the focus and information packaging follow the traditional approach of controlling

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cryptosporidiosis, salmonellosis, *Map,* leptospirosis, rota and corona viruses individually, the common principles are lost. In the end, the producer concludes that control of any of these agents is impractical, or of low priority, and moves on to more immediate, higher priority issues. If the starting point is, for example, how to manage the control points of a set of common risk factors for enteric infectious disease rather than understanding the control details of the infectious agents themselves, producer compliance is likely to be higher.

Ultimately, the control program must motivate most producers to change their behavior when such a change is needed. Even complete understanding without behavioral change is a failure. Some with long experience on control programs are not optimistic (Franks, 2001). At least for dairy producers, the Milk and Dairy Beef Quality Assurance Program appears to have been a failure: it has been adopted by only a small proportion of producers (Gibbons-Burgener et al., 1999, 2000). What is needed is an integrated approach to on-farm disease control that meets the needs of the livestock producer and motivates behavioral change. This is not a novel idea; several groups have proposed a more integrated approach to disease control by using a hazard analysis critical control point (HACCP) strategy (which targets control at the most critical points in the causal web) and establishing good farming practices (Noordhuizen and Welpelo, 1996; Weber and Verhoeff, 2001; Wells and Ott, 1998). Some are beginning to appear on the Internet, including those presented at http://www.gov.mb.ca/agriculture/foodsafety/gpp/ (Manitoba Agriculture and Food, 2001), which lists a manual of practices and offers' measurements for self-assessment. General outlines for a given type of management and husbandry situation will be the same but must be sufficiently flexible to be easily adaptable to the specific circumstances of each farm. Finally, the information must be packaged and delivered in a manner that is in harmony with the information management style of producers and that motivates them to change their practices. Motivation can require feedback signals in the form of market access or market price differentials established through testing by the downstream purchaser of the farm product.

Vaccination

As a component of biosecurity control programs, vaccination is problematic for several reasons. First, producers adopting vaccination may be less likely to employ other management practices that, although requires more work, might be more effective. A recent NAHMS report stated: “When considering disease control programs, many producers and veterinarians think primarily about vaccinating. Yet there are many other management practices that can be used to minimize both disease occurrence and the risk of introducing new diseases onto operations” (NAHMS, 1996a). Second, the proportion of producers voluntarily adopting vaccination may be low. A study of beef-cow calf producer biosecurity practices based on NAHMS Beef 1997 data found that although the producers importing purchased cattle into their herd were approximately twice as likely to vaccinate for two common viral infectious agents of cattle, IBR and BVD, as those who did not, only 25 percent did so

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(Sanderson et al., 2000). Third, evidence suggests that a significant proportion of producers often do not use existing vaccinations appropriately. In a study of BVD vaccination and biosecurity practices in 387 randomly selected Pennsylvania dairy herds after a regional outbreak of clinical BVD, Rauff et al. (1996) found that, although 82 percent of producers indicated that they routinely vaccinated for BVD, the authors regarded only 27 percent of the herds as adequately vaccinated. Because of these vaccination errors and weaknesses in associated biosecurity practices, the authors concluded that 30 percent of the herds were at high risk of a clinical BVD outbreak. Finally, some producer skepticism against vaccination may be warranted as the evidence of field efficacy for many of the currently available commercial enteric and respiratory disease vaccines is limited at best (Perino and Hunsaker, 1997; Radostits, 1991) and anecdotal reports of vaccine failures are common. Federal vaccine licensing standards do not require demonstration of field efficacy under circumstances in which producers could reasonably expect the vaccine to be an important component of disease control. Adoption of vaccination and other biosecurity practices may be limited if the producer perceives the risk for that specific disease to be low for their herd and is willing to take the risk or that the expense of implementing the biosecurity step will be higher than the actual cost of an outbreak of the disease (Sanderson et al., 2000).

Herd-Level Control

A herd plan provides the basis for organizing control strategies on a farm. Prerequisites are knowledge of herd goals and resources for voluntary programs and agreements between herd owners and their advisors on the intensity necessary to achieve herd goals with respect to control or eradication of *Map* (Rossiter and Burhans, 1996). Incorporating Best Management Practices concepts will facilitate control of other economically important diseases and could result in greater producer compliance overall.

Many states have adopted control programs for *Map* test-positive herds and status programs for test-negative herds. Control programs should be tailored to the individual herd, but working within state program standards will allow recognition of control progress or test status and could be necessary for access to state laboratory and technical resources.

Dairy Herds

Whitlock (2001) divides control principles into two categories. First is management practices that prevent highly susceptible newborn and young animals from ingesting manure from infected animals. Second is reducing farm contamination with *Map* by culling infected animals. Rossiter and Hansen (2000) list three management principles for control of *Map:* reducing infections by manure management, reducing infections by colostrum and milk management, and reducing infections by management of infected animals. Test-and-cull strategies are not likely, by themselves, to be effective in herd *Map*

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control. Better hygiene and management are more effective control tools (Groenendaal and Galligan, 1999).

Veterinarians and other consultants who work with dairy farmers to implement *Map* control programs should promote management recommendations that adapt recognized control principles to specific situations. Most control measures fall into one of three generally accepted categories of *Map* control (Rossiter and Hansen, 2000; Rossiter et al., 1998; Sockett, 1994):

1. Protect young stock from older animals and from feces-contaminated feed and water:
    - Clean and disinfect maternity and calf pens after each use.
    - Calve cows in clean, dry, dedicated maternity pens.
    - Remove calves immediately after birth to clean, dry calf pens, stalls, or hutches.
    - Feed colostrum only from test-negative cows.
    - After colostrum feeding, use pasteurized milk or use milk replacer.
    - Raise calves separate from the adult herd for at least the first year of life.
    - Do not allow shared feed or water between adults and young stock; do not offer feed refusals from adult cattle to young stock.
    - Avoid vehicular and human traffic from adult animal areas to young stock areas.
2. Prevent manure contamination of feed and water sources:
    - Use separate equipment for handling feed and manure.
    - Design and maintain feedbunks and waterers to minimize risk of contamination with manure.
    - Do not spread manure on grazing land.
3. Reduce total farm exposure to the organism:
    - Immediately cull all animals with clinical signs of JD.
    - Cull culture-positive animals as soon as possible; for cows with low or moderate fecal culture colony counts, removal at the end of lactation may be acceptable.
    - Test adult cattle at least annually by serum or fecal tests; positive serum test results should be confirmed by fecal culture.
    - Purchase replacement animals from test-negative herds; if this is not possible, assess the status of the herd of origin through owner or veterinarian statements, by negative serum ELISA tests of at least thirty adult animals, or both.

Beef Herds

Control plans for beef-cow calf herds follow the same principles as those for dairy herds, but must adapt the procedures to meet calf management

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needs. In addition to the management procedures suggested for dairy herds, some specific control measures for beef herds (adapted from Hansen and Rossiter, 2000) include the following:

- Avoid manure build-up in pastures and corrals where late-gestation cows are kept.
- Provide a clean calving area, with low cow density.
- Move cow-calf pairs to clean pasture as soon as bonding occurs.
- Move feedbunks, waterers, and creep-feed areas frequently to avoid exposing calves to manure build-up.
- Do not put weaned calves on pastures used by cows.
- Blood or fecal test the entire breeding herd annually; avoid calving-out and raising offspring from test-positive animals.
- If possible, calve first-calf heifers in an area separate from older cows.

Sheep Flocks

Sheep flock JD control or eradication programs have been more widely implemented in Australia than in other sheep-raising countries. The Australian program is based on negative serologic testing (ELISA or AGID [agar gel immunodiffusion]) of a sample of the adult flock (2 years or older) and management that includes boundary fencing and introducing flock additions only from flocks of similar *Map* status. The flock-sampling program, which is designed to detect 2 percent or greater infection prevalence with 95 percent confidence, requires testing 400–500 sheep from each flock. Fecal culture of samples from sheep with histologically confirmed JD has been unrewarding because of low test sensitivity. Recent use of Middlebrook agar or a modified BACTEC radiometric medium have greatly improved the sensitivity of detection and allowed the use of pooled (up to 50 animals per pool) fecal samples in control programs (Whittington et al., 1999).

Goat Herds

There are few organized programs for control of *Map* in goat herds. Elements to consider in developing a strategy for elimination of *Map* from infected herds include identification and removal of infected animals, reduction in the rate of new infections in susceptible animals through improved sanitation and kid-rearing techniques, and vaccination to increase host resistance to new infections (Smith and Sherman, 1994).

Zoos and Game Farms

A recent workshop addressed control programs for zoos accredited by the American Zoo and Aquarium Association (Proceedings of the Workshop on Diagnosis, Prevention and Control of Johne's Disease in Non-Domestic Hoofstock, 1998). Plans suited to the unique needs and management of game farms have not been developed, but they could be guided by relevant aspects of similar control plans for domesticated and zoo animals.

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CURRENT CONTROL PROGRAMS

National Programs

In the past decade, there have been several attempts to establish nationwide management programs to eliminate JD from animal herds. In 1993, a task force of the Johne's disease committee of USAHA (United States Animal Health Association) drafted a model herd certification program to provide states with an example from which to develop or standardize programs. Some states modified their programs to conform, but relatively few herd owners have elected to pursue herd certification. The primary deterrents cited were the amount of testing required and the associated costs. In 1997, the newly formed NJWG appointed a committee to design a scientifically sound and more affordable herd certification program. The program was evaluated by veterinarians, veterinary associations, cattle breed associations, and interested industry groups, and a revised version was adopted by USAHA in October 1998 as the U.S. Voluntary Johne's Disease Herd Status Program for Cattle (Whitlock et al., 2000a). The standards have been adopted in whole or in part by at least 20 states. In 1999, additional guidelines were developed to address JD-affected herds: the Minimum Recommendations for Administering and Instituting State Voluntary Johne's Disease Programs for Cattle. Although the proposed program standards are not required by the federal government, resources for national coordination of JD programs have been appropriated by Congress.

Two additional programs have been proposed. In 2001, the USDA Animal and Plant Health Inspection Service (APHIS) released for comment draft program standards for a voluntary control program. The National Milk Producers Federation has developed a federally funded program to provide reimbursement for producers with JD-related expenses.

Control programs have in general relied on management techniques to identify infected herds and then clear those herds of the disease, because effective treatment and vaccination strategies do not exist. In addition, because of the long subclinical phase and the limited sensitivity of diagnostics, eradication programs require a long-term commitment.

NJWG and Control Plans

The United States Animal Health Association has long had an interest in and concern about JD in domesticated animals. Its work is conducted primarily by committees, and until the formation of a separate JD Committee in 1987, the Tuberculosis Committee was the focal point for consideration of JD issues through the Johne's Subcommittee. By 1990, the Johne's Committee had created several subcommittees to address specific tasks.

In 1994, the USAHA's JD Committee recommended formation of NJWG, which was to be broadly representative of the many public- and private-sector groups with interest in control of JD. At its first meeting, NJWG adopted a mission statement:

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> The NJWG will serve as a resource for animal agriculture in assessing any association between Johne's and human health. Recognizing that JD has major economic implications for producers, the NJWG will develop and coordinate implementation of a National Johne's program. The program will be designed to protect the public and animal health, reduce the economic burden upon producers and bring about a uniform approach for control, herd certification, and eventual eradication of this insidious and costly disease in the United States, (approved 4/9/95, Colorado Springs, Colorado; USAHA, 2002).

NJWG set out the following initial objectives:

- Evaluate information about *Map* as a zoonotic pathogen and assess the likelihood that animals serve as a reservoir of infection.
- Evaluate the potential for *Map* to contaminate foods of animal origin.
- Identify and encourage research needed to develop a strategy for control and herd certification.
- Evaluate domestic and international economic consequences and suggest good management practices for prevention of entry and spread of *Map*.
- Develop policy objectives and goals for disease control and herd certification.

Subcommittees study economics, state control programs, research status and priorities, laboratory diagnostic issues, education, small ruminants, certification, strategic planning, serology quality control, validation of check tests, and certification of veterinary inspection. Through its subcommittees and the entire working group, NJWG has made significant progress toward meeting the initial objectives. In September 2000, a survey of NJWG members and others with interest in JD identified issues for additional committee concern and effort. The top three priorities were producer education, economic incentives for testing and adding value for “status” herds, and issues of serologic tests (quality control and false positives).

The National Johne's Working Group has emerged as a prime mover both in setting the agenda and in developing the tools needed to address issues of JD control. NJWG has set short-term, intermediate, and long-term goals. Because the group includes representatives of most of the major organizations with animal-agriculture interests, decisions made by NJWG are likely to be supported and promoted by those organizations.

NJWG 1998 Program

The 1998 NJWG program (U.S. Animal Health Association, 1998; see Appendix B) is designed as a model for improving the equivalency of state control programs, as a framework for the establishment of new state programs,

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and to assist state veterinarians and state JD advisory committees as they considered implementation of herd certification programs. The model is a voluntary herd status program, which identifies minimum requirements for operation of a scientifically sound approach to identification of low-risk herds. It provides for confidentiality of testing results at the discretion of the producer (within state limits), but it also encourages producers who have entered the program to reveal test results as a way to promote the value added by JD-free herd certification.

The program recommends an initial risk evaluation, to inform producers of existing on-farm factors associated with the spread of JD and to introduce to them the Best Management Practices that have been identified to prevent the introduction and spread of JD. Important practices that must be adopted before entry into the program are the prevention of commingling, the prevention of exposure to the manure or raw milk of susceptible species over a period of 12 months, and the permanent identification of individual cattle.

There are two paths through the program: the standard and the fast track. If the producer can certify that the herd has been free of JD for five years, that the property has been JD free for one year, and that no cattle have been introduced from JD-infected herds for five years, then the herd can be entered into the certification program through the fast track. This allows entry directly into Level 2, by-passing the first of four levels of certification. The fast track permits producers to proceed more quickly than the standard track does, but it requires a greater financial investment at program entry. The fast track will allow herds to reach Level 4 in two years with three tests; the standard track requires at least three years and four tests to reach Level 4. However, the standard track allows entry to the program with a minimal investment of funds and gradually increases the producer's investment in the program.

The four levels differ primarily by the number of herd animals tested and the types of diagnostic tests performed (Tables 4–1, 4–2). Previously infected herds and JD-vaccinated herds can enter the program after infected animals are removed or vaccination is discontinued and the number of nonvaccinated animals meets the testing criteria. There is also a provision for an appeal process, for example, if results from an ELISA or fecal-culture test are disputed.

On-farm biosecurity measures are required to prevent the spread of JD from animals of unknown status. Exposure to pooled milk (for calves) or to manure from untested cattle should be eliminated, and cattle should not be grazed or have other contact with other JD-susceptible species. Cattle being transported should be hauled in cleaned and disinfected trailers, and commingling should be avoided.

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Table 4–1. Standard-Track Certification from the U.S. Voluntary Johne's Disease Herd Status Program for Cattle

Level	Criteria
1	Program entry requirements have been met, and negative ELISA tests have been performed on 30 second- or higher-lactation animals. A sample size of 30 was selected to optimize herd sensitivity and herd specificity and to maintain a fixed cost for all herds entering the program.
2	The herd has met the requirements for Level 1, and negative ELISA on a statistical subset of second- or higher-lactation animals has been performed. The Level 2 testing must be completed between 10–14 months after any Level 1 testing.
3	The herd has met requirements for Level 2 and has negative fecal-culture-test results on a statistical subset of second- and higher-lactation animals. Bulls 2 years of age and older must be included in this testing. The fecal culture must be collected between 10–14 months after any Level 2 testing.
4	The herd has met the requirements for Level 3 and has a negative ELISA on a statistical subset of second- or higher-lactation animals. Level 4 testing must be completed between 10–14 months after any Level 3 testing. Level 4 status is maintained by achieving negative ELISA results on 30 second- or higher-lactation animals every 10–14 months.

SOURCE: USAHA, 1998

Table 4–2. Fast-Track Certification from the U.S. Voluntary Johne's Disease Herd Status Program for Cattle

Level	Criteria
2	Fast-track program entry requirements have been met, and negative ELISA results have been obtained on a statistical subset test of second- or higher-lactation animals.
3	The herd has met requirements for Level 2 fast-track and has negative fecal-culture test results on 30 second- or higher-lactation animals. (A history of JD freedom for 5 years before program entry adds sufficient confidence to allow fast-track herds to test 30 animals rather than the statistical subset used in the standard tack to obtain Level 3 status.) Level 3 testing must be completed 10–14 months after any Level 2 testing.
4	The herd has met the requirements for fast-track Level 3 and has negative ELISA results on a statistical subset test of second- or higher-lactation animals. Level 4 testing must be completed 10–14 months after any Level 3 testing. Level 4 status is maintained by achieving negative ELISA results on 30 second- or higher-lactation animals every 10–14 months.

SOURCE: USAHA, 1998

A key feature of the program is the status of herd additions. Young cattle are required to come from program herds of similar status. Older animals have more stringent testing and biosecurity requirements, if purchased from nonprogram herds. Replacement cattle must come from herds of equivalent status. Semen and embryos may be used from any herd.

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USDA 2002 Program

The USDA's recently approved "Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program" (USDA, 2001a) for beef and dairy cattle was developed with input from NJWG and USAHA (USDA, 2001a; see Appendix C). It is intended to be a federal-state-industry cooperative program, in which all levels have important roles. States will have primary administrative responsibilities through specified positions and groups, and will be supported by federal agencies and industry. The program is voluntary, so producer incentive for participation relies on the potential for the added market value associated with products from known-status herds.

As with the NJWG control program, the USDA draft program is intended to provide minimum national standards, which local authorities may exceed as warranted. The program has administrative, technical, and testing components.

Administratively, the USDA program mandates the appointment of a qualified designated state JD coordinator who assists producers, health officials, and veterinarians in combating JD through training, interpreting results, and helping to review and develop herd management plans. The coordinator also advises and informs other federal and state animal health officials, specifically USDA's JD program officials and a mandated state JD group, about JD activities and issues. The coordinator may also decide to incorporate the services of private veterinary practitioners in the development of technical program elements, such as herd management plans, and should then implement a JD certification program for those veterinarians to ensure their qualifications.

The program stipulates that the coordinator should serve as a member of the mandated JD advisory group and that a producer should be its chair. It is recommended that the membership should consist of dairy producers, beef producers, university or extension faculty, diagnostic-laboratory personnel, regulatory veterinary medical officers, and beef and dairy veterinary practitioners, and that they meet at least once a year.

There are three primary technical program elements: education of producers, development of good management strategies, and herd testing and classification. The education element of the program is the producer's entry point to the program. At this stage, the producer should receive information about the disease; about good management practices for the control, elimination, and prevention of JD; and about the JD control program itself.

The intermediate element involves establishing approved but voluntary management plans and practices that are producer- or market-driven. The first step is to conduct an on-farm risk evaluation, which can be used as the basis for a management plan. The JD risk assessment instrument assesses "maximum risk" values for each management group of animals on a farm. The numerical values constitute a consensus opinion of JD control experts; the numerical values attempt to weight the greatest risk to the youngest groups of animals in recognition of available information on transmission risks. The management plan should focus on biosecurity measures to minimize exposure to and spread of JD through infected animals and a contaminated environment. Minimum

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management practices, such as the prevention of commingling of infected and uninfected animals, the use of single-source colostrum, individual housing, and the permanent and individual identification of all cattle, should be put in place. Annual renewal of participation in the program is based on repeating the risk assessment and updating the herd management plan.

Herd testing and classification is the third element of the USDA draft control program. An initial screening test on a random sampling of a herd determines whether the herd will enter the test-positive or the test-negative track of this third element. There are different designated sampling schemes for testing, which vary by the number, age, and sex of the cattle selected for testing, and which are similar to the NJWG sampling scheme. Vaccinated herds are eligible to enter the program without additional testing or culling, as long as an organism detection method is used. Appeal of a positive test result for an animal is allowed.

Test-Positive Component

The USDA program has instituted a test-positive component to gather information about the prevalence of JD-infected herds. Test-positive herds must be enrolled in the program and abide by the education, herd management, identification, renewal, and appeal procedures as laid out in the first two elements of the program. They also must conform to guidelines for herd additions and testing, following a protocol similar to the random-sampling protocol laid out in the NJWG program.

Additionally, the USDA program has a four-level test-positive-classification scheme, ranging from Level A to Level D (Table 4–3). Herds can advance through the levels to enter the test-negative classification ladder. Level A indicates a negative herd-screening result, and producers whose herds qualify for Level A are encouraged to apply to enter the test-negative program component. Level D indicates greater than 15 percent positive herd prevalence.

Test-Negative Component

Entry into the test-negative component requires the same education, herd management, identification, renewal, and appeal procedures as in the test-positive track. The test-negative component has more stringent guidelines for herd additions and testing, similar to those of the NJWG program. Testing should be performed by or under the supervision of a recognized health official, and the tests must be done by an approved laboratory. As in the test-positive track, advancement is possible, and there are standard and fast tracks that are similar to those of the NJWG program (Tables 4–4, 4–5).

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Table 4–3. Test-Positive Requirements from the Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program

Level	Criteria
A	An annual herd test reveals no screening or official Johne's-test-positive animals. Can be maintained by achieving negative screening test results on 30 second- or higher-lactation animals every 10–14 months. Herds achieving Level A should be encouraged to enter the test-negative program at Level 1. The following qualifications must be met: • Negative test results on at least 30 randomly selected second- or higher-lactation animals (3 years old or older), or • Negative test results on the whole herd and on bulls over 2 years of age.
B	An annual whole-herd test and bulls over 2 years of age reveals less than 5% of animals positive to a screening or official JD test.
C	An annual whole-herd test and bulls over 2 years of age reveals at least 5%, but not more than 15%, of animals positive to a screening or official JD test.
D	Herds should be classified as Level D if either of the following apply: • A test on at least 30 randomly selected second- or higher-lactation animals (3 years or older) reveals one or more test-positive animals, or • A whole-herd test with the addition of bulls over 2 years of age reveals more than 15% of the animals positive to a screening or official JD test.

SOURCE: USDA, 2001a

Table 4–4. Standard-Track Test-Negative Components from the Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program

Level	Criteria
1	The herd owner has developed a herd management plan and agreed to abide by the requirements of the test-negative component. The herd has had negative screening-test results on 30 second- or higher-lactation animals.
2	Herds have met the Level 1 requirements and have had negative screening-test results on a statistical subset of second- or higher-lactation animals. Level 2 testing must be completed within 10–14 months of any Level 1 testing.
3	Herds have met the Level 2 requirements and have had negative fecal-culture-test results on a statistical subset of second- and higher-lactation herd members. Bulls 2 years and older must be included in this testing. The fecal culture must be collected within 10–14 months of any Level 2 testing.
4	Herds have met the Level 3 requirements and have had negative screening-test results on a statistical subset of second- or higher-lactation animals. Level 4 testing must be completed within 10–14 months of any Level 3 testing.

SOURCE: USDA, 2001a

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Table 4–5. Fast-Track Test-Negative Components from the Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program

Level	Criteria
1	Skip this level if the owner signs a declaration that no cows were seen or diagnosed with JD in the past five years and has an approved herd plan in place. The State may require the declaration to be cosigned by the herd veterinarian. The signed declaration must include the following statements: • I am fully aware of the management and disease history of the herd during the past five years. • JD is not known or suspected to have existed in the herd during the past five years or on the property during the past 12 months. • No cattle have been introduced from known infected herds during the past five years.
2	Herds have met the Level 1 requirements and have had negative screening-test results on a statistical subset of second- or higher-lactation animals. Level 2 testing must be completed within 10–14 months of any Level 1 testing.
3	Herds have met the Level 2 requirements and have had negative fecal-culture-test results on 30 second- or higher-lactation cows and all bulls 2 years or older. The fecal culture must be collected within 10–14 months of any Level 2 testing.
4	Herds have met the Level 3 requirements and have had negative screening-test results on a statistical subset of second- or higher-lactation animals. Level 4 testing must be completed within 10–14 months of any Level 3 testing.

SOURCE: USDA, 2001a

Laboratory Approval

Laboratories that are being used for JD testing must have official approval specifically for JD testing. Private, university, state, and federal laboratories can participate, but state animal health officials must be able to audit them. Whether they perform analytical or screening tests, laboratories must meet performance validation standards, as described in Boxes 4–1 and 4–2. Currently approved diagnostic tests and laboratories are listed in Tables 4–6 and 4–7.

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BOX 4–1 APPROVAL PROCESS FOR LABORATORIES PERFORMING OFFICIAL JOHNE'S DISEASE TESTS

- A laboratory seeking approval to perform official JD tests must contact the National Veterinary Services Laboratory (NVSL) for a test kit of 25 samples. (A valid check test sample from NVSL will be determined by a consensus of at least 70% of the participating laboratories.)
- The laboratory must correctly identify 100% of the negative test samples.
- The laboratory must correctly identify 100% of the TNTC (too numerous to count) test samples.
- The laboratory must correctly identify at least 70% of the test samples that were not classified as negative or TNTC.
- The laboratory must use the same procedure and materials during the check test that are used during routine testing.
- No retest is available within the same fiscal year.

BOX 4–2 APPROVAL PROCESS FOR LABORATORIES PERFORMING JOHNE'S DISEASE SCREENING TESTS (SEROLOGY TESTS)

- A laboratory seeking approval to perform screening tests must contact NVSL for a test kit of 25 samples. (A valid check sample will be determined by NVSL using available licensed ELISA kits.)
- The laboratory must correctly identify at least 90% of the serology check test samples.
- The laboratory must use the same procedure and materials during the check test that are used during routine testing.
- One retest is available if a laboratory fails the first time.

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Table 4-6. National Veterinary Services Laboratory Approved Johne's Disease Tests

Test	Description
Fecal, tissue culture[a]	Culture is the standard for organism-based tests, although culture methods are not currently standardized. Protocols for recommended methods are available from NVSL upon request. Sensitivity of fecal- and tissue-culture tests is estimated at 40%; specificity is 99% for tests that are done correctly.
Direct DNA probe (without PCR)	DNA probes can detect *Map* without culture. DNA tests are relatively rapid (3 days) but more expensive than culture methods. They can miss low shedders. Sensitivity is estimated at 40%; specificity is about 99.9%.
Radiometric culture	Radiometric-based methods have been adapted from those used to isolate the bacterium that causes human tuberculosis. Radiometric culture can detect small amounts of bacteria, and it is faster than standard fecal-culture methods (7 weeks vs. 16 weeks). It is more expensive than other methods, it requires technicians to handle radioactive materials, and it requires specialized instrumentation to read the culture vials. Sensitivity is estimated at 40%; specificity is about 99%.
Histology of tissue	*No check test is currently available*. Microscopic identification of characteristic changes and of *Map* in tissue is a definitive test for JD. Tissue changes and bacteria can be seen in the intestinal lining and in nearby ileum, mesenteric, and ileocecal lymph nodes of infected animals. Sensitivity, which depends on disease stage and the number and type of specimens examined, typically exceeds that of other methods. Specificity is considered to be 100%, based on defined criteria (granuloma with acid-fast bacteria observed by board-certified pathologist).
USDA-licensed ELISA	Animals identified as positive by ELISA should be considered infected until disease is ruled out by an official JD test. ELISA is used for screening and to inform management decision making. Sensitivity is estimated at 25% for nonclinical cases, 85% for clinical cases. Specificity is estimated at 98–99%.

[a] SOURCE: http://www.usaha.org/njwg/jdtests.html (8/1/02).

Table 4-7. Canadian and U.S. National Veterinary Services Laboratory Approved Laboratories for Johne's Disease (Nov. 1, 2001)

State/Province	City	Laboratory	Fecal	PCR	Serology
Canada					
Alberta	Edmonton	Alberta Agriculture, Food & Rural Development Food Safety Division	Yes	No	No
		Veterinary Pathology Laboratory Alberta Ltd.	No	No	Yes
		Virology-Serology Laboratory AHLB-Alberta Agriculture	No	No	Yes
	Lothbridge	Palliser Animal Health Laboratories	No	No	Yes

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State/Province	City	Laboratory	Fecal	PCR	Serology
Canada					
British Columbia	Abbotsford	Animal Health Monitoring Laboratory	Yes	Yes	Yes
Manitoba	Winnipeg	Veterinary Services Laboratory	No	No	Yes
Saskatchewan	Regina	Prairie Diagnostic Services	Yes	No	Yes
	Saskatoon	Prairie Diagnostic Services Bacteriology/Mycology	Yes	No	Yes
United States					
Alabama	Auburn	Charles S. Roberts Veterinary Diagnostic Laboratory Alabama Department of Agriculture	No	No	Yes
Arkansas	Little Rock	Arkansas Livestock & Poultry Arkansas State University	No	No	Yes
Arizona	Tucson	Arizona Veterinary Diagnostic Laboratory	No	No	Yes
California	Davis	California Animal Health & Food Safety Laboratory University of CA, Davis	Yes	No	Yes
	Fresno	California Animal Health & Food Safety Laboratory University of CA	Yes	No	Yes
	San Bernadino	California Animal Health & Food Safety Laboratory University of CA	Yes	No	Yes
Colorado	Denver	Rocky Mountain Regional Animal Health Laboratory CO Department of Agriculture	Yes	Yes	Yes
	Fort Collins	Veterinary Diagnostic Laboratory CO State University	Yes	No	Yes
	Rocky Ford	Colorado State University Veterinary Diagnostic Laboratory Rocky Ford Branch	No	No	Yes
Delaware	Dover	Delaware Department of Agriculture	No	No	Yes
Florida	Kissimmee	Animal Diagnostic Laboratory FL Department of Agriculture	No	No	Yes
	Live Oak	Live Oak Diagnostic Laboratory	No	No	Yes
Georgia	Tifton	Veterinary Diagnostic and Investigations Laboratory University of GA	No	No	Yes
Iowa	Ames	Veterinary Diagnostic Laboratory IA State University	Yes	No	Yes
		Diagnostic Bacteriology Laboratory, NVSL USDA/APHIS/VS	No	No	Yes
		National Animal Disease Center USDA-ARS	Yes	No	No
		Diagnostic Bacteriology Laboratory, NVSL USDA/APHIS/VS	Yes	No	No

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State/Province	City	Laboratory	Fecal	PCR	Serology
United States					
Idaho	Boise	Idaho Bureau of Animal Health Laboratories	No	No	Yes
	Caldwell	Caine Veterinary Teaching Center University of Idaho	No	No	Yes
Illinois	Centralia	Animal Disease Laboratory Illinois Department of Agriculture	Yes	No	Yes
	Galesburg	Animal Disease Laboratory Illinois Department of Agriculture	Yes	No	No
	Urbana	Labs of Veterinary Diagnostic Medicine College of Veterinary Medicine	Yes	No	Yes
Indiana	Dubois	Animal Disease Diagnostic Laboratory	Yes	No	No
	West Lafayette	Animal Disease Diagnostic Laboratory Purdue University	Yes	Yes	Yes
Kentucky	Hopkinsville	Breathitt Veterinary Center Murray State University	Yes	No	Yes
	Lexington	Livestock Disease Diagnostic Center University of Kentucky	Yes	No	Yes
Maryland	College Park	Animal Health Diagnostic Laboratory Maryland Department of Agriculture	No	No	Yes
	Frederick	Animal Health Diagnostic Laboratory	Yes	No	No
Maine	Westbrook	IDEXX Laboratories, Inc.	No	Yes	Yes
Michigan	East Lansing	Animal Health Diagnostic Laboratory Michigan State University	Yes	Yes	Yes
		Laboratory Division Michigan Department of Agriculture	Yes	No	Yes
	Lansing	Biostar Research, Inc. (Antell Biosystems)	Yes	Yes	Yes
Minnesota	St. Paul	Minnesota Veterinary Diagnostic Laboratory	Yes	No	Yes
Missouri	Cameron	NW Missouri Veterinary Diagnostic Laboratory Missouri Department of Agriculture	Yes	No	Yes
	Columbia	Veterinary Medical Diagnostic Laboratory University of Missouri	Yes	No	Yes
	Fayette	Allied Monitor, Inc.	Yes	No	Yes
	Gray Summit	Veterinary Services Laboratory Purina Research Center	No	No	Yes

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State/Province	City	Laboratory	Fecal	PCR	Serology
United States					
	Jefferson City	Animal Health Laboratory Missouri Department of Agriculture	Yes	No	Yes
	Springfield	Missouri Veterinary Diagnostic Laboratory Missouri Department of Agriculture	No	No	Yes
Mississippi	Jackson	Mississippi Veterinary Diagnostic Laboratory	No	No	Yes
Montana	Bozeman	Veterinary Diagnostic Laboratory Montana Department of Livestock	No	No	Yes
North Carolina	Raleigh	Rollins Animal Disease Diagnostic Laboratory	No	No	Yes
North Dakota	Fargo	North Dakota State Veterinary Diagnostic Laboratory North Dakota State University	Yes	No	Yes
Nebraska	Lincoln	Veterinary Diagnostic Center University of Nebraska, Lincoln	No	No	Yes
New Jersey	Trenton	Division of Animal Health Laboratory New Jersey Department of Agriculture	Yes	No	Yes
New York	Ithaca	Veterinary Diagnostic Laboratories New York State College of Veterinary Medicine	Yes	No	Yes
Ohio	Reynoldsburg	Animal Disease Diagnostic Laboratory Ohio Department of Agriculture	Yes	No	Yes
Oklahoma	Stillwater	Oklahoma Animal Disease Diagnostic Laboratory Oklahoma State University	Yes	No	Yes
Oregon	Salem	Animal Health Laboratory Oregon Department of Agriculture	Yes	No	Yes
Pennsylvania	Harrisburg	Pennsylvania Veterinary Laboratory Department of Agriculture	Yes	Yes	Yes
	Kennett Square	Johnes Research Laboratory University of Pennsylvania	Yes	No	Yes
	Quakertown	Quakertown Veterinary Clinic	No	No	Yes
South Carolina	Columbia	Clemson Veterinary Diagnostic Center Clemson University	No	No	Yes
South Dakota	Brookings	Animal Disease Research & Diagnostic Laboratory South Dakota University	Yes	Yes	Yes
Tennessee	Nashville	C.E. Kord Animal Disease Laboratory	Yes	No	Yes
Texas	Amarillo	Texas Veterinary Medical Diagnostic Laboratory Texas A&M University	Yes	No	Yes

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State/Province	City	Laboratory	Fecal	PCR	Serology
United States					
	College Station	Texas Veterinary Medical Diagnostic Laboratory Texas A&M University	Yes	Yes	Yes
Washington	Olympia	Washington State Department of Agriculture Lab Services	Yes	No	Yes
	Pullman	Washington Animal Disease Diagnostic Laboratory Washington State University	Yes	No	Yes
Wisconsin	Barron	Wisconsin Veterinary Diagnostic Laboratory	Yes	No	No
	Madison	Wisconsin Animal Health Laboratory Wisconsin Department of Agriculture	Yes	No	Yes
	Madison	Johnes Testing Center University of Wisconsin, Madison	Yes	No	Yes
West Virginia	Charleston	Animal Health Division West Virginia Department of Agriculture	No	No	Yes

SOURCE: USDA, 2002

NMPF 2002 Proposal

The National Milk Producers Federation (NMPF) has recommended plans for JD management and testing. NMPF along with other farm organizations, represents many of the milk-processing cooperatives in the United States. Although the NMPF plan has evolved over time and iterations have varied in programmatic cost and scope, the March 20, 2002, version of the NMPF proposal is representative of this group's current goals.

The proposal seeks federal funding to support state JD program activities. About $49 million in federal support is requested to fund JD testing by ELISA and fecal culture and to fund state infrastructure and pay laboratory costs. Funding would be earmarked for states with programs administered through state-approved JD advisory committees. Funding to support testing would be available only to those herds with Johne's herd management plans approved by the chief livestock veterinarian in the state and based on herd risk assessment conducted under the direction of a USDA-approved Johne's certified veterinarian. Fecal-culture-positive animals would be ear-punch identified and removed from the herd before drying-off (end of lactation). Culture-positive animals would be removed to slaughter only, except heavily infected animals (TNTC on fecal culture), which would be rendered.

The goal of the NMPF initiative is to encourage voluntary testing of herds and the establishment of herd plans that incorporate Best Management Practices for JD control and eradication. An earlier program initiative proposed

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additional funding for indemnity support to producers who remove fecal-culture-positive animals from their herds (NMPF, 2002, 2003).

State Programs

Many states have established, or are moving toward, JD programs that are compatible with national standards (Figures 4–1, 4–2, and 4–3; Table 4–8). A few states have JD programs that predate the national effort. Each program has some unique approaches to JD control.

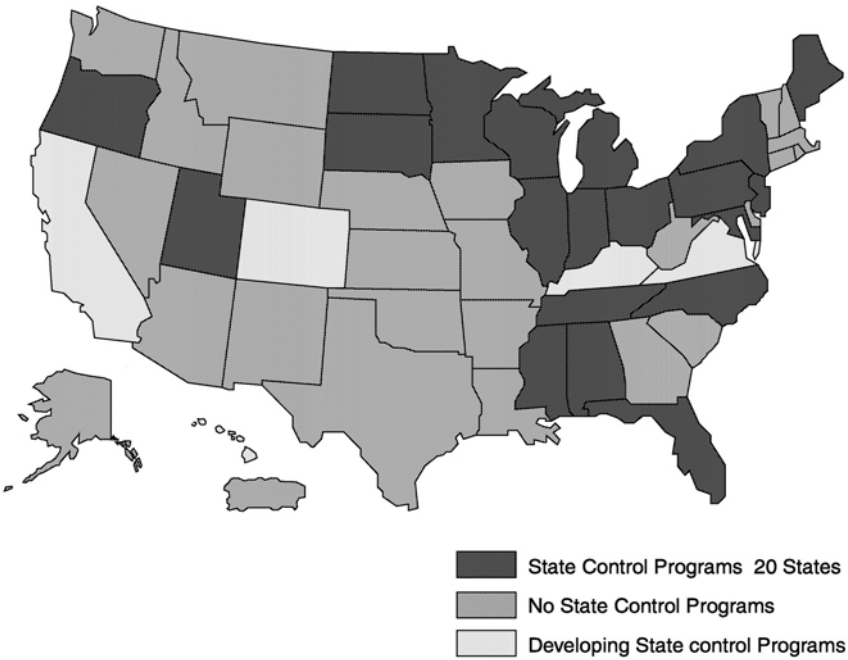

Figure 4–1. Johne's Disease State Control Programs, January 2002
SOURCE: USDA, 2003a

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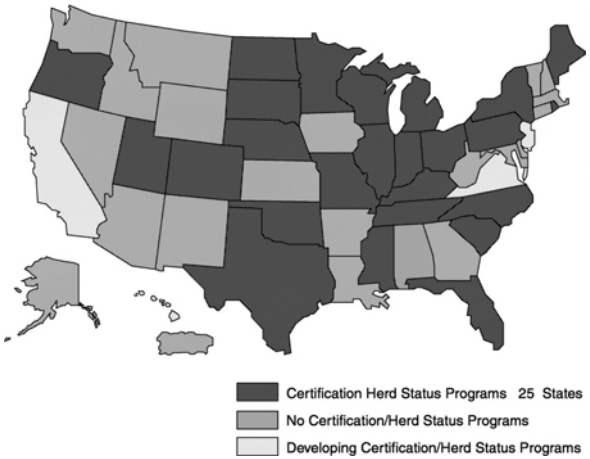

Figure 4–2. Johne's Disease State Certification and Status Programs, January 2002
SOURCE: USDA, 2003b

Figure 4–3. Johne's Disease State Advisory Committees, January 2002
SOURCE: USDA, 2003c

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New York

JD control programs are an integral part of the larger NYSCHAP, which is administered by the state's Department of Agriculture and Markets and by the New York State Animal Diagnostic Laboratory. NYSCHAP enrolls herds in three categories of participation:

- *Participating herd* A farm risk assessment is conducted and a herd plan outlines management procedures to reduce the risk of introducing *Map* to the farm or to prevent spreading of the organism to susceptible animals on the farm.
- *Control herd* In addition to risk assessment and management procedures, producers of these herds include testing in the control plan.
- *Status herd* Producers of herds that are negative on serum or fecal testing are encouraged to enter a herd status program that is similar to the national voluntary JD program. Special features of the New York program are the use of a kinetics-based enzyme-linked immunosorbent assay (KELA) that allows characterization of individual animal test results by level of risk for *Map* infection.

In 2001, the New York Animal Diagnostic Laboratory adopted a liquid-medium Trek system for fecal culture of *Map,* enabling a much reduced turn-around time (NYSCHAP, 2001). More than 400 New York herds are currently participating in some form of control program (Dr. John Huntley, New York State Department of Agriculture and Markets, personal communication, October 2001).

Pennsylvania

Pennsylvania has had JD control programs for more than 20 years. Early efforts were based on fecal-culture identification of infected animals with permanent quarantine or slaughter-with-indemnity of culture-positive animals. The effort in Pennsylvania includes three complementary programs:

- *Thirty Free Tests Program* Any dairy or beef producer may request serum ELISA on 30 animals. Testing is done within the state laboratory system at no charge to the producer. Producers are encouraged to sample 30 randomly selected second-lactation or older animals, but they may choose any 30 animals. Test results are informational for the producer only, but herd owners are encouraged to enter their herds in one of the other state programs, based on the results of the 30 tests.
- *Voluntary Johne's Disease Control Program* A voluntary JD control program is available for herds with at least one animal with JD-positive ELISA or other test results. The program is consistent with USAHA-recommended program standards, and it includes a participation stage without testing and a control stage with testing.

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- *Voluntary Johne's Disease Status Program* Pennsylvania has adopted the proposed national voluntary JD status program for test-negative herds (Pennsylvania Bureau of Animal Health and Diagnostic Services, 2000).

More than 30,000 animals from 1,100 herds have been tested through the Thirty Free Tests Program. About 500 herds are enrolled in management, control, or status programs. No indemnity is offered currently (Philip DeBok, Pennsylvania Department of Agriculture, personal communication, 2001).

Wisconsin

The current JD program in Wisconsin has been in place since July 2000. It is a voluntary program that encourages testing to establish a herd classification. Any cattle or goats offered for sale as breeding animals include an implied warranty that the animals are free of JD unless the seller offers, in writing, a Wisconsin Johne's Disease Management classification. Herd classification is established through testing of “test eligible” animals (bulls 24 months of age or older, cows 36 months of age or older, goats 18 months of age or older). The following classifications have been established:

- Entire eligible herd tested or test 30 eligible animals or 10 percent of the eligible animals, whichever is greater; no test positives
- Entire eligible herd tested; fewer than 5 percent test positive
- Entire eligible herd tested; 5–15 percent test positive
- Entire eligible herd tested; more than 15 percent positive or 30 eligible animals or 10 percent of the herd tested; one or more positives
- No testing done; maximum risk

Herd owners may elect one of three testing options:

- *Random herd test* Thirty eligible animals or 10 percent of eligible animals, whichever is greater
- *Entire herd test* All eligible animals tested at one time
- *Split herd test* With a state Department of Agriculture approved plan, test all eligible animals over a period not to exceed one year

Testing may be by fecal culture or by serum ELISA in cattle, or by fecal culture in goats (Wisconsin Department of Agriculture, Trade and Consumer Protection, 2000).

Although considerable program experience has been gained through the New York, Pennsylvania, and Wisconsin programs, these and other programs have not been objectively evaluated and reported. Each of these programs has changed significantly over time, making it difficult to evaluate program effectiveness and impact. Collection and publication of this information would be extremely valuable in the formulation of a successful control strategy.

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Table 4–8. Components of State Johne's Disease Herd Status/Control Programs

Program	New York	Pennsylvania	Wisconsin
Federal-state-industry partnership	+/`	+	+
Coordination with NJWG voluntary JD herd status program	+	+	+
Integral education component	+	+	+
On-farm risk assessment	+	+	`
Development of herd management plan	+	+	`
Subsidized testing for program entry	+	+	+
Promotion of likelihood ratios for interpreting ELISA results	+	+	`
Market-based incentives, disincentives	+	`	+
Best Management Practices for other fecal pathogens	+	`	`
Integral research component	`	`	`
Restricted animal movement from test-positive herds	`	`	`
Incremental progression to mandatory control, eradication	`	`	`
Laboratory proficiency testing	+	+	+
Program audit with review, correction mechanism	`	`	`

Notes: +, Included in published descriptions of program; ` , not included in published descriptions of program; +/`, status unclear based on published descriptions of program; NJWG, National Johne's Disease Working Group.
SOURCE: NYSCHAP, 2001; Pennsylvania Bureau of Animal Health and Diagnostic Services, 2000; Wisconsin Department of Agriculture, Trade and Consumer Protection, 2000

Non-U.S. Programs

Few regional or national JD programs have been established outside the United States because of legal, political, and economic issues (Table 4–9). Long-term success among national programs is limited. Iceland has effectively controlled JD in sheep through a national vaccination program (Fridriksdottir et al., 2000). Australia has implemented market assurance programs that involve certification of assessed herds and flocks of cattle, sheep, goats, and alpaca (Allworth and Kennedy, 1999; Kennedy and Allworth, 1999). Specific areas of Australia have been regionalized since 1999, when Western Australia was declared disease-free for ovine and bovine JD (Kennedy and Benedictus, 2001). The Australian program is a partnership between government and seven affected industries that began modestly as an effort to assess the status of herds and flocks to identify test-negative sources. The program includes identification of test-positive herds and flocks, and there are mandatory restrictions on the sale or movement of animals from affected premises. Depopulation and restocking is being pushed at different rates by various Australian states.

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Table 4–9. Components of Nationwide Johne's Disease Herd Status/Control Programs

Program Element	Australia	Netherlands	USAHA	USDA
Federal-state-industry partnership	+	+	+	+
Integral education component	+	+	+	+
On-farm risk assessment	+	+	+	+
Development of herd management plan	+	+	+	+
Subsidized testing for program entry	+	+	+	`
Promotion of likelihood ratios for interpreting ELISA results	`	`	`	`
Market-based incentives, disincentives	+	+/`	`	`
Best Management Practices for other fecal pathogens	`	+/`	+/`	`
Integral research component	+	+	`	`
Restricted animal movement from test-positive herds	+	`	`	`
Incremental progression to mandatory control, eradication	`	+	`	`
Laboratory proficiency testing	`	`	+	+
Program audit with review, correction mechanism	+	`	+	+

Notes: +, Included in published descriptions of program; ` , not included in published descriptions of program; +/` , status unclear based on published descriptions of program.
SOURCES: Kennedy and Allworth, 2002; Benedictus et al., 1999; USAHA, 1998; USDA, 2001a.

PRINCIPLES OF CONTROL IMPLEMENTATION

National-government-funded animal health programs typically are directed to exotic-disease exclusion and to the control or eradication of specific diseases of widely recognized economic or public-health importance. However, the emergence of the global food system and the expansion of trade in animals and animal products has increased national attention to diseases such as JD, that can affect national economic development by restricting international movement.

Despite the increased attention, the control and eradication of JD from beef and dairy cattle, sheep, and goats face formidable challenges, in part because of low awareness among producers, but also because of the insidious nature of infectious diseases. Despite the availability of adequate diagnostic tests and the increasing availability of disease-free replacement stock, there is still room for significant improvement in both areas. The economic pressure for increasing herd and flock sizes in U.S. commercial animal agriculture contributes to the spread of disease, as infected replacement animals are added to existing herds.

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Regional or national coordination of prevention and control programs is critical for effectively addressing these challenges. The responsibility for initiating, coordinating, and funding regional or national animal health programs can rest on government or industry, or on a partnership between the two. Existing programs rely on varying combinations of entities, and they have emphasized the importance of a coordinated response, regardless of which agency has primary responsibility for the program. There is consistent recognition that the involvement of producers is critical to program success. An integral education component that leads to on-farm risk assessment and development of herd management plan is essential for success. Some programs rely on market-based enrollment incentives and disincentives, but other ways to engage producers also have been developed—subsidies for testing and indemnification, for example. Another possibility for promoting producer involvement is through the incorporation of Best Management Practices for a wider range of fecal pathogens, but this concept has not been incorporated into current programs. Additional research on JD and control principles and practices, and the promotion of scientifically sound practices—such as the use of likelihood ratios for interpreting ELISA results—also are important elements that are not considered in some current programs.

Regulatory elements—restricting animal movement from test-positive herds, requiring laboratory proficiency testing, and program audits with review and correction mechanisms—have been introduced to bolster the effectiveness of JD control and eradication programs. Clearly, another effective step toward eradication will be to gradually make compliance mandatory. Although there is no current mandatory national JD control or eradication program, federal authorities have one additional but limited tool to control the spread of JD: there are regulations that control interstate transport of clinically affected animals. Historically, the federal government has mandated that animals and animal products that are transported from one state to another must be accompanied by a certificate of veterinary inspection identifying the animals, attesting to animal health, and documenting relevant diagnostic test results. The federal government establishes minimum animal health requirements for interstate movement, including the requirement that animals be certified by a federally recognized veterinarian as free of any visible signs of infectious or communicable disease. This is of little benefit in controlling the spread of JD however, because most *Map*-infected animals are subclinically affected. Cachectic animals or animals that exhibit clinical diarrheal disease would not be certifiable for interstate movement, except directly to slaughter. Individual states require additional tests or certification regarding specific diseases. In 2000, the USDA Animal and Plant Health Inspection Service Veterinary Service promulgated rules that prohibit the interstate movement of animals known to be test positive to an officially recognized JD test, unless the animals are moving directly to slaughter, however, testing of those animals prior to movement is not required.

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5

Economic Implications of Johne's Disease

Because most cases of Johne's disease (JD) are subclinical and prevalence data are lacking or imprecise, it is difficult to assess the economic consequences of this disease for individual cows, herds, or the nation. In the United States, several estimates of economic losses have been made, including an estimate of $15.4 million annually in New England (Chiodini et al., 1984), $54 million in Wisconsin (Arnoldi and Hurley, 1983), $5.4 million in Pennsylvania (Whitlock et al., 1985), and from $200 million to $250 million annually in U.S. dairy herds (Garry et al., 1999). By comparison, economic losses in Australia were estimated to be the equivalent of U.S. $2.1 million annually (Gill, 1989).

Economic consequences at the herd level were estimated as part of the 1996 National Animal Health Monitoring System (NAHMS) survey of U.S. dairy herds. For JD test-positive herds with fewer than 10 percent of cull cows showing clinical signs consistent JD, the adjusted annual value of production was $40 per cow lower than for JD test-negative herds that had no reported cull cows with clinical signs. For JD test-positive herds with 10 percent or more cull cows showing clinical signs, the economic impact was $227 per cow, and the majority of the loss was attributable to reduced milk production with additional losses from higher cow replacement costs and lower cull cow revenues (NAHMS, 1997a). Benedictus and colleagues (1987) apportioned JD losses into decreased production (32 percent), veterinary and treatment costs (2 percent),

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reduced value at slaughter (20 percent), loss due to idle production facilities (3 percent), and unrealized future income based on age at culling and prior production (43 percent). Groenendaal and Galligan (1999) used simulation models to predict average losses of $35 per cow per year, increasing to $52 and $71 per cow per year after 10 and 20 years, respectively. Finally, Ott and colleagues (1999) calculated average annual losses of $22–$27 per cow. The variations in these estimates are partly the result of differences in prevalence, herd size, management practices, and the varying assumptions that underlie the calculations. In all estimates of the economic impact of JD, however, the losses are significant.

TRADE

JD is spread most effectively through the movement of infected animals that contaminate a new environment, thus setting the stage for widespread exposure of more animals. *Map* also can be isolated from semen and embryos from affected breeding stock, although the effectiveness of this route of transmission is unknown. Consequently, JD has serious implications for domestic and international trade of live animals and germplasm. The identification of *Map* in animal products also can affect trade, especially if a link between *Map* and Crohn's disease is demonstrated conclusively.

Interstate Trade

There are currently no federal limitations on intrastate movement of animals between premises. Participants in voluntary JD status programs can agree to the limitations on introduction of new animals as a requirement for maintaining existing status in state programs. Current control programs allow only animals from herds of equal or higher JD status to enter participating herds without conditions.

Interstate movement requires that animals be accompanied by a certificate of veterinary inspection stating that they show no clinical signs of any disease (U.S. Department of Agriculture [USDA], 2001b), including JD. Rule changes promulgated by USDA's Animal and Plant Health Inspection Service-Veterinary Services (USDA/APHIS/VS) in 2000 also prohibit interstate movement of animals with a positive result to an official JD test, except movement directly to slaughter. Official JD tests detect the *Map* organism and must be completed by a laboratory approved by USDA/APHIS/VS/National Veterinary Services Laboratories. However, the rule changes refer only to animals with known test results and do not require JD testing of domesticated animals before interstate movement. Some states broadly prohibit entry of animals known to be affected by or exposed to any infectious or contagious communicable disease. Kentucky specifically defines communicable disease to include JD (Kentucky, 2002). However, no state currently requires *Map* testing or any specific certification as to the *Map* exposure status of the animal or its herd or flock of origin.

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International Trade

International trade of animals has contributed to the global dissemination of JD. Infected animals of European origin are cited as the original source of JD in several countries. Concern over the potential for spread of JD and other diseases has led to the development of international guidelines for testing and certification of animals destined for export. The veterinary administrations of 158 nations comprise the International Office of Epizootics (OIE), which has developed the *International Animal Health Code,* a compendium of specific recommendations concerning the international movement of animals and animal products (OIE, 2001a). OIE also promulgates the *Manual of Diagnostic Tests and Vaccines,* which provides standards for the application of diagnostic tests and quality assurance for vaccines (OIE, 2000).

The OIE considers JD (generally called paratuberculosis in official OIE documents) a disease of major global importance. JD is categorized as a List B transmissible disease, considered to be of socio-economic and/or public health importance within countries and significant in the international trade of animals and animal products (OIE, 2001a). Specific guidance on JD in all species is provided by *International Animal Health Code,* Chapter 2.2.6. Current code recommendations are limited to live ruminants and address individual animal certifications and testing as follows:

> Veterinary Administrations of importing countries should require (for domesticated ruminants for breeding or rearing), the presentation of an international veterinary certificate attesting that the animals:
>
> - showed no clinical sign of paratuberculosis (JD) on the day of shipment;
> - were kept in a herd in which no clinical sign of paratuberculosis (JD) was officially reported during the 5 years prior to shipment;
> - were subjected to diagnostic tests for paratuberculosis (JD) with negative results during the 30 days prior to shipment.

Current OIE recommendations provide only a limited protection against importation of *Map*-infected animals, because few affected animals show clinical signs, official reporting systems are not widely enacted or enforced, and no specific recommendations are provided for the choice of diagnostic tests or their interpretation.

Adoption of the sanitary and phytosanitary (SPS) provisions of the General Agreement on Tariffs and Trade in 1994 (World Trade Organization [WTO], 1994) and the dramatic increase in international trade in animals, germplasm, and animal products over the past 25 years have pushed OIE to revisit several current guidelines. The SPS trade agreements identified OIE as the international standards-setting agency for animal health issues with regard to trade. Therefore, the OIE code recommendations represent the de facto baseline for import requirements promulgated by national veterinary administrations. OIE recommendations cannot be challenged in international trade disputes

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before WTO. Countries have the right to require more stringent animal health import measures than those recommended by OIE, but SPS mandates that additional requirements are to be science based and justified by risk assessment. Additional requirements that exceed the code guidelines can be challenged on the basis of scientific validity.

Chapter 2.2.6 of the *Manual of Standards, Diagnostic Tests, and Vaccines 2000* (OIE, 2000) could be reconsidered by OIE in the near future. OIE recently commissioned a supporting document on JD that provides a comprehensive review of current knowledge focusing on controlling the spread of JD via trade. Supporting documents are usually the precursor to the revision of specific code chapters. Proposed revisions to the *International Animal Health Code and Manual* arise from recommendations of member countries and then proceed through several committees' review. Final disposition of proposed revisions is decided at the OIE annual meeting of the International Committee, a committee of the whole that consists of one vote for each member country. The OIE code tends to reflect broad consensus and to recognize the needs of developed and developing nations alike.

Impacts

JD is likely to become more of a trade issue in the near future, both here and abroad. The implementation of various state JD control programs sets the stage for differing import requirements by individual states. International import restrictions also could increase as more countries embark on JD control and eradication programs. Australia has identified several of its states as JD-free zones, restricting the movement of animals into those areas (Kennedy and Allworth, 1999). The implementation of a national control and assurance program that incorporates internal movement control empowers Australia to require similar assurances of JD status from its global trading partners. Similar import requirements that go beyond the OIE international standard could be implemented within the legal context of the SPS by other countries that have JD control and eradication programs in place. If it is established that *Map* can cause Crohn's disease, more restrictions on international movement of animals, animal products, and germplasm can be expected.

There are few restrictions on the interstate movement of JD-exposed or *Map*-infected animals other than those that show clinical signs of disease and those known to be organism-test positive. Because it is difficult to effectively and economically screen animals for *Map,* livestock is often bought and sold without consideration of *Map* status. Most buyers who seek large numbers of cattle, sheep, or goats currently have no practical options for ensuring JD-negative status for their purchases. Consequently, producers of rapidly expanding herds or flocks are at a very high risk of buying Map-infected animals. Current international standards for the safe movement of animals recommend JD testing, combined with clinical examination and herd history, although no specific guidance is provided for testing protocols or interpretation of results. Increased global concern about JD will encourage development of further trade barriers related specifically to JD and *Map*. In the absence of

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coordinated JD control programs, imports and exports will be affected. Restrictions on the importation of animals infected with *Map* will be limited by OIE and SPS requirements. Furthermore, export opportunities will be lost unless larger populations of JD-free or low-risk animals can be certified by government agencies.

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6

Johne's Disease and Crohn's Disease

Understanding the human health implications of Johne's disease (JD) will depend on resolution of a central question: Is the etiologic agent of Johne's Disease, *Mycobacterium avium* subsp. *paratuberculosis (Map),* a significant cause of disease in humans? More specifically, is *Map* a cause or the cause of Crohn's disease (CD), or is it an incidental bystander without clinical significance? This has been a controversial subject attracting strong adherents to both positions, but its resolution is essential for the development of rational approaches to disease management and prevention. Programs designed to limit human exposure to organisms from zoologic and other environmental sources will present enormous challenges if *Map* is found to be a significant human pathogen. However, the stated task of this committee is not to determine the etiology of CD, or even to determine the link between Johne's and Crohn's disease, but to review and make recommendations regarding the control and diagnosis of JD, which, of course, may include human health implications. The question of the JD/CD link was therefore examined in the context of the primary study focus. In addition, because there are two recent and thorough investigations of the relevant evidence by the National Institutes of Health (National Institute of Allergy and Infectious Diseases [NIAID], 1998) and the

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European Union (European Commission, 2000), these data are dealt with in more general terms.

CROHN'S DISEASE

Crohn's disease is an inflammatory disorder of the intestine of unknown cause. The disease is chronic, and patients tend to remit and relapse. Symptoms include general malaise, chronic weight loss, abdominal pain, and diarrhea. It is a life-long disease that has no cure. CD usually begins early in life, with peak incidence between the ages of 16 and 25, but it can occur in early childhood or later in life. Although the disease can occur in any portion of the gastrointestinal tract, it has a predilection for the terminal ileum. The three most common patterns of disease are the involvement of the terminal ileum and cecum, the ileum alone, and the colon alone. The disease is treated with various agents—aminosalicylates, corticosteroids, azathioprine, anti-TNF antibody, antibiotics—to inhibit the inflammatory response in the intestine. Unfortunately, therapy is imperfect and relapses are common. The disease can be complicated by perforation, abscess formation, fistula formation, strictures, and intestinal obstruction. Several of those complications can require surgery in which sections of the bowel are removed. Some patients suffer many intestinal resections which leave them with a “short bowel” (insufficient small bowel to maintain hydration, nutrition, and health). Those patients are sustained with total parenteral nutrition—intravenous feeding with a specialized formulation.

Etiology of Crohn's Disease

The cause or causes of Crohn's disease are unknown. Prominent hypotheses include an aberrant or autoimmune host inflammatory response to undefined antigens, infectious etiologies including *Map,* and aberrant immune response to a specific infectious agent. There is conflicting evidence for and against each of these proposed pathogenetic mechanisms for Crohn's disease. As it is currently understood, the disease could, in fact, be more than one malady (Gilberts et al., 1994; Mishina et al., 1996). If this is true, then the existing conflicts in interpretation of evidence for causation may be resolved by clear categorization of Crohn's disease into multiple syndromes having distinct etiologies. If *Map* is involved in some cases of Crohn's disease, it does not appear to be a simple case of infection by an agent in a susceptible host. The generally favorable response of Crohn's patients to profound immunosuppression and/or bone marrow transplantation supports the notion that immune dysfunction or dysregulation is an important element of the disease (James, 1988; Kashyap and Forman, 1998; Lopez-Cubero et al, 1998; Soderholm et al., 2002; Yoshida et al, 1996). This suggests that if *Map* is involved at all, it is as a result of an aberrant host immune response to the presence of the agent.

In addition to *Map,* various other causes of Crohn's disease have been proposed: chronic ischemia and microinfarction, persistent measles infection, chronic viral infection, infection with pathogenic *E. coli,* abnormal response to a

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dietary component, and abnormal inflammatory response to normal intestinal microflora, or components of the flora, in genetically predisposed individuals (Fiocchi, 1998; Podolsky, 1991; Rath et al, 2001; Sartor, 1997; Selby, 2000). Various polymorphisms of a human gene, NOD2, that confers increased susceptibility to CD, has recently been described (Hugot et al., 2001; Ogura et al., 2001). The role of this gene, which may function as an apoptosis regulator, is currently unclear.

Epidemiology of Crohn's Disease

The occurrence of Crohn's disease is not uniform throughout the world or even throughout the United States (Eisen and Sandler, 1994; Ekbom et al., 1991; European Commission, 2000; Kyle, 1992; Sonnenberg and Wasserman, 1991; Sonnenberg et al., 1991). In general, Crohn's disease is much more common in developed countries than in developing nations. Whether this reflects a true difference in prevalence or is the result of variations in disease diagnosis, definition, recognition, and reporting is not clear (Eisen and Sandler, 1994). However, most authorities believe there are true geographic differences (Garland et al., 1981; Sonnenberg et al., 1991; Yang et al., 2001). The incidence in the United States and in Sweden has increased over the past 60 years. The reasons for this are unclear. The highest rates in Europe are found in Sweden (Ekbom et al., 1991) and in the United Kingdom, especially Scotland (Kyle, 1992). Disease rates are lower in Mediterranean countries (European Commission, 2000; Shivananda et al., 1996). Prospective studies of European incidence show a variation of 0.9 to 9.5 per 100,000 population (European Commission, 2000; Shivananda et al., 1996). Prevalence rates may more accurately reflect the magnitude of the problem. Prevalence rates as high as 133 per 100,000 have been observed in Olmstead County, Minnesota (Loftus et al., 1998).

There is a substantial difference in rates throughout the United States (Sonnenberg et al., 1991), with as much as a three-fold variation in prevalence from state to state. A study of veterans cared for within the Veterans Administration hospital system (Sonnenberg et al., 1991) showed a marked difference in prevalence among states. The highest-prevalence states were Oregon, Idaho, Nebraska, Wisconsin, West Virginia, Maine, New Hampshire, Massachusetts, Connecticut, and Rhode Island (Sonnenberg et al., 1991). It has been suggested that there is a gradient of prevalence from northern Europe to Mediterranean countries (Eisen and Sandler, 1994; European Commission, 2000) and from northern to southern states in the U.S. (Sonnenberg et al., 1991). The significance of these observations is not clear.

In both the United States and Sweden, Crohn's disease is more common in urban areas than in rural areas (Eisen and Sandler, 1994). It is more common in white Americans than in blacks (Garland et al., 1981; Sonnenberg and Wasserman, 1991), in Jews compared with other ethnic groups (Eisen and Sandler, 1994; Yang et al., 1993), and in smokers versus nonsmokers (Calkins, 1989; Tobin et al., 1987). The disease can affect more than one member of a family, and rare clusters of Crohn's disease have been reported (Bennett et al.,

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1991; Lashner et al., 1986; Meucci et al., 1992; Yang et al., 1993). There could be a cohort effect of age in Sweden (Ekbom et al., 1991) and in Scotland (Kyle, 1992) where persons born between 1945 and 1950 have a higher prevalence of Crohn's disease.

IMPLICATIONS OF THE LINK

Whether there are important human health implications associated with the development of a JD control program depends on the degree to which clinically significant human disease results from exposure to *Map*. It follows that the influence of an animal control program on human health will correspond to its capacity to meaningfully reduce exposure of susceptible persons to strains of *Map* that can cause disease. There is a paucity of rigorous data on *Map's* role in human disease. Without such data, it is not possible to analyze the essential epidemiologic, pathologic, or environmental factors necessary for the rational design of programs that would interrupt transmission to humans. Even if *Map* is an important human pathogen, it might not be possible to significantly reduce human exposure through epidemiologic control measures alone. Early diagnosis and effective treatment could prove the only practical means of reducing human disease under such circumstances. Methods to improve the sensitivity, specificity, and speed of identifying *Map* are critical if gaps in current knowledge are to be filled. Any discussion of the human health implications of a JD control program without this information is speculative at best.

Map and Human Disease

Despite intense interest in the zoonotic potential role of *Map,* it has still not been unequivocally established as a cause of clinically significant human disease. There have, however, been two well-documented cases of *Map* infection in humans: a young boy with scrofula (Hermon-Taylor et al., 1998), and a case of widely disseminated mycobacterial disease in an adult male with AIDS (Richter et al., 2002). Because *Map* infection will be diagnosed only if it is specifically considered and if appropriate specialized laboratory tests are performed, it is possible that *Map* has passed unrecognized in an unknown number of similar cases. No systematic surveillance studies have been undertaken in humans to identify the extent of disease syndromes attributable to *Map* infection, nor is it known if the pathogenesis of the disease that may follow *Map* infection in humans is similar to or distinct from that occurring in other species. However, the clinical manifestations in the two cases cited above closely resemble syndromes associated with *M. avium* subsp. *avium* infection (Horsburgh et al., 1985), raising the possibility that *Map* can cause host damage by mechanisms similar to those of *M. avium*.

In considering whether human disease caused by *Map* is more widespread than currently documented, a parallel may be drawn with the increased recognition of previously unsuspected clinical disease attributable to infection with *M. avium* subsp. *avium* and other atypical mycobacteria of the *M. avium* complex (MAC) that followed their recognition as important human

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pathogens in AIDS (Zumla and Grange, 2002). For example, before its widespread recognition as a cause of disseminated disease in AIDS, MAC pulmonary disease was regarded as clinically similar to indolent tuberculosis, occurring primarily in men with underlying chronic pulmonary disease such as chronic obstructive pulmonary disease and silicosis. However, in the mid-1980s, MAC was identified as an important cause of nodular pulmonary disease, especially in middle-aged women who had no underlying predispositions (Prince et al., 1989). This recognition arose from an increased appreciation of MAC as a potential human pathogen, from the use of improved diagnostic methods, and from heightened clinical surveillance. It is possible that increased surveillance for *Map* as a potential etiologic agent will similarly lead to its association with clinical syndromes.

Because the frequency of the above described disease syndromes that could be *Map*-associated is low, it is impossible with available information to predict what effect JD control programs will have on the occurrence of human disease. However, the overall risk to the general public posed by exposure to *Map* is likely to be very low compared to that posed by the many other potentially zoonotic agents. Increased interest in *Map* as a potential human pathogen will help to provide valuable information about the possible spectrum and characteristics of human disease caused by *Map*. If the prevalence of human *Map* infection increases or is found to be greater than currently believed, independent consideration of JD control programs designed to limit human exposure could be warranted.

Map and Crohn's Disease

Important human health implications associated with the control of JD will be most strongly linked to determination of the effect of *Map* infection on the development of Crohn's disease. The evidence for a relationship is discussed in detail later, but of note is the case of the child with scrofula caused by *Map,* referred to above, who subsequently developed Crohn's disease. That case has been cited as supporting evidence for an etiologic role in at least some cases of Crohn's disease (Hermon-Taylor et al., 1998), although no similar cases have been reported. Temporal changes in the prevalence and distribution of Crohn's disease have been documented that could be the result of increased exposure of susceptible populations to the causative agent. If *Map* is etiologically implicated and if increases in its environmental ubiquity underlie the changing epidemiology of Crohn's disease, then successful reduction of exposure to *Map* could reduce rates of associated human disease.

There are three possible outcomes to the controversy that surrounds the question of whether or not *Map* has a clinically important role in the development of Crohn's disease, which are also relevant to JD control efforts:

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- If *Map* is the cause of all cases of Crohn's disease, this relationship will then drive animal control programs.
- If *Map* causes a subset of Crohn's disease cases, this linkage will have the same implications for animal control programs as if all cases were caused by *Map*.
- If *Map* is not a cause of Crohn's disease, recommendations regarding diagnosis and control of JD depend only on animal health and agricultural concerns.

If *Map* is shown to have no role in Crohn's disease, then considerations for control programs will be based on JD's effects on commercial animal production. The most practical and convincing evidence that *Map* is not implicated would be provided by demonstration of an alternative etiology and a highly effective treatment. So far, however, that has not happened.

However, if *Map* is etiologically linked to all or most cases of Crohn's disease, there will be far-reaching implications. The simplest way to demonstrate a causal relationship would be through significantly altering the clinical course of disease by treatments that are effective against *Map*. Clinical studies testing this hypothesis are currently under way in Australia, and their outcome will be followed with great interest. However, failure of the treatments used in these trials will not disprove an association between *Map* and Crohn's disease, because it is not known whether the regimens being evaluated are truly effective against *Map,* and the proportion of Crohn's cases caused by *Map* is unknown. The stage of disease at which treatment of *Map* might be optimally effective also has not been identified. However, a convincing response to treatment in all or even in a large proportion of cases in well-controlled studies would strongly support a connection with *Map* infection. For example, if a subset of Crohn's patients from which *Map* could be isolated or identified responded to anti-*Map* therapy, while a similar subset from which *Map* could not be isolated or identified did not, a causal role for *Map* would be strongly supported for the first subset. The clinical understanding of Crohn's disease would be modified by such studies, and the door would open to developing new diagnostic and therapeutic tools for managing disease caused by *Map,* and to undertake focused studies of disease pathogenesis. Of greatest importance, there would be new hope for treatment that could relieve the chronic suffering and debilitating manifestations that characterize the disease.

Environmental sources of *Map* may be ubiquitous, and they may include reservoirs that are traditionally subject to surveillance and management to reduce risk of pathogen exposure to the public. Dairy and beef cattle production have been most widely discussed as sources of *Map,* but it is clear that more diverse species could serve as sources of zoonotic infection. *Map* is hardy and has been found in open-drainage water systems (Manning and Collins, 2001). Contamination of public-water sources with *Map* has not been widely studied but, as with other environmental mycobacteria, it is likely to be

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present to some degree, depending on regional factors. Establishing how any of these reservoirs functions in transmission to humans will be a daunting task.

Recognition of *Map* as a possible human health concern should invigorate interest in areas of potential risk and result in public and private resources being directed toward finding ways to reduce the hazard to humans. However, there are insufficient data to determine what specific control measures will reduce transmission to humans. Efforts directed at improved diagnosis and effective treatment might more reliably and economically reduce the associated burden of human disease.

If *Map* is found to cause a subset of cases of Crohn's disease, it will be more difficult to demonstrate with certainty than if it is found to cause all or most cases. If the proportion is very small, it is not clear that a JD control program will have a significant effect on human disease. Efforts to improve diagnosis will be important in this setting. Several studies have found evidence of *Map* in up to two-thirds of cases studied (Collins et al., 2000; Dell'Isola et al., 1994, Lisby et al., 1994; Sanderson et al., 1992, Suenaga et al., 1995). If *Map* causes an equivalent proportion of cases and Crohn's disease is found to be a heterogeneous entity with diverse causes, discrimination of cases caused by *Map* will be a priority. Should a large percentage of cases be caused by *Map,* the general implications are similar to those described should most or all cases be etiologically related. There will, in addition, be new interest in identifying the cause of non-*Map* related cases of Crohn's disease.

The human health implications of JD control programs will remain speculative until more is known about the capacity of *Map* to cause human disease. This knowledge will depend on improvements in diagnostic methods and the conduct of well-designed human clinical studies. Provision of adequate resources to gain this information should be a priority. There is insufficient knowledge to determine whether reduction of disease caused by *Map* should be addressed through treatment of persons already infected, through epidemiologic control programs designed to prevent human exposure, or to a combination of those approaches.

Comparison of Johne's Disease and Crohn's Disease

Crohn's and Johne's diseases have been compared clinically and pathologically. Both are granulomatous diseases of the intestine with a predilection for the ileum. But the similarity of the two diseases has been overstated. When they are compared clinically and pathologically (Tables 6–1 and 6–2), several significant differences can be seen (European Commission, 2000; NIAID, 1998; Rubery, 2001; Selby, 2000; Van Kruiningen, 1999). The similarities and differences have been interpreted by experts both in favor of and in opposition to the view that *Map* is a cause of Crohn's disease (NIAID, 1998).

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Table 6–1. Clinical Features of Crohn's Disease and Johne's Disease

Feature	Crohn's Disease	Johne's Disease
Preclinical stage		
Symptoms and signs	Not known	Decreased milk yield
Incubation period	Not known	Minimum 6 months
Clinical stage		
Presenting symptoms and signs	Chronic diarrhea, abdominal pain, weight loss	Chronic diarrhea, dull hair, weight loss, decrease in lactation
Gastrointestinal symptoms and signs		
Diarrhea	Chronic (3 weeks+)	Chronic[a]
Blood in stool	Rare	Rare
Vomiting	Rare	No
Abdominal pain	Yes	Unknown
Obstruction	Yes	No
Extraintestinal manifestations		
Polyarthritis	Yes, but rare	No
Uveitis	Yes, but rare	No
Skin lesions	Yes	No
Amyloidosis	Yes, but rare	Yes[b]
Hepatic granulomatosis	Yes, but rare	Yes
Renal involvement	Yes, but rare	Yes[c]
Clinical course		
Remission and relapse	Yes	Yes

[a]Not seen in sheep
[b]Goats primarily
[c]Goats, deer, primates primarily, also camelids
SOURCE: Adapted from European Commission (2000)

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Table 6–2. Pathologic Features of Crohn's Disease and Johne's Disease

Feature	Crohn's Disease	Johne's Disease
Lesion location		
Esophagus and oral cavity	Yes	No
Ileum and colon	Yes	Yes[a]
Mesenteric lymph nodes	Yes	Yes
Rectum, anus	Yes	Advanced cases[b]
Segmental involvement of intestine	Yes	Yes
Macroscopic features		
Macroscopic appearance	Edema of affected bowel wall, "garden hose" like appearance	Thickened bowel wall[b]
Parietal edema	Yes	Yes
Stenosis	Yes	Rare
Perforation	Yes	Rare
Fistula	Yes	No
Pseudopolyps	Yes	No
Mucosal aspect	Cobble stone appearance	Corrugated[b,c]
Microscopic appearance		
Transmural involvement	Yes	Rare
Fibrosis	Yes	No
Lymphoid aggregates	Yes	Yes[c]
Granuloma	Yes (50–70% of cases)	Occasionally
Caseation	No	Usually not[d]
Fissures	Yes	No
Visible acid fast bacilli	No	Yes[e]

[a]Ileum and jejunum are the initial and most frequent locations
[b]Not always in sheep
[c]Predominant feature in lymphocytic-paucimicrobial form
[d]Varies with species
[e]Scarce or absent in lymphocytic-paucimicrobial form
SOURCE: Adapted from European Commission (2000)

The data obtained from human studies directed at the cause of Crohn's disease are varied and conflicting:

- Culture of mycobacteria (including, but not limited to *Map*) from Crohn's tissues (Chiodini et al., 1984; Collins et al., 2000; Coloe et al., 1986; Gitnick et al., 1989; Graham et al., 1987; Haagsma et al., 1988; Moss et al., 1992; Schwartz et al., 2000; Thorel et al., 1990a)

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- Serum antibody responses to various *Map* antigens (Cohavy et al., 1999; Elsaghier et al., 1992; El-Zaatari et al., 1999; Naser et al., 2000; Olsen et al., 2001; Suenaga et al., 1999; Vannuffel et al., 1994)
- PCR of Crohn's tissues with primers directed against IS900 (thought to be specific for *Map;* see Chapter 3) (Al-Shamali et al., 1997; Cellier et al., 1998; Chiba et al., 1998; Collins et al., 2000; Dell'Isola et al., 1994; Dumonceau et al., 1996; Fidler et al., 1994; Frank et al., 1996; Kanazawa et al., 1999; Lisby et al., 1994; Mishina et al., 1996; Murray et al., 1995; Rowbotham et al., 1995; Sanderson et al., 1992; Schwartz et al., 2000; Suenaga et al., 1995)
- Hybridization studies of Crohn's tissues in situ using probes directed against IS900 (Hulten et al., 2000b, 2001)
- Treatment of Crohn's patients with anti-mycobacterial therapy (Table 6–3) (Borody et al., 2002; Grahamand Al-Assi, 1995; Gui et al., 1997; Hampson et al., 1989; Prantera et al., 1994; Rutgeerts et al., 1992; Schultz et al., 1987; Shaffer et al., 1984; Swift et al., 1994; Warren et al., 1986). The work has been summarized by Collins, 1998; Van Kruiningen, 1999; Hulten and colleagues, 2000a; Goswami, 2000; and Chamberlin and colleagues, 2001.

Table 6–3. Antimycobacterial Therapy Studies for Crohn's Disease

Drug	Number of Patients	Duration (months)	Outcome	Reference
Rifampicin, Ethambutol	14	12	No improvement	Shaffer et al., 1984
Rifampicin, Ethambutol, Isoniazid, Pyrazinamide	1	7	Remission	Warren et al., 1986
Rifampicin, Ethambutol, Isoniazid, Pyrazinamide	1	4	Remission	Schultz et al., 1987
Dapsone	1		Remission	Prantera, 1988
Rifampicin, Ethambutol, Isoniazid, Pyrazinamide	20	9	Remission, 50%	Hampson et al., 1989
Ethambutol, Isoniazid, Pyrazinamide, Rifampicin	5	Up to 12	Patients not stabilized; recurrence of symptoms during treatment	Jarnerot et al., 1989

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Drug	Number of Patients	Duration (months)	Outcome	Reference
Rifampicin, Ethambutol	10	6	No clinical improvement	Rutgeerts et al., 1992
Clofazimine, Dapsone, Ethambutol, Rifampicin	40	9	Remission in some patients	Prantera et al., 1994
Ethambutol, Isoniazid, Rifampicin	130	24	Treatment ineffective	Swift et al., 1994
Clarithromycin	15	6	Remission, 50%	Graham et al., 1995
Rifabutin, Macrolide	46	18	Remission, 93% for more than 2 years	Gui et al., 1997
Rifabutin, Clarithromycin	12	24	Full remission for 6 patients; partial response from 2 patients	Borody et al., 2002

SOURCE: Adapted from Collins, 1998.

The lack of uniform results in pathogen isolation studies, serologic response, or responses to antimycobacterial therapy could reflect the possibility that only a subgroup (of unknown size) of Crohn's disease cases is related to *Map*. Studies of human disease tend to be small and highly selected, and studies of the effectiveness of antimycobacterial chemotherapy in Crohn's disease have frequently been uncontrolled and not based upon in vitro antimicrobial sensitivity. The finding of *Map* in Crohn's tissues by culture, PCR, in situ hybridization, or other methods may reflect several possibilities (an innocent bystander has merely colonized the intestine; a secondary infection that is not the cause of Crohn's disease; a surrogate marker for another agent that has not been detected; a false positive result especially in the case of PCR; an actual cause of Crohn's disease). For instance, PCR will be necessary to identify sequences from a normal commensal bacterium in the tissues to control for increased intestinal permeability in IBD, and another pathogen not felt to be an etiologic agent in IBD should also serve as a control (e.g., *Salmonella*).

When considering treatment studies, based upon experience with MAC, isoniazide and pyrazinamide are unlikely to contribute meaningfully to therapeutic regimens, while rifampin and ethambutol by themselves may have limited potency and durability (Young et al., 1986). Regimens using clarithromycin or azithromycin plus rifabutin and ethambutol have been the mainstay of effective treatments for MAC (Shafran et al., 1996) and there is evidence of similar efficacy against *Map* in vitro (Williams et al., 1999). Their

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empiric use for treating *Map* understandably follows from this evidence, but there has been little rigorous preclinical testing that might provide strong assurances of success. Confirmation of the effectiveness of these regimens against *Map* has not been confirmed in a suitable animal model, since no such model exists. There are important precautionary notes to interpretation of studies that use these regimens, for which the experience with MAC is again instructive. The contribution of rifabutin to effective treatment of disseminated MAC in AIDS is far less than predicted (Gordin et al., 1999), and the fluoroquinolones, which appear very potent in vitro, are either of limited benefit or counterproductive (Fernandes et al., 1989; Shafran et al., 1996). Additionally, the optimal length of treatment for serious MAC disease, which is much easier to diagnose and follow clinically than JD or CD, has not been established and relapses have been described both on and after treatment (Dunne et al., 2000). Finally, these agents may have activity against a wide variety of organisms whose role in CD may be underappreciated. Consequently, if there is a striking response to these treatment regimens in clinical trials, the role of *Map,* while strongly supported, will not be proven. For those who are afflicted, this will be a detail that can be sorted out later. However, if response rates are marginal or of limited durability, a role for *Map* will not be excluded since the anti-microbials used may not have been sufficiently potent or durable.

The lack of agreement among studies could be attributable to the low sensitivity and questionable specificity of the microbiologic and immunologic methods. Moreover, laboratory techniques vary widely and have exhibited poor reproducibility, thus making it difficult to compare results among studies. The result is a body of scientific literature that is confusing and often contradictory. Furthermore, it is possible that Crohn's disease as currently defined is not a single entity (Gilberts et al., 1994; Mishina et al., 1996). The lack of uniform results in pathogen isolation studies, serologic response, or responses to antimycobacterial therapy could derive, in part, from the possibility that only a subgroup is related to *Map.*

Evidence Required to Support a Cause-and-Effect Relationship

What evidence would establish *Map* as a human pathogen and as a cause of Crohn's disease? The generally accepted standard for establishing a specific infectious agent as the cause of a human disease has been the fulfillment of Koch's postulates (Koch, 1884). Briefly stated, with current status by available evidence, these are:

1. The microorganism must be found in all cases of the disease.
    - *Partially fulfilled* Met for some but not all cases. Wide range of association in different studies employing diverse methodologies.
2. It must be isolated from the host and grown in pure culture.
    - *Fulfilled*
3. It must reproduce the original disease when introduced into a susceptible host.

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- *Partially fulfilled* Experimental animals have been infected with human isolates but do not reproduce the disease syndrome.

4. It must be found in the experimental host so infected.

- *Fulfilled*

If all of the criteria are fulfilled, then the organism isolated is the definitive etiological agent of the disease syndrome in question. When such a link has been established, clinical diagnosis of the disease is sufficient to implicate the presence of the specific organism before it is confirmed in the laboratory. This is not presently the case for *Map*.

Partial fulfillment of Koch's postulates support an association with disease but may or may not support causation. It is commonly the case that different pathogens may be responsible for a single clinical syndrome. Koch's postulates are not fulfilled for a single organism in such diseases as pneumonia, meningitis, endocarditis, hepatitis, and urinary tract infection among many others. Thus, many well-recognized etiological agents have only partially satisfied Koch's postulates for the diseases they cause. Koch's postulates can be difficult or impossible to apply in chronic diseases of complex or multifactorial etiology. Many parasitic illnesses and viral diseases have unique host-dependent clinical manifestations, such that animal models imperfectly reflect human clinical disease syndromes. The most notorious recent example of this was the claim that HIV did not cause AIDS because the third postulate was not fulfilled in that human viruses do not reproduce the disease in animal models (Duesberg et al., 1989). Only the tragic accidental infection and disease of laboratory workers and recent dramatic responses to effective antiretroviral therapy has settled this controversy. Finally, lack of even partial fulfillment of the criteria does not rule out an etiological relationship between an organism and a disease. This is exemplified by leprosy whose etiological agent, *M. leprae,* has never been isolated in pure culture, leaving the second criterion unsatisfied to this day. A reasonable interpretation of Koch's postulates is that evidence supports an association but is insufficient to prove or disprove definitively an etiological relationship between *Map* and Crohn's disease.

The Hill-Evans criteria have been formulated (Evans, 1976) as epidemiologically based criteria for evaluating the causal link between environmental factors, including infection, and disease. While fulfillment of all ten of the Hill-Evans criteria would strongly incriminate *Map,* fulfillment of all ten is not likely. A listing of the Hill-Evans postulates, with the committee's interpretation of the available data and whether they satisfy the attendant criteria, illustrates the point:

1. Prevalence of Crohn's disease should be significantly higher in those exposed to *Map* than those not.

- *Direct data insufficient* Geographic differences in rates of CD and emergence of disease in unaffected populations migrating to areas of higher prevalence suggest that there

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are environmental factors influencing rates of CD (Probert et al., 1996; Shivananda et al., 1996). Furthermore, no systemic studies that identify regional human exposures to *Map* with which to correlate CD incidence have been performed. However, it should be noted that Sweden has one of the highest prevalence rates of Crohn's disease in the world yet has little Johne's disease (Ekbom, 1991; Viske, 1997).

2. Exposure to *Map* should be more frequent among those with Crohn's disease than those without.

    - *Data insufficient* No studies specifically evaluate the exposure of patients with CD to *Map*. Detailed surveys of potential environmental sources of *Map* would be required to conduct these studies and this information is not available.

3. In prospective studies, the incidence of Crohn's disease should be higher in those exposed to *Map* than those not.

    - *Data insufficient* Such prospective studies have not been conducted.

4. Crohn's disease should follow exposure to *Map* with a normal or log-normal distribution of incubation periods.

    - *Data insufficient* Knowing the time of exposure of a susceptible population is necessary to satisfy this condition.

5. A spectrum of host responses along a biologic gradient from mild to severe should follow exposure to *Map*.

    - *Data insufficient* Specific and sensitive assays of host response to *Map* that are necessary to address this criterion are not available (see Chapter 3).

6. A measurable host response should follow exposure to the *Map* in subjects who lack this response before exposure, or it should increase in those with the response before exposure. The response should be infrequent in those not exposed to *Map*.

- *Data conflicting and insufficient* Specific and sensitive assays of host response to Map that are necessary to address this criterion are not available (see Chapter 3).

7. In experiments, Crohn's disease should occur more frequently in those exposed to *Map* than in unexposed control subjects.

    - *Data insufficient* It is unethical to perform this experimentally in humans; however, disease caused by *Map* does occur in non-human primates (McClure et al., 1987), but no experimental studies have been conducted in these species.

8. Reduction or elimination of *Map* should reduce the risk of the disease.

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- *Data insufficient* Evidence for this criterion is unlikely to be available in the foreseeable future.

9. Modifying or preventing the host response should decrease or eliminate the disease.
    - *Various therapeutic trials performed, but data not conclusive* See Table 6–3.
10. All findings should make biologic and epidemiologic sense.
    - Johne's and Crohn's diseases have many clinical and pathological similarities, but there are significant differences as well. It seems biologically plausible that *Map* could cause at least a subset of Crohn's disease.

It is unlikely that the data needed to satisfy all the criteria will be obtained soon. What data could be obtained to moderately or strongly support a causal role for *Map*? The following specific lines of evidence would provide varying degrees of support for a cause-and-effect relationship.

1. Epidemiologic Association between *Map* Exposure and Crohn's Disease
    - *Strong evidence of causation:* A higher incidence of Crohn's disease among those in direct contact with *Map* than in those without such exposure. Requires detailed knowledge of environmental sources of exposure currently not available.
    - *Strong evidence of causation:* Low prevalence of Crohn's disease in populations with no potential exposure compared to those with potential exposure. Requires detailed knowledge of environmental sources of exposure currently not available.
    - *Moderate evidence of causation:* A higher incidence of Crohn's disease among people who work with *Map*-infected animals or herds. Conditions conferring host susceptibility are not well understood.
    - *Moderate evidence of causation:* A higher incidence of Crohn's disease among susceptible people who consume products containing or contaminated with *Map*. Conditions conferring host susceptibility are not well understood.
    - *Moderate evidence of causation:* Identification of a common susceptibility gene in animals and humans.
    - *Moderate evidence of causation:* Identification of similar responses to a specific treatment in affected animals and humans.
    - *Moderate evidence of causation:* More *Map* seroreactivity among Crohn's disease patients than in controls with

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other causes of diarrhea or intestinal disease or in normal control subjects. Currently available assays lack adequate specificity to address this question.

2. Pathologic Evidence
   - *Strong evidence of causation:* Identification of *Map* in tissues from patients with Crohn's disease, but not in controls
   - *Moderate evidence of causation:* Isolation of *Map* from human milk in Crohn's-affected mothers, but not from unaffected control subjects.
3. Inoculation Studies
   - *Moderate evidence of causation:* Inoculation studies in humans are unethical and cannot be done. However, inoculation studies are possible in primates. Because it is unlikely that all of these studies will be done, two lines of evidence would provide strong evidence to support a role for *Map* in the causation of Crohn's disease. Briefly stated, the regular isolation or identification of *Map* or of *Map* RNA-DNA from Crohn's disease tissues and not from appropriate control groups, with the unequivocal therapeutic response of patients with Crohn's disease to therapy directed at *Map,* would strongly implicate *Map* as a human pathogen and as a cause of Crohn's disease. A large-scale therapeutic trial is being conducted in Australia. If properly designed and large enough, and if the anti-microbial regimen selected has potent and durable activity against *Map,* it could help to provide an answer to the question of therapeutic response.

CONCLUSIONS

The critical questions outlined above are not new. They have been debated for at least the past 10 years, and they are the subject of innumerable publications. Furthermore, they have been addressed in depth by three authoritative bodies in the past four years: the National Institute of Allergy and Infectious Diseases Division of Microbiology and Infectious Diseases: *Crohn's Disease—Is There a Microbial Etiology? Recommendations for a Research Agenda, 1998* (NIAID, 1998); the Scientific Committee on Animal Health and Animal Welfare, European Commission: *Possible Links Between Crohn's Disease and Paratuberculosis* (European Commission, 2000); and the United Kingdom Food Standards Agency: *A Review of the Evidence for a Link Between Exposure to Map and Crohn's Disease in Humans* (Rubery, 2001). All reached similar conclusions:

- There is insufficient evidence to prove or disprove that *Map* is a significant human pathogen.

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- There is insufficient evidence to prove or disprove that *Map* is a cause of Crohn's disease.
- The problem is of such importance and the hypothesis sufficiently plausible to warrant increased research to resolve the questions.

The committee has not seen evidence to warrant any other conclusions; however, a new approach to resolving the issue is needed and is proposed by the committee in this report.

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7

Conclusions and Recommendations

Based on its review and synthesis of the literature, evaluation of existing control and herd status programs, and input received at the public workshop, the Committee on the Diagnosis and Control of Johne's Disease reached the following general conclusions:

CONCLUSION 1. Johne's Disease (JD) is a significant animal health problem that warrants implementation of control programs tailored to specific animal species and specific segments of the agriculture industry. Furthermore, JD control deserves high priority from the USDA, individual states, and industry. The significance of the disease derives primarily from its consequences for animal and herd health, for the agriculture industry, and for national and international trade. The current concern about JD in industry and government agencies of a potential link with Crohn's disease in humans provide additional support for making JD control a high priority.

CONCLUSION 2. There remains insufficient evidence to prove or disprove that *Mycobacterium avium* subsp. *paratuberculosis* is a cause of some or all cases of Crohn's disease (CD) in humans; a new approach is needed to resolve the issue—one that is based on a research agenda that will

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provide answers to specific criteria set forward in Hill-Evans postulates. A causal link between *Map* and Crohn's disease remains a plausible hypothesis that warrants a new research approach and steps by industry and government agencies to identify and mitigate avenues of.

CONCLUSION 3. Available diagnostic tests and information about the biology of JD and methods to control it are adequate for immediate implementation of control programs.

CONCLUSION 4. There are significant gaps in knowledge about some areas relevant to control, and are discussed below under recommendations. The committee emphasizes that closing those gaps will improve control programs, although the need for information should not delay their implementation.

CONCLUSION 5. Control of JD will require a long-term commitment and iterative program implementation to maximize the chance of success. This commitment must come from all constituencies, including USDA, state agencies, and industry.

CONCLUSION 6. Because JD is currently of greatest concern to the dairy industry much of the emphasis in control recommendations is directed there. Other industries, however, should consider this an opportune time to deal aggressively with the disease, before infection prevalence increases and the disease becomes more widespread.

CONCLUSION 7. The USDA National Animal Health Monitoring System (NAHMS) prevalence surveys for JD have been a critical element in laying the groundwork for control programs.

CONCLUSION 8. The Voluntary Bovine Johne's Disease Control Program proposed by the National Johne's Working Group (NJWG) has most of the elements necessary for a successful control program, but prospects for success are and will be limited by a lack of uniform implementation among individual states.

CONCLUSION 9. The committee endorses the NWJG's efforts in educating producers and veterinarians, and advocates the expansion of these efforts.

CONTROL PROGRAMS

JD control programs are based on knowledge about the transmission of *Map,* the organism's persistence in the environment, and methods for reducing exposure to *Map* in animal environments. Minimum requirements for control therefore include identification of major transmission points, diagnostic and

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screening tests capable of identifying shedders, mechanisms for intervention at major transmission points, individual animal identification and trace-back capabilities, sources of disease-free replacement stock, and follow-up surveillance strategies (Kennedy and Benedictus, 2001). Available data suggest that these requirements have been met for JD in domesticated hoofstock and lead to the following conclusions:

- Most transmission of *Map* is through a fecal-oral route, but transmission also can occur in utero or via colostrum or milk.
- Infected adult ruminants are the major transmission source of the organism.
- Animals in the late preclinical or clinical stage of infection are more likely to shed high numbers of organisms than are early-stage infected animals.
- Young animals (less than 6 months of age) are most susceptible, but older animals can be infected by heavy or prolonged exposure
- *Map* can persist in water, manure, or other environmental sites for extended periods (one year or longer).
- Introduction of new *Map*-infected animals is a primary means by which a herd or flock becomes infected.
- Diagnostic and screening tests are adequate for detecting shedders at the individual animal and herd level, but tests are used best at the herd level.
- Reporting ELISA results as likelihood ratios, rather than as a positive or negative test result, will be beneficial for control programs and could increase confidence in the tests.
- Removal of late preclinical and clinical animals will greatly reduce *Map* organism numbers in the environment of an infected herd.
- Removal of newborn calves from dams at birth (before suckling) can interrupt an important avenue of transmission.
- Prevention of fecal contamination of feed, water, and the environment will reduce the opportunity for transmission of *Map*.
- The NAHMS prevalence surveys should continue, with attention given to maximizing the data obtained from the samples collected through add-on projects and investigations.

National and International Control Programs

Compelling arguments exist for an expanded federal role in JD prevention and control in the United States. First, animal agriculture has recognized the serious economic consequences of the disease and identified it as a major animal health issue. U.S. animal agriculture has requested assistance addressing JD. Individual states have implemented JD programs, but the likelihood of their success is diminished by the lack of consistency across states and by the absence of federal program guidelines.

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Second, the emergence of regional and national JD programs in Australia, Japan, and parts of Europe to certify the JD status of herds, flocks, and zones will increase pressure for additional government assurances of the JD status of animals and animal products destined for export. Japan is a major market for U.S. products. Australia and Europe are major competitors with the U.S. for animal and animal product markets. The U.S. must implement a national JD program in order to compete effectively in this emerging trade environment.

Third, there is some evidence that JD could be linked to Crohn's disease in humans. Definitively answering this question will require years of additional research. In the interim, pressure for JD prevention and control is likely to increase. Implementing a national JD prevention and control program will be a pragmatic step for U.S. government policy to address industry requests and trade competitiveness. Regardless of the eventual scientific conclusion regarding the putative Johne's-Crohn's link, a national program will improve the overall health of U.S. domesticated animals and the public's trust in the food supply.

Johne's disease is widespread in U.S. animal agriculture. A national prevention and control program can be implemented most successfully in an iterative fashion, each step building on the one before and setting the stage for the next. Much of the groundwork has come in NJWG's collaborative efforts. Industry organizations and university experts have cooperated with state and federal government officials to develop guidelines for state status and control programs that have been adopted in whole or in part by most states. Federal regulations have been adopted to limit the interstate transport of *Map*-positive animals. USDA's National Veterinary Service Laboratory has established a quality assurance program to ensure more consistent testing and greater confidence in results.

CONTROL RECOMMENDATIONS

While the committee strongly endorses a Best Management Practices approach to control, it also felt that control programs should initially focus on JD to take advantage of growing support for control of the disease. In addition, it is anticipated that funding for various aspects of control will be more readily available through government-industry partnerships if control has an easily identifiable target, such as JD, rather than a broader concept of Best Management Practices. As control efforts progress, an incremental transition to a Best Management Practices approach should be more feasible.

RECOMMENDATION 1. *An integrated, bottom-up approach to on-farm disease control is needed that meets the needs of the livestock producer and motivates behavioral change, with support at broader industry, state, and federal levels. Components of such a control program are described in Box 7–1.*

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General outlines for a given type of management and husbandry situation will be the same, but must be sufficiently flexible to be easily adaptable to the specific circumstances of each farm. Finally, the information must be packaged and delivered in a manner that is in harmony with the information management style of the producer and motivates them to change their behavior. This motivation may require feedback signals in the form of market price differentials established through testing by the downstream purchaser of the farm product.

BOX 7–1 COMPONENTS OF A COORDINATED INDUSTRY/ GOVERNMENT JD CONTROL PROGRAM

The following elements are important for a successful control program. At the farm level:

1. On-farm risk assessment and development of a farm plan.
2. Manure management that minimizes potential for transmission of pathogens by the fecal-oral route.
3. Protection of young stock.
4. Acquisition of replacement animals free from infection by *Map* and other significant pathogens that are shed in the feces.
5. Removal of infected animals from the farm.
6. Reduction of environmental contamination by *Map*.

At the state-federal program level:

1. Minimum national standards for program implementation.
2. Performance-based criteria for diagnostic testing and laboratory accreditation.
3. Rapid identification and protection of JD-free herds that can be used to provide *Map*-free replacement animals.
4. Incremental implementation, progressing from a voluntary herd status program to a system of strong (preferably market-based) incentives for participation and disincentives for nonparticipation, culminating in a mandated herd control program, if JD eradication is the ultimate goal.
5. A gradual transition from an exclusive focus on *Map* to a broader health and market assurance program that emphasizes Best Management Practices to prevent the spread of all pathogens by the fecal-oral route.
6. A mechanism for periodic program review and self-correction.
7. A program to prevent the reemergence of disease after low prevalence or eradication is achieved.

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Recommendations for a stepwise expansion of the federal role in JD prevention and control:

1. The government should promulgate uniform methods and rules (UM&R) for voluntary JD status and control programs; the federal standards would provide consistent definitions and program guidelines for a baseline across all states; individual states could mandate additional requirements.
2. The federal government should provide control infrastructure, including support and incentives to upgrade diagnostic laboratories across the United States, to promote large-scale testing.
3. All states should be required to implement a control program that is voluntary for producers in accordance with the UM&R.
4. Producers should be encouraged to test all herds and register them either in status programs or in control programs, based on test results; federal subsidies may be needed to cover the cost of initial testing in order to encourage participation.
5. Federal restrictions should continue on interstate and international transport of cattle from *Map*-positive herds.
6. A federal plan should be established to monitor the success of the control program; the plan should provide for periodic program review and self-correction.

While control programs for dairy herds may be of highest priority, control programs for beef cattle, sheep, goats, and captive cervids should be developed and implemented. Control programs for zoo animals and wildlife should also be monitored to ensure that a non-domesticated animal reservoir does not compromise control efforts for any species. The committee also urges USDA to develop a system that encourages staff to publish data on the evolution, progress, and determinants of success of animal disease control programs.

EDUCATION AND TRAINING RECOMMENDATIONS

The National Johne's Working Group has made education of cattle producers and veterinarians a high priority. However, the educational ‘push' from the herd veterinarian and other herd advisors, would be more effective when accompanied by a market incentive ‘pull' from food processors, distributers, and consumers for control to be implemented at the farm level. The committee endorses NJWG's effort, but offers the following additional recommendations for action by federal and state authorities to expand and improve cattle-education programs and initiate them for other species.

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RECOMMENDATION 2. *Commodity-oriented (dairy, beef, sheep, goat, llama, etc.) materials should be developed and standardized nationally and there should be a rationale and guidelines for development of control programs and certification plans. These should not be considered the same as national program standards, but they should provide an information base for participation in national programs.*

RECOMMENDATION 3. *Informational resources should be developed for practicing veterinarians that include guidance on diagnostic test selection, sample size and selection of animals for testing, interpretation of test results, risk assessment methods, writing herd plans, and monitoring compliance and progress.*

RECOMMENDATION 4. *Educational resources that emphasize control of risk factors (Best Management Practices) should be developed instead of materials that emphasize control of a single etiologic agent.*

RECOMMENDATION 5. *Training programs are needed for state Johne's coordinators, USDA personnel, practicing veterinarians, and laboratory personnel to ensure a uniform base of knowledge and practice.*

RESEARCH RECOMMENDATIONS

The committee identified significant gaps in the current state of knowledge of the pathophysiology, immunology, diagnosis, and control of JD in domesticated livestock and wildlife. Choosing research projects needed to fill those gaps will be important to the success of any JD herd status and control programs, and is sufficiently complex for the committee to recommend the convening of a USDA panel to formulate consensus methods to address these research questions. The committee considered ongoing research to be important for the success of any control program and therefore felt that a research element should be integral to future program development. The committee developed recommendations in several areas described below.

Epidemiology of Map Infection and Johne's Disease

The committee felt that significant gaps exist in our understanding of the epidemiology of JD, which could affect the success of control programs being proposed. In particular, the committee recommends additional research on:

RECOMMENDATION 6. *Age-dependent dose-response curves are needed to clarify the magnitude and significance of age-related susceptibility or resistance to infection and on the degree to which horizontal transmission occurs in different age groups. Use of*

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epidemiological modeling would help to determine the best measure of infectivity.

With the increase in concentrated calf-rearing operations, there is an urgent need to investigate the possibility of horizontal transmission in young animals. Current control strategies also assume that horizontal transmission is insignificant in adults, but this should be confirmed because the success of control strategies could depend on this issue.

RECOMMENDATION 7. *The effects of chronic, low-level exposure on infectivity and on the outcome of infection should be identified.*

Much of the data on infectivity and age susceptibility has been derived from decades-old studies in which one or a few large infective doses typically were administered. Although this provides valuable information, because it does not mimic natural exposure, the conclusions that can be drawn from such studies are limited. It would be helpful to have a better understanding of the outcome of chronic, low-level, or intermittent exposure to *Map* in the environment.

Results of the two investigations proposed above will help regulators to establish a scientific basis for several control measures, such as how long to wait before restocking contaminated land, whether environmental decontamination can expedite the restocking process, and whether removal of calves from dams at birth is the best means of breaking the transmission cycle.

RECOMMENDATION 8. *Experimental studies and field investigations of natural infection in nonruminant and ruminant wildlife in the United States should focus initially on native lagomorphs and other small mammals prevalent on or around livestock operations with endemic JD.*

Recent investigations on the role of wildlife in the epidemiology of JD in livestock in Scotland, Australia, and the Czech Republic have yielded interesting results. The identification of endemic *Map* infection in Scotland has important ramifications for control programs there. Little work has been done in the United States on the susceptibility of nonruminant wildlife to *Map* infection. Determining the prevalence of *Map* infection in wildlife on or around livestock operations will be important to understanding the success or failure of any livestock JD control programs.

As control programs are implemented, they present several opportunities to take advantage of resulting "natural experiments." The committee recommends that these opportunities not be lost.

RECOMMENDATION 9. *Results of diagnostic testing, control practices, and other epidemiologic data should be evaluated, and used to answer remaining research questions, and to refine and optimize control programs.*

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RECOMMENDATION 10. *The USDA NAHMS prevalence surveys should continue, with attention paid to maximizing the data obtained from the samples collected through "add-on" projects and investigations.*

Diagnostics and Immunology

Although the committee acknowledges that available diagnostic tools are sufficient to implement control programs, significant deficiencies still exist. To address these important gaps, the committee recommends the following:

RECOMMENDATION 11. *Epidemiologically sound sampling and sample-pooling protocols should be developed and validated to facilitate screening and monitoring of large cattle herds and sheep flocks.*

Recent research in Australia suggests that pooling of sheep fecal samples could enable more cost-effective flock screening for control programs. This work should be repeated in the United States and expanded to include cattle. Another important need is for a rapid, sensitive test to detect the presence of *Map* in bulk-milk samples. This would promote more efficient and cost-effective collection of herd prevalence data, which will be important for control. Some promising studies have been conducted, but additional research is needed in this area.

RECOMMENDATION 12. *Sensitive and specific serologic and fecal-culture methods should be developed and validated for use in sheep and goats.*

The development of diagnostic tests for JD in sheep, goats, and other species has trailed that for cattle. Although much of the current control emphasis is on dairy cattle, development of more sensitive and specific tests is needed for these other species. Recent reports suggest that the difficulty in isolating sheep strains of *Map* by fecal culture has largely been overcome, but methods are still slow and not ideal for use in control programs.

RECOMMENDATION 13. *Methods for detecting an early, specific immune response to* Map *should be developed.*

There are no reliable tests to identify animals in the early stages of infection, before fecal shedding of *Map* begins. Early identification of infected animals would support control programs, especially for prepurchase testing of replacement animals. Exposure to other mycobacteria, such as *M. avium* subsp. *avium,* is likely to be common in cattle, so it is essential that any test to identify animals in the early stages of infection be highly specific for *Map*. This might require identification of unique antigenic epitopes in *Map,* against which an early immune response is generated.

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Map Genome Studies

As the committee evaluated knowledge gaps and research needs, the importance of complete sequencing of the *Map* genome became evident. Sequencing would yield many benefits, including identification of unique *Map* antigens for development of diagnostic tests and vaccines, improvement of diagnostic methods based on polymerase chain reaction (PCR) and identification of potential virulence factors. The *Map* genome sequencing project at USDA's National Animal Disease Center is nearing completion, and the committee strongly recommends the following:

RECOMMENDATION 14. *USDA and other agencies should seize the opportunity presented by the completion of the* Map *genome project to accelerate progress in JD research, diagnostic test improvement, and vaccine development.*

Information about the completion of the project should be disseminated to the international research community for Johne's disease and for Crohn's disease, and the sequence data should be made available as soon as possible. Research funds should be directed to research and development projects that use the results of the *Map* genome project. The paucity of funds available for JD research has limited progress in several important areas. The completion of the *Map* genome project provides a unique opportunity to correct this oversight, and it should not be neglected.

Vaccine Development

Current vaccines for *Map* are highly problematic. There are conflicting data on their ability to reduce shedding of *Map,* and the fact that they generate cross-reactions to intradermal tests for *M. bovis* makes them unsuitable for widespread use in control programs. Because vaccines can expedite the reduction of disease prevalence, the committee recommends the following:

RECOMMENDATION 15. *Research should be done on the nature and evolution of the immune response to* Map, *and ways to modulate the immune response to elicit protection should be studied.*

RECOMMENDATION 16. *Research is needed on the feasibility of using recombinant-vaccine technology to create a vaccine that generates a specific, protective immune response in livestock without interfering with diagnostic tests for JD, bovine tuberculosis, and other diseases.*

Development of an efficacious vaccine will require identification of unique *Map* antigens that will elicit a protective immune response without generating cross-reactions to other mycobacteria, such as *M. bovis.* The *Map* genome project has the greatest potential for providing the basis for these advances.

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Human and Animal Health

After evaluating all of the available evidence for a causal role for *Map* in Crohn's disease, the committee was of the unanimous opinion that the evidence was insufficient to either establish or refute a causal connection. However, a causal link between *Map* and Crohn's disease remains a plausible hypothesis that warrants additional study. The committee considered the following research to be important elements for the resolution of this question:

RECOMMENDATION 17. *A blinded study should be done for the detection of* Map *and* Map *RNA-DNA in identical coded intestinal tissue samples sent to various laboratories using standardized methods for* Map *culture and detection. This will help clarify the degree to which conflicting research results have been the result of variations in methods versus operators.*

RECOMMENDATION 18. *A large-scale, double-blind, multicenter study should be done to detect the presence of* Map *and* Map *RNA-DNA in tissue specimens from patients with Crohn's disease, using the same standardized methods as above. The specimens should be stratified by type of disease, duration of disease, and treatment. Control subjects without Crohn's disease should be included in the study.*

RECOMMENDATION 19. *A large-scale, double-blind, multicenter study of the treatment of Crohn's disease patients with anti-*Map *combination antimicrobial therapy should be undertaken. The patients should be stratified by type of disease, duration of disease, and treatment, presence or absence of known susceptibility genes, treatment, and presence or absence of* Map *by culture or PCR methods. There also should be an appropriate control group of patients with Crohn's disease who do not receive anti-*Map *therapy.*

RECOMMENDATION 20. *A multicenter, double-blind study is needed to search for* Map, Map *antigens, and* Map *RNA-DNA in breast milk of lactating women with Crohn's disease compared to controls. Finding such in lactating women with Crohn's disease would provide strong support for the proposed connection between* Map *and Crohn's disease.*

If a subset of CD patients responds to anti-*Map* therapy, or *Map* is otherwise implicated as a cause of CD in a subset of patients, research on methods to better identify this subset will be needed. Other research considered important by the committee included on-going studies of Crohn's susceptibility genes and familial tendencies, gene microarray studies to determine which genes are up- and down-regulated in Crohn's disease and animal models, especially genetically altered animals.

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RECOMMENDATION 21. *The National Institutes of Health or similar body should convene a panel with experts in gastroenterology, Crohn's disease, infectious disease, mycobacteriology, biostatistics, epidemiology, etc., to define the precise study designs and to rank order the various studies to be done.*

Although the committee did not find sufficient evidence to implicate *Map* as a cause of Crohn's disease, there was consensus that efforts to identify and mitigate avenues of exposure to *Map* would be prudent while awaiting definitive resolution of this important question. Identifying environmental sources of *Map* also is an important element of JD control in livestock, so there is additional justification for such investigations. The committee therefore recommends researching the following projects:

RECOMMENDATION 22. *Research should be conducted to determine the prevalence of viable* Map *in potable-water supplies, streams, ponds, and other bodies of water with potential for* Map *contamination. This could require development of better methods for identifying and quantifying* Map *in environmental samples.*

RECOMMENDATION 23. *Additional studies are needed to determine whether* Map *is present in retail milk or other dairy products, as well as in pasteurized colostrum or commercial colostrum replacers that are fed to calves.*

RECOMMENDATION 24. *Research should be done to determine the prevalence of viable* Map *in peripheral lymph nodes, muscle, and other tissues that are processed for human consumption.*

RECOMMENDATION 25. *Research should be done to determine the prevalence and concentration of* Map *in other environmental materials likely to be contaminated with ruminant manure and associated with exposure to humans or susceptible animals. Those materials could include composted manure, fruits and vegetables, pastures, and crops fed to livestock.*

If a causal relationship is established between human *Map* infection and a subset of Crohn's disease cases, the above research recommendations will be essential for implementation of new control programs aimed at protecting public health by minimizing exposure to *Map*. Additional research would then be needed to develop methods for routine screening of dairy products, meat, and meat products for *Map*.

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Appendix A

INTERPRETATION OF DIAGNOSTIC RESULTS

ESTABLISHING AND COMPARING TEST PERFORMANCE

Properly establishing and then understanding the performance of diagnostic tests is crucial to the design and operation of infection control and eradication campaigns incorporating these tests. Most diagnostic tests are imperfect, so that often a test result cannot be interpreted with certainty, and properly establishing test performance is technically demanding and expensive. This is particularly true for many Johne's disease (JD) tests, which undoubtedly has impeded JD control and eradication efforts.

A test result is often classified into a category, such as positive or negative. For bacterial culture, a positive test may be the appearance of colonies on the medium that has a colony morphology consistent with that of the bacteria of interest within the appropriate timeframe. A negative test would be the failure of growth of any colonies with the appropriate morphology to appear by the time incubation is ended. For a serologic test such as Enzyme-Linked

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Immunosorbent Assay (ELISA), a positive test result might be the appearance of a particular color marker of sufficient intensity (optical density) that it is above a cutoff value. In this example, a negative test result would be when the color intensity is below the cutoff value. Further, because of the many variables affecting the test mechanics, the actual cutoff value for each run usually has to be calculated from control results within that test run. The mechanics of many of these tests are sufficiently complex and resource intensive that their performance is often laboratory- and operator-dependent, making protocol standardization difficult at best.

When test outcomes are classified as positive or negative, present or absent, the result can be wrong in two ways. A positive result can be a true positive, correct, or a false positive, wrong. A false positive occurs when the condition being tested for is actually not present in the animal, but the test indicates that it is. For example, in the case of fecal bacterial culture, a false positive could occur when organisms in contaminated feed or water are consumed by an animal, and then pass through the animal rather than infect it (e.g., Sweeney et al., 1992a). Alternatively, false positive cultures could occur from accidental laboratory contamination or other error. In the case of a serologic test such as ELISA, the false positive result could occur because the animal responded to an antigen in their environment that is immunologically similar to the target antigen from *Map* that is used in the test.

A negative test result can be a true negative or a false negative. A false negative test means that the condition is present in the animal but the test indicates that it is not. In the case of fecal culture, a false negative could occur because the number of organisms in the fecal specimen is too few to be detected, but the animal is infected (e.g., Whitlock et al., 2000b). In the case of serologic tests, the test could be a false negative because an infected animal has not mounted the particular immune response that the test detects, or has mounted a weak immune response that is below the threshold of detection as a positive result.

The measure that is most useful when interpreting an uncertain test result is the predictive value, which is the likelihood that a test result is correct. The positive predictive value (PPV) is defined as the likelihood that a positive test result is a true positive and the negative predictive value (NPV) is defined as the likelihood that a negative test result is a true negative (Last, 1995). These predictive values depend on the prevalence of the condition being tested for in the population being tested, meaning both PPV and NPV are different when the test is used in an uninfected group compared to when the test is used in an infected group with a high prevalence. Intuitively, a positive test is more likely to be a false positive in a herd without any history of having any animals diagnosed with the infection. Similarly, a negative test is more likely to be a false negative in a herd with a history of having many animals with confirmed disease. As a consequence, predictive values are not useful for comparing test performance across groups of animals with significantly different infection prevalences.

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Because of the dependence of predictive values on the actual infection prevalence (as opposed to the apparent test prevalence) in the group being tested, they are generally not useful as a basis for comparing test performance even though they are often reported. Instead, tests are most usefully compared on the basis of their respective epidemiological sensitivity and specificity values. Sensitivity is defined as the probability that an animal with the infection will test positive (true positive,) and specificity is defined as the probability that an animal without the infection will test negative (true negative), respectively (Last, 1995). It is important to note that test sensitivity depends on the spectrum of disease in the group of individuals in which the test is being used. Because the disease process is more advanced in an individual with advanced clinical disease, a test will usually be more sensitive in that animal than in an individual that was just infected and pathological changes are not as advanced. Similarly, a test will work better in a herd with long-established disease than in a herd with recently introduced disease, since the long-infected herd will have both a higher prevalence and more individuals with advanced disease. An important note is that in addition to changing prevalence, prior testing with removal based on the test results can markedly change the spectrum of disease and thus test sensitivity and specificity in that group.

Bayes Theorem relates predictive values and prevalence to sensitivity and specificity (Last, 1995). Understanding these relationships is critical to making appropriate testing decisions, both in deciding which tests to employ and how to interpret positive and negative test results. Note that although the concepts are related, epidemiologic sensitivity and specificity are different from analytic sensitivity and specificity. Analytic sensitivity is the ability of the test procedure to detect an analyte that is the result of the target infection, such as an antigen or an antibody. Analytic specificity is the ability of the procedure to discriminate against other analytes that are not the result of the target infection but are closely related, such as cross-reacting antigens or their antibodies. For example, the analytical sensitivity of conventional culture procedures is the probability of detecting a given number of organisms per gram of a particular type of sample. With a greater number of organisms per gram, the analytic sensitivity increases. The type of diagnostic sample, such as feces versus lymph node tissue, has a major impact on analytical sensitivity because feces have many more competing organisms and contaminants than do lymph node tissues. Because the number of organisms in feces is quite variable and may be sufficiently low as to have a very low probability of detection in a given fecal sample, even in clinical animals, epidemiologic sensitivity of fecal culture in clinical animals is less than 100 percent. Epidemiologic sensitivity will be even lower in subclinically infected animals because of their lower level of shedding.

Establishing epidemiologic sensitivity and specificity values properly is an expensive process. First, a sufficient number of animals in two groups, the infected and the infection-free, must be studied to provide reasonable confidence intervals on the estimates. Second, the disease spectrum of the study animals must be representative of the disease spectrum in the animals to which the test will be applied. For establishing sensitivity, the disease spectrum for JD ranges

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from the clinically affected animals, which are usually older, to animals that are silently infected with little pathological change or immunological response, which tend to be younger. Typically, there are far more of the latter than the former. If the group of study animals is biased toward those that are more severely affected, the estimate of test sensitivity will be biased upward. For establishing specificity, the infection-free animals must have been exposed to the cross-reacting or competing conditions at the same frequency as the groups in which the test will be used. For example, for immunologically based tests such exposures may be similar environmental mycobacteria or other bacteria with cross-reacting antigens. One of the difficulties of establishing test specificity is that the exposures that lower specificity are likely different in different geographic regions and different livestock species. Third, the appropriate gold standard, a definitive reference testing procedure with very high sensitivity and specificity, must be used to establish the infection status of the study animals. At present, the gold standard is necropsy followed by extensive culture and histological examination of multiple sections of lower small intestine and associated lymph nodes to reliably establish the infection status of each study animal.

Based on the assumption that acquisition of infection as an adult is rare, an alternative approach is to follow previously tested animals to slaughter, allowing the progression of the natural history of the disease to a more advanced and thus easier to detect state. Often, rather than such an intensive, expensive investigation, other strategies are used, such as using combinations of antemortem tests. The problem with this compromise is that it changes the assessment of test performance from an absolute measure to a relative measure of unknown bias. Given that the spectrum of disease is weighted toward those subclinical animals that are difficult to detect, this approach likely biases estimates of test performance upward. The result is that as technology improves, the estimations of conventional test performance have been moving downward.

It is also important to note that unless the gold standard test is applied, the resulting prevalence estimate is an apparent prevalence. Deriving a valid estimate of true prevalence from an apparent prevalence requires that both the disease spectrum and competing condition exposure be the same in the tested group as the group from which the estimates of test performance were derived. At best, these are tenuous assumptions.

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Appendix B

USAHA VOLUNTARY JOHNE'S DISEASE HERD STATUS PROGRAM FOR CATTLE

United States Animal Health Association 1998 Committee Reports—Johne's Disease U.S. Voluntary Johne's Disease Herd Status Program for Cattle

PREFACE

Johne's disease (paratuberculosis) is a growing concern to U.S. cattle industries. Years of research effort have produced several new diagnostic tests to detect *Mycobacterium paratuberculosis*-infected cattle. These tests have been evaluated independently by several research groups and been found sufficiently accurate to reliably be used in a program to certify cattle herds as having negligible risk of infection.

In 1993, a task force of the Johne's disease committee of USAHA drafted a model Johne's disease herd certification program (USAHA, 1993). Some states modified their Johne's disease certification programs to conform to

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this model. However, relatively few herd owners have elected to pursue herd certification citing the amount of testing required and the associated costs as the main deterrent. Consequently, in 1997 the USAHA National Johne's Working Group (NJWG) appointed a committee to try to design a more affordable and yet scientifically sound herd certification program.

Multiple meetings were held to design this program and input was solicited from experts and all stake holders. The program was submitted for discussion at NJWG meetings held in conjunction with the Livestock Conservation Institute's meeting March 1998 and the National Cattlemen's Beef Association meeting in July 1998. In August 1998, the program was sent to all state veterinarians, area-veterinarians-in-charge, veterinary associations, cattle breed associations, and interested industry groups for comment. The program was adopted by USAHA in October 1998.

The program presented in this document is intended as a model. The guidelines are considered minimal requirements for operation of a scientifically sound program to identify herds of low risk of *M. paratuberculosis* infection (Johne's disease). This model program was developed to assist State Veterinarians and Johne's disease advisory committees, or their equivalent, in each state as they consider implementation of Johne's disease herd certification programs. It is hoped that the model program will promote greater similarity and equity among different state programs.

NJWG Herd Certification Subcommittee members:

Leslie Bulaga, *Co-chair,* USDA/APHIS/Veterinary Services

Michael Collins, *Co-chair,* University of Wisconsin School of Veterinary Medicine

Ian Gardner, University of California, School of Veterinary Medicine

William Hartmann, Minnesota Board of Animal Health

Karen Jordan, National Milk Producers Federation

Richard Keene, National Holstein Association

Lee McPhail, Ohio Department of Agriculture

Ken Olson, American Farm Bureau Federation

Boyd Parr, American Farm Bureau Federation

William Rotenberger, Steele Veterinary Clinic and Chair USAHA Johne's Committee

Joseph VanTiem, USDA/APHIS/Veterinary Services

Gary Weber, National Cattlemen's and Beef Association

Scott Wells, USDA/APHIS/Veterinary Services

Robert Whitlock, University of Pennsylvania, College of Veterinary Medicine

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U.S. VOLUNTARY JOHNE'S DISEASE HERD STATUS PROGRAM FOR CATTLE OCTOBER 1998

DEFINITIONS

Herd: a group of cattle managed as a separate and discrete unit not commingled with other groups of susceptible species. All cattle on two or more premises geographically separated but on which cattle have been interchanged or where there has been contact between the premises is considered one herd. Contact of animals between separated premises under common management is assumed to have occurred unless complete separation and biosecurity measures between premises can be established by the herd owner or manager.

Herd member: any susceptible species of animal that is commingled with the herd.

Commingling: physical contact or exposure to manure or raw milk of susceptible species. For example, all cattle and other susceptible species grazed together or on the same area of a property or farm, at any time during any 12-month period, are considered to be commingled. Susceptible species include domesticated and exotic ruminants such as sheep, goats, cervids, and camelids. Exposure to manure via contaminated water or feed sources is also considered commingling.

Biosecurity: animal husbandry and hygiene practices designed to limit opportunities for exposure to *M. paratuberculosis*.

Animal identification: all cattle in a Program herd must be permanently and individually identified using an identification method approved by the State Johne's Advisory Committee. However, Level 4 herds must individually identify all cattle using a USDA approved official identification system.

Accredited Veterinarian: a veterinarian approved by the Deputy Administrator of USDA/APHIS/VS to perform functions required by State-Federal-Industry cooperative programs. For the Voluntary Johne's Disease Herd Status Program (VJDHSP) these duties include annual herd visits, animal testing, and producer education. All samples for Program testing must be collected by an accredited veterinarian or State or Federal animal health official.

Accredited Laboratory: a laboratory that has passed an annual check test for Johne's disease administered by the National Veterinary Services Laboratories. All program testing must be conducted by a laboratory approved for the specific test being used.

Johne's Epidemiologist: a State or Federal regulatory health official who has demonstrated the knowledge and ability to perform the functions specified by the VJDHSP. The Johne's epidemiologist should be selected in consultation with the State Johne's Advisory Committee, State animal health official, Area-Veterinarian-in-Charge, and the National Program Coordinator.

National Program Coordinator: a USDA staff veterinarian who will assist State Johne's epidemiologists, State Johne's Advisory Committees, and the

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USAHA Johne's Committee with the administration and review of the Voluntary Johne's Disease Herd Status Program.

Herd Status Levels: herds may achieve status Levels of 1, 2, 3 or 4. Each level of increase indicates higher confidence in the Johne's disease free status of the herd. (Percentages shown on the flow diagrams in Appendix II represent a mathematical estimate of the probability herds at each level of certification are free of Johne's disease based on serial testing.)

Level achievement year: the year in which a herd met Program standards to be granted a specific status Level. For example, a herd completing Level 2 testing in 1998 that elects to remain at Level 2 would have Level 2 1998 status. Level achievement year is noted because continued monitoring increases confidence the herd is not infected.

ELISA or Fecal Culture Statistical subset: an ELISA on or fecal culture of a statistically determined number of animals. The table in Appendix I shows the number of animals to include in Program ELISA and fecal culture statistical subset testing.

State Johne's Advisory Committee: an appointed committee which is the authority responsible for overseeing and coordinating the State's Voluntary Johne's Disease Herd Status program. The Committee may be comprised of any or all of the following:

Dairy producers—purebred, commercial and commodity groups

Beef producers—purebred, commercial and commodity groups

University Extension—beef and dairy

Veterinary practitioners—beef and dairy

Regulatory veterinary medicine—state, federal, and/or field services

Responsibilities of the committee should include, but are not limited to:

1. Informing and educating the industry regarding Johne's disease
2. Overseeing financial needs for state Johne's disease activities
3. Recommending state policies with approval of the appropriate State authority on operating a Johne's disease program which enhances a Johne's disease herd status program, reduces the spread of Johne's disease and assists infected herds in managing or controlling the infection
4. Setting standards for release of information on Program herd status
5. Overseeing appeals of Program herd status
6. Providing input to the USAHA Johne's Committee National Johne's Working Group for evaluation and revision of the VJDHS Program

The duties of the Johne's Advisory Committee could be assumed by an existing animal health committee in the State.

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PROGRAM PROTOCOL

Confidentiality

Within the limits of each state's laws, it is important to maintain as much confidentiality of testing results as possible. At the same time, to promote the program, stimulate the market place to assign added value to animals from program herds and demonstrate the benefits of buying cattle from Johne's disease status program herds, it is desirable for owners of status level 1–4 herds to disclose their herd status. When possible, program herd owners should have the option of publicly withholding or promoting their herds' status level.

Entry to the Program

Herds may enter the VJDHSP by two methods—Standard and Fast Track. All samples for Program testing must be collected by an accredited veterinarian or State or Federal animal health official and submitted to an accredited laboratory.

It is recommended that a farm or herd risk evaluation be done before completing herd testing for Level 1 Standard Track or Level 2 Fast Track. This evaluation would inform producers entering the program of existing herd risk factors for the spread of Johne's disease. A farm risk checklist may also be used as a yearly reminder of existing herd risk factors.

Additionally, Program herds should be encouraged to implement Best Management Practices as provided by the National Johne's Educational package to prevent the introduction and spread of Johne's disease in their herd.

Standard Track

The herd must meet identification and commingling requirements described in the definitions. Herds enter the Standard Track Program by Johne's ELISA testing 30 second lactation or higher animals. No declaration of prior disease freedom is required. Negative test results on this initial test qualifies the herd for Level 1 status.

Fast Track

The herd must meet identification and commingling requirements described in the definitions. Additionally, the herd owner must submit a signed statement that:

1. I am fully aware of the management and disease history of the herd and the property during the past five years
2. Johne's disease is not known or suspected to have existed in the herd for the past five years or on the property during the past twelve months

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3. Cattle are not known to have been introduced from known infected herds during the past five years

The above written statement and a negative ELISA test on a statistical subset of second or higher lactation animals qualifies the herd for Level 2 status. States may additionally require the herd veterinarian of record to co-sign the owner statement.

Previously Infected Herds

Cattle herds previously culture positive for *M. paratuberculosis* may enter the program by completing Standard Track entry requirements. Infected (positive on an organism detection test) and/or test positive (positive on any Johne's test) animals must be removed from the herd before Program entry.

Johne's Disease Vaccinated Herds

Herds previously vaccinated for Johne's disease may enter the Program once vaccination has been discontinued. These herds must utilize fecal culture as the only test until enough non-vaccinated natural additions qualify for ELISA testing. The number of animals to test at each level remains the same.

Herds Tested Negative Prior to Implementation of this Program

Herds Johne's disease tested negative prior to the implementation of this program may be entered at an assigned Program Level determined after a review by the Johne's epidemiologist. That review must include:

1. Verification that the herd meets the minimum standards for testing (fecal culture results can be used in place of ELISA results); Future Program tests must be performed in accredited laboratories
2. Risk assessment of the number, source, and testing history of herd additions made after the first qualifying test

Additionally, producers wishing to use the Fast Track must make the required written statements for the time period (five years) prior to the first qualifying test.

Animals to Test

Random sampling will give the most confidence and should be used to select animals for testing when feasible. When possible, the same animals should not be tested in consecutive testing rounds. Animals should be selected to be representative of the herd population. This program uses second or higher lactation animals as the most obvious indication of animal age. Detailed requirements for sample handling and submission must be provided by the testing laboratory.

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Maintaining a Status Level

Producers may elect to remain at any level of confidence in either Track by conforming with the program standards and performing an ELISA test on 30 randomly selected animals of second or higher lactation every 10–14 months. A level achievement year for each herd should also be noted as continued monitoring increases confidence the herd is not infected.

Testing Intervals

Testing intervals are every 10–14 months from the date the test samples are taken. Herds will be removed from the program if the testing interval requirements are not met, unless an extension has been received from the Johne's Epidemiologist and/or State Cattle Committee.

BIOSECURITY

A program herd must have biosecurity measures in place in order to avoid exposure to manure or milk from ruminants of unknown Johne's disease status. These measures include:

1. Pooled milk from cows of unknown Johne's disease status should not be used to feed baby calves
2. Manure from Embryo Transfer donors or other “visiting” cows (e.g., transport cows that lay over at program farms for rest or to be milked) should not be allowed to come in contact with the program herd and this manure should not be disposed of on pastures or in a manner which would contaminate pastures or animal feed
3. Exhibition cows and calves (especially under 6 months old) should be hauled in cleaned and disinfected trailers and avoid commingling; (Animal exhibition, consignment sales and transport are considered situations of low *M. paratuberculosis* infection transmission risk, however prudent care and diligence about biosecurity is recommended)
4. A program herd must not be commingled with or grazed behind susceptible species, (e.g., sheep, goats, farmed deer, camelids, non-program cattle)

PROGRAM LEVELS

Flow diagrams depicting progression through each status level appears as Appendix II. Percentages on the diagrams represent mathematical estimates of the probability herds at each level or certification are free of Johne's disease infection based on serial testing. This program does not certify animals free of Johne's disease. Owners may elect for their herd to remain at any status level by

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ELISA testing 30 second or higher lactation animals every 10–14 months. Maintenance of Level 4 status gives the producer a high level of certainty that their herd is free of infection. With continual maintenance of Level 4 status, it could be assumed that there is negligible risk of infection from Level 4 herds.

All samples for Program testing must be collected by an accredited veterinarian or State or Federal animal health official and submitted to an accredited laboratory. If an animal is removed from the herd while ELISA results are pending, a fecal culture should be collected and submitted on hold to the laboratory. This will allow, if the owner wishes, an appeal of herd status to be made if the animal tests ELISA positive (see Appeal Process).

Standard Track

The standard track is designed to allow entry to the program with a minimal investment of funds and gradually increases the producer's investment in the program. The standard track will require at least three years and four tests to reach Level 4.

Level 1—program entry requirements met, negative ELISA on 30 second or higher lactation animals. A sample size of thirty was selected to optimize herd sensitivity and herd specificity and maintain a fixed cost for all herds entering the program.

Level 2—met requirements for Level 1, and negative ELISA on a statistical subset of second or higher lactation animals (see Appendix I for the Herd Subset Testing chart). The Level 2 testing must be completed within 10–14 months of any Level 1 testing.

Level 3—met requirements for Level 2 and have negative fecal culture results on a statistical subset of second and higher lactation herd members. Bulls two years of age and older must be included in this testing (see Appendix I for the Herd Subset Testing Chart). The fecal culture must be collected within 10–14 months of any Level 2 testing.

Level 4—met requirements for Level 3 and have a negative ELISA on a statistical subset of second or higher lactation animals. Level 4 testing must be completed within 10–14 months of any Level 3 testing. Level 4 status is maintained by achieving negative ELISA results on 30 second or higher lactation animals every 10–14 months.

Fast Track

The fast track allows producers to proceed to a higher status level of confidence more quickly than the standard track, and requires greater financial investment at program entry. The fast track will allow herds to reach Level 4 in two years with three tests.

Level 2—program entry requirements for Fast Track met, negative ELISA statistical subset test of second or higher lactation animals (see Appendix I for the Herd Subset Testing chart).

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Level 3—met requirements for Level 2 Fast Track and have negative fecal culture results on 30 second or higher lactation animals. Level 3 testing must be completed within 10–14 months of any Level 2 testing.

Level 4—met requirements for Fast Track Level 3 and have negative ELISA results on a statistical subset test of second or higher lactation animals. Level 4 testing must be completed within 10–14 months of any Level 3 testing. Level 4 status is maintained by achieving negative ELISA results on 30 second or higher lactation animals every 10–14 months.

HERD ADDITIONS

Heifers that have not calved and bulls less than 2 years of age:

from herds of equal or higher program levels

-or-

from program herds which are one level below the purchasing herd.

Animals in this category may not be added to Program herds from non-program herds.

First and higher lactation cows, bulls greater than or equal to 2 years of age:

Level 1–3 herds—from herds of equal or higher level

-or-

from any other herd as follows:

1. ELISA test of addition(s) in the herd of origin within 30 days prior to entry to the program herd

   -and-

2. Submission of fecal culture from addition(s) within 30 days of arrival

   -and-

3. Testing of herd addition(s) in addition to required animal sampling numbers for the next required annual testing. Herd additions are not granted the same status as the receiving herd until this additional testing is negative.

Additional risk of infection is incurred when animals are purchased from non-program herds. Non-program herds should be encouraged to ELISA test 30 second or higher lactation animals before a Program herd will purchase from them (i.e., non-program herds should be encouraged to achieve Level 1 status). When possible, additions from non-program herds should be isolated from the program herd and biosecurity maintained until fecal culture results are reported negative.

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Level 4 herds—from herds of equal level
-or-
from Level 2 or 3 program herds as follows:

1. ELISA test of addition(s) in the herd of origin within 30 days prior to entry to the program herd
   -and-
2. Submission of fecal culture from addition(s) within 30 days of arrival
   -and-
3. Testing of herd addition(s) in addition to required animal sampling numbers for the next required annual testing. Herd additions are not granted the same status as the receiving herd until this additional testing is negative.

Additional risk of infection is incurred when animals are purchased from herds of lower status.

Replacements raised elsewhere:

Replacements may only be raised with animals from equivalent status Level herds. Replacements must not be commingled with lesser status level animals or herds.

Embryo Transfer/Artificial Insemination (AI):

Program herds may utilize semen and embryos from any other cattle herds. However, embryos must be processed according to International Embryo Transfer Society protocols. Embryo transfer recipient cows must meet herd addition requirements.

The risk of transmission of *M. paratuberculosis* from semen is unknown. AI centers are encouraged to routinely test their bulls for Johne's disease and to remove for sale all semen from bulls found to be infected. All semen used in program herds must be processed according to Certified Semen Services standards.

CHANGES IN HERD STATUS

Herd additions:

The status of the herd will revert to the status of the lowest animal if Program herd addition requirements are not met.

Change of ownership:

Herd status is determined by the status of the lowest herd member. Therefore, when ownership of a herd or part of the herd changes and no new animals are added to the herd, the herd level remains the same. When ownership of a herd changes and the herd is commingled with another herd or

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herds, the resulting new herd shall be assigned the lowest level and achievement year of the herds combined to make the new herd.

APPEAL PROCESS

Appealing ELISA positive results:

Status of herds with ELISA positive test(s) is "suspended pending confirmation." Producers may elect to either leave the program or confirm the test results.

Confirmation of ELISA-positive cattle will be done by fecal culture. Fecal culture must be submitted within 45 days of notification of ELISA results. If found to be culture-negative, the herd may advance or retain its status Level. Additionally, the animal(s) must be included in the next round of Program testing if still resident in the program herd.

Appealing Fecal culture positive results:

Fecal culture-positive cattle will be assumed to be infected with *M. paratuberculosis* and the herd will have its status revoked. Herds may reenter the program at Level 1 by following program standards.

If the animal owner wishes to appeal this decision, the herd is assigned a status of "suspended pending appeal." Appeal may be made using one of three means of proving the animal in question is not infected:

1. Necropsy of the animal with culture and histopathology of at least the ileum, mesenteric lymph node and ileocecal lymph node

   -or-

2. Biopsy of the ileum (full thickness) and mesenteric or ileocecal lymph node with histopathology and culture of the tissues and culture of a fecal sample taken at the time of biopsy

   -or-

3. Six separate fecal cultures from the animal on samples collected not less than 30 days and not more than 45 days apart

The herd Johne's disease status will be suspended until all testing is completed. Only negative results on all tests on all samples will allow the herd to advance or retain its Program Level.

Appeal of status for other reasons:

Herd owners may appeal any decision or discuss extenuating circumstances that prevent compliance with the program rules to the State Johne's Advisory Committee:

1. A sample size of 30 was selected to reduce cost while maintaining acceptable accuracy

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2. A history of Johne's disease freedom for five years prior to program entry adds sufficient confidence to allow Fast Track herds to test 30 animals rather than the statistical subset used in the Standard Track to obtain Level 3 status

APPENDIX I Herd Subset Sampling

PLEASE NOTE: The sample numbers below have been calculated based on the following assumptions:

1. The cattle to be tested are in second or higher lactation
2. For these calculations, 25 percent test sensitivity of the ELISA and 40 percent test sensitivity of the fecal culture were assumed (this were the consensus estimates of the Herd Status Committee for subclinically infected cows in first of higher lactation, and no changes were made for the older population sampled
3. For these calculations, 100 percent test specificity of the ELISA and fecal culture was assumed (given follow-up of all ELISA positives with fecal culture)
4. The confidence of detecting infection (at least 1 test-positive cow), if present at a true prevalence of 2 percent, is 95 percent
5. Sampling without replacement (hypergeometric distribution)

Table B-1. Testing Sample Size

Number of cows in herd of 2nd or higher lactation	**Number of cattle to sample (2nd lactation or higher)**	
Less than 300	Test all	Test all
400	Test all	313
500	Test all	324
600	531	332
700	540	338
800	547	342
900	552	345
1000	580	360

Note: In smaller herds, all cattle second or higher lactation must be tested. In herds with fewer than 30 second and higher lactation animals, first lactation animals must also be tested.

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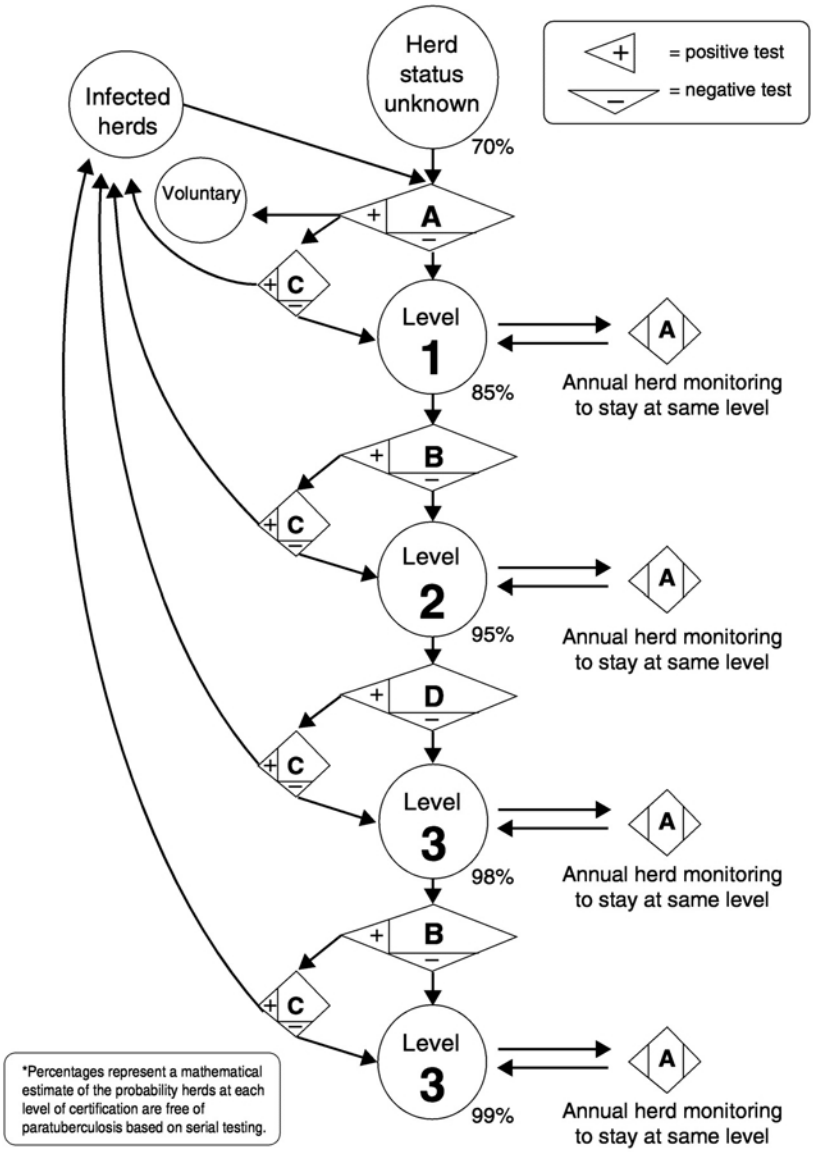
APPENDIX II Voluntary Johne's Disease Herd Status Program Standard Track



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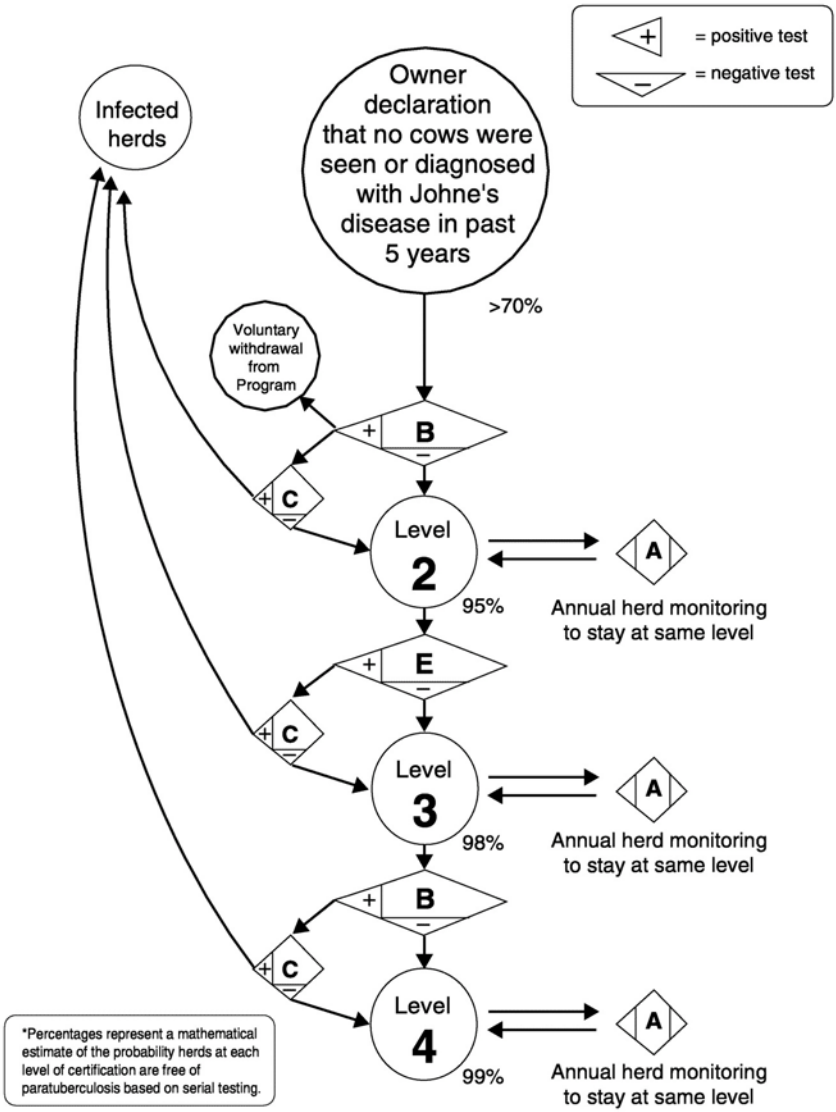
Voluntary Johne's Disease Herd Status Program Fast Track



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Voluntary Johne's Disease Herd Status Program Key to chart—Definition of tests

ELISA on 30 second or higher lactation cows

ELISA on a statistical subset of second or higher lactation cows (see charts for number to be tested)

Confirmatory test:
for ELISA-positive cows, fecal culture
for fecal culture-positive cows, see details in program literature

Fecal culture a statistical subset of second or lhigher lactation cows and all bulls 2 years or older
(see charts for number to be tested)

Fecal culture on 30 second or higher lactation cows

Positive test

Negative test

The interval between tests is 10 to 14 months, except for confirmatory testing.

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Appendix C

USDA/APHIS DRAFT JOHNE'S DISEASE CONTROL PROGRAM

Draft—Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program

CONTENTS

Introduction
Part I **Definitions and Abbreviations**
Part II **Administration of the Program**
1. Designated Johne's Disease Coordinator
A. General
B. Qualifications
C. Responsibilities
2. State Johne's Disease Group
A. General
B. Recommended Members
3. Johne's Certified Veterinarians

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A. General
B. Qualifications
C. Responsibilities
Part III **Program Elements and Procedures**
1. Education
A. General
B. Requirements
2. Management
A. General
B. Risk Assessment
C. Herd Management Plan
D. Renewal
3. Herd Testing and Classification
A. General
B. Requirements for Entrance
C. Test-Positive Component (Control and Testing)
D. Test-Negative Component (Herd Status)
Part IV **Laboratory Procedures**
1. Approved Laboratories
A. General
B. Approval Process for Official Johne's Disease Testing
C. Approval Process for Screening Testing
2. Approved Program Tests
A. Official Johne's Disease Tests
B. Screening Tests
Appendix Table C-1. Herd Subset Sampling

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INTRODUCTION

This document describes the cooperative State-Federal-Industry Voluntary Bovine Johne's Disease Control Program, to be administered by the State and supported by Industry and the Federal government. It is intended as a working document that will change as the Program develops.

The objective of this Program is to provide minimum national standards for the control of Johne's disease. The Program consists of three basic elements: (1) Education, to inform producers about the cost of Johne's disease and to provide information about management strategies to prevent, control, and eliminate it; (2) Management, to work with producers to establish good management strategies on their farms; and (3) Herd Testing and Classification, to help separate test-positive herds from test-negative herds.

The Program has been developed in conjunction with the National Johne's Working Group, with advice and recommendations from the Johne's committee of the United States Animal Health Association. The Program has been approved by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS).

The minimum national standards described in this document do not preclude the adoption of more stringent methods and rules by any geographical or political subdivision of the Unites States with regard to activities within their boundaries. However, regulations dealing with interstate movement must still conform to Federal regulations.

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Part I:

Definitions and Abbreviations

Accredited veterinarian: A veterinarian approved by the Administrator in accordance with the provisions of 9 CFR part 161 to perform functions required by State-Federal-Industry cooperative programs.

Administrator: The Administrator of APHIS or any person authorized to act for the Administrator.

AIN: American Identification Number: A unique animal identification number consisting of 12 alphanumeric characters plus a 3-character country code. The AIN System is administered through the National AIN Oversight Board.

Animal health official: A full time employee of the State animal health department or of APHIS who has authority from the State veterinarian or the AVIC to carry out Program activities.

Anniversary date: The date on which the DJC gave final approval for initial Program participation.

APHIS: Animal and Plant Health Inspection Service.

Approved laboratory: A private, State, Federal, or university laboratory that has passed an annual check test for Johne's disease administered by NVSL. All Program testing must be done in a laboratory approved by NVSL for the specific test being used in a State's testing program.

AVIC: Area Veterinarian in Charge: The veterinary official of VS/APHIS/USDA, who is assigned by the Administrator to supervise and perform the official animal health work of APHIS in the State or States concerned.

CFR: Code of Federal Regulations.

Commingling: Physical contact with susceptible species. For example, all cattle grazed together on the same area of a property or farm will be considered commingled.

DJC: Designated Johne's disease coordinator: A person who has demonstrated the knowledge and ability to perform the functions required under these Program standards and who has been selected for this position by the State animal health official and the AVIC. The VS regional Johne's disease epidemiologist and the VS Johne's disease staff should concur in the selection and appointment of the DJC.

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ELISA: Enzyme Linked Immunosorbent Assay.

Exposure: Contact with known infected animals; contact with the manure or raw milk of infected or exposed animals of susceptible species; or contact with infected herds via contaminated water or feed sources.

Herd: A group of animals that has been managed as a separate and discrete unit. This may include two or more geographically separated groups of animals under common ownership or supervision, but which have an interchange or movement of animals without regard to health status. The State animal health official will make the final determination of the herd status of a group of animals.

Herd member: An animal of any susceptible species that is commingled with the herd.

Herd management plan: A written plan, produced by the certified veterinarian or animal health official in conjunction with the producer, that includes animal husbandry and hygiene practices specific to that herd and that is designed to limit opportunities for exposure to *Mycobacterium avium* ss *paratuberculosis*.

Infected animal: An animal that has been confirmed by an official Johne's disease test to be infected with *Mycobacterium avium* ss *paratuberculosis*.

JD: Johne's disease: An intestinal bacterial disease caused by *Mycobacterium avium* ss *paratuberculosis*. Clinical signs, which appear after a long incubation period, include long-lasting or chronic diarrhea and weight loss despite a good appetite.

Johne's certified veterinarian: An accredited veterinarian who has received training approved by the DJC for Johne's disease epidemiology and herd management plan development.

Known infected herd: A herd in which at least one herd member has been determined to be infected with *Mycobacterium avium* ss *paratuberculosis* based on an official Johne's disease test during the previous 12 months.

Level achievement year: The year a herd in the Herd Testing and Classification element of the Program obtained its current classification.

Management herd: A herd that has completed a risk assessment and herd management plan that satisfies the requirements of the DJC, but has not completed the requirements for the test-negative or test-positive levels.

National AIN Oversight Board: A group that is selected by USDA/APHIS/VS, and that is responsible for selection and retention of Official AIN Administrators and supervision of the AIN System.

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NVSL: National Veterinary Services Laboratories.

Official AIN Administrator: Any organization, agency, or private enterprise selected by the National AIN Oversight Board to administer the distribution of Official AIN identification devices.

Official AIN identification device: A visual tamper-resistant identification device, such as an eartag, which is issued by an Official AIN Administrator and which bears a unique AIN, the 2-character postal code of the State where the animal is located at the time of identification, and the U.S. Shield.

Official eartag: An identification eartag approved by APHIS as being tamper-resistant and providing unique identification for each animal. An official eartag may conform to the alphanumeric National Uniform Eartagging System, or it may bear the valid premises identification that is used in conjunction with the producer's livestock production numbering system to provide a unique identification number. When the final rule concerning the AIN System is published in the Federal Register, Official AIN identification devices will be used instead of currently used identification systems.

Official Johne's disease test: An organism-detection test approved by the Administrator and conducted in a laboratory approved by the Administrator. The Administrator approves laboratories to conduct an official Johne's disease test only after determining that the laboratory meets the check test proficiency requirements prescribed by NVSL. Approval continues as long as such check test proficiency requirements are met on an annual basis.

Premises identification number: A unique number assigned by the State animal health official to a livestock production unit that is, in the judgment of the State animal health official and AVIC, epidemiologically distinct from other livestock production units. The premises identification number consists of the State's two-letter postal abbreviation followed by the premises' assigned number or code.

Program: Voluntary Bovine Johne's Disease Control Program.

Screening test: A Johne's disease test approved by the Administrator for use in the Voluntary Bovine Johne's Disease Control Program and conducted in a laboratory validated through an approval process by NVSL. Screening tests are tools that have been developed to aid in determining the presence or absence of *Mycobacterium avium* ss *paratuberculosis* within a herd. Animals found positive to these tests should be considered suspect unless they show clinical

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signs of Johne's disease (in which case they are considered positive) or they are confirmed positive or negative by an official Johne's disease test.

State: Any of the 50 States, the Commonwealth of Puerto Rico, the Commonwealth of the Northern Mariana Islands, the District of Columbia, and any territories and possessions of the United States.

State animal health official: The State official who is responsible for the livestock and poultry disease control and eradication programs in a State.

State Johne's Disease Group: A group of interested persons organized by the State animal health official to assist in the oversight and coordination of the State's Johne's Program.

Susceptible species: Domesticated and exotic ruminants, such as cattle, bison, sheep, goats, cervids, and camelids, that are capable of natural infection with *Mycobacterium avium* ss *paratuberculosis.*

Test-negative herd: A herd that is enrolled in the Program, and meets the test-negative component requirements described in this document.

Test-negative level: Level 1, 2, 3, or 4 with each increase indicating a lower probability of Johne's disease in the herd.

Test-positive herd: A herd that is enrolled in the Program and that meets the test-positive component requirements described in this document.

Test-positive level: Level A, B, C, or D with Level A indicating zero or an extremely low prevalence and D indicating the highest prevalence of Johne's disease in the herd.

VS: Veterinary Services: The division of APHIS in charge of animal health activities within the United States.

USDA: United States Department of Agriculture.

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PART II. ADMINISTRATION

1. Designated Johne's Disease Coordinator (DJC)

A. General

Each State must have one person to act as its DJC. This person should be selected jointly by the State animal health official and the AVIC, and be approved by the VS regional Johne's disease epidemiologist/regional director and the Johne's disease staff of APHIS/VS/National Animal Health Programs. States have a 1-year grace period to allow the chosen DJC candidate to meet the education and training requirements. During this time period, the candidate is considered to be the acting DJC.

B. Qualifications

Each DJC candidate must:

1. Be a State, Federal, or university veterinarian.
2. Successfully complete a Johne's disease (JD) epidemiology course that includes on-farm risk assessments and herd management plan development.
3. Have at least 80 hours of experience in assessing risk, developing herd plans, and classifying JD test-positive animals and herds.

C. Responsibilities

The DJC has the responsibility to:

1. Interpret laboratory test results and classify animals and herds based on the use of official and screening tests.
2. Provide training for State personnel performing Program work.
3. Provide training for certified veterinarians and develop a mechanism within the State to evaluate/monitor the involvement of the certified veterinarians.
4. Review the risk assessments and herd management plans submitted by herd owners and certified veterinarians.
5. Periodically audit the Program to determine if it is adequately controlling JD in the State.
6. Assist animal health officials, herd owners, and the herd owner's veterinarian with developing herd management plans as requested/needed.
7. Participate in the Program activities as a member of the State Johne's Disease Group.

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8. Provide a quarterly report to the VS regional Johne's disease epidemiologist and the Johne's staff of VS, National Animal Health Programs, on the progress of the Program.

2. State Johne's Disease Group

A. General

A Johne's disease group or an equivalent must be formed to assist the State in Program development, implementation, and review. A representative at the producer level, for either the beef or the dairy industry, is recommended as the chairperson for the group. The DJC must be a member. The group must meet at least once a year.

B. Recommended members

This group should include, but not be limited to:

1. Dairy producers—purebred, commercial, and commodity groups
2. Beef producers—purebred, commercial, and commodity groups
3. University/extension faculty
4. Animal health diagnostic laboratory personnel
5. Regulatory veterinary medical officers—State, Federal, and/or field services
6. Veterinary practitioners—beef and dairy

3. Johne's Certified Veterinarians

A. General

States may elect to use the services of private practitioners in addition to State or Federal personnel to assist herd owners in conducting risk assessments and developing herd management plans. States using these veterinarians must determine that they meet the qualifications listed below. The DJC needs to develop a process to closely monitor the herd management plans developed by new Johne's certified veterinarians. For example, the DJC may require the new certified veterinarian to develop the first one-to-five herd management plans in conjunction with an experienced animal health official. Johne's certified veterinarians will be required to take a JD refresher course approved by the DJC at least once every 3 years.

B. Qualifications.

Johne's certified veterinarians must be accredited veterinarians and demonstrate to the DJC that they have the knowledge needed to:

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1. Develop herd management plans
2. Provide JD risk assessments
3. Understand JD epidemiology, testing, and test interpretation
4. Understand State and Federal Program requirements
5. Collect and submit fecal, tissue, and blood samples for JD testing

C. Responsibilities

Johne's certified veterinarians have the responsibility to:

1. Provide risk assessments and develop herd management plans that will meet the approval of the DJC when requested by the herd owners
2. Collect and submit samples according to the requirements set by the DJC

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PART III. PROGRAM ELEMENTS AND PROCEDURES

1. Education

A. General

The education element in each State serves as the entry level for producer participation in the State's voluntary Program. The education element must provide producers with basic Mine's disease information, management strategies for controlling and eliminating the disease, and information on the various aspects of the State's Program. Education can take place through group workshops or through one-on-one sessions with the producer's veterinarian. A record of participation should be kept. In the education element of the Program, producers should receive information concerning the following:

B. Requirements

1. Basic JD information—cause, clinical stages, transmission, etc.
2. Management strategies for:

a. Manure
b. Colostrum and milk
c. Replacements
d. Infected animals

3. Control and testing strategies:

a. Testing
b. Test interpretation

4. The State Program

2. Management

A. General

Producers informed about Johne's disease may wish to participate in the management element, an intermediate step in the Program. This recognizes producers for putting approved management practices and plans into place. At this stage, herd testing is an option available to the producer. The following components must be completed to the satisfaction of the DJC:

B. Risk assessment

Prior to developing an individual herd management plan, a risk assessment must be conducted in order to identify aspects of management likely to spread *Mycobacterium avium* ss *paratuberculosis* throughout the herd. A copy of the risk assessment must be submitted with the herd management plan to the DJC.

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C. Herd management plan

The Johne's certified veterinarian or an animal health official, in conjunction with the herd owner, will develop a herd management plan to prevent the introduction of JD into the herd and to reduce transmission of the disease among animals within the herd. A copy of the herd management plan and risk assessment must be submitted to the DJC for review and final approval. Guidelines for developing a herd management plan can be reviewed by reading "Johne's Disease, a Plan for Pathogen Reduction: Manual for Veterinarians: 1st Edition". The herd management plan should address management practices that prevent the calves and young stock from becoming infected with *Mycobacterium avium* ss *paratuberculosis*. The herd management plan must discuss:

1. Minimum biosecurity measures—These should be in place in order to reduce exposure to manure or milk from susceptible species of unknown JD status. These biosecurity measures, incorporated into the herd management plan, should require the producer to:

a. Never use unknown sources of milk or colostrum to feed calves.
b. Minimize exposure of livestock to susceptible animals that are infected or have been exposed to infected animals.
c. Minimize exposure of feed and water to manure.
d. Minimize exposure of young stock, when practical, to manure from adult animals. This will vary depending on management of the cattle located on the premises.
e. Never commingle Program herds with, nor graze Program herds behind, susceptible species that are infected or exposed to infected animals (e.g., sheep, goats, farmed deer, camelids, non-Program cattle).
f. Never allow manure from Embryo Transfer donors or other "visiting" cows (e.g., transport cows that are kept at Program farms for rest or to be milked) to come into contact with herd animals. This manure should not be disposed of in areas that would contaminate pastures or animal feed.
g. Always haul exhibition animals (especially under 6 months old) in clean trailers and never commingle with susceptible species of unknown JD status.

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2. Minimum management practices—dairy herds

a. A single animal calving area separate from other adult animals must be used.
b. Each calf must be separated from adult animals within 12 hours of birth.
c. Colostrum must be from a single source; no pooled colostrum may be used.
d. Cows with positive screening tests or official Johne's disease tests must have colostrum discarded; each calf must be fed colostrum from test-negative animals.
e. Calves must be fed milk replacer or pasteurized milk.
f. Each calf must be in an individual hutch or crate until weaning.
g. Animals must be housed by age, separate from older animals, until 15 months of age.
h. All calves must be permanently identified.

3. Minimum management practices—beef herds

a. Cow/calf pairs must be kept separate from the non-calving portion of the herd.
b. Colostrum must be from calf's dam or other single source; no pooled colostrum may be used.
c. Cows with positive screening tests or official Johne's disease test must have colostrum discarded; each calf must be fed colostrum from test-negative animals.
d. All calves must be permanently identified.

4. Animal identification

All cattle must be permanently and individually identified using an identification method approved by the State Johne's group. It is recommended that all animals in participating herds should be individually identified using an official eartag. Any previous regulations listed in Title 9 CFR regarding animal identification with other animal health programs still apply.

D. Renewal

To continue in the Program, a herd owner and certified veterinarian must annually repeat the risk assessment and make appropriate changes to the herd management plan. The updated risk assessment and herd management plan must be submitted to the DJC.

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Herd Testing and Classification

A. General

Herd testing and classification constitute the third successive Program element. The purpose of this element is to publicly recognize producers in the Program for putting approved management practices and plans into place, as well as for separating test-negative herds from test-positive herds. Herds at this stage will continue undergoing herd risk assessments and be subject to herd management plans that were developed under the management element. After initial testing, participating herds may participate in either the test-positive or test-negative component of this element according to the test results.

B. Requirements for entrance

Herds enrolling in the herd testing and classification element must have completed a risk assessment and developed a herd management plan using the guidelines established in the management element.

1. Testing

   Initial testing is required to determine the herd's test status. This can be accomplished by doing a screening test on a minimum of 30 randomly selected animals at second lactation (3 years old) or higher. In herds with fewer than 30 animals at second lactation or higher, first-lactation animals (2 years old) must also be included until 30 animals are tested, or until all animals at first lactation and higher have been tested. Herd owners should be encouraged to test statistical subsets (see Table 1) or greater numbers of cattle when possible. All samples must be collected by or under the supervision of an accredited veterinarian or a State or Federal animal health official. Vaccinated herds are eligible. Vaccinated herds must be tested by an organism-detection test. All samples must be submitted to a laboratory approved by NVSL.

2. Appealing the status of a test-positive animal

a. For animals found positive to a screening test, a herd owner may elect to confirm the test results as follows:

1) An official Johne's disease test must be submitted within 45 days of notification of the screening test results.

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2) If the official Johne's disease test is negative, the herd may retain its test-negative status, but that animal must be included in the next round of Program testing if that animal remains in the herd.

b. For animals found positive to an official Johne's disease test, a herd owner may appeal the results by submitting a written statement to the DJC within 30 days of the positive results requesting an appeal, and must arrange for:

1) Conducting a necropsy of the animal with culture and histopathology of the ileum and of the mesenteric and ileocecal lymph nodes; OR
2) Conducting a full-thickness biopsy of the ileum and biopsy of the mesenteric or ileocecal lymph nodes with histopathology and culture of the tissues and a fecal culture sample taken at the time of biopsy; OR
3) Submitting six separate fecal cultures from the animal on samples collected between 30 and 45 days apart. All six cultures must be negative for the animal to be considered a test-negative animal. The herd JD status will be suspended until all testing is completed. Only negative results on all tests will allow the herd to retain a test-negative status.

3. Placement

Herds that test positive must remain in the management element or be enrolled in the test-positive component. Herds that test negative will be eligible to enter the test-negative component.

C. Test-positive component (Control and Testing)

The purpose of the test-positive component is for the State to maintain a record of infected herds that are being tested with approved risk assessment and herd management plans in place. States may include assessment levels for herd prevalence. Herds enrolled in this component must use testing protocols approved by the DJC.

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1. Requirements for herds in the test-positive component

a. Application—The herd owner enrolling the herd must sign an agreement to abide by the requirements concerning minimum biosecurity and management, identification, testing, and herd addition strategies. This agreement must be renewed every 10 to 14 months from the anniversary date.

b. Herd additions;

1) All purchased animals must be from herds with approved herd management plans.

2) Heifers raised off the premises must be raised with the proper biosecurity and control measures in place.

c. Animal identification—All animals must be individually identified using an official eartag. Any previous regulations listed in Title 9 CFR regarding animal identification for any other APHIS programs still apply.

d. Testing—The herd owners along with the certified Johne's veterinarian must develop a testing protocol as follows:

1) All samples must be collected by or under the supervision of an accredited veterinarian or a State or Federal animal health official.

2) Vaccinated herds must be tested by an organism-detection test.

3) All samples must be submitted to a laboratory approved by NVSL.

4) All animals specified in the test protocol must be tested within 10 to 14 months of the anniversary date.

5) Herds not adhering to the prescribed testing requirements will be placed in the management element.

6) It is recommended that animals positive in an official Johne's disease test be identified as such and restricted to the premises. When infected cattle leave the herd, it is

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recommended that they go directly to slaughter or rendering.

7) A test-positive animal status may be appealed using the same appeal process described above for entry into the herd testing and classification element. If an animal is removed from the herd while screening test results are pending, a fecal culture should be collected, submitted, and held at the laboratory. This will allow the owner to appeal the herd level if the animal tests positive to a screening test.

e. Optional—assessment levels.

The State's test-positive component may use assessment levels. Herds in a State program may achieve Level A, B, C, or D. Each level classifies a herd based on the known prevalence of Johne's disease within the herd. The level achievement year should also be indicated. For example, a herd that completed Level B testing in 1998 and elects to remain at Level B would have a Level B 1998 status. The level achievement year should be noted because continued monitoring increases confidence that the herd prevalence is within that category. States that use assessment levels must follow the test-positive level requirements listed below.

1) Level A—An annual herd test reveals no screening or official Johne's test-positive animals. Level A can be maintained by achieving negative screening test results on 30 second or higher lactation animals every 10–14 months. Herds achieving Level A should be encouraged to enter the test-negative program at Level 1. Qualifying herds have:

a) Negative test results on at least 30 randomly selected second or higher lactation animals (3 years old or older),

b) Negative test results on the whole herd and on bulls over 2 years of age.

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2) Level B—An annual whole-herd test with the addition of bulls over 2 years of age reveals less than 5 percent of animals positive to a screening or official Johne's disease test.
3) Level C—An annual whole-herd test with the addition of bulls over 2 years of age reveals at least 5 percent, but not more than 15 percent, of animals positive to a screening or official Johne's disease test.
4) Level D—Herds should be classified as Level D if either of the following apply:

a) test on at least 30 randomly selected second or higher lactation animals (3 years old or older) reveals one or more test-positive animals, OR
b) a whole-herd test with the addition of bulls over 2 years of age reveals more than 15 percent of the animals positive to a screening or official Johne's disease test.

2. Renewal/Advancement

A herd will remain in this classification for up to 14 months. For continuation, the herd owner must reapply with a copy of the test results, updated herd management plan, and an agreement to follow the test-positive component requirements. If the herd owner wishes to renew/advance but is waiting for confirmation of screening test results and therefore cannot meet the 14-month deadline, the herd owner can send the DJC a letter of intent to renew/advance. If the letter of intent is received by 30 days after the deadline, the DJC may allow the herd to retain its status for up to 5 months. Herds for which the necessary test results have not been supplied by the 5-month deadline must be placed in the management element. Owners of removed herds may reapply after the herds complete the required testing.

D. Test-negative component (Herd Status)

The test-negative component must include a herd management plan based on the requirements in the management element. The test-negative component includes a series of levels; owners may

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improve their herd status (achieve a higher level) by additional testing and biosecurity measures. Each higher level represents a greater probability that the herd is free from JD. However, this does not certify that a herd is free of JD. Herds in the test-negative component may remain at any given level by doing monitoring testing or may advance to a greater surveillance level with additional testing. Requirements for herds in the test-negative component include:

1. Application

   The herd owner enrolling the herd must sign an agreement to abide by the requirements concerning minimum biosecurity and management established in the management element as well as the identification, testing, and herd addition requirements listed below.

2. Herd additions

a. Purchased heifers and bulls less than 2 years of age may be added to the herd provided that:

1) The animal was purchased from a herd with a test-negative level that is equal to or higher than the herd it is entering, OR

2) The purchased animal is tested in the next herd test after it reaches 3 years of age. The addition's status will remain at its entry level until it has tested negative at that herd test.

b. Purchased or replacement animals 2 years of age or older may be added to the herd provided that:

1) For test-negative Level 1, 2, or 3 herds:

a) The animal was purchased from a herd with a test-negative level that is equal to or higher than the herd it is entering, OR

b) The animal has a screening test within 30 days prior to entering the test-negative level herd with negative results, and

c) Fecal cultures are collected from each animal and submitted within 30 days of arrival, and

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d) The herd addition is tested on the next herd test. The addition's status will remain at its entry level until it has tested negative at the next herd test.

2) For test-negative Level 4 herds:

a) The animal was purchased from a herd with a test-negative level that is equal to the herd it is entering, OR

b) The herd of origin has a test-negative level of 2 or 3 and the purchased addition has a screening test with negative results within 30 days prior to entry into the program herd, and

c) Fecal cultures have been collected from each animal added and submitted within 30 days of arrival, and

d) The herd addition is tested on the next herd test. The addition's status will remain at its entry level until it has tested negative at the next herd test.

c. Heifers raised off the premises must be raised with the proper biosecurity measures in place and raised with animals at an equal or greater test-negative level.

d. Test-negative program herds may use semen and embryos from other cattle herds provided that the semen used is processed according to Certified Semen Services standards and the embryos are processed according to International Embryo Transfer Society protocols. Embryo transfer recipient cows must meet herd addition requirements.

3. Animal identification

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All animals must be individually identified using an official eartag. Any previous regulations listed in 9 CFR regarding animal identification for any other APHIS program still apply.

4. Testing

a. All samples must be collected by or under the supervision of an accredited veterinarian or a State or Federal animal health official.

b. Vaccinated herds will be eligible for the test-negative component after vaccination has been discontinued. All testing must be done by an official Johne's disease test until enough non-vaccinated natural additions qualify for serology testing. The number of non-vaccinated animals will be the sample size required for that size herd for a statistical subset for serology.

c. All samples must be submitted to a laboratory approved by NVSL.

d. Herd removal provisions—If an animal in a test-negative herd tests positive to a screening test or an official Johne's disease test, or if the testing requirements are not followed, the herd must be removed from the test-negative component and placed in the test-positive component or in the management element (unless an appeal is pending).

e. Appealing the status of a test-positive animal must be done using the same appeal process as for entry into herd classification and testing.

5. Herd Advancement—Test negative

Test negative herds may achieve Level 1, 2, 3, or 4. Each higher level represents a greater probability that the herd is free from JD. However, this does not certify that a herd is free of JD. The level achievement year should also be indicated. For example, a herd completing Level 2 testing in 1998 and electing to remain at Level 2 would have a Level 2 1998 status. All levels can be maintained by achieving negative screening test results on 30 second or higher lactation animals every 10–14 months. The level achievement year should be noted because continued monitoring increases confidence the herd is not infected. To advance from one level to the next, a statistical subset must be tested (Table IV–1), and the herd must meet the level requirements listed below for Standard Track or Fast Track test-negative component levels.

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a. Standard Track—The standard track is designed to allow entry into the Program with a minimal investment of funds and gradually increase the producer's investment in the Program. The standard track will require at least 3 years and 4 tests to reach Level 4.

1) Level 1—The herd owner has developed a herd management plan, and has agreed to abide by the requirements of the test-negative component, and the herd has had negative screening test results on 30 second or higher lactation animals.
2) Level 2—Herds have met the requirements for Level 1, and have had negative screening tests on a statistical subset of second or higher lactation animals. Level 2 testing must be completed within 10–14 months of any Level 1 testing.
3) Level 3—Herds have met the requirements for Level 2 and have had negative fecal culture results on a statistical subset of second and higher lactation herd members. Bulls 2 years of age and older must be included in this testing. The fecal culture must be collected within 10–14 months of any Level 2 testing.
4) Level 4—Herds have met the requirements for Level 3 and have had a negative screening test on a statistical subset of second or higher lactation animals. Level 4 testing must be completed within 10–14 months of any Level 3 testing.

b. Fast Track—The fast track allows producers to proceed to a higher level of confidence more quickly than the standard track, but requires greater financial investment at program entry. The fast track will allow herds to reach Level 4 in 2 years with three tests.

1) Level 1—Skip this level if owner signs a declaration that no cows were seen or

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diagnosed with Johne's disease in the past 5 years and has an approved herd plan in place. The State may require the declaration to be cosigned by the herd veterinarian. The signed declaration must include the following statements:

- I am fully aware of the management and disease history of the herd during the past 5 years.
- Johne's disease is not known or suspected to have existed in the herd during the past 5 years or on the property during the past 12 months.
- No cattle have been introduced from known infected herds during the past 5 years.

2) Level 2—Herds have met requirements for Level 1, and have had a negative screening test on a statistical subset of second or higher lactation animals. Level 2 testing must be completed within 10–14 months of any Level 1 testing.
3) Level 3—Herds have met the requirements for Level 2 and have had negative fecal culture results on 30 second or higher lactation cows and all bulls 2 years or older. The fecal culture must be collected within 10–14 months of any Level 2 testing.
4) Level 4—Herds have met the requirements for Level 3 and have had a negative screening test on a statistical subset of second or higher lactation animals. Level 4 testing must be completed within 10–14 months of any Level 3 testing.

6. Renewal/Advancement

A herd will remain at any level for up to 14 months. For continuation of this classification, the herd owner must reapply with a copy of negative test results, an updated herd management plan, and an agreement to follow the test-negative component requirements. If a herd owner wishes to renew/advance but is waiting for confirmation of screening test results and therefore cannot meet the 14-month deadline,

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the herd owner can send the DJC a letter of intent to renew/advance. If the letter of intent is received by 30 days after the deadline, the DJC may allow the herd to retain its status for up to 5 months. Herds for which the necessary test results have not been supplied by the extended 5-month deadline must be removed from the test-negative component. Herds removed from the test-negative component may reapply at the test-negative Level 1.

PART IV. LABORATORY PROCEDURES

1. Approved Laboratories

A. General

All official Johne's disease and screening tests used for the Program may be conducted in a private, university, State, or Federal laboratory that has been specifically approved for conducting JD testing. The State animal health official has the authority to decide if private laboratories may participate in the Program. States must have the authority to periodically audit the JD diagnostic laboratories participating in the Program. If a laboratory lies outside of the State, the State may rely on audits conducted by the animal health officials from the State in which the laboratory is located.

B. Approval process for laboratories performing official Johne's disease tests

1. A laboratory seeking approval to perform official Johne's disease tests must contact NVSL for a test kit of 25 samples. (A valid check test sample from NVSL will be determined by a consensus of at least 70 percent of the participating laboratories.)
2. The laboratory must correctly identify 100 percent of the negative test samples.
3. The laboratory must correctly identify 100 percent of the Too Numerous To Count (TNTC) test samples.
4. The laboratory must correctly identify at least 70 percent of the test samples that were not classified as negative or TNTC.
5. The laboratory must use the same procedure and materials during the check test as is used during routine testing.
6. No retest is available within the same fiscal year.

C. Approval process for laboratories performing screening tests (serology tests)

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1. A laboratory seeking approval to perform screening tests must contact the NVSL for a test kit of 25 samples. (A valid check sample will be determined by NVSL using available licensed ELISA kits.)
2. The laboratory must correctly identify at least 90 percent of the serology check test samples.
3. The laboratory must use the same procedure and materials during the check test as is used during routine testing.
4. One retest is available if a laboratory fails the first time.

2. Approved Program Tests

A. Official Johne's disease tests

1. Fecal/tissue culture—Culture is the standard for organism-based tests although culture methods are not currently standardized. Protocols for recommended methods can be obtained from NVSL upon request. Sensitivity is estimated at 40–10 percent; specificity is considered to be 99 percent if done correctly.
2. DNA probe—DNA probes can detect the presence of *Mycobacterium avium* ss *paratuberculosis* without having to grow it. The test has the advantage that it takes a short time (less than 3 days) but has the disadvantages of higher cost and missing low shedders. Sensitivity is estimated at 40 percent; specificity is about 99.9 percent.

a. Radiometric culture—Radiometric culture is a radioisotope-based method adapted from one used to isolate the bacteria that causes human tuberculosis. The method can detect low numbers of bacteria, and it is faster than standard fecal culture methods (7 weeks versus 16 weeks). The disadvantages are higher cost, the handling of radioisotopes, and the requirements for specialized instruments to read the culture vial. Sensitivity is approximately 40 percent; specificity is about 99 percent.

b. Histology of tissue—No check test is available at this time. Microscopic identification of the characteristic pathological changes and of *Mycobacterium avium* ss. *paratuberculosis* organisms in tissue is a definitive test for JD. Tissue changes and bacteria can be observed in the intestinal lining and in nearby ileum, mesenteric, and ileocecal lymph nodes in infected animals. Sensitivity depends on the stage of disease and the number and type of specimens collected, but is typically considered to be greater

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than for other laboratory tests. Specificity is considered to be 100 percent based on defined criteria (e.g., granuloma with acid fast bacteria seen by a board-certified pathologist).

B. Screening test

1. USDA licensed Enzyme Linked Immunosorbent Assay (ELISA)—All animals found positive in ELISA tests should be considered suspect until confirmed using an official Johne's disease test. ELISA tests are to be used as screening tools or for helping make management decisions. Sensitivity has been estimated at 25 percent for nonclinical cases and approximately 85 percent for clinical cases; specificity is between 98 and 99 percent.

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Table C-1. Herd Subset Sampling

Number of Cattle in Herd (2nd or higher lactation)	**Number of Cattle to Sample (2nd or higher lactation)**	
	ELISA	Fecal culture
<300	Test all	Test all
301–400	Test all	313
401–500	Test all	324
501–600	531	332
601–700	540	338
701–800	547	342
801–900	552	345
µ 901	580	360

Notes: In smaller herds, all cattle second or higher lactation must be tested. In herds with fewer than 30 second- and higher-lactation animals, first-lactation animals must also be tested. Sample numbers above have been based on the following assumptions:

- The cattle to be tested are in second or higher lactation.
- For these calculations, ELISA tests was assumed to have 25% sensitivity and fecal cultures was assumed to have 40% sensitivity (These were consensus estimates of the Herd Status Committee of the NJWG, USAHA for sub-clinically infected cows in first or higher lactation, and no changes were made for older populations sampled.)
- For these calculations, 100% test specificity of the ELISA and fecal culture was assumed (given follow-up of all ELISA positives with fecal culture).
- The confidence of detecting infection (at least 1 test-positive cow), if present at a true prevalence of 2%, is 95%.
- Sampling without replacement.

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About the Authors

BRUCE A.RIDEOUT, *Chair,* serves as Associate Director of Conservation and Science and Head of Pathology of the Zoological Society of San Diego, where he has been working as a pathologist since 1991. In addition, he has been an Adjunct Assistant Professor in the Department of Biology at San Diego State University since 1992, and is a member of the University of California at Davis Veterinary Advisory Board. Dr. Rideout has served as Pathology Consultant and Advisor to numerous animal studies, including Chair of the Pathology Working Group of the 1998 Johne's Disease Workshop. Prior to his work at the San Diego Zoo, he was a Resident in the Department of Pathology at the National Zoo in Washington, DC, and in the Department of Pathology of the School of Veterinary Medicine at UC Davis. He has previous experience with the National Research Council (NRC), having served on the Committee on the Scientific Bases for the Preservation of the Mariana Crow in 1996. Dr. Rideout earned his B.S. in veterinary science with highest honors in 1985, his D.V.M. in 1986, and a Ph.D. in comparative pathology in 1991, all from the University of California, Davis.

SHELDON T.BROWN has been Chief of the Infectious Disease Section at the Bronx Veterans' Affairs Medical Center since 1997 and is Clinical Associate Professor of Medicine at the Mt. Sinai School of Medicine of New York University. He also has served as Infectious Disease Consultant to the Department of Medicine at the Veterans Affairs Hudson Valley Health Care

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Center since 1996. Dr. Brown is experienced in the conduct of clinical trials, having been co-investigator on numerous multi-center trials and site Principle Investigator on seven clinical trials for treatment of HIV and opportunistic infections, and has participated in studies investigating the relationship between *M. avium* subsp. *paratuberculosis* and Crohn's Disease. His laboratory interests focus upon investigations of mycobacterial infections, including *M. tuberculosis* and non-tuberculous mycobacteria. He has particular expertise in atypical mycobacterial infections of the immunocompromised host. Dr. Brown has been a Diplomate of the National Board of Medical Examiners since 1984, and of the American Board of Internal Medicine since 1987, and was certified by the American Board of Internal Medicine in the Subspecialty in Infectious Disease in 1990. He received the Meritorious Service Award from the Bronx Veterans' Affairs Medical Program in 1997. Dr. Brown graduated with a B.A. from Antioch College in 1974, received a Premedical Certificate from the Columbia University School of General Studies in 1980, and was awarded an M.D. with honors from New York University School of Medicine in 1984.

WILLIAM C.DAVIS is a Professor in the Department of Veterinary Microbiology and Pathology at Washington State University, where he has been on faculty since 1968. His expertise is the cellular immunology of animals, and has almost 300 journal publications on a broad range of topics in this field. Dr. Davis's work on Johne's disease has involved cellular immune responses during infection by *Mycobacterium avium* subsp. *paratuberculosis*. He is a member of the American Society for Investigative Pathology, the Transplantation Society, the American Association of Veterinary Laboratory Diagnosticians, and the American Association of Veterinary Immunologists, by whom he was selected Distinguished Veterinary Immunologist in 1993. In the same year, Dr. Davis was honored during the Bicentennial Celebration of the Faculty of Veterinary Medicine of Madrid, Spain as Bicentennial Professor. Dr. Davis has had previous experience with the NRC, having served on the Subcommittee on Animal Sciences of the Committee on Bioscience Research in Agriculture from 1984 to 1985. Dr. Davis earned a B.A. in biology from Chico State College in 1955, and his M.A. in biology and Ph.D. in medical microbiology transplantation immunology from Stanford University in 1959 and 1967, respectively.

JOHN M.GAY is currently an associate professor in the Field Disease Investigation Unit of the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University. Previous positions include Assistant Professor at the Veterinary Medicine Teaching and Research Center of the University of California at Davis and Large Animal Clinical Sciences Resident at the University of Minnesota. He has spent over five years in private practice with beef and dairy cattle. He is also board certified by the American

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College of Epidemiology and the American College of Veterinary Preventive Medicine. Dr. Gay's research interests include the clinical epidemiology and diagnostics of Johne's disease in cattle. His work with Johne's disease combines applied epidemiology and laboratory research (helping practitioners understand and implement herd control plans to break the transmission cycle, as well as evaluating the associations between ELISA titers at dryoff and subsequent herd performance). Dr. Gay's experience with animal production and health extends to his childhood on his family's 1,400-head commercial cow-calf ranch in eastern Montana. Dr. Gay earned his B.S. in mechanical engineering in 1973 from the University of Washington, his D.V.M. in 1978 from Washington State University, and his Ph.D. in veterinary science in 1988 from the University of Minnesota.

RALPH A.GIANNELLA has been on the faculty and has been serving as Director of the Division of Digestive Diseases at the University of Cincinnati College of Medicine since 1980. He is also a Staff Physician at the Veterans Affairs Hospital in Cincinnati and an attending physician at the University of Cincinnati Hospital. He has authored over two hundred journal articles, books, abstracts, chapters, and letters on a broad range of topics in his field, and is on the editorial board of the Edizione Minerva Medica (Italy), GastroHep.com (UK), Revista Espanola Enfermedades Digestivas (Spain), and Gastroenterologia Integrada (Spain). Additionally, Dr. Giannella serves on the Board of Directors for the Foundation for Digestive Health and Nutrition, and the American Gastroenterological Association Foundation. Dr. Giannella is a Fellow of the American College of Physicians, a member of the American Society of Microbiology, a member and former President of the American Gastroenterological Association, and has served on the Crohn's and Colitis Foundation of America's Research Training Awards Committee. Dr. Giannella has been elected to membership in the Central Society for Clinical Investigation, the American Society for Clinical Investigation, and the Association of American Physicians, among other organizations. He is also a Diplomate of the American Board of Internal Medicine and the American Board of Gastroenterology. Dr. Giannella earned his A.B. from Cornell University in 1961, and his M.D. from the Albany Medical College in 1965.

MURRAY E.HINES II is an Associate Professor at the Veterinary Diagnostic and Investigational Laboratory of the Department of Veterinary Pathology at the University of Georgia's College of Veterinary Medicine. His research has involved ruminant paratuberculosis and swine mycobacteriosis (tuberculosis) including evaluation of virulence factors, vaccine efficacy, cytokine expression, diagnostic tests and control methods. He is also Chief of the Necropsy, Immunohistochemical Staining, and Pathology Research Resources Sections. He has been a section moderator at two Colloquia of the International Association for Paratuberculosis and Other Intestinal Mycobacterioses (1991

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and 1996) and at the American Association of Laboratory Animal Veterinarians in 1993. He is licensed in Tennessee and Georgia and is a Diplomate of the American College of Veterinary Pathologists. Prior to assuming his position at the University of Georgia, Dr. Hines was an Assistant Research Professor at the University of Miami, School of Medicine, and was in private practice for seven years. He received his D.V.M. from the University of Tennessee in 1980 and his Ph.D. in veterinary pathology from Louisiana State University in 1991.

WILLIAM D.HUESTON currently serves as Professor and Director of the Center for Animal Health and Food Safety in the College of Veterinary Medicine at the University of Minnesota, and adjunct Professor of Epidemiology in the School of Public Health. Previously, he was Associate Dean and Professor of the Virginia-Maryland Regional College of Veterinary Medicine in College Park, Maryland. Before joining the Regional College faculty, Dr. Hueston was Director of the Operational Support Staff and Director of the Center for Animal Health Monitoring of the Centers for Epidemiology and Animal Health at USDA/APHIS, where he designed, implemented, and managed a national system for monitoring animal health in the United States. During his tenure at USDA, he also held an adjunct faculty position at Colorado State University. Prior to USDA, Dr. Hueston was a faculty member at the Ohio State University. He was also an industry and clinical veterinarian for four years, and is the author and co-author of numerous refereed journal publications. He is board certified with an epidemiology specialty by the American College of Veterinary Preventive Medicine, and is a Diplomate in the American College of Veterinary Preventive Medicine. Dr. Hueston received his B.A. in biology from University of Virginia, and his M.S. in preventive medicine, D.V.M., and Ph.D. from the Ohio State University.

LAWRENCE J.HUTCHINSON brings 25 years of applied research, extension services, and producer education activities to this study. His work on Johne's disease has led to a better understanding of its epidemiology, transmission, diagnostic detection, and control. Dr. Hutchinson has conducted field studies that have involved beef packing plants, dairy herds, and diagnostic test development, and which have led to a comprehensive risk assessment tool for Johne's disease, as well as programs for control and test-negative status recognition. He is currently a Professor of Veterinary Science and Extension Veterinarian at the Pennsylvania State University, where he has been on faculty since 1976. Prior to his academic appointment, Dr. Hutchinson was in general and large animal veterinary practice in Vermont, New York, and Pennsylvania for fourteen years. He has a significant number of publications and research grants on the subject of Johne's disease and has developed many new courses, extension programs, and other educational materials. He is also a member of numerous professional organizations, such as the American Veterinary Medical Association, the Pennsylvania Dairy Sanitarians, the U.S. Animal Health

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Association, and the National Extension Dairy Quality Assurance Committee, and is immediate past president and a member of the American Association of Bovine Practitioners. Dr. Hutchinson has also been selected as the Extension Veterinarian of the Year by the American Association of Extension Veterinarians, and the Pennsylvania Veterinarian of the Year by the Pennsylvania Veterinary Medical Association. Dr. Hutchinson received a B.S. in pre-veterinary studies in 1959 from the Pennsylvania State University, and his D.V.M. from New York State Veterinary College in 1962.

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