Workshop on Mycobacterium avium subsp. paratuberculosis in North American Bison (*Bison bison*)

PROCEEDINGS AND WORKSHOP REPORT

Alberta Cooperative Conservation Research Unit (ACCRU)

National (Canadian) Wood Bison Recovery Team

Editors: Murray Woodbury, Elena Garde, Helen Schwantje, John Nishi, and Brett Elkin with contributions from Michelle Oakley, Jenny Powers, Jennifer Sibley and workshop participants.

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ABSTRACT

Many of the emerging diseases worldwide are shared between domestic livestock, free ranging wildlife, and humans. Infection by mycobacterial organisms is not a recent or newly emerging issue, but the increasing frequency of disease caused by *Mycobacterium* species, where wildlife, domestic livestock, and humans are epidemiologically linked is cause for concern for wildlife management, agricultural and public health agencies alike. Human tuberculosis, including that caused by *Mycobacterium bovis*, once thought to be a well understood disease of lifestyle and socioeconomic consequences is once again a global concern. Johne's disease (*Mycobacterium avium* subspecies *paratuberculosis* infection), previously considered a production limiting disease important only in domestic ruminants, has recently been implicated in the pathogenesis of Crohn's disease in humans. Furthermore, wildlife and livestock reservoirs of infection are now considered to be important to the epidemiology of both of these mycobacterial diseases.

Innovations in diagnostic testing and laboratory techniques have led to the discovery of many infectious agents and evidence of non clinical infection in previously unrecognized species and circumstances. Among the innovations, molecular DNA detection methods demonstrate the presence of mycobacterial organisms in new species not known to show classic signs of clinical disease and also permit the identification and characterization of mycobacteria to a much higher degree than previously possible.

The generation of information is usually followed by its evaluation and analysis, and a search for the meaning and consequences of the new knowledge. At times, new information is merely supportive of current and conventional thinking, but often it forces change and the adoption of new principles and ideas. The same is true with the rapid evolution of disease diagnostics. New, more sensitive tests, and testing in previously impossible circumstances are generally desirable activities, but what can or should one do about interpreting and acting on the results?

The increased intensity of research into *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infections in domestic and wildlife species, and concern about the zoonotic potential of MAP organisms is leading to new information on the distribution of this organism in wildlife and natural ecosystems. MAP DNA has been identified in fecal samples from free-ranging and captive Wood and Plains bison in northern Canada, without signs of clinical disease.

A workshop, entitled ***Mycobacterium avium* subsp. *paratuberculosis* in North American Bison (*Bison bison*)** was held in Edmonton, Alberta, on Friday, February 18 and Saturday, February 19, 2005. Invitees included scientists, veterinarians, wildlife and agricultural workers and managers, and students from universities, institutes and governments in Canada and the United States. Following an information session, the

group discussed how to assess the risks and consequences associated with this discovery and the implications to wildlife management and translocation activities.

The results of the workshop are contained in this report. Key research needs include better surveillance to determine the distributions and prevalence of the organism in wood bison across Canada, identification and characterization of this organism (by culture), and and determination of the significance of this organism to wood bison health at an individual and population level. The need remains to follow up on these plans: this will require a collaborative effort between the varied stakeholders involved in wild bison conservation and wildlife disease research, and the identification of research funding to commit to this work.

About this report . . . .

This workshop summary and report was funded by the British Columbia Ministry of Environment and was compiled from material provided by several workshop participants. **Michele Oakley** from the Department of Environment, Government of Yukon and **Helen Schwantje** took notes during the workshop, which are paraphrased here, and **Jenny Powers**, Wildlife Veterinarian, U.S. National Park Service, provided an initial summary. Material from some of the PowerPoint presentations used at the workshop is included. The Department of Veterinary Microbiology at WCVM kindly granted permission to use portions of an MSc thesis by **Jennifer Sibley** as background information on MAP and fingerprinting methods. Sincere thanks to all who provided material and participated in the workshop.

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EXECUTIVE SUMMARY

The Alberta Cooperative Conservation Research Unit (ACCRU), in cooperation with the Bison Health Management Subcommittee of the National (Canadian) Wood Bison Recovery Team, sponsored this workshop to discuss the implications of *Mycobacterium avium* subspecie*s paratuberculosis* (MAP) infection to the conservation and management of wild bison in Canada.

A number of geographically distinct, free-ranging Wood and Plains Bison herds in northern Canada have molecular (DNA) evidence of a strain of *M. avium paratuberculosis* (MAP) from ground-collected fecal samples. However, there is evidence that the organism varies genetically from the MAP found in clinical Johne's disease and no living mycobacterial organism has been isolated by culture of these samples. No clinical disease, manifested as weight loss or diarrhea, as typically seen with Johne's disease in commercial bison, has been observed in any of the captive or free-ranging herds sampled.

The goals of the meeting were to summarize what is known about this organism, describe its identified distribution and the clinical signs it can cause in bison (i.e. Johne's disease), as well as identify knowledge gaps and future research priorities regarding MAP infection and its significance to bison management. Additionally, the group was to provide standardized herd monitoring and testing protocols for free-ranging bison and discuss management recommendations for potentially MAP infected bison herds. These recommendations would be expected to help guide national wood bison conservation and genetic management efforts, which might include translocation and /or reintroduction of wood bison from existing herds. Finally, this document is produced as a summary for communication and education purposes.

Summary of conference proceedings:

WELCOME, OPENING REMARKS

The group was warmly welcomed by **Dr. Helen Schwantje,** and **Dr. Bill Samuel** representing the sponsors of the meeting, the Bison Health Subcommittee of the National Wood Bison Recovery Team, and the Alberta Cooperative Conservation Research Unit (ACCRU), University of Alberta respectively.

WORKSHOP GOALS AND OBJECTIVES

Dr. Helen Schwantje, Chair, Bison Health Subcommittee, National Wood Bison Recovery Team, and Wildlife Veterinarian, Biodiversity Branch, Ministry of Environment, Victoria, BC. led a discussion about the material that needed to be shared by workshop participants and some objectives were set to provide format and structure to the meeting (see introductory paragraphs above).

DISTRIBUTION OF MAP IN FREE-RANGING BISON

The speakers in this session provided information describing the known distribution of MAP in North American captive and free ranging bison populations. Veterinarian **Dr. Brett Elkin** and bison ecologist **John Nishi** from the Government of the Northwest Territories presented a geographic accounting of the northern bison populations and described those populations where *M. bovis* was present and where MAP DNA has been found. **Dr. Becky Manning**, a senior scientist from the Johne's Testing Center at the School of Veterinary Medicine in the University of Wisconsin at Madison, WI, was unable to attend the meeting in person but was linked via telephone and provided a teleconference presentation discussing the expression of Johne's disease in wild and farmed US bison. Veterinarian **Dr. Jenny Powers** from the U.S. National Park Service at Fort Collins, Colorado talked about the status of known MAP infections in elk and bison in US National Parks, focussing on endemic Johne's disease in Tule elk at Point Reyes, California.

DIAGNOSTICS

Dr. Becky Manning presented material via teleconference on current diagnostic approaches to MAP infection and Johne's disease. Diagnostic difficulty in general is compounded by species differences in test suitability and interpretation of results. **Dr. Greg Appleyard**, a molecular biologist from the Western College of Veterinary Medicine in Saskatoon informally discussed the recent finding of MAP DNA in captive and free-ranging Canadian Wood and Plains bison fecal samples and gave information about innovations in PCR and molecular typing techniques useful in the identification of MAP. **John Nishi**, provided general information on sampling theory and explained the sampling protocols developed for free ranging bison conservation herds in Canada.

CLINICAL EFFECTS AND SIGNIFICANCE OF MAP IN BISON

Dr. Morgan Scott, an epidemiologist now at Texas A&M University, College Station, Texas, but formerly from Agri Systems Support Branch, Alberta Agriculture, Food, and Rural Development, Edmonton, Alberta opened the session with a discussion about the epidemiology and control of Johne's disease, using Alberta's Johne's disease control program as an example. He was followed by epidemiologist **Dr. John Berezowski** from Agri Systems Support Branch, Alberta Agriculture, Food, and Rural Development, Edmonton, Alberta who, having extensive clinical practice experience with commercial bison herds, discussed the clinical expression of MAP in domestic bison herds and the interpretation of Johne's test results in these animals.

MANAGEMENT OF MAP IN CAPTIVE AND FREE RANGING BISON

Phillip Merchant, a wildlife technician with the Fish and Wildlife Branch, Department of Environment, Government of Yukon in Whitehorse, Yukon Territory shared his experiences with Johne's disease at the Yukon Wildlife Preserve. Clinical Johne's disease is recognized and considered endemic in several species at this multi-species game farm. A disease management plan with the goal of creating a MAP-free population and environment at the preserve is being followed. **Dr. Murray Woodbury**, Research Chair in Specialized Livestock Health and Production at the Western College of Veterinary Medicine in Saskatoon, gave information about how Johne's infection might be handled in captive bison and discussed some alternatives for wild populations. Because of the nature of bison and bison populations the removal of MAP from wild populations could be very difficult. **Dr. Gary Wobeser**, a wildlife pathologist at the Western College of Veterinary Medicine in Saskatoon, SK and representing the Canadian Cooperative Wildlife Health Centre, gave his views on the significance of MAP in free ranging wildlife and outlined the implications of wildlife infections. The workshop information sessions were finalized with a discussion of policy and management implications of MAP for the conservation of free-ranging bison populations by **Dr. Brett Elkin**, and **John Nishi**. It was their belief that, should the identification of this strain of MAP prove to signal clinical infection of these bison herds with Johne's disease, the long term management of wildlife populations and the recovery efforts for Wood bison will be adversely affected.

INFORMATION GAPS AND RESEARCH NEEDS

Led by facilitator **Dr. Helen Schwantje**, there was an open discussion of the potential problems posed by the finding of MAP DNA in northern bison and a consensus that the problem needs to be studied more intensively. It was agreed that the Wood Bison Recovery Team and other organizations interested in bison need to further discuss the issue and to participate in planned and coordinated research activities.

THE ISSUE AT HAND

A number of geographically distinct, captive, semi-captive and free ranging wood and plains bison in northern Canada have molecular (DNA) evidence of *M. avium paratuberculosis* (MAP) from polymerase chain reaction (PCR) tests of ground-collected fecal samples. However, at this time, despite multiple attempts, no living mycobacterial organism has been isolated through culture of these samples. Preliminary molecular typing work suggests that the PCR is detecting a MAP that has a molecular sequence (IS1311) that has not previously been described. IS1311 typing is quite new and very few IS1311 sequences have been examined, so it is not known how common or unique this particular pattern is among MAP or how this pattern relates to other characterized typing schemes. The evidence indicates that while the identity of the organism is not known, it is different from the Johne's organism known to cause disease in cattle and other species.

No clinical disease, manifested as weight loss or diarrhea, has been observed in any of the herds tested. Two captive/semi-captive herds, the Hook Lake recovery project herd and the Elk Island National Park herd, are observed regularly and their health status is monitored intensively, but this type of clinical disease monitoring is not possible in free-ranging herds. The Hook Lake bison are kept in a small enclosure at a density higher than many typical livestock management situations. These circumstances would be expected to create a high risk of disease if the Johne's organism (MAP) was present. However, no clinical signs of disease have been observed during the eight years the herd has been in existence. Elk Island National Park (EINP) holds plains and wood bison in separate semi-captive naturalistic paddocks. The bison have not shown signs of Johne's and neither have sympatric white-tailed deer, elk, or moose at EINP. These ungulates have coexisted with wood bison at densities of 30-40 ungulates per square mile for decades. All species are frequently observed by park staff and visitors, and large numbers of elk and bison are handled annually at the park, again without finding any indication of symptoms typical of Johne's disease.

Canadian and American wildlife managers are considering proposals to translocate a subset of bison from these herds to supplement existing bison herds and restore wood bison to other parts of Canada and the United States (Alaska) within their natural range. Managers need to use the best available information to understand the potential health risks to receiving herds, other sympatric wildlife, and domestic livestock before translocations proceed. This workshop was designed to provide a venue to discuss the current knowledge of Johne's disease in bison and to provide an opportunity to exchange information about the newly recognized organism

PROCEEDINGS AND WORKSHOP REPORT

Feb 18, 2005

Workshop Goals and Objectives

Facilitator: Helen Schwantje, Chair, Bison Health Subcommittee, National Wood Bison Recovery Team, and Wildlife Veterinarian, Biodiversity Branch, Ministry of Environment, Victoria, BC.

Dr. Schwantje led a discussion about the material to be shared by workshop participants and presented them with the following goals and objectives for the workshop:

- Discuss the current state of knowledge on the distribution, effects and significance of MAP in bison
- Provide recommendations on herd monitoring and testing protocols for bison
- Identify knowledge gaps and set priorities for research and management actions
- Produce a summary document highlighting the meeting discussions

DISTRIBUTION OF MAP IN FREE RANGING BISON

Current Status of MAP Infection in Canadian Bison Populations

Brett Elkin, Wildlife Disease Specialist, Wildlife Division, Government of NWT Environment & Natural Resources, Yellowknife, NWT.
John Nishi, Bison Ecologist, Natural Resources, Wildlife and Economic Development Government of NWT, Fort Smith, NWT.
Greg Appleyard, Microbiologist, Dept. of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK.

Dr. Elkin revealed that there have not been any documented clinical cases, although evidence of MAP organisms (DNA) has been found in free-ranging bison. The Hook Lake captive Wood bison herd is now considered free of brucellosis and tuberculosis. (Editors note: It has been discovered subsequent to this meeting that the Hook Lake bison herd is now considered *M. bovis* postive). In order to use this captive herd for conservation reintroductions, it was necessary to look at what other pathogens they might have, and fecal testing produced genetic evidence of MAP. The point was made that the origin of the infection is uncertain, but these results were also found in the free-ranging herd of origin, in the Slave River Lowlands. The NWT team continues to look for clinical cases, and positive serology, fecal culture or PCR tests. All positives to date are from PCR testing and limited serology and the organism has not been cultured. Positive

fecal PCR tests have been obtained in Slave River Lowlands (Hook Lake and Grande Detour), Wood Buffalo National Park (WBNP), Mackenzie Bison Sanctuary, where the largest free-ranging tuberculosis and brucellosis-free herd exists, EINP, (in both Plains and Wood bison) and Hook Lake Wood Bison Recovery Project . We need to look at the details of translocations, positive samples and the associated strains found to detect where this MAP might have come from. Cattle had been grazed for 30 years in WBNP, and on mission farms where wild bison range. There was contact between bison and cattle across fences in EINP, especially on the north side. Animals from WBNP were used to establish the Mackenzie and Elk Island National park herds in 1963 and 1965 respectively.

Dr. Greg Appleyard spoke about the work of graduate student Jennifer Sibley who used a nested PCR technique with no enrichment directly from bison feces, to find the MAP DNA in samples related to her thesis. Her isolations were confirmed with BACTEC culture at the BC provincial laboratory in Abbotsford. PCR of IS900 and locus 251 were used and Jennifer found no positives unless a nested PCR is done. The DNA isolates were not positive on culture. There are various potential causes of this such as age of samples, or samples having undergone multiple freeze/thaw cycles. Two strains were found from northern samples; one corresponds to sheep and one to cattle. There is a variable region (IS1311) in MAP which was different in each strain tested by Jennifer, (eg. US bison, northern Wood bison, cattle, sheep, etc). Sampling from the Grand Detour site (in WBNP) yielded more PCR fecal positives than any other herd of bison sampled. Further work is required to understand the biological significance of this finding.

Fecal inhibitors were not considered to be a problem in the lab technique used. Real time PCR has not been tried. Real time PCR would increase sensitivity drastically because of an additional probe and fewer inhibitors; compounds in feces like phytoenzymes, heparin, etc, which will inhibit the PCR process. Dr. Appleyard was not sure how real time improvements compare to improvements seen with nested PCR.

A copy of the PowerPoint presentation used by Dr Elkin is included in **Appendix VII**.

Current Status of MAP Infection in US Bison Populations
Becky Manning, Senior Scientist, Johne's Testing Center, School of Veterinary Medicine,
University of Wisconsin, Madison, WI.

Dr. Manning was unavailable due to illness but sent along her PowerPoint presentation so that participants might follow her comments which were provided through email and telephone connections. A copy of the PowerPoint presentation used by Dr. Manning is included in **Appendix VII**.

Current Status of MAP infection in Wildlife in US National Parks
Jenny Powers, Wildlife Veterinarian, U.S. National Park Service, Fort Collins, CO.

Dr. Powers described the situation in the Tule elk herd at Point Reyes, California, where the National Park Service MAP research has focussed. As a result, Johne's disease is largely unmanaged in these wild elk. Tule elk were restored to the area in 1968. They are native and considered of special concern. The Park is fenced but the land was previously used for livestock grazing by neighbouring dairy farms. Elk showing clinical diarrhea and weight loss are seen in this herd. There is high prevalence and eradication of the disease is not considered feasible. The herd is growing, and there are no predators. She spoke about the National Park Service policy on the occurrence of disease. "Natural" processes are favoured, with intervention only if there are unacceptable impacts on species of special concern, or risk to the public is associated with the organism. Native species, including pathogens, are generally left as is, but "exotic organisms or species", especially if human activity caused the problem are managed if feasible and necessary. "Native" pathogens can also be managed for various philosophical reasons, including if public health is at risk or there is a transmission risk to species of concern. Testing for the presence of MAP in the Parks Service is currently through fecal culture and testing using PCR techniques is not done. Native wildlife such as elk, bison, deer, bighorn sheep, and mountain goats are tested in various parks. Rocky Mountain goats adjacent to Rocky Mountain National Park are culture positive, but in the parks are culture negative. There is concern regarding livestock bordering many parks such as domestic goats, cattle, and llamas.

MAP has been cultured from some carnivore species. One opinion is that the probability of transmission across fences is low and direct fecal contact (ingested) is needed to cause infection, especially in young animals. Dr. Powers wondered "What is the significance of MAP in wildlife? Why are we worrying about this? She suggested that there may be no significance in free-ranging situations, which are very different from feedlot or commercial farming situations. It must be made clear that we may have the Johne's disease-causing organism in free-ranging bison but we do not yet know if we have the disease. We need to consider the significance now because of management initiatives to reintroduce animals. In some populations the prevalence of Johne's disease might be high, but incidence could be very low. This is an important distinction in this disease.

DIAGNOSTICS

MAP Diagnostics: Current Approaches and Challenges

Becky Manning, Senior Scientist, Johne's Testing Center, School of Veterinary Medicine,
University of Wisconsin, Madison, WI.

Dr. Manning was unavailable due to illness but sent along her PowerPoint presentation so participants could follow her comments which were provided through email and

telephone connections. A copy of the PowerPoint presentation used by Dr. Manning is included in **Appendix VII**.

***Mycobacterium avium* spp. *paratuberculosis*: Surveillance strategies for bison conservation herds in Canada – some considerations**

John Nishi, Bison Ecologist, Resources, Wildlife and Economic Development Government of NWT, Fort Smith, NWT.

There are many things to consider in designing disease surveillance programs, and there are many references and resources out there to consult. In this presentation, we briefly outline some of the basic issues that need to be considered in order to develop useful surveillance strategies for MAP in bison herds. Our approach in this presentation is to ask the relevant questions where they apply to disease surveillance.

There are three important questions to address prior to designing and implementing a surveillance strategy for MAP:

- What is feasible?
- What are the sampling objectives?
- How many do I need to sample?

Feasibility of a surveillance program will be tied to whether the population you intend to sample is captive, semi-captive, or free-ranging. The reason is that there are direct implications for the types of samples you will be able to collect. For example, in a captive or semi-captive herd with access to a handling facility, you would be able to repeatedly sample live animals.

It is also important to recognize the distinction between anti- and post-mortem examination of animals, because it has direct bearing to the type of field work required, the samples you could collect, and the diagnostic tests you could conduct. For example, anti-mortem inspection of animals would allow you to conduct a Johnin skin test (although it is unvalidated in bison), and run diagnostic tests on whole blood, sera, and fecals. Post-mortem examination of bison carcasses through field collections or opportunistic sampling of hunter-kills facilitates internal examination of the carcass, as well as diagnostic testing of the blood, sera, fecals, and tissues (i.e., distal ileum, and ileal-cecal lymph node).

It is also important to recognize that bias will affect surveillance strategies of free-ranging wildlife depending on the how the samples are obtained. On the one hand, if you bias your sampling to individual animals that show classic symptoms of disease – such as emaciation through chronic wasting and lethargy – you would have a smaller sample size but would have a higher likelihood of detecting the disease organism. On the other hand, if you were to sample the wild population at random (therefore unbiased), you would need to sample many more animals, and would have a lower probability of detecting the disease.

When we consider the sampling objectives of a surveillance program, we need to recognize that the question of “Is the disease present” is very different from the question of “How much disease is present?”

The first question focuses our sampling efforts at the population or herd level. For example, if we are collecting fecal samples from a free-ranging bison herd, we may need to consider the benefits of pooling fecal samples. The second question focuses our sampling efforts at being able to sample individual animals, and therefore independent samples of individuals are required.

The question of “How many to sample?” from a population, should be firmly based on sampling theory and statistical power. For example, the classic sampling power curve from Cannon and Roe shows that in order to be 95% confident that you would have achieved a minimum detectable prevalence of 10% in a population of 2000 animals, you would have to randomly sample at least 33 individuals. However, having said this, it is as important to recognize that no diagnostic tests are 100% accurate. Consequently, the sensitivity and specificity of diagnostics tests are almost always less than 1.

Another important complication to this story, especially when it comes to using fecals samples for MAP is the fact that Johne’s disease can present in three different ways in infected animals:

- No clinical disease (non-shedder)
- Clinically diseased (non-shedder)
- Clinically diseased (shedder).

What this means is that sensitivity and specificity of the fecal test for individual, animal will be further affected by the stage (or class) of infection in the animal.

Therefore, it becomes difficult to interpret true within-herd prevalence, because we usually assume that infected animals are uniformly distributed in the herd. The stage of infection can skew this distribution, and make it very difficult to estimate true prevalence. The main consideration is that large sample sizes are needed to confidently detect MAP in wild populations.

We suggest that further work will be required to improve our understanding of MAP in conservation herds of bison.

- We need to determine which herds are priorities for sampling. This may be as straight forward as ranking according to whether we feel the data at a herd level are sufficient for now, limited, or completely absent.
- We need to recognize that at the herd-level, the emphasis should be on determining the presence or absence of MAP. At the animal-level, we need to better understand the implications of basic test performance, combined with stage of infection, as these two factors interact to affect our ability to determine prevalence.

- Given the difficulties of poor test performance, it is very important to define absence or freedom from disease at both the herd level and animal level. For example, how confident would we be based on 25, test-negative, fecal samples collected from a herd of 400? How confident are we that an individual animal is not diseased, if we collect one fecal sample from it and it is test-negative? It is likely, that we will need to consider these definitions within a risk-based framework because there will be no black and white answers.

A copy of the PowerPoint presentation used by John Nishi is included in **Appendix VII.**

Clinical Johne's Disease and Diagnosis in Bison
John Berezowski, Epidemiologist, Agri Systems Support Branch, Alberta Agriculture, Food, and Rural Development, Edmonton, Alberta

Effects of Johne's disease on reproduction have been documented in cattle by Dr. John VanLeuwen at Atlantic Veterinary College and others. The accuracy of serological (ELISA) testing varies with the herd prevalence. If clinical disease has not been seen, care must be taken with the interpretation of ELISA test results. ELISA accuracy has been evaluated with cattle, but not bison. Prevalence and where the herd is on the epidemic curve, both affect the accuracy of the test. Because MAP is an intracellular infection, antibody production develops later in the course of disease. The most sensitive way to test individuals appears to be culture and PCR of tissue samples. There is some work in bison to show this is the most sensitive testing method — although we don't know how specific (false positives) this would be in bison.

Dr. Berezowski discussed various Johne's tests and diagnostic approaches used. Serological tests such as ELISA's are used in cattle, sheep and elk. There are 2 varieties of serologic tests, those that measure cell mediated immunity (CMI) and those that measure humoral immunity (antibody detection—IDEXX or Biocore). A gamma interferon test measuring CMI is used by the US Department of Agriculture (Bovigam). Antibody tests use 2 types of *M. avium* antigen; *M. avium* subsp *avium* or subsp *paratuberculosis*. There are 2 commercial tests: 1) an IgA based test (Australia) and 2) the protein G conjugate (not just IgG) based test which is more specific. Many ruminants will show a cross reaction with tuberculosis positive samples. The specificity is increased because some cross reacting species are removed in process.

A comparison was made between IDEXX and Biocore tests. IDEXX has been available for 10 yrs. It was originally used for herd testing, not for the diagnostic testing of single animals. It is important to note that ELISA tests can miss positives because there is no antibody production in the early stages of infection. ELISA results are a colorimetric reaction when antibody and antigen bind and these are compared to a positive control. A question arises about the suitability of bovine positive controls when compared to other species positives, i.e. bison. It may not be significant. It is unknown if different species' (bison, elk, bovine) antibodies bind to the same prepared antigen, but probably

not. This may present a problem when using cattle ELISA test kits on wildlife samples. Reference was made to Becky Manning who is purported to be working on a bison specific ELISA. If one is testing bison and can do serology, Becky's test may be superior. On the other hand, gamma interferon testing might be better if prompt access to a lab is available (samples must be analysed within 12 hours of sampling). Gamma interferon should provide positive results earlier in the course of disease than ELISA testing, but it is a non-specific test and should not be used as a screening tool. Perhaps the two can be combined; the gamma interferon first, followed by ELISA antibody testing. These applications are likely not feasible in free-ranging herd situations.

Do we need to be able to actually culture this organism in bison to say that it is there? We can get positive results on PCR based tests with negative cultures but at this time we are not sure what this means. We really need some information on the actual presence of disease in the herd to help interpret the accuracy of the results. For wildlife it is very hard to know if there are any true negatives so it is difficult to find a 'baseline'. Should culture be the standard for wildlife species? For various reasons we may not be able to culture organisms from all species. A culture positive is very likely positive but negative cultures may not mean negative results (high specificity and low sensitivity). For free-ranging wildlife, we are at point where we know an organism is there but don't know if the disease is there. If some free ranging wildlife are infected with an organism but never develop disease, the condition only becomes a problem if they are put in farmed or other confinement situations where management conditions may lead to higher infection levels and conditions more likely to produce disease. So, are we looking for freedom from infection or freedom from disease? We might be able to say they are free from disease because we will not put them in situations where disease will develop.

Are we willing to accept some positive (infected) animals? This may be easier to manage. Why have we never cultured the organism from these free-ranging herds? We have not looked before or could there be another *M. avium* subspecies that we have not yet identified and are not yet able to grow. We know that bison can show clinical symptoms of Johne's disease since clinically sick bison have been seen in farmed bison. We don't know why wild bison aren't showing clinical signs. One possibility is that there are different strains of MAP infecting each bison population. The difference in strains identified in the northern bison so far is a genetic difference in 3 base pairs, but mycobacterial species are so homogenous, we may not need much difference for it to be significant. So if wild bison have a non-pathogenic strain infecting their gastrointestinal tracts what are the effects of the genetics of the organism, the genetics of host and the effects of the environment? Elk Island National Park and Hook Lake Recovery Project have not seen clinical cases despite high concentrations of animals and older animal classes.

CLINICAL EFFECTS AND SIGNIFICANCE OF MAP IN BISON

Prevalence and Ecological Risk of MAP in Bison

Morgan Scott, Epidemiologist, Texas A&M, College Station, Texas, formerly from Agri Systems Support Branch, Alberta Agriculture, Food, and Rural Development, Edmonton, Alberta.

Dr. Scott began the presentation by discussing the seroprevalence of Johne's in dairy and beef cattle in various regions of Alberta. The seroprevalence appears to be tied to environmental variations affecting the survival of MAP and the type of herd operation. Testing showed that beef and dairy have similar prevalence. There is the possibility that the test behaves differently in different production systems. Pooling of fecal samples (ranked by age) gave a measure of agreement (Kappa) between ELISA and fecal culture tests that is poor for dairy testing but good for beef. Risk factor studies for Johne's disease in beef cattle suggested that open water sources lower the risk of being serologically positive in heifers but researchers are not sure why. Purchases of animals into the herd had little effect, possibly because the overall prevalence was so low in beef herds. Johne's disease may manifest differently between beef and dairy. The average age of dairy animals is much lower than beef (4yrs for dairy; 6 yrs for beef). In the US, one lactation may be the average in dairy production because of intense culling. These are demographic differences. Some material in dairy manure in animals fed silage may inhibit shedding or survival of bacteria or bacteria's ability to be found by tests. Acidified feed (such as silage) reduced bacterial growth (i.e. *E. coli)* in the intestinal tract. The low recovery of MAP from infected animals might not be just a function of testing. Big cattle herds have opted to live with Johne's. In cattle, there is some discussion that pastures are so heavily contaminated with MAP, that some positives are from bacteria passing through the gut, showing positive on direct PCR testing (animal is not actually infected, test found organisms eaten and moving through gut). They do get positive cultures on seronegative animals but not a huge number.

MANAGEMENT OF MAP IN CAPTIVE AND FREE RANGING BISON

Management of MAP in Captive Bison Herds

Murray Woodbury, Research Chair, Specialized Livestock Health and Production, Western College of Veterinary Medicine, Saskatoon, Saskatchewan.

Management controls that appear to be the most effective for control of MAP should interfere with fecal-oral transmission, especially in young animals. The infective dose through ingestion must be high. There may be some *in utero* transmission. Researchers are not sure if reservoirs such as rabbits, ground squirrels, and foxes are mechanical, dead end hosts or actually are infected and can transmit the bacteria. The organism persists in the environment, but we are not sure of the degree of infectivity (risk) of a contaminated environment over time. Some control options in captivity are to cull adult

animals and bottle-raise their young, fallow the environment, or test and repopulate with the clean bottle raised offspring. Environmental disinfection with phenolic compounds is best, as chlorinated products may not work as well in these situations. It is unknown whether stress can induce the manifestation of disease in bison. Vaccination against Johne's disease has been suggested for captive herds to reduce the incidence. This may help to reduce the severity of clinical symptoms in cattle, but does interfere with testing for tuberculosis, and there is no evidence that it is effective in bison.

The presence of an organism in bison in the Hook Lake Wood Bison Recovery project provides some evidence that orphaning neonatal calves (up to 2 weeks of age) may not result in animals free of all disease. However, dairy cow colostrum was used as feed for these calves, and calves can be infected in utero by MAP. Testing for all mycobacterial infections can be very variable in non-domestic species and this is probably so in MAP testing also.

There has been much research on the survivability of the Johne's disease organism in the environment, some from 1950's, and some more recent. Australians depopulated flocks of sheep and measured how long the organism persisted in the environment of these farms. It did eventually disappear, but because it is hard to culture, even when environment reads "negative", it may have fallen below the threshold to detect it, but could still be present and infective. Slightly different results occur in different environments, in terms of organism survivability. Organisms may disappear faster under environmental conditions than in lab conditions. UV and desiccation will have a large impact on survivability of the organism.

A copy of the PowerPoint presentation used by Dr Woodbury is included in **Appendix VII.**

Dealing with Johne's Disease at the Yukon Wildlife Preserve.
Phillip Merchant, Wildlife Technician, Fish and Wildlife Branch Laboratory, Department of Environment, Government of Yukon, Whitehorse, Yukon.

Johne's disease is endemic in some species on the Yukon Wildlife Preserve. Phil Merchant discussed the husbandry practices of the facility which may have led to the high prevalence on the farm. There was some sharing of feeding/housing and handling facilities, domestic livestock milk was used to feed hand reared animals and pastures were "swapped" between species. However, there have been no bison positive for Johne's disease yet, despite testing. Some species have showed clinical Johne's disease (Dall's sheep, caribou, muskox). There were no clinical signs in test positive elk or moose calves. The interpretation of test results has been difficult. There are some positives from DNA testing; and there have been culture positive feces and tissues. Some culture positives have tested negative on DNA tests. For example, a wild, orphaned moose calf tested positive soon after arrival, but was not mixed with other animals or placed on "contaminated" pastures. HealthGene in Toronto did the DNA

testing. HealthGene says their test has a high (99%) sensitivity, but has not provided details about how effective their test is in all animal species or if there are publications to support this. All animals have not been tested for tuberculosis. Only animals/species that have been sold and shipped have been TB tested, such as Rocky Mountain goats, thinhorn sheep, and bison. The purpose of the once privately owned facility was game farm/wildlife viewing but it is now under public ownership and will be used as a public educational facility. It is hoped that the future role will include research/conservation projects as well as pursue interests in rehabilitation of ungulates, raptors, and other species. Why are they concerned about having Johne's disease? The Preserve management board wants to build animal numbers to increase the quality of the wildlife viewing experience. Obviously, this will result in a surplus of animals, and Johne's disease makes it difficult to deal with the sale of surplus animals. There are clinical signs in some species that cause public concern about disease spread from the facility either via escapes (there have been escapes in the past) and the fact that free-ranging bison, sheep, elk, and deer can range very close by. In addition, there is concern about scavengers and runoff water etc. spreading infection.

Significance of MAP in Free Ranging Wildlife

Gary Wobeser, Canadian Cooperative Wildlife Health Centre and Wildlife Pathologist, Dept of Veterinary Pathology, Western College of Veterinary Medicine, Saskatoon, SK

Dr. Wobeser declined to give a formal presentation but had some information and opinions to share with participants. He warned not to expect this disease to look the same in all species. There could be a variety of expression, all the way from subclinical to very clinical disease. Elk are killed quickly, and wild sheep may not show obvious gross pathology even after clinical signs have appeared. Often, when you think you have a multi-host pathogen, it turns out to be a group of related strains, for example with the morbilliviruses. The distinction between infection vs. disease is really important. The broader version of that idea is the PCR positives vs. subclinical infections vs. clinical infections. Dr. Wobeser noted that there is almost never more than one test applied to the same animal. This was done in a study of Saskatchewan farmed deer, with poor agreement between the tests. This has also been done in Yellowstone National Park on some animals.

***Mycobacterium avium* spp. *paratuberculosis*: Implications for bison conservation and wildlife management**

John Nishi, Bison Ecologist, Resources, Wildlife and Economic Development Government of NWT, Fort Smith, NWT.

As described in a previous presentation, we first became aware of the presence of a MAP organism in the Hook Lake Wood Bison Recovery Project (HLWBRP). Since we had not seen any clinical signs of Johne's disease in the HLWBRP herd and the MAP organism could not be cultured, we did not know how to interpret these initial results.

Our first efforts were directed at sampling the free-ranging wood bison in the Slave River Lowlands because this was the source population from which we captured the founder animals. We also initiated sampling of other herds including the Mackenzie, WBNP, and Nahanni herds. Preliminary PCR results showed that a MAP organism was detected on a proportion of fecal samples collected from these wild herds as well.

Two questions came up:

1) What do these results (PCR +ve fecal samples) mean?
2) Is MAP an emerging infectious disease in northern bison (and other wildlife) or is it widespread & only just recently recognized?

These are our perspectives on these questions according to three general themes:

- the implications for moving individual bison through translocations;
- the potential significance of MAP at the interface between livestock and bison;
- the implications of legislation and policy on Johne's disease in the cattle industry, and what this might mean to management of bison.

From a genetic conservation perspective, there is increasing recognition that managing gene flow between isolated populations may be a useful long-term management strategy. In fact, the importance of genetic augmentation has been identified in the National Wood Bison Recovery Plan, as well as wood bison management plans for British Columbia and the Yukon. What this means is that animals may be moved between herds to achieve genetic management objectives.

Therefore, an important implication of MAP in a bison herd is that the risk (i.e., Johne's disease) of moving individual animals from one herd to another may exceed the potential benefit (i.e., genetic augmentation) of conducting the translocation. In addition to better understanding the biological and epidemiological significance of MAP as it relates to Johne's disease, we think that a risk assessment approach will be required to assist in making these decisions.

A risk assessment approach could be used to develop a methodology that would be implemented to establish non-infected animals from an infected herd. It will also clarify our definition of a "non-infected" population/herd of animals, and require the definition to be based on a quantitative and empirical treatment of surveillance data.

A risk assessment approach can also be used to help make decisions on translocations. For example, if we have a source population that is considered infected with MAP (based on PCR +ve fecal samples), it will be important to determine the status of the potential recipient herd. If both the source and recipient herd are considered as infected with the same MAP organism, then the potential benefit of genetic augmentation through translocation probably exceeds the disease risk. Conversely, if the source herd is considered infected and the potential recipient herd is considered non-infected (based on rigorous sampling), than the risk of introducing the MAP organism may exceed the

benefit of genetic augmentation. This example can be further expanded to consider the MAP infection status of sympatric wildlife species. This approach requires a clear and empirical basis for determining the MAP infection status of a population/herd of interest. It also highlights the importance of collecting sufficient data on potential recipient herds, and sympatric wildlife.

There are serious concerns within the livestock industry that Johne's is a production limiting disease that may warrant mandatory and regulatory-based management policies. Currently, management policies for Johne's disease in commercial livestock within Canada and the United States are on a voluntary basis. However, if Johne's disease were to become a reportable disease in the United States, there would likely be strong implications to US trading partners, and a push for Canada to follow suit. Arguably, one of the main reasons for the push to list Johne's as a reportable disease is the potential - although as yet unsubstantiated - link to Crohn's disease in humans. If this link to human health were to be well established and grounded in solid evidence, it is likely that Johne's would be listed as a reportable disease. The potential role of wildlife populations to act as a reservoir for Johne's disease (or a MAP organism) is unknown, but given the potential implications to livestock and human health, there may be substantial pressure to change management objectives for wildlife populations because of the potential for spill-over and spill-back of this disease organism. As wildlife managers, we do not well understand the significance of the MAP organism to free-ranging bison populations.

We think that Johne's disease would present serious implications for management and conservation of free-ranging wood bison in Canada. However, since we have not observed clinical Johne's disease in the free-ranging herds, our current problem is not entirely clear-cut because we have only been able to detect the presence of the DNA of MAP through fecal PCR. There has not been a clear link made between the presence of the MAP organism in these bison herds and Johne's disease. Consequently, the implications for translocations and reintroductions of wood bison will depend on our ability to define the biological significance of the test positive results (this will likely include improving our diagnostic tests), and to collect good data on other herds (& sympatric wildlife species) where reintroductions or augmentations are being considered. We need to be able to address the issues of:

- the risk of introducing MAP in to a "clean" herd or area;
- whether disease management will be required to reduce risks, and
- whether the occurrence of MAP in northern bison will be an issue if all herds are found to be infected.

Management of MAP in captive bison herds suggests the need for prevention, control, or elimination of the disease organism. We need to determine whether control or elimination of herd-level MAP infection is required, because there will likely be implications to genetic management. Based on work in the cattle industry, it is likely that control or elimination of MAP in captive bison will be difficult and take much time and money.

We suggest that there are at least five key information gaps that we face with respect to MAP in bison.

- There is a need to determine the MAP infection status of all captive and free-ranging bison populations. We need to be able to better define the geographic distribution of this disease organism in bison, as well as the prevalence rates within populations.
- The strain of MAP that has been found in wood bison herds needs to be isolated, cultured, and identified.
- The biological significance of MAP to the health of captive and free-ranging bison populations needs to be understood.
- There is a need to determine whether the strain of MAP found in bison, is found and can cause disease in other sympatric species of wildlife.
- We need to understand whether voluntary Johne's disease programs may have implications to bison management. Can the strain of MAP found in bison cause clinical disease in cattle?

We outline a few succinct recommendations that should be considered for further discussion.

- Agencies currently managing either free-ranging or captive wood bison herds should implement sampling programs and determine whether MAP is present in their populations. These sampling programs should be standardized (comparable) across jurisdictions and be based on appropriate sampling intensity.
- Dedicated efforts and resources should be found to culture the strain of MAP found in the northern bison herds. This would lead to timely and useful research on strain typing of this organism.
- Agencies should develop and use a risk assessment / management strategy to facilitate informed and transparent decision making as it applies to MAP in bison. We need to understand and determine whether and how the presence of MAP in these bison herds is a problem that requires management.

MEETING OUTCOMES

Information Gaps and Research Needs
Recommendations and Development of a Meeting Report
Facilitator: Helen Schwantje, Chair, Bison Health Subcommittee, National Wood Bison Recovery Team, and Wildlife Veterinarian, Biodiversity Branch, Ministry of Environment, Victoria, BC.

Outcome 1 - Identification knowledge gaps and set priorities for research and management action

- Encourage research relating to the different strains of North American MAP (share and compare molecular approaches) and their ecology in wildlife populations.
- Determine through the application of a variety of well-characterized typing techniques if the MAP identified by Greg Appleyard's lab is a unique strain.
- Can the strain of MAP identified in wild bison in Canada be isolated through culture and is it capable of causing clinical Johne's disease and if so, in what species.
- Continue to culture the organism containing the distinctive IS1311 sequence.
- Gain a clear understanding of the health impacts of MAP on bison herds, and determine the implications for translocations.

Outcome 2 - Recommendations on herd monitoring and testing protocols

- Improve monitoring efforts for MAP infection in bison using multiple diagnostic tests (histopathology, serology, PCR, culture).
- Build an accessible North American free-ranging species biological sample (tissue, serum, fecal) repository to enable research scientists to investigate the presence and distribution of MAP strains and their potential health impacts in wild populations.
- Document exposure (or lack thereof) to MAP and other possibly pathogenic organisms in resident wildlife before restoration with alternate, potentially MAP susceptible, wildlife to the same habitat.
- Understand the strengths and limitations of diagnostic tests before using them for MAP surveillance in a herd. This will clarify how the information can best be used in wildlife management decisions.
- Coordinate MAP programs using an epidemiologically sound sampling program to understand the scope of this issue in free-ranging and captive bison herds across North America.
- Evaluate the MAP status of sympatric wildlife and possible reservoir hosts as a key component to defining the distribution of MAP in the environment.
- Where there are significant livestock/wildlife interactions (especially when either species are at high densities) implement risk management practices and monitor for clinical as well as sub-clinical Johne's disease.

- Wildlife managers should be attentive to government agricultural policy regarding MAP control. While wildlife translocation and management practices may not currently be restricted by the presence of MAP organisms in wild populations, this may become a future issue.
- Until the significance of finding MAP DNA in wildlife is made clear, wildlife managers should coordinate and communicate with appropriate agencies prior to restoration programs and create sensitivity to the possible existence of MAP organisms in animals used in restoration projects.

Appendix I

Background Information on MAP in Bison

Adapted from Sibley, Jennifer A. Molecular tools for the characterization of Mycobacterium avium subspecies paratuberculosis. 2005. MSc Thesis. University of Saskatchewan, Saskatoon, Canada. Copyright by University of Saskatchewan 2005, used with permission.

Mycobacterium avium-intracellulare complex

Taxonomically, mycobacteria belong to a single genus *Mycobacterium*, within the family Mycobacteriaceae and the order Actinomycetales. The order Actinomycetales includes a large and diverse group of micro-organisms, but Mycobacteria are easily distinguished by their ability to synthesize mycolic acids found within their cell wall. Mycobacteria are bacterially defined as aerobic, acid-fast, rod-shaped, facultative intracellular organisms that are non-motile (Rastogi et al., 2001). Many *Mycobacterium* species, such as *Mycobacterium avium*, are pathogenic in both human and animal populations. *Mycobacterium avium* and a closely related species, *Mycobacterium intracellulare,* belong to a group of bacteria classified as the *Mycobacterium avium-intracellulare* complex (MAC). MAC is a large cluster of genotypically and phenotypically related organisms which can be divided into 28 serovars. Serovars 1-6, 8-11, 21 and 28 are considered to belong to the species *M*. *avium*, while serovars 7, 12-20 and 25 are assigned to *M*. *intracellulare* (Saito et al., 1990; Wayne et al., 1993). Based on biochemical and DNA analysis, *M*. *avium* can be further subdivided into 3 subspecies corresponding to pathogenicity and host-range characteristics: *Mycobacterium avium* subspecies *avium*, *Mycobacterium avium* subspecies *silvaticum* and *Mycobacterium avium* subspecies *paratuberculosis* (Thorel, 1989)

MAC Diseases

Mycobacterium avium subspecies *avium* (*M. avium*) is ubiquitous and the causative agent of avian tuberculosis. *M. avium* infections are found in all mammals, including humans, but disease is sporadic and rarely transmissible (Thorel et al., 2001). In humans, *M. avium* can cause pulmonary disease in adults (Prince et al., 1989), submandibular adenopathies in children (Danielides et at., 2002), and disseminated infections in the immuno-compromised (Liao et.al, 2004). *Mycobacterium avium* subspecies *silvaticum* (*M. silvaticum*) isolates are obligate pathogens of animals causing tuberculosis in birds (Collins et al., 1985) and a paratuberculosis-like disease in mammals (Matthews and McDiarmid, 1979). The inability to grow on egg-based culture medium is characteristic of this subspecies (Thorel et al., 1990). *Mycobacterium avium* subspecies *paratuberculosis* (*M. paratuberculosis*) causes granulomatous enteritis in ruminants, commonly referred to as Johne's disease. The requirement of mycobactin for growth is a characteristic of this subspecies (Thorel et al., 1990).

MAP virulence, hosts and susceptibility

Mycobacteria are known to be extremely resistant to both physical and chemical damage due to the high impermeability of the mycobacterial cell wall (Russekk, 1996). This permits *M. paratuberculosis* to survive in the environment for long periods of time, which is an important factor in Johne's disease transmission. *M. paratuberculosis* can survive for up to 1 year in feces and soil (Lovell et al., 1944) and from 9 to 17 months in water, depending on temperature and pH (Larsen et al., 1956; Lovell et al., 1944). Heat tolerance is another virulence factor that may be important in the transmission of *M. paratuberculosis* to humans. Heat treatment at 63C for 30 minutes will kill 100% of *Mycobacterium bovis* isolates (Chiodini and Hermon-Taylor, 1993). Under the same conditions, 5 – 9% of *M. paratuberculosis* isolates will survive and as a consequence, may be resistant to commercial pasteurisation conditions (Chiodini and Hermon-Taylor, 1993). *M. paratuberculosis* has a higher heat tolerance than other milk-borne pathogens, such as *Listeria monocyotgenes* and *Coxiella* species (Sung and Collins, 1998). This knowledge has recently stimulated an increase in research into the survival of *M. paratuberculosis* in milk products. *M. paratuberculosis* bacteria can be isolated from raw milk (138) from both clinically (Taylor et al., 1981) and subclinically infected cattle (Streeter et al., 1995; Sweeney et al., 1992). *M. paratuberculosis* DNA can be found in retail pasteurized milk (Merkal, 1973).

M. paratuberculosis infects ruminant livestock animals, such as cattle, sheep and goats. *M. paratuberculosis* has also been cultured from a number of wild ruminant (Chiodini and Van Kruiningen, 1983; De Lisle et al, 1993; Reddacliff and Whittington, 2003) and non-ruminant species (Beard et al., 2001a; Beard et al, 2001b; Daniels et al., 2001; Daniels et al., 2003; Greig et al., 1999) such as foxes, wood mice, brown hares and crows. Viable *M. paratuberculosis* has been found in lesions associated with Crohn's disease in humans (Thorel, 1989).

In experimental infection studies with cattle, animals less than 6 months of age are more susceptible to *M. paratuberculosis* infection than older animals (Larsen et al., 1975). This age-related susceptibility is thought to be due to immature cellular immunity found in very young animals. Adult animals are susceptible to infection but require a higher infectious dose than younger animals (Whitlock and Buergelt, 1996).

Resistance to intracellular pathogens, such as mycobacteria, has been demonstrated in mice and suggested in humans, to be linked to the Natural Resistance Associated Macrophage Protein 1 (NRAMP1) (Abel et al, 1998; Gomes and Appelberg, 1998). There is also evidence to support an association between increased susceptibility to Crohn's disease and a defect in the NOD2 gene of humans (Hugot et al., 2001).

Johne's disease

Johne's disease is characterized by a long incubation period and clinical signs vary depending on the stage of infection (Chiodini et al., 1984). There are 3 stages of Johne's disease, the first being the subclinical carrier stage where infected ruminants show no clinical signs of disease and do not shed *M. paratuberculosis* in their feces. The second stage is the subclinical shedder stage where there are still no clinical signs of infection, but infected animals are shedding *M. paratuberculosis* in their feces. The final stage of the disease is the clinical stage where infected animals are showing clinical signs of disease and are profusely shedding *M. paratuberculosis* in their feces. Under conditions of stress, more of the infected animals may develop clinical disease. Once clinical disease develops, affected animals eventually die

MAP transmission, pathogenesis and pathology

The main route of *M. paratuberculosis* transmission is fecal-oral via contaminated soil, water, teats or fomite surfaces. The introduction of Johne's disease into a population generally occurs when an infected animal contaminates the grazing area with feces containing live *M. paratuberculosis* bacteria. Uninfected animals can also acquire infection by ingesting *M. paratuberculosis* contaminated feed or milk (Sweeney et al., 1992b) or through in utero transmission (Seitz et al., 1989). Wildlife species have been demonstrated to harbour *M. paratuberculosis* bacteria and could therefore be reservoirs of infection (Daniels et al., 2003). Domestic ruminants may contact wildlife and/or their excreta when grazing in an area contaminated with wildlife feces. Transmission from wildlife to domestic animals has not been documented, but can be postulated on the basis of genetic studies that demonstrate no differences between domestic and wildlife isolates (Greig et al., 1997). Transmission of strains between domestic species (Pavlik et al., 1995; Power et al., 1993; Riemann et al., 1979) and from domestic species to wildlife has been well documented (Cook et al., 1997).

The pathogenesis of Johne's disease has been well described by Manning and Collins (Manning and Collins, 2001). The classical pattern of the host immune response is strongly biased towards a cell-mediated response during the early, subclinical stages of infection and later shifts to a humoral response during the late clinical stages of the disease. Once ingested, *M. paratuberculosis* bacteria cross from the lumen of the small intestine into the lymphoid system via M-cells of the Peyer's patches and are then taken up by epithelial macrophages, which become activated to drive a T cell response. The initial response is a TH1 or tuberculoid response which is characterised by the production of cell-mediated cytokines (IFNã, IL-2, TNFá) and an infiltration of lymphocytes into the tissues. Typically, the number of *M. paratuberculosis* bacteria seen in the tissues at this stage is very low. The TH1 response or subclinical stage of Johne's disease can last for months to years. At some point in the disease, for unknown reasons, the TH1 response gives way to a TH2 or lepromatous response, which is characterised by IL-4, IL-5, IL-6 and IL-10 cytokine production and an influx of inflammatory cells to the site of infection. Inflammation of the intestine causes a thickening of the intestinal wall leading to poor nutrient absorption in affected animals

(Shulaw et al., 1993; Williams et al., 1979). The TH2 response is responsible for activation and sustained antibody production. By this stage of the disease, clinical signs, such as weight loss and diarrhea, are evident.

The pathologic changes occurring during an *M. paratuberculosis* infection have also been well described (Whittington and Sergeant, 2001). The earliest lesions begin as small granulomas in the Peyer's patches of the ileum and eventually extend to the adjacent lamina propria and villi. The fully-developed lesion is a chronic, granulomatous enteritis where macrophages, packed with bacteria, invade the lamina propria and submucosa of the intestine (Sigurdardottir et al., 1999). *M. paratuberculosis* bacteria are then shed from the lamina propria into the lumen of the gut and can be found in the feces. Fecal shedding of *M. paratuberculosis* is sporadic but can occur quite early in the course of Johne's disease, before any detectable antibody response or observable clinical signs (Merkal, 1973).

MAP prevalence, treatment and control

The spread of *M. paratuberculosis* from one geographic area to another is generally related to the trading of infected animals. The true prevalence of infection and the economic losses associated with Johne's disease are unknown because there are no practical diagnostic tests that reliably detect subclinical infections of *M. paratuberculosis*. National surveillance programs have been established in Australia (Nicholls, 1999), and proposed in Canada and the United States, to provide assurance that herds have a low probability of being infected. But because of the lack of a rapid, sensitive diagnostic assay, the reported prevalence of Johne's disease in each country is really a reflection of the quantity and strength with which testing is done. Effective surveillance programs will require that monitoring is ongoing, with repeated testing at specified intervals.

Several studies across various regions of the United States of America have yielded seroprevalences of *M. paratuberculosis* infection in cattle populations ranging from 1.6 to 55% (Braun et al., 1990; Johnson-Ifearulundu and Kaneene, 1998; Kreeger, 1991; Whipple et al., 1991). Based on histological lesions, the prevalence of *M. paratuberculosis* infections in randomly selected Canadian slaughterhouse sheep was determined to be 3% in 2003 (Arsenault, 2003). A survey of commercial cow-calf operations in Saskatchewan, Canada demonstrated a herd prevalence of 3% (Waldner et al., 2002). The seroprevalence of *M. paratuberculosis* infections in 90 dairy herds in the Maritimes provinces of Canada was 3.4% (Chi et al., 2002).

Infections due to *Mycobacterium* species are among the most difficult to treat because of the high lipid content and complexity of the bacterial cell wall (Rastogi, 1991). Also, mycobacteria are capable of surviving within host macrophages, which are largely responsible for eliminating pathogenic microbes (Barrow, 1997). The chronic nature of *M. paratuberculosis* infections requires prolonged therapy, with multiple drug regimens

of low toxicity. Suitable drugs are therefore expensive, making the treatment of *M. paratuberculosis* infections economically unfeasible.

As therapeutic measures have proved inefficient, identification of subclinically infected animals and their eradication form the basis of treatment and control. To prevent new infections, animals should be born in a clean, dry environment, free of fecal contamination. Newborn animals should be fed only colostrum from animals that routinely test negative for the presence of *M. paratuberculosis*. The control of Johne's disease could conceivably be affected by the ability of wildlife species to acquire and then pass infection back to domestic animals, but this relationship has never been characterised. The prevalence of *M. paratuberculosis* in wild ruminants is relatively low (Pavlik, 2000), whereas the prevalence in farmed animals has been demonstrated to be higher, probably due to the higher density of animals in farmed situations (Pavlik, 2000). Other factors influencing the ecology of *M. paratuberculosis* infections are the increase in the number of susceptible hosts and environmental pollution leading to the acidification of soils and water (Manning and Collins, 2001). Eradication of *Mycobacterium tuberculosis* and *Mycobacterium bovis* from human and animal populations may have left animals with increased *Mycobacterium* susceptibility. Environmental pollution may have opened a niche for *M. paratuberculosis* since it has been demonstrated that *M. paratuberculosis* is far more resistant to inactivation by low pH than other pH-resistant pathogens, such as *Yersinia enterocolitica* and *Listeria monocytogenes* (Sung and Collins, 2000)

Diagnosis of MAP infection

Clinical signs of Johne's disease are manifested by weight loss and sometimes diarrhea (Chiodini and Van Kruiningen, 1984). Mastitis, infertility and decreased milk production are additional signs of Johne's disease (Chiodini and Van Kruiningen, 1984). The symptoms of Johne's disease were first described in 1895 by H. A. Johne and L. Frothingham, who demonstrated a connection between cattle enteritis and the presence of acid-fast micro-organisms in sections of the intestinal mucosa (Cocito et al., 1994). Diagnosis of Johne's disease is usually based on clinical signs and the presence of *M. paratuberculosis* is confirmed by an established diagnostic test. Control of infection using this diagnostic protocol has proven to be difficult because of the different stages of Johne's disease. It is important to identify infected animals before they reach the subclinical shedder stage and become an unapparent source of infection to other animals. It is unfortunate that most diagnostic tools available for the detection of *M. paratuberculosis* are less than satisfactory (Cocito et al., 1994). It is customary to perform whole herd culture tests to detect subclinical shedders and to determine the prevalence of the infection. As no test is 100% sensitive, control of the Johne's disease depends on repeated tests at 6 month or yearly intervals over a number of years to eliminate positive animals.

There are 2 main types of tests used to diagnose Johne's disease: Tests for the detection of *M. paratuberculosis* bacteria and tests that detect an immunological response to infection.

1. Detection of MAP bacteria

Cultivation of *M. paratuberculosis* bacteria from feces or tissues remains the most reliable method of detecting infected animals (Sockett et al., 1992b; Sweeney et al., 1992a). The estimated sensitivity of fecal culture is roughly 33% and the specificity has been accepted as 100% (Whitlock et al., 2000). A disadvantage of using conventional culture methods is the long incubation time and the variation in sensitivity as some strains are more difficult to isolate than others. The addition of certain amino acids or biochemical products, such as pyruvate, to culture media has been shown to both enhance (Whitlock et al., 1996) and inhibit (Paolicchi, 2003) the growth of certain *M. paratuberculosis* isolates.

Traditional culture techniques are usually performed using culture slants of Herrold's Egg Yolk Media (HEYM) supplemented with mycobactin J, a chelating agent which aids the bacterium in acquiring iron needed for growth (Thorel, 1984). This method requires up to 20 weeks of incubation, depending on the host origin of the isolate. An increasing number of *Mycobacterium* species have been identified possessing similar, if not identical, biochemical characteristics. This renders identification by classical culture methods ineffective. In the past, *M. paratuberculosis* was distinguished from *M. avium* and *M. silvaticum* by its dependence on mycobactin but recently, *M. paratuberculosis* isolates that are not mycobactin-dependent have been described (Aduriz et al., 1995).

Radiometric methods of culture, with automated monitoring of ^{14}C-labelled bi-products from bacterial metabolism, are a less time-consuming form of bacterial growth because a positive culture can be detected before the visual formation of bacterial colonies. Radiometric culture has a higher sensitivity and specificity when compared to conventional culture methods (Sockett et al., 1992; Whittington et al., 2000).

Broth-based cultivation of *M. paratuberculosis* bacteria from feces has also been described. A comparison of 7H10 Tween broth and HEYM yielded minimum *M. paratuberculosis* detection times of 27 and 49 days, respectively (Damato and Collins, 1990). However, evaluation procedures for the growth of *M. paratuberculosis* in broth media have not been performed with actual clinical specimens, therefore the high background turbidity of fecal and tissue specimens subsequent to processing may change the detection times. Stich *et al* (Stick et al., 2004) evaluated a broth-based, non-isotopic automated system, called MB/BacT, and found it to be considerably more sensitive and rapid than using HEYM for the detection of *M. paratuberculosis* in bovine feces. Unfortunately, automated assays require a higher equipment investment.

Another method for the direct detection of *M. paratuberculosis* bacteria is acid fast staining of fecal smears. This is a rapid method for detecting the presence of mycobacteria, but it is not as specific or sensitive as culture (Benazzi et al., 1996). The direct detection of *M. paratuberculosis* bacteria in feces or tissues should always be confirmed with other detection methods to rule out the existence of other *Mycobacterium* species that can be present in these sample types.

2. Detection of host immunological response to infection

An immunological response to an *M. paratuberculosis* infection can be detected by measuring host antibody production. Antibody detection tests, such as Agar Gel Immunodiffusion (AGID), Complement Fixation (CF) and Enzyme-linked Immunosorbent Assay (ELISA), can be very sensitive when antibody production is at its highest, as in the late stages of disease. But, because the sensitivity of antibody detection tests increases with the progression of disease, actual clinical signs may be evident before a positive test result is achieved. The ELISA assay can be performed in as little as a few hours, but the overall sensitivity has been estimated at only 45% since antibodies may not be detectable until late in infection (Collins, 1996). Ferreira *et al. (2002)* evaluated the AGID assay for its possible adoption as a diagnostic test in field conditions. AGID was demonstrated to be unsatisfactory as a screening diagnostic test for subclinically infected animals, though it may be useful as a confirmatory test for suspect animals demonstrating the clinical signs of Johne's disease. Several studies have demonstrated the low sensitivity of CF when applied to subclinically infected animals (Collins, 1996; Sherman et al., 1990; Sockett et al., 1992b). The consensus in the literature is that antibody detection assays are of little help in preventing the spread of infection because of the associated lag time between *M. paratuberculosis* shedding and a positive result.

Detection of the subclinical stage of the disease can be done by measuring the hosts' cellular response with such assays as delayed type hypersensitivity (DTH) or gamma interferon (IFNã). DTH tests measures a cutaneous T-cell-mediated inflammatory response due to the increase in skin thickness produced by the intradermal inoculation of the Johnin antigen (Chiodini et al., 1984). A positive reaction is indicative of prior exposure to antigen or *M. paratuberculosis* infection. Since this test detects a cell-mediated immune response, it is useful for the detection of the early stages of an *M. paratuberculosis* infection. However, DTH has a low specificity because of cross-reactions with other *Mycobacterium* species. In the IFNã assay, the release of the cytokine, IFNã, from host derived, sensitized lymphocytes after an overnight incubation with antigen is quantified by immunodetection. This assay may be useful for detection of the subclinical stage of an *M. paratuberculosis* infection, but is plagued with non-specific reactions (Reddacliff and Whittington, 2003).

A newly marketed commercial ELISA kit (Johne's Absorbed EIA, CSL Pharmaceuticals, Parkville, Vic) was evaluated for test sensitivity and specificity for the detection of *M. paratuberculosis* in subclinically infected cattle (Collins et al., 1991). The kit demonstrated a sensitivity of 47.3% and a specificity of 99.0%. The comparison of the sensitivity and specificity of this test with other tests for the detection of subclinically infected animals indicated that this test is, at present, the most efficient commercially available test for Johne's disease in cattle.

Molecular methods of detection

Several polymerase chain reaction (PCR) assays, targeting the IS900 region, have been developed for the detection of *M. paratuberculosis* DNA (Bull et al., 2000; Corti and Stephan, 2002; Djonne et al., 2003; Englund et al., 2001; Marsh and Whittington, 2001; Vary et al., 1990; Whittington et al., 1998; Whittington et al., 1999). IS900 is an insertion sequence or small mobile genetic element containing genes related to transposition. Unfortunately, PCR-based detection of *M. paratuberculosis* can be limited by poor recovery of DNA due to the highly specialized, lysis-resistant *Mycobacterium* cell wall (Brennan and Nikaido, 1995) and by the presence of inhibitory substances in preferred clinical specimens, such as fecal samples (Abu Al-Soud and Radstrom, 2000; Jiang et al., 1992; Uwatoko et al., 1996; Wilde et al., 1990). To address some of these limitations, different methods of cell wall analysis and removal of inhibitors have been developed. Immunomagnetic bead separation coupled with bead beating and real-time PCR was found to be an effective procedure for the detection of *M. paratuberculosis* DNA in fecal samples (Khare et al., 2004). The use of buoyant density centrifugation and sequence capture PCR has also proved to be an efficient means of purifying and concentrating *M. paratuberculosis* bacteria from fecal samples (Halldorsdottir et al., 2002). Recently, a real-time PCR method was developed (Fang et al., 2002) with a sensitivity equal to that of fecal culture and the ability to detect amplified products without electrophoresis. Real-time PCR has the advantage of being a more rapid assay and can be performed in a single tube to limit cross-sample contamination.

The discovery of the *M. paratuberculosis* specific genetic element, IS900, and the use of PCR based techniques have greatly improved the diagnosis of Johne's disease (Moss et al., 1991; Vary et al., 1990). But limited numbers of *M. paratuberculosis* bacteria in subclinically infected animals and the high concentration of inhibitors in clinical specimens, means that accurate detection of infected animals is still relatively poor making further improvement a necessity.

Currently favoured assays for *M. paratuberculosis* detection vary. Studies in Austria indicate that a combination of serological examination and detection of causative agent (PCR or culture) should be used for *M. paratuberculosis* diagnosis (Gasteiner et al., 2000). Researchers in Argentina suggest using a combination of ELISA for screening and culture for confirmation of *M. paratuberculosis* infection, but emphasize the importance of continued IFNã test development for the early detection of Johne's disease (Paolicchi et al., 2003). The Australian state of Victoria has a voluntary bovine

Johne's disease test and control program that relies on the use of an absorbed ELISA (Johne's Absorbed EIA Kit, CSL Pharmaceuticals, Parkville, Vic), but recent studies suggest that pooled fecal culture is more economical (Whittington et al., 2000). The University of Minnesota (Minnesota, USA) also supports pooled fecal culture as a valid and cost-effective method for the detection of *M. paratuberculosis* infection in dairy cattle herds (Wells et al., 2003). Each country has preference towards certain brand-name assays, but the underlying technique remains the same.

Definitive diagnosis should follow the Office International des Epizooties suggestions that confirmation of *M. paratuberculosis* infection be based on the finding of gross or microscopic pathognomonic lesions and isolation of *M. paratuberculosis* bacteria in culture.

Genetics and strain typing

The entire *M. paratuberculosis* genome was recently sequenced by University of Minnesota researchers with collaborators at the United States Department of Agriculture's (USDA) National Animal Disease Center in Ames, Iowa. The *M. paratuberculosis* genome contains nearly 5 million base pairs in a circular chromosome with more than 4,500 predicted genes.

Widely studied genes in the *M. paratuberculosis* genome include the rDNA genes, the internal transcribed spacers and IS900. In mycobacteria, ribosomal genes are linked in a single operon starting with 16s rRNA, then 23s rRNA and finally 5s rRNA. This operon is present as a single copy in slowly growing mycobacteria, such as *M. paratuberculosis*, and as two copies in the more rapidly growing *Mycobacterium* species (Bercovier et al., 1986). Sequencing of the rDNA genes has been used to distinguish between different species of bacteria (Da Costa et al., 2004; Elshahed et al., 2003), but the rDNA genes are fairly conserved within species and are not useful for differentiating between isolates of the same species.

Between the 16s and 23s rRNA genes and between the 23s and 5s rRNA genes are 2 internal transcribed spacers ITS1 and ITS2, respectively. This arrangement is present in all bacteria (Snyder and Champness, 1997). Ribosomal spacers have proven to be extremely useful tools for typing and identifying closely related bacteria due to their high size and sequence variability (Giannino et al., 2003).

A subspecies-specific region of the *M. paratuberculosis* genome has been identified and well characterized. Each *M. paratuberculosis* cell can contain 15 to 20 copies of a putative transposase known as IS900 (Whipple et al., 1990). This insertion sequence is said to be the sole genetic element that distinguishes *M. paratuberculosis* from *M. avium* and therefore gene-based diagnostics have tried to capitalize on this. It was originally thought that IS900 was specific to *M. paratuberculosis* (Collins et al., 1989; Moreira et al., 1999), but it has since been demonstrated that IS900-like sequences exist in other *Mycobacterium* species (Englund et al., 2002).

Restriction fragment length polymorphism analysis (RFLP)

Due to the above mentioned difficulties associated with the detection of *M. paratuberculosis*, additional molecular knowledge has been sought that might aid in the understanding of Johne's disease. A common way of differentiating among closely related species is to exploit differences found in the genome. Many molecular methods of genotyping, known as DNA fingerprinting or strain typing, have been described. RFLP analysis combined with southern blotting using various probes such as 5s rRNA (Chiodini, 1990), IS900 (Thoresen and Olsaker, 1994) and IS1311 (Whittington et al., 1998) were the first of these to be described. This form of strain typing involves the digestion of the whole genome with restriction enzymes, fixation of the resultant DNA fragments onto a nylon membrane and then the detection of specific sequences with various, *M. paratuberculosis*-specific probes. The RFLP assay has been standardized by Pavlik and coworkers (Pavlik et al., 1999). An analysis of 1008 isolates of *M. paratuberculosis* resulted in 28 distinctive RFLP profiles or strain types, using a section of IS900 as a probe. Although RFLP analysis is an accepted form of strain typing, drawbacks associated with this type of analysis include labour intensiveness and technical difficulties, such as high DNA concentration requirements. RFLP analysis is also limited in its ability to provide epidemiological information mainly because one RFLP pattern seems to predominate within a group of infected animals and a recent study demonstrated a poor relationship between RFLP type and species of ruminant host or clinical status (Pavlik et al., 2000).

Other fingerprinting techniques

Amplified fragment length polymorphism analysis (AFLP) is a new tool for the detection and evaluation of genetic variation. AFLP's are a more rapid and less labour intensive alternative to the RFLP technique. The AFLP technique is used to selectively amplify a subset of DNA fragments obtained after the genomic digest of a micro-organism. Genetic differences between organisms are displayed through the different patterns of DNA fragments on a polyacrylamide gel.

The difference between RFLP analysis and the AFLP technique is that only a proportion of the DNA fragments generated by restriction digest will be amplified in the AFLP technique. This reduces the complexity of the initial DNA fragment mixture and therefore DNA fragment patterns are easier to distinguish on polyacryalmide gels. Genetic differences are detected as the absence or presence of DNA fragments due to mutations in restriction sites and insertions and/or deletions within a DNA fragment. AFLP analysis has been used to strain type different bacterial (Jonas et al., 2004; On et al., 2003), plant (Keiper et al., 2003; Park et al., 2004) and parasite (Blake et al., 2003; Claes et al., 2003; Grech et al., 2002) species. Whether AFLP offers better discriminatory power over PFGE depends on the micro-organism studied (D' Agata et al., 2001; Lindstedt et al., 2000). AFLP analysis is technically preferred over RFLP analysis and has a higher discriminatory power than RFLP (Chemlal et al., 2001).

Randomly amplified polymorphic DNA analysis (RAPD) is a popular typing technique because of its simple and straightforward protocol. This technique is similar to a standard PCR protocol, except RAPD analysis uses only a single, randomly chosen oligonucleotide that acts as both the forward and reverse primer. This single primer will hybridize to many different sites within the sample DNA, but a PCR fragment will only be generated if the primer anneals at 2 sites on opposite strands of the DNA, within 2 kb, the maximum length of a PCR product (Edwards, 1998). Genetic variation is determined by changes in the pattern of amplification products displayed after agarose gel electrophoresis. RAPD analysis has been used to study the genetic diversity of different bacterial (Andrzejewska et al., 2003; Kageyama et al., 2002), viral (Comeau et al., 2004; Stemmler et al., 2001), parasite (Prugnolle et al., 2002; Sreekumar et al., 2003) and plant (Sandhu et al., 2002; Yang et al., 2004) species. RAPD is technically less-demanding than RFLP analysis (Smith et al., 2002), but problems of interpretation due to inconsistent intensity of bands in different polymerase chain reaction runs may arise (Stemmler et al., 2001) and reproducibility between laboratories is questionable (Jones et al., 1998).

PCR-Restriction endonuclease assay (PCR-REA) is a more time efficient form of stain typing. Through RFLP analysis, using the insertion sequence IS1311 as a probe, it was discovered that IS1311 exists in many *Mycobacterium* species (Keller et al., 2002)**,** but specific sequence differences could be used to distinguish between and within species (Whittington et al., 1998). An assay was developed which requires PCR amplification of a region of IS1311 and subsequent digestion of the PCR product with restriction enzymes. This form of strain typing permits distinction between cattle and sheep associated strains of *M. paratuberculosis* and has since been expanded to include a bison strain (Whittington et al., 2001). PCR-REA has been demonstrated to be a rapid, reliable method for strain typing of *M. paratuberculosis* isolates, but this technique also provides limited epidemiological information in that most *M. paratuberculosis* isolates are typed by this technique as cattle strains.

It is important to point out that “strain” refers to the genetically characterized digestion pattern associated with a particular group of *M. paratuberculosis* isolates and in this case, the “strain” has been named for the host in which it was originally isolated or most often found.

Pulse-field gel electrophoresis analysis (PFGE) has been adopted and used as a variation of the RFLP assay in an attempt to resolve the different restriction digest profiles and to lessen the technical time required of a typical RFLP. To date, 16 PFGE profiles have been described from 93 isolates (Stevenson et al., 2002) and when compared to RFLP, PFGE could segregate the previously established RFLP profiles into smaller, more epidemiologically useful groups. The drawback to this method of strain typing is the requirement of large amounts of un-sheared DNA and time required (up to 6 months) for the growth of *M. paratuberculosis* bacteria in a liquid media. Strain typing or genotyping of micro-organisms may provide insight into the epidemiology of Johne’s disease. The same molecular techniques used for strain typing

bacterial genomes can be applied to host genomes and result in information that could explain why some hosts are more susceptible to disease than others. The recent identification of Johne's disease-like lesions in the intestines of carnivores in Scotland demonstrates that disease caused by *M. paratuberculosis* is not limited to ruminant animals (Daniels et al., 2003). Strain typing of the carnivore isolates demonstrated that the same strain was found in both cattle and carnivores in Scotland. It can be hypothesized that the carnivores acquired this infection by eating rabbits, which have also been demonstrated to be carriers of this same strain. Furthermore, this unusual report of disease in carnivores could also be due to the pathogenic characteristics of this particular strain. It is in this way that strain typing identifies transmission patterns and pinpoints particular areas of *M. paratuberculosis* research that will aid in the overall understanding of Johne's disease.

Appendix II

National Park Service Summary

The following material was created by *Dr. Jenny Powers, Wildlife Veterinarian, U.S. National Park Service, Fort Collins, CO.* for a report to the National Park Service regarding the objectives of the workshop.

Current knowledge on distribution, effects and significance of MAP in bison:

- Using a PCR assay directly on previously frozen northern bison fecal samples, DNA was detected in a pattern consistent with MAP (i.e. IS900 and 251 positive). Another portion of DNA, belonging to MAP and other *Mycobacterium avium* organisms (IS1311), displayed a distinctive sequencing pattern found in feces from a number of northern Canadian bison herds.
- Body condition monitoring, via observations by wildlife personnel as well as hunters, has not found evidence of clinical signs resembling Johne's disease in these herds.
- All samples were cultured but no organisms were isolated. Therefore, it is not known whether the DNA results indicate (Abba and Golijow, 2004) the presence of living MAP organisms (of a known or previously unrecognized strain), (Abel et al., 1998) whether the bison are truly infected by the organism that the DNA belongs to, or (Abu Al-Soud and Radstrom, 2000) if infection with this organism is capable of causing disease.
- The MAP DNA has also been identified in other free-ranging ungulate feces in Canada and Alaska, including Dall's sheep, Stone's sheep and mountain goats. No clinical cases have been reported in source herds, though it is important to note that limited body condition monitoring has been completed.
- The Yukon has a captive game park which was formerly private and is now in public ownership. Conventional clinical and sub-clinical Johne's disease has been diagnosed in multiple ungulate species at the game park, using a variety of diagnostic tests including culture. Direct PCR on feces as well as serum antibody assays have yielded positive results. The source of the Johne's disease is under investigation but it appears to be associated with livestock sources.
- There is confirmed Johne's disease in Canadian cattle and clinical cases of conventional Johne's disease ('cattle strain') have been identified in Canadian commercial bison herds. Similarly, there is Johne's disease in U.S. cattle herds and clinical Johne's disease has been documented in U.S. commercial bison herds due to both conventional 'cattle' and slow growing 'bison' strains of MAP.
- USDA has a voluntary Johne's disease control program for domestic cattle. Presently, this is a 'risk management' not an infection eradication program. The Canadian Food Inspection Agency (CFIA) has Johne's disease on their 'annually notifiable diseases' list. Several provincial governments (e.g. Alberta) have voluntary Johne's control programs. CFIA and USDA representatives have noted that risk management is the key to managing this infection in domestic livestock.

- Clinical Johne's disease has been identified via fecal and tissue culture as well as serology in Tule elk at Point Reyes National Seashore, CA. Fallow deer, axis deer and domestic cattle are also affected by this disease at PORE. Minimal testing has been performed in other U.S. National Park Service units.
- Infection and clinical disease has also been confirmed in Key deer at the National Key Deer Refuge in Florida (U.S. Fish and Wildlife Service) via fecal culture and tissue culture. They also have serologic test-positives.
- MAP infections have been identified, at a very low prevalence, in free-ranging/ semi-captive bison at the National Bison Refuge in Moiese, Montana. Additionally, clinically affected bison have been identified in private semi-captive wild bison herds.
- It is presently unknown whether free-ranging bison in the U.S. have evidence of this currently un-culturable strain of MAP DNA in their feces, similar to that found in the northern Canadian herds. Further investigation is needed.

Appendix III

Workshop Agenda

WELCOME, OPENING REMARKS

Helen Schwantje, Chair, Bison Health Subcommittee, National Wood Bison Recovery Team, and Wildlife Veterinarian, Biodiversity Branch, Ministry of Environment, Victoria, BC.

Bill Samuel, Unit Leader, Alberta Cooperative Conservation Research Unit (ACCRU), and Professor of Biological Sciences and Associate Dean (Research), Faculty of Science, University of Alberta, Edmonton, Alberta

WORKSHOP GOALS AND OBJECTIVES

Facilitator: Helen Schwantje

DISTRIBUTION OF M. PARATB IN FREE RANGING BISON

Current Status of *M. paratb* Infection in Canadian Bison Populations
Brett Elkin, Wildlife Disease Specialist, Wildlife Division, Government of NWT Environment & Natural Resources, Yellowknife, NWT.

John Nishi, Bison Ecologist, Resources, Wildlife and Economic Development Government of NWT, Fort Smith, NWT.

Greg Appleyard, Molecular Biologist, Dept. of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK.

Current Status of *M. paratb* Infection in US Bison Populations
Becky Manning, Senior Scientist, Johne's Testing Center, School of Veterinary Medicine,
University of Wisconsin, Madison, WI.

Current Status of *M. paratb* infection in Wildlife in US National Parks
Jenny Powers, Wildlife Veterinarian, U.S. National Park Service, Fort Collins, CO.

DIAGNOSTICS

M. paratb Diagnostics: Current Approaches and Challenges
Becky Manning, Senior Scientist, Johne's Testing Center, School of Veterinary Medicine,
University of Wisconsin, Madison, WI

Sampling Protocols For Free Ranging Conservation Herds in Canada
John Nishi, Bison Ecologist, Resources, Wildlife and Economic Development Government of NWT, Fort Smith, NWT.

Clinical Johne's Disease and Diagnosis in Bison
John Berezowski, Epidemiologist, Agri Systems Support Branch, Alberta Agriculture, Food, and Rural Development, Edmonton, Alberta

CLINICAL EFFECTS AND SIGNIFICANCE OF M. PARATB IN BISON

Prevalence and Ecological Risk of *M. paratb* in Bison
Morgan Scott, Epidemiologist, Texas A&M, College Station, Texas, formerly from Agri Systems Support Branch, Alberta Agriculture, Food, and Rural Development, Edmonton, Alberta.

MANAGEMENT OF M. PARATB IN CAPTIVE AND FREE RANGING BISON

Management of *M. paratb* in Captive Bison Herds
Murray Woodbury, Research Chair, Specialized Livestock Health and Production, Western College of Veterinary Medicine, Saskatoon, Saskatchewan.

Dealing with Johne's Disease at the Yukon Wildlife Preserve.
Phillip Merchant, Woldlife Technician, Fish and Wildlife Branch Laboratory, Department of Environment, Government of Yukon, Whitehorse, Yukon.

Significance of *M. paratb* in Free Ranging Wildlife
Gary Wobeser, Wildlife Pathologist, Dept of Veterinary Pathology, Western College of Veterinary Medicine, Saskatoon, SK

Policy and Management Implications of *M. paratb* for Conservation of Free-ranging Bison Populations
Brett Elkin, Wildlife Disease Specialist, Wildlife Division, Government of NWT Environment & Natural Resources, Yellowknife, NWT.

John Nishi, Bison Ecologist, Resources, Wildlife and Economic Development Government of NWT, Fort Smith, NWT.

INFORMATION GAPS AND RESEARCH NEEDS

Facilitator: Helen Schwantje

Open Discussion

RECOMMENDATIONS AND DEVELOPMENT OF MEETING REPORT

Open Discussion

Appendix IV

Contact Information for Participants

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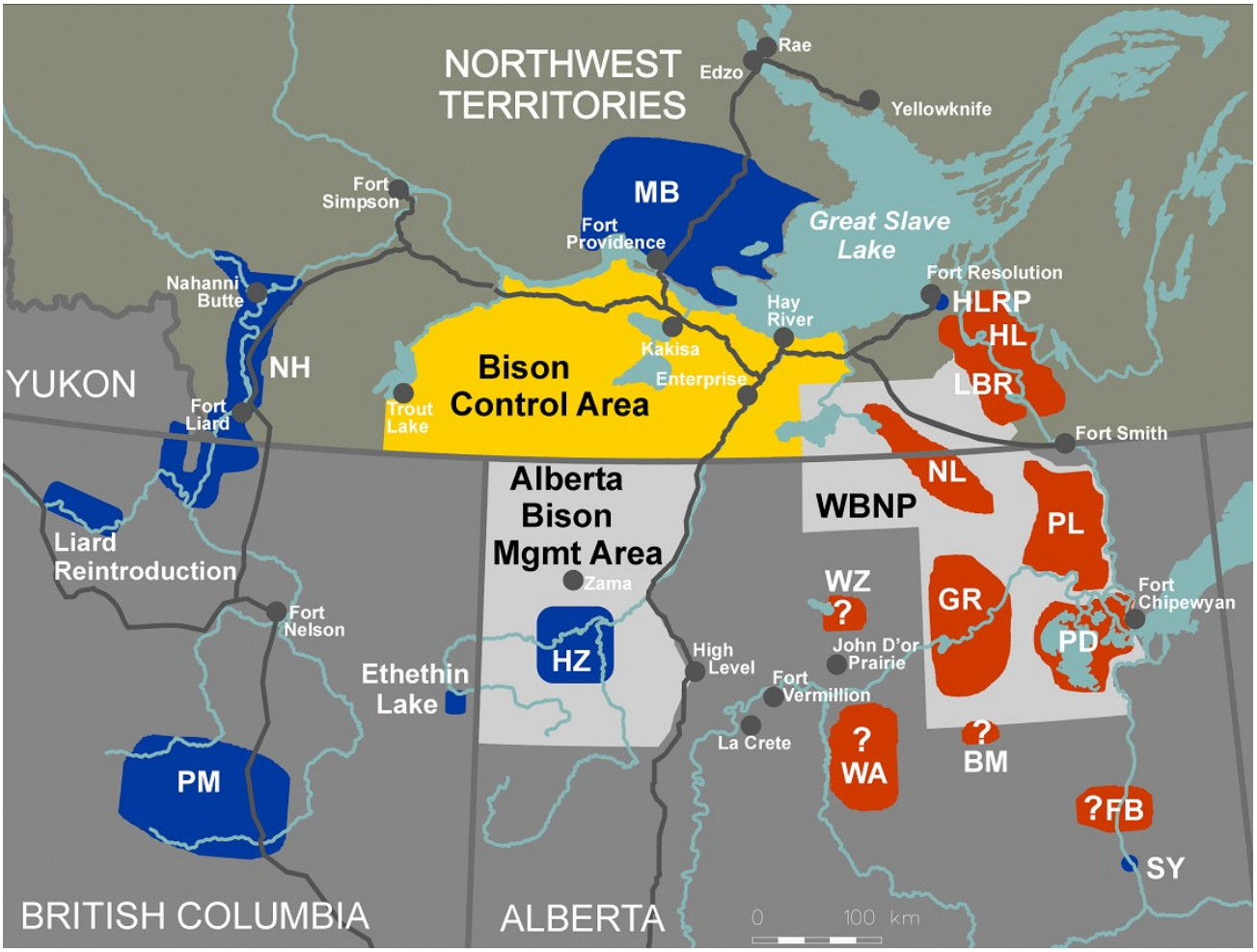
Helen Schwantje	BC Environment	helen.schwantje@gov.bc.ca

Appendix V

Map of Free-Ranging and Semi-Captive Wood Bison Herds in Canada.



Map courtesy of Environment and Natural Resources, Government of the NWT

Map Legend

Red: Wood bison herds that are known to be infected with bovine brucellosis and/or bovine tuberculosis.

WBNP - Wood Buffalo National Park
GR – Garden River
PD – Peace Athabasca Delta
FB – Firebag
WA – Wabasca
HL – Hook Lake (free-ranging)
LBR – Little Buffalo
NL – Nyarling River
PL – Pine Lake
WZ – Wentzel

Blue: Free ranging Wood and Plains bison herds that are known or believed to be free of bovine brucellosis and bovine tuberculosis.

MB – Mackenzie bison herd
HLRP – Hook Lake Recovery Project (captive)
HZ – Hay Zama
EL – Etthithun Lake
NH – Nahanni
PM – Pink Mountain (Plains Bison herd)

Herds that have been sampled and are fecal PCR positive for *Mycobacterium avium paratuberculosis* but without isolation of a living organism:

Hook Lake Wood Bison Recovery Project (also positive on ELISA)
Slave River Lowlands (Hook Lake and Grande Detour)
Mackenzie Bison Sanctuary
Wood Buffalo National Park (Pine Lake, Sweetgrass, Salt Plains)
Elk Island National Park
Pink Mountain (Plains Bison herd)

Herds that have been sampled and are fecal PCR negative for *Mycobacterium avium paratuberculosis*:

Yukon (small sample size)
Nahanni (small sample size)
Delta Junction plains bison (Alaska)

Appendix VI
Northwest Territories Basic Sampling Protocol for MAP: Field Post-Mortem Examination of Bison.

B.T. Elkin and J.S. Nishi

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the bacterium that causes Johne's disease. In the Northwest Territories, although we have not observed clinical cases of Johne's disease in either free-ranging or captive bison, we have found indirect evidence of MAP (or an MAP-like organism) in bison feces based on DNA extraction techniques. As culture confirmation of MAP is the gold standard for confirming whether an individual animal is infected with the organism, we have designed a basic sampling protocol for collection and preservation of tissues during routine post-mortem examination of bison in the field. This field protocol (summarized in Table 1) is centered on the ileo-cecal junction in bison, because MAP is most often isolated from this region of the intestinal tract in ruminants with Johne's disease.

Table 1. Sample collection and diagnostic tests used to detect MAP in bison

Tissues / Samples collected in field	*Fresh / Frozen*	Fixed (10% buffered formalin)
Serum	Serology (ELISA)	n/a
Feces	Culture & PCR	n/a
Distal ileum	Culture and DNA extraction (PCR)	Histology (H&E and Ziehl-Neelson staining)
Cecum		
Ileocecal lymph node		

1) SERUM SAMPLES:

Blood samples can be collected by either venipuncture (live animal) or by cutting the jugular vein (freshly dead bison). When cutting the jugular vein, caution should be taken to dissect down to the vein and avoid contamination with esophageal contents or skin/hair contaminants. Blood should be collected in red top Vacutainer® tubes, and kept above freezing until the sample can be processed. The blood should be allowed to clot and/or centrifuged, and serum separated off into 1 ml aliquots in storage vials. If serum samples cannot reach a diagnostic laboratory within 24 hours of collection, the aliquots should be frozen (-20° C) until they are processed.

2) FECAL SAMPLES:

Fecal samples should be collected as fresh as possible. Samples should be collected per rectum if possible. Otherwise, collect from the ground as soon after defecation as

possible, minimizing contamination of the sample with material from the environment (soil, plant material, etc.). Separate gloves should be used for each sample to prevent cross contamination. One sample should be collected from each animal, with each sample being at least 3 grams (approximately one teaspoon of feces). Samples should be placed in individual Whirpak® bags, labeled with animal identification information, date/time, and collection site. If samples can be shipped to the lab within 24 hours, the samples should be kept cool and shipped (on ice packs) as soon as possible. If not, samples should be frozen as soon as possible and kept frozen until they reach the laboratory.

3) TISSUE SAMPLES:

Tissue samples should be collected as aseptically as possible. It is best to collect the ileocecal lymph nodes first if possible to minimize contamination occurring when the GI tract is opened. Paired samples should be collected for each tissue – one in 10% neutral buffered formalin, and one fresh sample. If samples can be shipped to the lab within 24 hours, the fresh samples should be kept cool and shipped (on ice packs) as soon as possible. If not, the fresh samples should be frozen as soon as possible and kept frozen until they reach the laboratory. The fixed tissues should be kept and shipped at room temperature.

Appendix VI

PowerPoint Presentations

Some of the PowerPoint presentations used in the workshop can be found in this appendix, in the order in which they are listed below.

Current Status of *M. paratb* Infection in Canadian Bison Populations
Brett Elkin, Wildlife Veterinarian, Wildlife Division, Government of NWT Environment & Natural Resources, Yellowknife, NWT.

***M. paratb* Diagnostics: Current Approaches and Challenges**
Becky Manning, Senior Scientist, Johne's Testing Center, School of Veterinary Medicine, University of Wisconsin, Madison, WI.

Sampling Protocols For Free Ranging Conservation Herds in Canada
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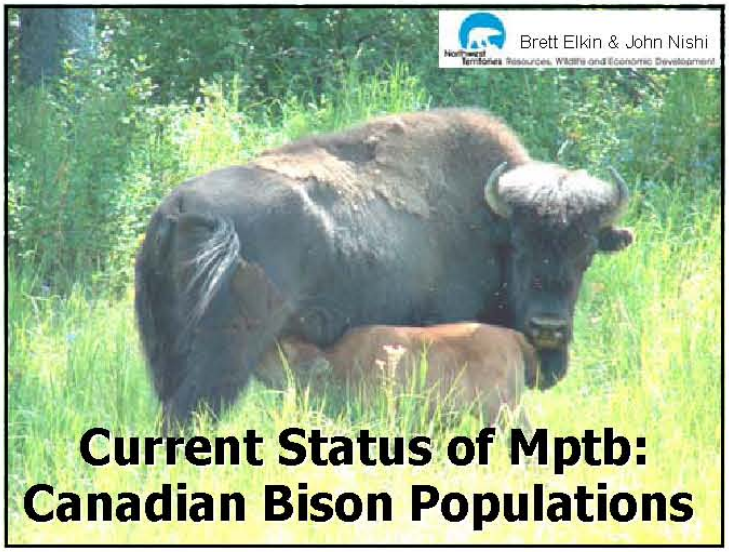
Policy and Management Implications of *M. paratb* for Conservation of Free-ranging Bison Populations
John Nishi, Bison Ecologist, Resources, Wildlife and Economic Development Government of NWT, Fort Smith, NWT.



Johne's Disease in Bison

- Reports in bison are rare
- Clinical cases documented in captive bison
- Not a reportable or program disease
  - producers often don't test
  - results not officially reported
- Prevalence & distribution: not documented

Photo from http://www.johnes.org

Hook Lake Wood Bison Recovery Project

Fecal PCR:

- Confirmed positive

Serology:

- 1999: 15/67 (22%)
- 2001: 1/100 (1%)
- 2002: 1/81 (1%)

Slave River Lowlands: Fecal PCR

Hook Lake Range:

- July 2000: 4/70 (6%)

Grande Detour:

- July 2002: 19/33 (58%)
- November 2002: 23/153 (15%)
- February 2004: 19/33 (55%)
- July 2004: 11/60 (18%)

Distribution of Mptb in Other Herds?

Diagnostic Challenges

- Long sub-clinical phase after infection
- Infected animal may not appear ill
- Detection of clinical cases in the wild
- Antibody production is late stage event
- Fecal shedding of organism is intermittent
- Difficult & slow to grow on on culture

Mptb Survey
Wood Bison Conservation Herds

- Clinical Cases
- Serology
- Fecal Culture & PCR*
- Tissue Histo, Culture & PCR

Mackenzie Bison Herd:

Fecal PCR:

- August 2001:
  9/105 (9%)

Wood Buffalo National Park: Fecal PCR

Pine Lake:

- July 2002: 19/33 (58%)

Sweetgrass:

- July 2002: 2/41 (5%)

Salt Plains:

- July 2004: 0/62

Elk Island National Park: Fecal PCR

Wood Bison:

- July 2000: 16/48 (33%)

Other Conservation Herds: Fecal PCR

Nahanni (NWT):

- 0/27

Yukon Herd (Yukon):

- 0/?

(BC):

- Confirmed present

Delta Junction (Alaska):

- 0/240

Management Implications

- Translocation & reintroduction of bison
  - introduction of Mptb to clean herds?
  - disease management to eliminate risks?
  - an issue if all herds infected?
- Mptb management within captive herds
  - implications for genetic management
  - time required for elimination or control

Key Information Gaps

1. Johne's status of all captive & free-ranging wood wood & plains bison populations
   - geographic distribution
   - prevalence rates

2. Strain of Mptb present in wood bison
3. Significance of Mptb to wood bison health
   - captive herds & free-ranging populations
4. Significance of Mptb to other wildlife species

Recommendations

- Implement sampling program for all captive & free-ranging wood bison herds
  - standardized across jurisdictions
  - statistically significant sample sizes
- Dedicated efforts to culture bison Mptb
- Research on strain typing
- Risk Management Strategy

Johne's disease in bison: United States

Elizabeth Manning MPH, MBA, DVM
Johne's Testing Center - School of Veterinary Medicine

Johne's disease in US bison…

- There are about 150,000 bison in private and public herds in the United States
- And yes, cases in bison have been diagnosed in herds besides those managed by *Dave Hunter* !
- No prevalence guesses possible: zero to minimal surveillance
- Wildlife managers doing a better job of looking than domestic bison raisers however (better awareness)
  - **T. Roffe has been testing in the National Bison Refuge, MT (~400 bison in the refuge; has tested ~ 5-10 bison annually for the past 4 years – v. low incidence)**
  - In *Dave's* bison, the incidence is now very low as well.

Johne's disease in farmed US bison…

- The prevalence is higher in this group vs. free ranging for the usual reasons:
  - Often managed on premises that used to be dairy farms
  - High animal density
  - Frequent exchange of animals among herds via auction
  - Infection doesn't get diagnosed and spreads
    - Economics of testing in depressed industry
    - Inadequate animal handling facilities for sampling
    - Not a management topic for the industry (and head-in-the-sand approach by some producers as in all animal groups - sheep, goat, llama, elk, etc.)

Johne's disease in farmed US bison

- 99% managers cull clinical animals without testing
- 1% "test" by gross necropsy (which can appear completely normal)
- Those virtuous few who collect tissues often find nothing
  - Histo. can be unrewarding
  - Tissue culture a challenge for labs expecting standard bovine *Mptb* strain growth patterns

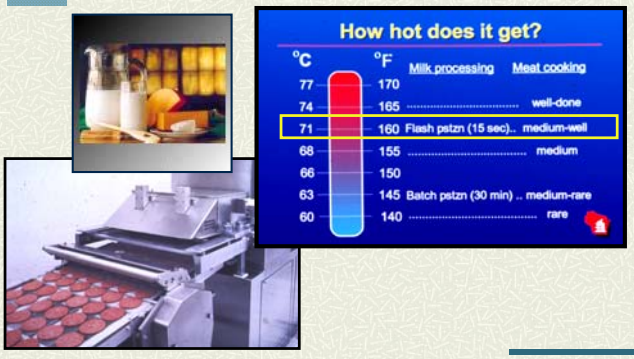
M. paratuberculosis infection: easy to ignore

- For both wildlife and domestic bison managers, this infection is easy to put on the bottom of the list
  - Lack of JD (1) awareness and (2) knowledge
  - Clinically too easy to define as something else (vague signs common to other ailments)
  - Diagnostics too expensive and too uncertain
  - Squeakier wheels

M. paratuberculosis infection: soon **less** easy to ignore?

- Additional factors now raising attention :
  - Zoonosis (Crohn's) ?
  - Non-ruminant wildlife vectors / reservoirs ?
  - Environmental contamination

Heat resistant



Environmentally hardy

- We know the organism dies off all the time but what we don't know is at what point we can stop worrying about new cases.

- Survival was found up to 55 weeks in a dry shady environment, with much shorter survival times in unshaded locations. Moisture levels, soil liming made no difference. (It appears to hate *changes* in temperature)

- Survived up to 24 weeks on grass that germinated through contaminated manure in completely shaded boxes and for up to 9 weeks on grass in 70% shade.

Whittington, 2004.

Wildlife exposure to *M. paratuberculosis*

Bacteria shed extensively by infected ruminants in:
- feces (ground, feed, water contamination)
- Manure management
  - Soil injection
  - Lagoon
  - Crop field spreading
- Preying on infected animals and ingesting organism
- Grooming in contaminated premises

M. paratuberculosis infects :

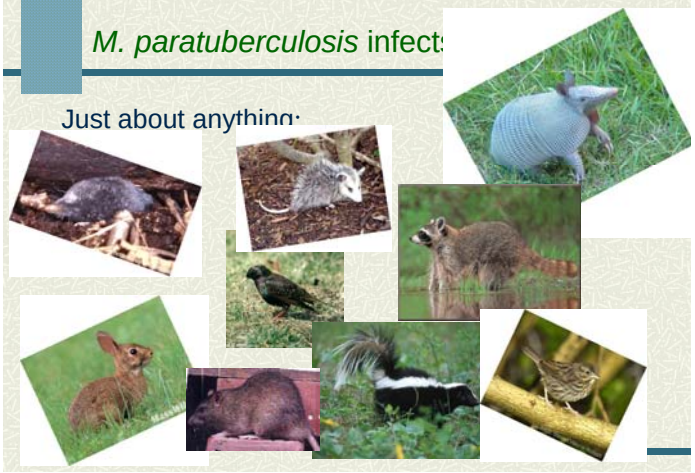
- **Non-ruminant species**
  - Extent and character of disease less clear
    - Omnivores
      - E.g. primates (mandrill, macaque); humans (Crohn's)?
    - Carnivores
      - E.g. fox, badger, cats, etc.

Wildlife exposure to *M. paratuberculosis*

- Environmental sampling
  - Bacteria isolated from 78% of herds with fecal culture positive cows
  - Alleyways, manure storage, calving areas, sick cowpen, water runoff
  - No surprise: the more heavily infected farms produced more test-positive environmental samples (Raizman, et al)

M. paratuberculosis infects

Just about anything:



Draft results

Cat	2
Snipe	1
Sparrow	1
Shrew	1
Raccoon	7
Starling	7
Rat	2
Skunk	1
Cottontail	1
Armadillo	2
Opossum	1

Total animals collected	688
Total samples inoculated	2752
Total animals Mptb +	26
Total samples Mptb +	36
Number shedding:	**8**

Test-positive wildlife: 26/688 = 3.8%.

Diagnostics for *M. paratuberculosis* infection in bison

Elizabeth Manning MPH, MBA, DVM
Johne's Testing Center - School of Veterinary Medicine

JD concerns for free-ranging wildlife

- Wildlife, endangered species health
  1. Wildlife reservoir for the infection
  2. Ruminant, carnivore, omnivore hosts
  3. *M. paratuberculosis* amplification
  4. Environmental contamination
- Possible interference with some TB assays (shared antigens)
- Hindrance of domestic agriculture control programs and political fall-out from same

Diagnostics – first steps

- Establish a case definition, meaning what are you diagnosing?
  - All infected animals?
  - Fecal shedders?
  - Infectious animals?
  - Animals with decreased productivity?
- **THEN**, evaluate the diagnostic test.
- Are fecal culture-positive animals that never progress to clinical disease and do not have pathology at necropsy epidemiologically important?

Diagnostics – next steps

- Define the goal:
  - *Disease* eradication?
  - *Infection* eradication?
  - Find and remove infectious animals?
  - Limit range contamination?
- **Decide what you will do with the results beforehand! (will a test-positive really affect management? Will a test-positive that cannot be confirmed affect management too much??)**
- *Then* establish surveillance protocols
  - Test type(s), frequency, species, ages, interpretation algorithms

Clinical assessment

- The usual signs:
  - Chronic weight loss
  - Diarrhea?
- Herd evaluation:
  - Too many open cows?
  - Too many in poor condition for range/season?
- Nothing too specific here…

Diagnostic options

- The usual samples:
  - Fecal
  - Blood: serum, lymphocytes
  - Tissue
  - Milk
  - Environmental (water, soil)
- The usual assays:
  - Serology
  - Culture (liquid or conventional)
  - Molecular (conventional, real-time, IMS, beacon)

Diagnostics - gross pathology

- *Range* of pathology due to stage of infection at death and type of species
- There may be *no lesions* at gross necropsy in a clinical animal or lesions may not look like those found in a Holstein:

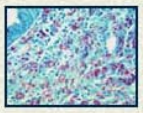
Normal ileum

Abnormal ileum

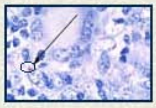
ALWAYS collect tissues for culture and histopathology!

Diagnostics - microscopic pathology

- *Range* of pathology due to stage of infection at death and type of species
  - Granulomatous inflammation in the intestinal tract
  - Acid-fast (pink) rods within macrophage cytoplasm
  - Or, few to no visible organisms or lesions
  - "Lymphoid depletion" keeps being reported in suspect cases



Pluribacillary



Paucibacillary

Diagnostic assays - culture

- Always be sure to take a large enough sample!
- Fecal samples
  - Directly
  - From ground
    - Take freshest available, don't pick up dirt too
- Tissue samples
  - Mesen./IC LN, ileum, (liver?)
  - In the US we have not seen enough cases to be confident we know the "best" single tissue sample
- And of course, get as specific as you can about what you have isolated

Diagnostic assays - culture

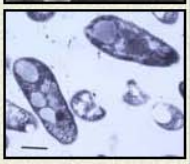
- Liquid culture is more promising than conventional
- Culture provides the living organism for further identification
  - Phenotypic and genotypic analysis
  - Basis for future diagnostic assays
  - Epidemiologic analysis
- Important especially for the first confirmation in a population – I like to see the organism alongside PCR or serologic data prior to labeling a herd as infected
- There are strains from bison that are very challenging to isolate, *but* the standard bovine strain gets them too, and the latter is easy to find
- Sample concentration methods (centrifugation, filter, immunoseparation) can improve sensitivity

Diagnostic assays – culture *again*

- Handling for each sample type may need its own optimization (e.g. milk fat gets in the way, environmental sample types from new regions may perform differently)
- Keep ALL acid-fast isolates, whether identified as *Mptb* with current methods or not and BEWARE of MIXED CULTURES (multiple mycobacteria types)
- Try buffy coats!

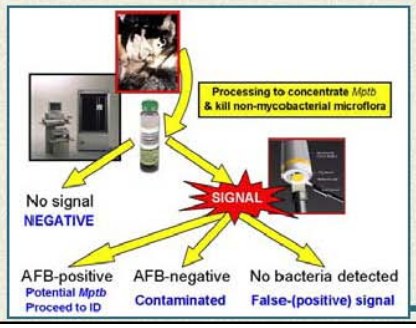
WARNING: How the organism is grown affects how it acts!

- How many forms does *Mptb* take?
  - Vegetative
  - Dormant
  - Persistent
  - CW deficient
  - Protease resistant
  - Viable & unculturable
- What effect does culture medium and age have on biological properties?
- Which form occurs in animals, humans, in foods or in the environment?
- Classical microbiologic studies are lacking to clarify these issues.



Rough – smooth colonies changed by a single medium component.

Automated liquid culture systems demand fast and accurate ID methods

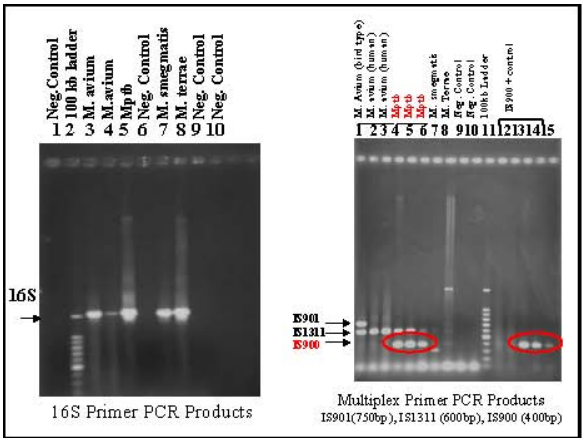


Molecular identification and genetic analysis

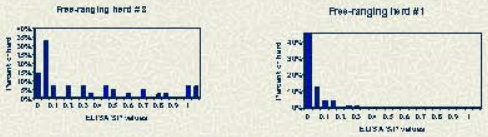
- Standard and real-time PCR
- Molecular beacons
- short sequence repeats
- AFLP
- Etc.

Many molecular methods have been and are under development that will provide useful data in describing the epidemiology of this infection in bison.

The same caveats re rational use and careful interpretation apply to these as to any diagnostic method.

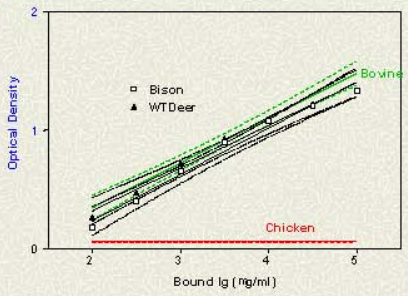


Diagnostic assays - serology



- If possible, establish a **population** baseline
- The usual false-negative (no antibody being produced to be detected) and false-positive problems
- Useful if feasible for whole herd testing to establish an index of suspicion for the population

Bison-antibody specific conjugates are nice, but protein G commercial ELISA works too



Johne's disease protein G ELISA

Bison

Transmission "control" program free advice

- Be careful about setting up control standards based on individual animal (vs. herd) results
- Try to avoid diagnostic results as "proof" of freedom of infection
- Focus on the changes over time in populations' results
- No matter how many times you explain about false-positives, your audience will hang you unless you have prepared them for the fact beforehand (see slide 4)

Remember *relative* risks

- Diagnostic tests can help find *most* of the *most* infectious bison – very useful but don't expect perfection.
- Don't worry whether there might be a few organisms on the truck's tires: focus on core risks.

Mptb: Surveillance Strategies for Bison Conservation Herds in Canada - Some considerations...

John Nishi & Brett Elkin

Northwest Territories Resources, Wildlife and Economic Development

Surveillance Strategies

What is Feasible?

What are the Sampling Objectives?

How Many To Sample?

What is Feasible?

Captive & Semi-captive vs. Free ranging herds

Anti-mortem inspection		Post-mortem inspection	
Handling facilities available	Handling facilities unavailable	Planned field collections	Opportunistic sampling of Hunter-kills
Animals rounded up and processed through a chute-tub-squeeze system.	Animals live captured in the field using a helicopter-based capture system, i.e., net gun or chemical immobilization.	Organized field collections with specific sampling of individual animals.	Opportunistic sampling of hunter-killed bison. Sampling is limited with poorer quality control.
Johnin skin test	Johnin skin test	-	-
Whole Blood & Sera	Whole Blood & Sera	Whole Blood & Sera	Whole Blood & Sera
Fecals	Fecals	Fecals	Fecals
-	-	Tissues	Tissues

Relative Strengths & Weaknesses

Surveillance Strategies

Few samples → Many samples

High probability of detection ← Lower probability of detection

BIASED — UNBIASED

Symptomatic targeting	Natural mortalities Roadkills Primitive weapons hunts	Random sampling (harvest)

M. Miller

What are the Sampling Objectives?

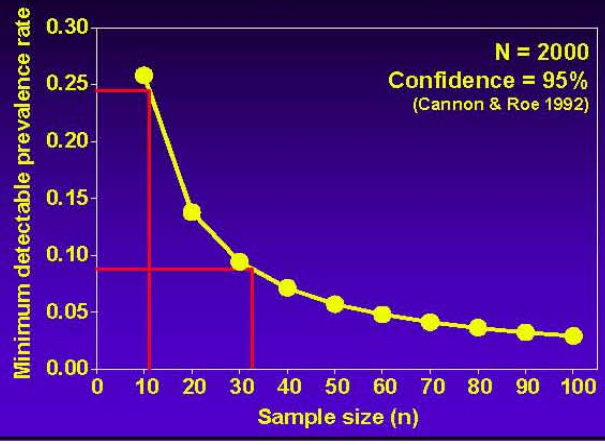
Surveillance Objective
(3 levels of stratification)

Is Disease present?

How much disease is present (prevalence)?

- **Herd**
- **Age Class**
- **Individual animal**

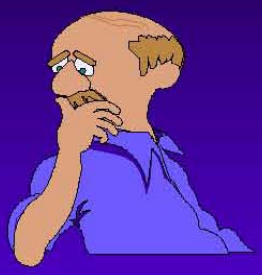
How Many to Sample?



Implications of Diagnostic Test Performance

1. Sensitivity

2. Specificity



		True Disease Status	
		+	-
Diagnostic Test Result	+	TP	FP
	-	FN	TN
		TP+FN	FP+TN
		Sensitivity = TP/(TP+FN)	Specificity= TN/(FP+TN)

Sensitivity: ability of a test to accurately identify those with disease

Specificity: ability of a test to accurately identify those who do not have disease

Implications of Disease Biology

1. Non-Infected
2. Infected
   - Non-clinical (non-shedder)
   - Clinical (non-shedder)
   - Clinical (shedder)

Probability of Detecting Infection Depends on…

- **Sensitivity and specificity of animal-level tests**
  - Varies with stages of infection in herd
  - Sensitivity
    - ELISA ~ 15-88% , Fecal culture ~ 33-100%
  - Specificity
    - ELISA ~ 95%, Fecal culture ~ 100%
- **True within-herd prevalence**
  - Usually assumes that infected animals are uniformly distributed in the herd
- **Sample size**

Herd-Level Testing:

Summary of herd sensitivity and specificity of current testing methods (blood & fecals) in cattle

Testing method	Hse	Hsp	Samples	Reference
ELISA	70-93%	89%	30 samples	Wells et al., 2002
ELISA followed by fecal culture	33-84%	100%	30 samples	Wells et al., 2002
Culture of individual fecal samples	64%	100%	Whole herd testing	Kalis et al., 2000
Culture of pooled fecal samples	94%	100%	5 samples/pool, Whole herd testing	Wells et al., 2002
Culture of samples from cow alleyway	78%	100%	2 samples per herd	Raizman et al., 2004

S. Tavornpanic et al.

Recommendations...

Sampling Priorities

- Data gaps: sufficient, limited, or absence of data

Two - Tiered Approach

- Herd-Level: determine presence / absence
- Animal-Level: evaluate implications of test performance & stage of infection on prevalence estimates

Define absence of disease

- Herd vs. animal level
- Quantitative risk-based criteria

Management of *M. paratuberculosis* in Captive Bison Herds

Murray Woodbury
Specialized Livestock Health and Production
Western College of Veterinary Medicine
Saskatoon, Saskatchewan, Canada

Transmission

- Fecal – oral transmission
  - Ingestion of feed, water, milk
- In utero transmission
- Wildlife reservoir?

Manage the Risk Factors

- Age
- Birth from infected dam
- Exposure to
  - Contaminated environment (theoretical)
  - Feces from infected animals
  - Contaminated feed/water
  - Milk, colostrum from infected animals

Control Program

depends on the level of calf management possible

Cow-Calf Control Program

- **Test and cull positive bison**
  - ELISA screening and fecal culture
- **Cull offspring of positive bison cows**
  - Star with last calf and work back
- **Breed uninfected bison**
  - Use AI or use biosecure bulls from tested herds
  - Consider ET from valuable cow
- **Manage calf rearing**
  - Consider hand raising calves from valuable cows

Cow-Calf Control Program

- **Improve sanitation of watering, feed facilities**
  - Disinfect things with phenolic compounds
  - Avoid manure contamination
    - Ponds are a significant infection risk
    - Drain standing water
    - Don't feed bales in the same spot all the time
- **Avoid muddy contaminated pastures for calving**
  - Move pairs to clean dry pasture ASAP
- **Avoid overstocking**

Mptb: Implications for Bison Conservation & Wildlife Management

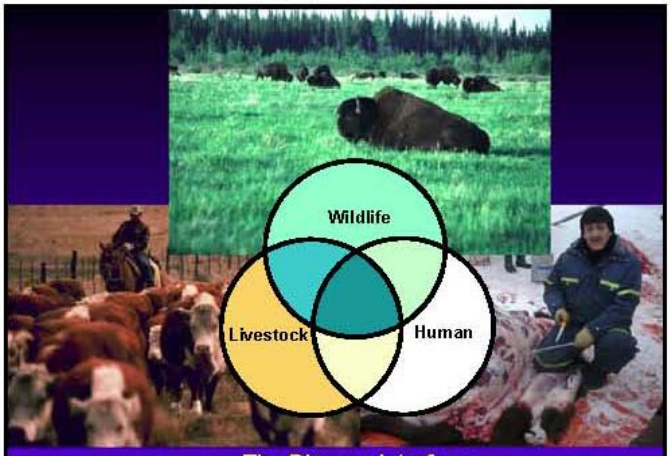
- *Mptb* (PCR +ve) present in a bison population NOW WHAT…?
- Is *Mptb* an EID or is it probably long widespread & now recognized?

John Nishi & Brett Elkin

Northwest Territories Resources, Wildlife and Economic Development

Presentation Themes

- Translocations
- Managing the Interface
- Legislation & Policy



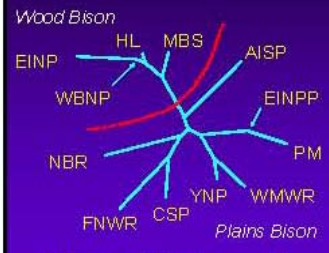
The Disease Interface:

Adapted from Daszak et al. 2000

Translocations:

Genetic Conservation & Management

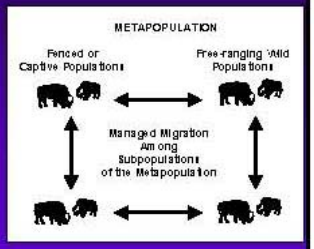
Genetic Salvage & Captive Breeding



Neighbour-joining tree created with D_A genetic distances

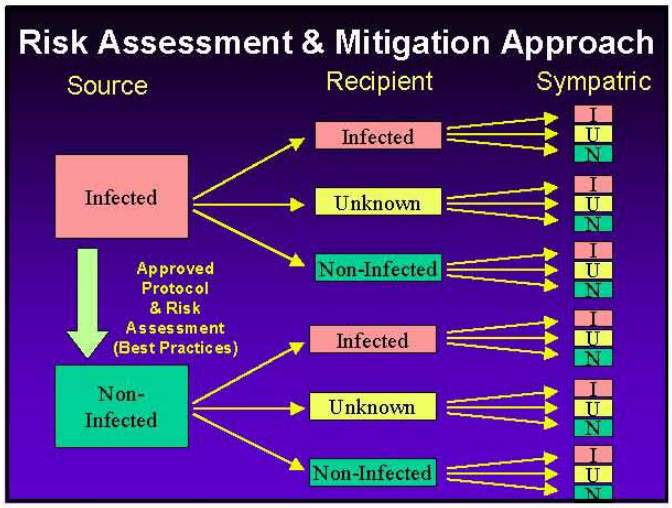
Wilson and Strobeck 1999, G. Wilson 2007

Gene Flow within a Metapopulation

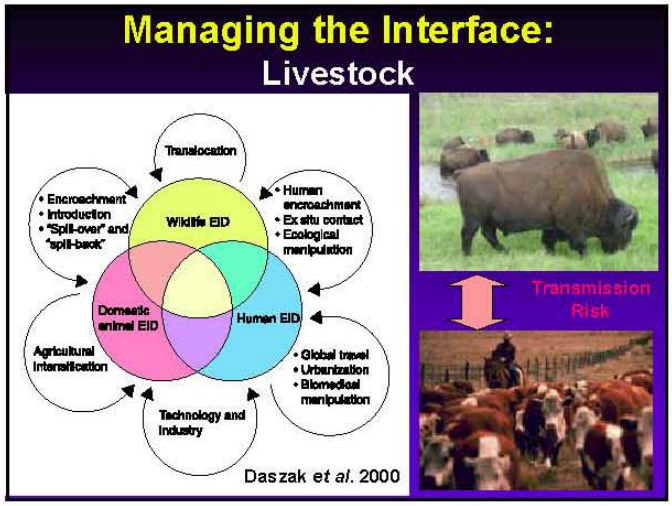


Adapted from Ballou et al. 1995


Transfer Priorities for Wood Bison
Use of wood bison from breeding herds (WBRT 2001)
Reintroductions
Conservation projects
Commercial Sales
Within Canada
Outside Canada
6) Captive operations (First Nations)
9) Livestock industry (donate proceeds) Breeding Stock - livesales Slaughter - meat sales
1) Establish wild herds in original range (Hook Lake Area)
4) Establish / augment wild herds in orig range
7) Scientific research institutions
2) Augment existing disease-free wild herds in orig range
5) Establish / augment wild herds outside orig range
8) Zoos (Captive breeding & display)
3) Establish / augment wild herds outside orig range
adapted from Gates et al. 2001, National Recovery Plan for Wood Bison p. 38


Risk Assessment & Mitigation Approach
Source
Recipient
Sympatric
Infected
Approved Protocol & Risk Assessment (Best Practices)
Non-Infected
Infected
Unknown
Non-Infected
Infected
Unknown
Non-Infected
I
U
N


Risk Assessment & Mitigation Approach
• Management Decisions…
• Is translocation warranted given uncertainty in Mptb?
– Yes
– No
– Maybe (Wait & collect more data)


Managing the Interface:
Livestock
Translocation
• Encroachment
• Introduction
• "Spill-over" and "spill-back"
Wildlife EID
• Human encroachment
• Ex situ contact
• Ecological manipulation
Domestic animal EID
Human EID
Agricultural intensification
• Global travel
• Urbanization
• Biomedical manipulation
Technology and industry
Daszak et al. 2000
Transmission Risk

Livestock

- Production limiting disease
- Risk of spillover
- Disease control measures
  - Voluntary versus Mandatory efforts
  - Regulatory implications

Managing the Interface: Human

- Link to Crohn's disease?

"Support for the hypothesis that there is a link between CD and MAP varied widely amongst those I spoke to. Judgement on the persuasiveness of the evidence to some extent at present appears to depend on one's scientific background. Thus clinicians and immunologists tend to be sceptical about the possible link. Microbiologists and public health specialists are more cautious about discounting the hypothesis. No one discounted the possibility of a link for some cases of CD entirely; neither did anyone suggest it was now proven.

Rubery 2001

Legislation & Policy

- Agricultural programs
  - Voluntary Control Programs
  - Reportable disease… (implications to potential and existing wildlife reservoirs)

Management Implications

- Translocation & reintroduction of bison
  - introduction of Mptb to 'clean' herds?
  - disease management to eliminate risks?
  - an issue if all herds infected?
- Mptb management within captive herds
  - implications for genetic management
  - time required for elimination or control

Key Information Gaps

1. *Mptb* Status of all captive & free-ranging wood wood & plains bison populations
   - geographic distribution
   - prevalence rates
2. Strain of Mptb present in wood bison
3. Significance of Mptb to wood bison health
   - captive herds & free-ranging populations
4. Significance of Mptb to other wildlife species
5. Implications of Agricultural control programs

Recommendations

- Implement sampling program for all captive & free-ranging wood bison herds
  - standardized across jurisdictions
  - appropriate sample sizes
- Dedicated efforts to culture bison Mptb
- Research on strain typing
- Risk Management Strategy (is Mptb a real problem)?

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