Article

Diagnostic detection methods for *Mycobacterium avium* subsp. *paratuberculosis* in white-tailed deer

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Abstract — This study compares the results and suitability of serological testing, microscopic examination, deoxyribonucleic acid (DNA) detection, and bacterial culture for detecting *Mycobacterium avium* subsp. *paratuberculosis (Map)* infection in asymptomatic farmed white-tailed deer (WTD) *(Odocoileus virginianus)*. Deer were classified as infected if culture slants from their feces, lymph nodes, or ileum were positive, or if a polymerase chain reaction (PCR) assay detected *Map* DNA in any of its tissues. Deer identified as positive by agar gel immunodiffusion (AGID) testing or enzyme-linked immunosorbent assay (ELISA) but not by bacterial culture, Ziehl-Neelsen staining, or PCR assay were classified as suspect. Culture of tissues classified 10/16 (62.5%), histopathologic examination 1/16 (6.3%), tissue smears 4/16 (25%), culture slant (CS)-PCR on feces 12/15 (80%), CS-PCR on tissue 13/16 (81.3%), and direct PCR on uncultured tissues 5/16 (31.3%) deer as infected. The ELISA classified 2/15 (13.3%) deer as positive and therefore suspect. The AGID test was negative for all deer. Fifteen of 16 deer were positive by 1 or more tests; only 1 deer was negative on all 11 assays. The CS-PCR gave superior results on antemortem fecal testing as well as postmortem tissue testing and can be recommended for improving the detection of *Map* in WTD at every stage of infection.

Résumé — Méthodes diagnostiques dans la détection de *Mycobacterium avium subsp. paratuberculosis* chez le cerf de Virginie. Cette étude vise à comparer les résultats et la pertinence des épreuves sérologiques, des examens microscopiques, de la détection de l'acide désoxyribonucléique (ADN) et de la culture bactérienne dans la détection des infections à *Mycobacterium avium subsp. paratuberculosis* (Map) chez le cerf de Virginie d'élevage (CV) *(Odocoileus virginianus)* asymptomatique. Les cerfs ont été considérés infectés si les cultures inclinées de fèces, les nœuds lymphatiques ou l'iléum étaient positifs ou si une épreuve d'amplification en chaîne par polymérase (ACP) avait détecté l'ADN de Map dans n'importe lequel de leurs tissus. Les cerfs identifiés positifs par méthode d'immunodiffusion en gélose (IG) ou par titrage immunoenzymatique utilisant un antigène adsorbé (ELISA) mais classé négatif par culture bactérienne, coloration de Ziehl-Neelsen ou ACP ont été considéré suspects. La culture de tissus a certifié que les cerfs étaient infectés dans 10 cas sur 16 (62,5 %), les examens histopathologiques dans 1 cas sur 16 (6,3 %), les frottis de tissus dans 4 cas sur 16 (25 %), les cultures inclinées (CI) -ACP sur fèces dans 12 cas sur 15 (80 %) et les CI — ACP sur tissus dans 13 cas sur 16 (81,3 %). Le titrage par ELISA a établi que 2 cerfs sur 15 (13,3 %) étaient positifs, donc suspects. Le test IG s'est révélé négatif chez tous les cerfs. Quinze des 16 cerfs ont été classés positifs par au moins 1 test; un seul cerf est demeuré négatif aux 11 tests. La CI-ACP a donné de meilleurs résultats à la fois dans les épreuves fécales antémortem et dans les épreuves tissulaires postmortem et peut être recommandé pour améliorer la détection du Map sur le CV à n'importe quel stade d'infection.

(Traduit par Docteur André Blouin)

Can Vet J 2008;49:683–688

Introduction

Paratuberculosis (Johne's disease) is a chronic enteritis of herbivores caused by *Mycobacterium avium* subsp. *paratuberculosis (Map)*. In North America, its presence is reported frequently in traditionally farmed species, but less commonly in specialized livestock, such as deer and elk (1–3). The susceptibility of deer to *Map* infection has been recognized and well described in countries where deer are farmed intensively, including New Zealand (4), Canada (5), the USA (2), and several European countries (6–8). The most common clinical sign of paratuberculosis in deer is sudden and extreme weight loss. Diarrhea, often the 1st sign of the disease in cattle, may not occur in deer. Paratuberculosis has been known to occur in outbreak form in young deer and elk, and to progress more rapidly in these species than in cattle (2).

Paratuberculosis is essentially untreatable (9), and the only effective control measures are farm management strategies aimed

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This research was supported by a grant from the Canada-Saskatchewan Agri-Food Innovation Fund (AFIF).

at the reduction of *Map* transmission and the culling of infected deer, which requires an accurate diagnostic test for detection (10). Poor sensitivity and specificity of current diagnostic tests have been major obstacles for the control of paratuberculosis in deer, as well as in other ruminants.

This paper reports the results of enzyme-linked immunosorbent assay (ELISA) and agar gel immunodiffusion (AGID), special staining, and microscopic examination of tissue and tissue smears; 2 different approaches for *Map* DNA detection; and bacterial culture for detecting *Map* infection in asymptomatic farmed white-tailed deer (WTD) *(Odocoileus virginianus)*.

Materials and methods

A pen of 16 female WTD, aged 2 to 9 y, in which a clinical case of paratuberculosis had previously occurred, was depopulated to control the spread of the disease on a Saskatchewan deer farm. All animals appeared to be healthy and in good body condition when euthanized by an IV injection of barbiturate. Serum was collected from each animal prior to euthanasia and frozen at -20°C until processed for ELISA and AGID testing. The serum from 1 animal was lost and not tested serologically. Samples of feces, mesenteric lymph nodes, and ileum were taken from each animal at necropsy, placed in individual sterile plastic bags, and processed the same day. Physical separation and careful sanitation were used to prevent cross contamination of samples. Laboratory testing of ileal sections, mesenteric lymph nodes, serum, and feces included bacterial culture, polymerase chain reaction (PCR), serologic testing, histologic examination, and staining of fresh smears.

Bacterial culture

Feces, mesenteric lymph nodes, and ileum from each animal were processed following a previously reported protocol for *Map* culture (11). Tissue preparations were decontaminated for 3 h with 0.75% hexadecylpyridinium chloride (HPC). Fecal preparations were also decontaminated with HPC, but they were incubated overnight at 37°C. Five Herrold's egg yolk agar (HEYM) slants (Becton Dickinson, Cockeysville, Maryland, USA) were inoculated with 0.2 mL of the processed material. Four slants (tubes) were supplemented with mycobactin J; 1 tube without mycobactin J remained as a negative control. The remaining inoculum was stored in transfer pipettes at -70°C. All tubes were incubated at 37°C for 16 wk and examined every week for *Map* growth. Fecal and tissue samples were classified as culture positive if *Map* was isolated on any of the 4 mycobactin J supplemented HEYM slants. Confirmation of *Map* growth included mycobactin dependence and the testing of a colony typical of *Map* from each specimen by Ziehl-Neelsen staining and PCR assay for the DNA sequence at IS900 (12).

Enzyme-linked immunosorbent assay

Sera were tested for antibody to *Map* by an ELISA (IDEXX; Portland, Maine, USA). The ELISA optical density results were obtained by following the manufacturer's instructions. All samples were tested in duplicate. Samples with a sample-to-positive (S/P) ratio of 0.25 or higher, using the ELISA kit bovine serum controls, were classified as positive.

Agar gel immunodiffusion assay

Serum samples were tested, using a commercially available AGID assay (ImmuCell Corporation, Portland, Maine, USA). Any visible precipitation that developed between the antigen and the test serum wells within 48 h of incubation was recorded as a positive AGID test.

Polymerase chain reaction assay on culture slants

A diagnostic approach combining the growth and harvest of bacteria from culture media and nucleic acid detection (CS-PCR) was described by Secott et al (12). Using this concept, cells were harvested from fecal and tissue culture slants from each animal after 16 wk of incubation; DNA was extracted from the cells by using a lysis buffer wash (100 mN NaCl, 500 mM Tris, pH 8, 10% SDS) and sequential treatment with lysozyme (5 mg/mL) at 37°C for 30 min and proteinase K (20 mg/mL) at 65°C for 2 h, as previously described (13). Following incubation at 65°C, 2 phenol/chloroform extractions were performed, and DNA was precipitated with ethanol. Five microliters of the DNA extracts from each culture slant, originating from the same sample, were combined for a PCR assay to detect IS900. A sample was considered positive by CS-PCR if it yielded a detectable PCR product of the expected size [278 base pairs (bp)].

Direct PCR assay

Extraction of DNA from lymph node and ileum samples from each animal was achieved by macerating approximately 1 cm^3 of tissue, placing it into a 1.5-mL tube containing 600 μL of lysis buffer, and extracting DNA by using the procedure described previously for culture slants (13). A tissue sample was considered positive by PCR if it yielded a detectable PCR product of the expected 278 bp size.

Primers and PCR conditions

Each 50 μL PCR reaction mixture was prepared by using sterile, ultrapure water, 3 mM $MgCl_2$, PCR buffer (Invitrogen, Burlington, Ontario), 25 mM dNTP's, 25 pM of each primer; IS900/P36 (5′-GGCCGTCGCTTAGGCTTCGA-3′) and IS900/P11(5′-CGTCGTTAATAACCATGCAG-3′) (14), 5 U/μL *Taq* DNA polymerase and mixed with 2 μL of DNA extract. In addition, each batch run included positive (*Map* extracted DNA from ATCC strain # 19698) and template-negative (water) controls. Following denaturation (4 min, 92°C), the PTC-200 Peltier thermal cycler (MJ Research, Waltham, Massachusetts, USA) was run for 35 cycles of 45 s at 94°C, 1 min at 55°C, and 1.5 min at 72°C. The reaction was finished with an extension phase (5 min, 72°C), then kept at 4°C. Ten microliters of each amplified PCR product was mixed with 5 μL of bromphenol blue and tracking dye, loaded to a 1.5% agarose gel, and electrophoresed.

Histopathologic examination

Fresh smears of all tissue specimens were stained with Ziehl-Neelsen stain and examined for the presence of acid-fast bacilli. Sections of ileum and lymph nodes were fixed in formalin, embedded in paraffin, sectioned, and stained with

Table 1. *Mycobacterium avium* subsp. *paratuberculosis* diagnostic test results for 16 farmed white-tailed deer

Deer ID	Culture (feces)	Culture[a] (LN)	Culture (Ileum)	CS-PCR (feces)	CS-PCR (LN)	CS-PCR (Ileum)	Direct PCR (LN)	Direct PCR (Ileum)	ELISA	Tissue smear stain	Histologic examination	AGID
Blue 87	—[c]	[b]P++	P+++	P	P	P	P	P	P	P	P	—
Black 6250	—	P++	P+	P	P	P	NA	—	—	P	—	—
J 10	—	P++	—	P	P	—	—	—	—	P	—	—
Green 10	—	P+	P+	P	P	P	—	P	—	P	—	—
Blue 212	—	P+	—	P	P	P	—	—	—	—	—	—
01-24225	—	—	P+	NA	—	P	P	P	—	—	—	—
Blue 130	—	—	P+	P	—	P	—	—	—	—	—	—
Blue 211	—	—	P+	P	—	P	—	—	—	—	—	—
Green 17	—	P+	—	P	P	—	—	—	—	—	—	—
Yellow 52	—	P+	—	—	P	—	—	—	—	—	—	—
White 55	—	—	—	P	P	P	P	—	P	—	—	—
Black 6255	—	—	—	—	P	—	P	—	NA	—	—	NA
Blue 7	—	—	—	P	P	—	—	—	—	—	—	—
Blue 209	—	—	—	P	—	—	—	—	—	—	—	—
J 7	—	—	—	—	—	—	NA	—	—	—	—	—
White 70	—	—	—	P	—	—	NA	—	—	—	—	—

[a] Average colony count per tube:
+ indicates < 10 colonies
++ indicates between 10 and 60 colonies
+++ indicates > 60 colonies
— indicates no colony growth
[b] P — positive test result
[c] — negative test result; fecal cultures were overgrown and presumed negative
NA — no test result available
LN = lymph node
CS-PCR = Polymerase chain reaction performed on bacterial culture slant growth
Direct PCR = Polymerase chain reaction performed directly on tissue
Culture = Bacterial culture on selective media
ELISA = Enzyme-linked immunosorbent assay
AGID = Agar gel immunodiffusion

hematoxylin-eosin and Ziehl-Neelsen stain. Lymph nodes, as well as lamina propria and submucosa sections of ileal wall, were examined for the presence of granulomas and acid-fast bacteria. Tissues were classified as *Map* positive according to the presence of granulomatous cells and acid-fast bacilli. The presence of lesions consistent with *Map* infection, such as infiltration of epithelioid cells, macrophages, and multinucleated giant cells, but without acid-fast organisms, would classify the tissue as suspect. A tissue was considered to be negative by this test if granulomas and acid-fast bacilli were not detected.

Case definition

Individual deer were classified as infected with *Map* if any one of the culture slants from their feces, lymph nodes, or ileum were positive, or if a CS-PCR or direct PCR assay detected *Map* DNA in any of their tissues. Deer identified as positive for *Map* by indirect tests such as AGID and ELISA, but not by bacterial culture, Ziehl-Neelsen staining, or PCR assay were classified as suspect cases.

Results

Mycobacterium avium subsp. *paratuberculosis* was cultured from the tissues from 10 of 16 deer (62.5%) but not from their feces. Fecal culture slants suffered heavy overgrowth of organisms other than *Map* and were presumed negative for *Map* on the basis of visual inspection. Four animals had positive lymph nodes only, 3 had positive ileum only, and 3 had positive lymph nodes and ileum (Table 1). The number of colonies varied from 1 to over 200 per slant and took between 4 and 10 wk to grow. All culture positive results were confirmed to be *Map* by mycobactin dependence, positive Ziehl-Neelsen staining for organisms, and IS900 PCR. Further PCR testing at IS1311 later identified the organisms as a "cattle strain" of *Map*.

Microscopic examination of tissue smears yielded small numbers of acid-fast bacilli in 4 animals. Three deer had positive ileum only, and 1 deer had positive ileal and lymph node smears. Histopathologic examination detected characteristic *Map* infection lesions in only 1 deer, which, coincidentally, had high numbers of cultured organisms (> 200 colonies per slant) in the same tissues. Intracellular acid-fast organisms with morphology typical of *Map*, numerous multinucleated giant cells, and epithelioid macrophages were demonstrated in both the lymphoid tissue and ileum of that deer.

Culture of tissues classified 10/16 (62.5%), histopathologic examination 1/16 (6.3%), tissue smears 4/16 (25%), CS-PCR on feces 12/15 (80%), CS-PCR on tissue 13/16 (81.3%), and direct PCR on uncultured tissues 5/16 (31.3%) deer as *Map* infected. Two of 15 (13.3%) deer had positive ELISAs and, therefore, were classified as suspect (S/P ratio 0.42 and 0.28, respectively). The AGID assay was negative for all deer tested. Fifteen of 16 deer were identified as positive by 1 or more tests; only 1 deer was negative on all 11 assays/tests.

Discussion

Bacteriological culture of *Map*, with its relatively low sensitivity but high specificity, has been considered the definitive diagnostic test for *Map* infection. Culture of tissue in particular is regarded as the postmortem diagnostic "gold standard" (15). Culture of

tissues identified more *Map*-positive deer than did direct PCR, agreeing with CS-PCR results in 10 of 13 deer (77%). Only 5 of the 13 (38.5%) CS-PCR tests that were positive in 1 or both ileum and lymph node were also positive on direct PCR. In our study, neither culture nor direct PCR performed as well as CS-PCR on feces or tissue. Fecal culture results were difficult to interpret due to the overgrowth by contaminants. In animals where there was most agreement among tests, the number or organisms cultured was relatively high, and in animals where culture of tissue was negative, only CS-PCR identified tissue, feces, or both as positive. This suggests that CS-PCR may have a lower detection threshold for *Map* organisms and, in that sense, is a superior test to either simple culture or direct PCR.

There has been little evaluation of tests, developed for cattle and sheep, used in WTD. The limited number of deer available in this study made comparative test evaluation difficult. Calculation of epidemiologic sensitivities was attempted, using CS-PCR as the gold standard, but there were wide confidence intervals for the resulting values and more than 1 included a value of zero, perhaps reflecting the small sample size. Ideally, a larger study, including deer with known *Map* infection status, is required to precisely estimate sensitivity and specificity of these tests. We are, therefore, unable to report meaningful values for the sensitivity and specificity of the tests used in this study; however, discussion of our results can include the findings from other similar studies.

Fecal culture yielded no positives in our study. Sensitivity of fecal culture depends on the stage of the disease in the animal tested and is reported to vary from 45% in subclinically to 91% in clinically affected deer (10), so the subclinical status of our subjects decreased the likelihood of successful fecal cultures. Culture of mesenteric lymph nodes, since they are colonized by *Map* in the early phase of infection, is considered more sensitive than fecal culture (8). However, a long incubation period and bacterial and fungal contaminants that overgrow culture slants are serious disadvantages of bacterial culture, regardless of the nature of the test material. In this particular study, there was significant overgrowth of all fecal culture slants, rendering them diagnostically useless. The CS-PCR assay offers a solution to these problems. The ability of PCR to detect the presence of DNA on slants containing bacterial growth not visible to the eye has the potential to reduce incubation time to as little as 6 wk. Since PCR detection of *Map* DNA is not inhibited by the presence of bacterial and fungal contaminants, a large proportion of samples that are inconclusive on standard culture can be found to be positive with PCR (12).

The ability of PCR to detect both viable and nonviable *Map* bacilli on the culture slant or the presence of "non culturable," cell wall-deficient forms of *Map* strains in the samples (16) may account for more infected animals being detected by CS-PCR than by traditional methods. Cell wall-deficient organisms or spheroplasts are extremely slow growing and difficult to culture, which helps to explain why organisms were not isolated from CS-PCR positive tissues. Also, spheroplasts do not stain with acid-fast stain and, therefore, are not visible in lesions. Paucibacillary lesions associated with cell wall-deficient organisms detected only by electron microscopy or IS900 PCR have been reported (17,18). The paucibacillary (tuberculoid) form of paratuberculosis is characterized by having only a few bacilli present and a strong cell-mediated immune response. Since positive serological test results are usually associated with a high mycobacterial load and pluribacillary (lepromatous) lesions (17), this also helps to explain the predominantly negative serological results in this study.

Since direct PCR assay of tissues was positive for only 5 deer, it appeared to be not as sensitive as has been previously reported (7,19). Since the assay is able to detect small numbers of bacteria, its performance in this study was confusing. However, no single tissue can be expected to consistently determine infection status, especially in lightly infected animals, and a wider collection of samples from our deer might have yielded more accurate results (20). The poor correlation between the culture results from lymph nodes and ileum in the same animal suggests that both sites and perhaps others should be included when sampling for the purposes of culturing the bacterium from tissues in deer.

Since all animals were negative by AGID, this test cannot be recommended for paratuberculosis testing in asymptomatic WTD. These findings are consistent with those of investigations in other species (21,22), suggesting that the AGID assay, optimized for use in cattle, lacks sensitivity when applied in some other species. While AGID has been described as a sensitive serological test for diagnosing clinical paratuberculosis in red deer, it was found to be inaccurate when detecting subclinical cases (23). Also, because of its inability to differentiate between *M. avium* subsp. *paratuberculosis* and *M. avium* subsp. *avium,* the assay lacks the specificity to be of use in WTD (23).

The deer that was test-positive on all assays, including the ELISA, was without doubt heavily infected. Positive serological test results are usually associated with a high mycobacterial load and pluribacillary lesions (17). The other ELISA-positive result was confirmed by positive direct PCR of tissues and fecal and tissue CS-PCR, but not bacterial culture of tissues. This may be explained by falsely positive PCR and ELISA results, or by the inherent difficulty of culturing some *Map* strains (24,25). Another explanation for the poor performance of ELISA in this study may be that the protocol had not been optimized for use in WTD. While a published study had demonstrated that the protein G conjugate used in the assay binds WTD immunoglobulin similarly to bovine immunoglobulin (26), validation of the entire ELISA protocol for WTD has not been completed. Avenues for improved performance in WTD may exist through different sample dilutions, diluent characteristics, solid phase antigen preparation, and validation of appropriate "cut-off" values.

The poor performance of serologic tests on WTD in this study was consistent with results in previous studies of paratuberculosis in fallow and key deer (21,27). The antibody conjugate used in those studies was also protein G, but since antibody binding characteristics are not uniform across cervid species, direct comparison may be inappropriate (26). Griffin et al (28) developed a more sensitive, customized ELISA that employs unique test antigens for the serodiagnosis of *Map* infection in red deer; this could prove useful in WTD testing.

Early *Map* infection is characterized by a strong cell-mediated immune (CMI) response, while advanced stages with progressive lesions are associated with a humoral, antibody producing, immune response (1). The simplest explanation for the poor performance of ELISA might be that since the deer tested in our study were in the preclinical stage of infection, antibody to *Map* was simply not present. The application of a test that detects a CMI response may be more useful for the detection of subclinical cases than are assays focused on the production of antibody.

Only the deer with the highest colony counts was classified as *Map* infected by histopathologic examination. Microscopic detection of *Map* specific lesions in tissues is sensitive in advanced clinical stages of paratuberculosis, but it is less reliable for detection of early or subclinical infections (18,22), and a lack of correlation between histopathologic and culture results for *Map* in subclinically affected deer has previously been shown (29). Frequently, neither lesions typical of paratuberculosis nor acid-fast organisms can be seen in tissue sections from early cases, even though *Map* can be cultured from these tissues (29). Perhaps the inability of histopathologic examination to detect *Map* infection in this study was because deer with low to moderate number of colonies were not clinically ill and, accordingly, had yet to develop detectable histological changes resulting from infection. Also, *Map* lesions are not homogeneously distributed in tissue, so it is possible to simply miss sampling lesions in truly infected animals.

Considering that, in deer, lesions typical of paratuberculosis may also be found in the upper ileum and jejunum (2,23), it is possible that more animals would have been detected in this study if more samples from those parts of the intestinal tract had been analyzed, but this approach would not be practical in most diagnostic work-ups. It is notable that *M. avium* and *M. bovis* infections in deer produce lesions, such as caseation necrosis and mineralization of lymph nodes, that may be indistinguishable from those caused by *Map* (30), making it impossible to differentiate these 3 infections from lesions by histopathologic examination alone. This would be of greater significance in geographic areas where tuberculosis is present.

In the 5 deer where microscopic examination of stained impression smears from tissues collected at postmortem detected acid-fast bacilli, which were subsequently confirmed by bacteriological culture of tissues, the procedure was more sensitive than histopathologic examination. Ziehl-Neelsen staining of impression smears of postmortem samples can be useful as an ancillary test, since it is inexpensive and simple to perform.

This study demonstrated differences in the performance of the currently available tests for *Map* infection in deer. Since little is known about diagnostic methods for paratuberculosis in WTD, we hope that these data will provide valuable information to the WTD industry. An increased awareness of the relative accuracy of diagnostic tests may help veterinarians to decide which tests are appropriate for the detection and control of paratuberculosis in WTD. The ELISA is inexpensive and easy to perform, but because it lacks sensitivity early in *Map* infections, it is perhaps best used only as a screening test in herds where culling has not yet occurred. Fecal culture has been recommended for antemortem testing of individual animals, confirmation of infection in ELISA positive animals, or subsequent testing of ELISA-positive herds (2). However, in this study, bacterial culture of tissue specimens was a more reliable (albeit postmortem) diagnostic test for paratuberculosis in WTD. Superior results on antemortem fecal testing, as well as postmortem tissue testing, were obtained with CS-PCR, which can be recommended for improving the detection of *Map* in deer at every stage of infection.

Authors' contributions

Dr. Woodbury conceived and designed the project, provided the case material and the samples, and wrote the manuscript. Dr. Chirino-Trejo contributed to the conception and design of the project, analyzed and interpreted the data, and contributed to the preparation of the manuscript. Ms. Mihajlovic generated most of the test results, analyzed and interpreted the data, and assisted in the preparation of the manuscript.

Acknowledgments

The authors are grateful to Dr. Elizabeth Manning at the Johne's Testing Center in Madison, Wisconsin for serological testing and advice on the preparation of this manuscript. We also thank the PCR laboratory staff at the Prairie Diagnostic Services, Saskatoon, Saskatchewan and Dr. Fei Huang for technical assistance with this project. CVJ

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Book Review
Compte rendu de livre

Mycobacterium bovis Infection in Animals and Humans, 2nd ed.

Thoen CO, Steele JH, Gilsdorf MJ, eds. Blackwell Publishing, Ames, Iowa, USA, 2006. 329 p. ISBN-13:978-0-8138-0919-9. CDN$149.99.

This is the 2nd edition of a book originally published in 1995. The purpose stated in the preface is "*to provide medical professionals, allied health scientists, research workers, diagnosticians, and graduate students with current information on the significance of* M. bovis *in the control and eradication of tuberculosis in animals and humans.*"

The book contains a great deal of important information and will be a very useful reference source. It consists of 29 chapters contributed by 56 authors. Documentation of the global extent of human infection by *Mycobacterium bovis* is a strong item in the book and will be of value to those concerned with public health and regulatory veterinary medicine. As with many multi-authored books, the chapters vary considerably in quantity and coverage. For instance, bovine tuberculosis in China is covered in 1.5 pages, while 26 pages are devoted to bovine tuberculosis in Russia and the former states of the Soviet Union. Similarly, tests for diagnosing bovine tuberculosis in live animals, a subject of importance to veterinarians, is covered within 3 pages with only 5 references from this century, while the description of infection by *Mycobacterium pinnipedii* was 11 pages. The book might be easier to use if the chapters had a common format, related subjects were grouped into sections, and there was some form of critical overview of each section, perhaps by the editors. For instance, the effect of wildlife on control programs in Italy and Ireland is covered in chapters 13 and 14, while similar information from the USA, Canada, and South Africa occurs in chapters 20, 21, and 22, respectively. While the individual chapters are very good, they could have been grouped with an overview of the problem and linked with other information about tuberculosis in wild animals scattered elsewhere in the book. Other groupings might have included information on human infection with *M. bovis,* economics of tuberculosis, pathogenesis and epidemiology, review of tuberculosis in different areas of the world, and diagnostic and control techniques, all of which are covered in the book. The text is adequately illustrated with tables and graphs. Approximately one-third of the small number of photomicrographs lack contrast. There are a few omissions including lack of coverage of bovine tuberculosis in Australia and New Zealand, and discussion of Bacille Calmette-Guérin (BCG) vaccines that are being actively researched in some countries for use in a variety of species.

The preface concludes that the information in this book "*is of value to public health officials, state and federal regulatory veterinarians, practitioners, and producers interested in the importation of animals for herd additions.*" The book will be of great interest to the first 2 of these groups, as well as to researchers, but of limited value for practitioners or producers.

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