A Review of Animal Health Policies and its Implications for Salvaging a Captive Breeding Herd of Disease-free Wood Bison (*Bison bison athabascae*)

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ABSTRACT

The Hook Lake Wood Bison Recovery Project (HLWBRP) was initiated in 1996 as a co-management project between the Government of the Northwest Territories (GNWT) and the community of Fort Resolution, NWT. The overall objective of the project was to salvage genetically representative wood bison (*Bison bison athabascae*), from a wild, free-ranging herd in the Slave River Lowlands that was infected with bovine tuberculosis (*Mycobacterium bovis*) and brucellosis (*Brucella abortus*), and to use the salvaged herd as a source of healthy stock for future re-introductions. The HLWBRP used a variety of techniques in an attempt to establish a captive 'disease-free' herd, which primarily comprised capturing newborn wild calves, intensive prophylactic treatment of calves with antibiotics, hand rearing calves in an isolation facility, and intensive testing of calves using diagnostic blood and skin tests for brucellosis and tuberculosis. A principal challenge of the HLWBRP was to define the criteria that would be used to establish the health status of the captive herd – interpreted from a regulatory perspective as "disease-free" and an epidemiological perspective as infection-free. In this report, we conducted an extensive review of the scientific literature and interviewed key individuals with expertise and knowledge of livestock and wildlife diseases. Our intent was to understand current livestock and wildlife regulations and policies within Canada and abroad in order to outline a policy framework for disease eradication and develop the basis for defining the health status of the HLWBRP. By conducting this review, we concluded that there are important opportunities and advances at the interface between wildlife and livestock health issues that could be gained by improving interdisciplinary and inter-agency collaboration. We recommended that the GNWT undertake a more

formalized and integrated approach for discussion and resolution of the health status of the HLWBRP by establishing a working group that includes representation from the Canadian Food Inspection Agency and the National Wood Bison Recovery Team. This approach would engage a federal livestock health regulatory agency as well as other wildlife management agencies in Canada that may have a vested interest in the management of the HLWBRP and/or the criteria used to define the health status in this conservation project. We think that the issue of health status for the captive HLWBRP herd provided a specific example of the challenges at the interface between conservation biology and veterinary medicine, and further suggest that although the HLWBRP was terminated[1] in 2006, the project will contribute to development of a consistent and integrated approach for wildlife and livestock health policy in the NWT and Canada, where genetic salvage and management of diseased wild bison is concerned.

[1] Unfortunately, a single case of bovine tuberculosis was confirmed in the HLWBRP in June 2005 during a routine cull of a captive born male. Subsequent disease testing and culling into winter 2006 confirmed that an additional 12 bison were infected with *M. bovis*. In March 2006 all remaining HLWBRP bison were destroyed.

Table of Contents

List of Figures

List of Tables

Introduction

Background

In 1990, following extensive stakeholder consultation and intense public debate, the federal Northern Diseased Bison Assessment Panel recommended depopulating free-ranging bison (*Bison bison)* in and around Wood Buffalo National Park (WBNP) that were infected with bovine tuberculosis [*Mycobacterium bovis* (BTB)] and brucellosis *(Brucella abortus)* and replacing them with disease-free wood bison (*B. b. athabascae*) (Connelly et al. 1990). The Hook Lake Wood Bison Recovery Project (HLWBRP) was initiated in 1996 through a collaborative effort between the community of Fort Resolution, NWT, and the Government of the Northwest Territories (GNWT). In conjunction with habitat restoration (see Chowns et al. 1998, Quinlan et al. 2003), the HLWBRP was conceived as a first step towards reestablishing healthy bison in the Slave River Lowlands (SRL) (Deninoo Wildlife and Resources Committee 1991, Deninu Kue' First Nation 1996).

A fundamental objective of the HLWBRP was to salvage a genetically representative, captive, disease-free herd of wood bison from the wild population in the SRL that was infected with BTB and brucellosis (Wilson et al. 2005). The healthy captive herd would then be used as source stock to re-establish a disease-free wild population in the SRL (see Gates et al. 1998, Nishi et al. 2001, Nishi et al. 2002a), and to develop long-term economic opportunities for the community of Fort Resolution (Deninoo Wildlife and Resources Committee 1991). Because of the national significance of this conservation project, contingencies also included the option of translocating healthy captive-born HLWBRP bison to other jurisdictions to meet genetic management objectives (McFarlane et al. 2006) within the framework of a national wood bison recovery plan (see Gates et al. 2001b).

The HLWBRP was based on an adaptive approach and a combination of techniques to propagate a healthy and genetically diverse captive herd. The approach was adaptive because it evolved over the period of time it was implemented and because it incorporated a new approach to address the issue of latency for brucellosis (i.e. isolation of founder females in the last trimester of pregnancy followed by serologic testing of dam and calf) and incorporated new diagnostic tests (i.e. are not currently validated for use in bison) for both tuberculosis and brucellosis. The combination of techniques used in the HLWBRP were: 1) orphaning newborn wild-caught calves that may have been exposed to *B. abortus* and *M. bovis*; 2) field-testing calves for maternal antibodies to brucellosis prior to entry into the isolation facility; 3) initial 6-month isolation period for calves in pairs to prevent potential spread of disease; 4) hand-rearing and prophylactic treatment of captive calves using a combination of anti-mycobacterial and anti-*Brucella* drugs; 5) isolation and serological testing for brucellosis of first time calving founder females and their captive-born calves at three days and four weeks post-calving; and 6) intensive whole-herd testing for both diseases and removal of any suspicious reactors.

From 1996 to 1998, 62 calves were captured from the wild (see Figure 1 in Wilson et al. 2005). As a result of early mortalities and removal of a suspicious caudal fold reactor (and its pen mate) in February 1997 (Gates et al. 1998 and see Nishi et al. 2001, Nishi et al. 2002a) the founder herd was reduced to 57 individuals that matured to breeding age. The first cohort of captive-born calves was born at the HLWBRP in spring 1999. In subsequent years, herd management focused on continued health monitoring, disease testing, and genetic management through determination of parentage and culling of genetically less important offspring (Wilson et al. 2003).

Need for Policy Review

The HLWBRP used an adaptive approach to salvage a healthy herd of wood bison. The project was undertaken by the GNWT and the community of Fort Resolution, NWT to achieve community-based objectives in bison conservation (Deninoo Wildlife and Resources Committee 1991, Deninu Kue' First Nation 1996). However, because of its potential to contribute to the recovery of wood bison in Canada and the United States (i.e. Alaska), a broader challenge for the HLWBRP was to establish that the captive herd was "disease-free" with respect to BTB and brucellosis at a national (and international) level. By achieving disease-free status, the HLWBRP would also establish the requirements for salvaging healthy bison from the diseased northern herds in and around WBNP.

However, because the specific technical criteria and decision-making process for achieving disease-free status for a wildlife project of this nature had not been fully established in Canada prior to initiation of the HLWBRP, it was uncertain as to how this status would be defined, determined, and recognized by other provincial/territorial, national, and international agencies. Therefore, it was critically important to review and understand livestock and wildlife disease policies[2] both within Canada and abroad

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[2] Policy is a defined plan or course of action, as of a government, political party, or business, intended to influence and determine decisions, actions, and other matters (Nelson Canadian Dictionary of the English Policy refers to a definite course or method of action, selected from among alternatives and in light of given conditions to guide and determine present and future decisions. Policy includes a high level overall plan, embracing the general goals and acceptable procedures, especially of a governmental body (Merriam-Webster online dictionary http://www.m-w.com/cgi-bin/dictionary?policy).

because these established policies would likely influence and determine the criteria that would be used to establish disease-free status in the HLWBRP.

Our goal in this report was to review pertinent wildlife and livestock disease policy both in Canada and abroad to identify policy options and needs for the HLWBRP in achieving disease-free status. We anticipated that the policy review would also guide strategic management and operational activities pertaining to disease-free status at the HLWBRP.

Our specific objectives were:

1) To review relevant literature on national and international government policies on reportable diseases occurring in domestic livestock and free-ranging ungulates as they pertain to defining, establishing, and maintaining disease-free status; and

2) To summarize previous attempts at eradicating a reportable disease(s) in free-ranging wild ungulates, with an emphasis on bovine tuberculosis and brucellosis.

Proviso

Unfortunately, in spring 2005, bovine tuberculosis was confirmed in the HLWBRP during a routine cull of a 2.5-year-old captive-born bull (Lutze-Wallace et al. 2006). Subsequent epidemiological investigation efforts based on additional disease testing and post-mortem examinations revealed that 12 animals in one of the project pens were infected with tuberculosis (Elkin and Nishi, unpublished data). In February and March 2006, all remaining bison at the HLWBRP were either culled on site or transported to abattoirs in Alberta. Post-mortem examinations, serology, and laboratory analysis of tissues indicated that no bison were infected with brucellosis (Elkin and Nishi, unpublished data).

Although the HLWBRP has been terminated, we have prepared this report within its original context to provide an overview of wildlife health policies and to develop strategic recommendations for addressing eradication of reportable diseases in wildlife conservation projects. The options and considerations we outline in the report are largely presented in a context where tuberculosis had not yet been observed in the HLWBRP. Therefore, the relevance of these options and considerations are linked more to future wildlife conservation and disease eradication projects, and represent options considered and implemented by the HLWBRP prior to its termination.

The occurrence of tuberculosis and termination of the HLWBRP underscores the importance (see McFarlane et al. 2006) and difficulties of genetic salvage of diseased wood bison populations. It also reinforces the need for a broad understanding of current wildlife and animal health policies, and development of an integrated and specific policy framework to support and design wildlife disease eradication projects in Canada.

Methods

We used three sources of information to compile this report. First, we conducted a computer assisted literature review using PubMed, Agricola and CAB databases. Second, we reviewed the websites of provincial and state departments of agriculture and wildlife for selected jurisdictions to try to locate relevant legislation (Appendix A). Third, we contacted professional colleagues (Appendix B) via telephone or e-mail to supplement the first two sources of information. Due to a lack of response to some of these personal communications, we cannot guarantee that all pertinent policies and program information was made available for this report. We also note that the majority of our research was initiated and conducted between 2000 and 2001, and that we have included recent revisions to relevant policy and legislation.

Results and Discussion

Northern Diseased Bison and Agricultural Livestock Health Issues

The decision to undertake a disease eradication or control program depends on the net social benefits of the campaign (Ekboir 1999). In the case of the HLWBRP, an important motivation was to mitigate against two potential costs that are inherently tied to the northern diseased bison issue (see Connelly et al. 1990, Nishi et al. 2006): (1) the loss of genetic diversity if wild wood bison in the SRL were depopulated without adequate genetic salvage of replacement stock (Wilson et al. 2005), and (2) reduction in Canada's international trading capacity in livestock if *M.* bovis and/or *B. abortus* spilled back from infected wild bison to domestic cattle or commercial bison herds in northern Alberta (Essey and Koller 1994, Kellar and Dore 1998, Koller-Jones et al. 2005). However, the lack of a comprehensive disease management and control policy for wildlife, and the partitioning of responsibilities and regulations between livestock and wildlife interests between provincial/territorial and federal jurisdictions intensify the problem of striking a balance between the single focus of disease eradication and the broader social and ecological concerns of bison conservation and management (see Nishi et al. 2002b).

Although the GNWT is responsible for management of wood bison in the NWT (GNWT 1988) and coordinates co-management activities in close consultation with local communities, bison management issues in the NWT often overlap with mandates of federal jurisdictions. Bovine tuberculosis and brucellosis are listed as "reportable diseases" (Department of Justice Canada 1990b) under the federal *Health of Animals Act* because they pose serious threats to international trade in livestock and have important socioeconomic and human health consequences. The Canadian Food Inspection Agency

(CFIA) (Department of Justice Canada 1997) is the federal agency responsible for policy implementation and enforcement of regulations arising from the *Health of Animals Act* (Department of Justice Canada 1990a) and pertaining to reportable livestock diseases. The mandate of the CFIA's National Animal Health Program is to protect "the health of food producing animals, domestic pets, and the Canadian public by preventing the introduction and spread of certain important animal diseases" (CFIA 2007). Similarly, federal agencies such as Environment Canada – Canadian Wildlife Service and Parks Canada Agency are also involved in management of wood bison (see Gates et al. 2001b, Nishi et al. 2006, Shury et al. 2006) because of their respective responsibilities under federal legislation and the designation (Department of Justice Canada 1998, 2000, 2002) (threatened status)[3] of wood bison populations in national parks, i.e. Elk Island National Park and WBNP. The recent development of a National Wildlife Disease Strategy in Canada (Environment Canada 2004) may provide a forum and model for addressing major wildlife disease issues that involve multiple stakeholders with differing mandates, authorities and interests.

Brucellosis and Tuberculosis Eradication in Canada

Canada began programs to eradicate tuberculosis and brucellosis from domestic livestock in 1907 and 1928 respectively (Connelly et al. 1990, Essey and Koller 1994, Kellar and Dore 1998). Canada was declared free of brucellosis in domestic animals in 1985 (see Kellar and Dore 1998), but continues to have cases of tuberculosis in farmed cervids and sporadic cases in cattle (Rothwell 2000, Koller-Jones et al. 2005). Bison in

[3] In Canada, wood bison are listed as threatened under the federal *Species at Risk Act*. Wood bison are listed under Appendix II of the Convention for International Trade in Endangered Species, and are considered an endangered subspecies under the United States *Endangered Species Act*.

and around WBNP remain the primary nidus of brucellosis in Canada (Tessaro et al. 1990, Doré 1999), while WBNP bison and wild cervids (wapiti and deer) in Riding Mountain National Park (RMNP) in Manitoba (Lees 2004, Lees et al. 2003) are the wildlife reservoirs for BTB in Canada (see Nishi et al. 2006).

Part IX of the *Health of Animals Regulations* (*Health of Animals Act*) (Department of Justice Canada 1990a) establishes criteria for the eradication of tuberculosis and brucellosis in livestock and farmed ungulates in Canada. In 2000 (when we initiated this review), this section of the regulations outlined the following requirements:

> "The Minister may declare an eradication area or part thereof to be a tuberculosis-accredited area for a period of one year, if he is satisfied from tests or statistical analysis that the number of <u>bovines</u>, in the area or part thereof, affected with tuberculosis does not exceed 0.2% of the number of animals in the area or part thereof and that a surveillance program sufficient to assure the discovery of *Mycobacterium bovis* in the area or part thereof, will be maintained during that period.
>
> The Minister may declare a tuberculosis-accredited area or part thereof to be a tuberculosis-free area if no case of *Mycobacterium bovis* has occurred among <u>bovines</u> in the area or part thereof during the previous five years.
>
> The Minister may declare an eradication area or part thereof to be a brucellosis-accredited area for a period of three years if he is satisfied
>
> *(a)* from tests and statistical analysis that the number of <u>cattle</u>, in the area or part thereof, affected with brucellosis does not exceed 0.2% of the number of cattle in the area or part thereof; and
> *(b)* that a surveillance program sufficient to assure the discovery of *Brucella abortus* in the area or part thereof will be maintained during that period.
>
> The Minister may declare a brucellosis-accredited area or part thereof to be a brucellosis-free area if no case of brucellosis has occurred among <u>cattle</u> in the area or part thereof during the previous five years".

However, following the emergence of BTB in wild cervids and additional cases of tuberculosis-positive cattle in the area in the RMNP area (Lees 2004, Lees et al. 2003), the CFIA amended the *Health of Animals Regulations* by zoning Manitoba and Canada into BTB (TB)-Free, TB-Accredited, and TB-Accredited Advanced areas (CFIA 2002a, 2002b and see Nishi et al. 2006). This amendment (see sections 74 and 75 in Part IX of the *Health of Animal Regulations*) was done in order to maintain trade status with the United States and harmonize with United Stated Department of Agriculture (USDA) rules and regulations (USDA 2005) and international standards as set out by the World Trade Organization (Chapter 1.3.5 – OIE 2006b; and see Zepeda et al. 2001, 2005).

Establishing health status in livestock and captive bison

A government order posted in 1979 listed the following conditions as criteria for establishing British Columbia and the Peace River area of Alberta as being brucellosis-free: (1) "the last brucellosis field strain infection identified through epidemiological study or bacteriological analysis has been eliminated" and (2) "the bovine herd that contained the last infection has proved negative to a test for brucellosis performed at least 120 days after the last infected animal was removed" (Department of Justice 1979). The same criteria were used for similar orders posted for Manitoba, Saskatchewan and Alberta (with noted excepted Improvement Districts) (Department of Justice Canada 1984). The criterion used to declare Ontario as being a brucellosis low incidence area in 1980 was that "the number of brucellosis infected bovine herds does not exceed 0.3% of the herds within the region" (Department of Justice Canada 1980).

It is important to note that, within the definitions provided in the *Health of Animals Regulations*, "bovines" are defined as "cattle or bison domestically raised or kept and, for the purposes of Part II, does not include a bison that has ever been in contact with or part of a wild herd." Part II of the regulations involves the importation of animals into Canada and states, "No person shall import a bovine into Canada from a country other than the United States unless the certificate required by paragraph 10(1) (*b*) shows that the animal proved negative to tests for brucellosis and tuberculosis" (Department of Justice Canada 1990a).

Although recently ended, the Captive Ungulate Policy outlined relatively stringent and specific testing criteria for establishing and maintaining herd health status for a growing captive commercial bison industry (and farmed cervids) from 1985-2001 (Table 1). The Captive Ungulate Policy required that any additions to a negative herd must either be from natural increase or from another herd of negative status; any additions of bison from a herd that was not of negative status would result in the entire herd losing its negative status.

As the HLWBRP founder population was established from calves of an infected wild herd, the recovery project did not meet the criteria for eradication of tuberculosis or brucellosis as outlined in the Captive Ungulate Policy. This interpretation was substantiated by Dr. Maria Koller-Jones (pers. comm. – Appendix B) who stated that "instances of reportable diseases when they occur in free-roaming animals of these species are not subject to federal control/eradication measures", and that the CFIA has "no authority over/mandate in free-ranging herds or their movement while these animals are 'owned' by the public" and it "does not have a specific policy framework for defining disease-free status in a captive herd established from wild populations. Each case is assessed individually."

Table 1. History of surveillance for BTB in bison in Canada (adapted from Doré 2003)

Time Period | **Summary of Events**

1980s

- Bison farming industry was becoming established.
- Canada in final stages of bovine TB eradication program for traditional livestock.

1985

- Captive Ungulate Program (CUP) was established as an effort to expand the National BTB Eradication Program to include other potential reservoirs of infection, i.e. commercial game farms, public zoos, private ungulate collections.
- CUP required the identification, inventory, and testing of all commercially farmed herds of captive ungulates (bison, elk and deer).

1988-1996

- CUP required periodic and regular testing (intradermal caudal fold tuberculin ‘skin’ test) of all farmed bison.

1990

- CUP was strengthened through addition of movement controls, and amendment of import conditions for live captive ungulates from the United States and other countries.

1991-1996

- CUP required a federal permit for farmed bison to be traded in Canada. Permit issuance was based on the herd of origin having negative status for BTB. Negative status was defined as having been tested for BTB with negative results within three years prior to movement.

1995

- The interval for defining negative status under the CUP was increased to five years.

1996

- In consultation with Canadian Bison Association (CBA), requirements for disease surveillance and definition of negative herd status for farmed bison were amended. A herd of negative status for BTB could be attained and maintained by:
  a) BTB testing (tuberculin skin test) all bison in herd 18 months of age and older within the previous five years with negative results;
  b) BTB testing of at least 10% of the bison in the herd 18 months and older, with negative results;
  c) slaughter inspection (under federal or provincial inspection) of at least 10% of the bison in the herd 18 months and older, with negative results;
  d) or having completed a combination of b) and c) above.

1997

- Requirement for a federal permit to move bison within Canada was removed, and with it the federal requirement for a bison herd to have negative status for BTB. Mechanism for maintaining health status was based on market and industry requirements as opposed to federal regulatory requirements.

2001

- Termination of a separate captive ungulate program for bison.
- BTB surveillance program was re-named the Canadian Bison Association Negative Status Herd Program.
- The Canadian Food Inspection Agency and CBA identified issues for review of BTB surveillance activities for farmed bison. These issues included:
  a) verification that the surveillance program (through periodic whole herd testing or a combination of slaughter monitoring and trace-back investigation, and tuberculin testing) would meet international requirements of the Office International des Epizooties (OIE) by ensuring discovery of BTB, should it be present at a level of 0.1% or greater.
  b) Evaluation of slaughter monitoring of bison to determine whether an abattoir-based surveillance program could confirm BTB freedom, without the need for supplementary tuberculin testing of bison herds
  c) Evaluation of "higher risk" bison herds as a result of their geographic location (proximity to WBNP or RMNP), or the uncertain health status of their animal sources (i.e. menageries and zoological collections).
- Introduction of the industry-led, Canadian Cattle Identification Program through amendment of *Health of Animals Regulations*. The program is administered by the Canadian Cattle Identification Agency (CCIA) and involves the individual identification of all commercial cattle and bison that move beyond their herds of origin with approved ear tags (CCIA Tag).

2004

- The *Health of Animals Regulations* were further amended to enhance the national cattle (and bison) ID program and increase the efficiency and the effectiveness of trace back investigations performed by the CFIA for health or food safety reasons.

Nevertheless, previous experience with bovine brucellosis and tuberculosis in plains and wood bison in Elk Island National Park during the 1960s illustrated that disease eradication could be achieved and disease-free status could be established for bison salvaged from the wild (this report and see Nishi et al. 2002b). Unfortunately, it is difficult to translate the lessons learned from the Elk Island National Park experience into specific criteria for designating disease-free status in the HLWBRP.

Implications of International Policies and Regulations

World Trade Organization

As a signatory to the World Trade Organization (WTO), Canada is subject to disease control guidelines governing international trade in livestock as set out in the Agreement on Sanitary and Phytosanitary Measures (SPS agreement) (WTO 1995, Thiermann 2000, Zepeda et al. 2001, 2005). The WTO delegated the task of developing these guidelines to the Office International des Epizooties (OIE) – the World Organization for Animal Health (Kellar and Inch 1998, Willis 2000, Zepeda et al. 2005).

As with the *Health of Animals Act*, OIE guidelines regarding tuberculosis (Chapters 2.3.3 – OIE 2006b) and brucellosis (Chapter 2.5.1 – O.I.E. 2006b) focus predominantly on cattle and base their definition of disease freedom on the disease status of source herds and zones (or compartments – see Zepeda et al. 2005). An officially disease-free herd can take in additions from disease-free herds if they are not vaccinated and are negative to a series of tests. In turn, the requirement for herd additions to a disease-free herd can be waived if they have been isolated and subjected to a series of serological tests. This allows for a progressive establishment of an officially disease-free herd, but may require the establishment of a series of isolated herds.

OIE guidelines for tuberculosis and brucellosis do not refer to wildlife specifically, but wildlife is explicitly considered for other diseases. Sutmoller et al. (2000) reported that the OIE considers any territory on which African buffalo are infected with Foot and Mouth Disease (FMD) viruses to be an infected area. Indeed, several sections of Chapter 2.1.1 of the OIE's Animal Health Code refer to wild ruminants, wild pigs and wildlife in their FMD controls. Other sections of the Animal Health Codes, such as those dealing with Rinderpest and Rift Valley fever also discuss rules and regulations regarding the implications of wildlife reservoirs for animal translocations and how this would affect the status of a country or zone. In each case, there is a reliance on taking animals from disease-free zones and the use of quarantine and testing to reduce risk.

In short, the OIE has developed guidelines for international livestock by which a country (zone or compartment) can be recognized for being historically free from disease. It has established criteria by which countries can declare disease freedom from diseases that have never occurred or that ceased to occur without having to apply extensive, active surveillance. Similarly, the OIE has developed guidelines for the recognition of disease freedom for a few selected diseases – FMD, Rinderpest, contagious bovine pleuropnemonia, and bovine spongiform encephalopathy. However, guidelines to recognize disease freedom after eradication are still lacking for most diseases (Zepeda et al. 2005).

International Union for Conservation of Nature - Species Survival Commission

Concomitant with the development of international policy on livestock diseases and trade, there has been increased awareness of disease issues in wildlife management and conservation as they relate to the translocation, reintroduction, and recovery of individual animals and populations (Scott 1988, Karesh 1993, Karesh and Cook 1995, Lyles and Dobson 1993, Woodford and Rossiter 1993, Cunningham 1996, Woodroffe 1999, Deem et al. 2000, Deem et al. 2001, Lafferty and Gerber 2002, Leighton 2002, Wobeser 2002). Movement of wildlife across international borders and international trade in wildlife is subject to laws concerning animal health, animal welfare, and international movements of endangered species (Cooper and Rosser 2002). Similar to livestock trade, the laws and recommendations regarding animal health for international movement of wildlife are based on the Animal Health Code developed by the OIE.

The World Conservation Union (IUCN) is a science-based organization that facilitates international conservation initiatives and has developed general policy guidelines concerning the regulation of disease and the movement of wild animals. The IUCN's first policy statement on the translocation of living organisms (IUCN 1987) covered the general topics of introductions, re-introductions and re-stocking, and emphasized a pre-planned approach to anticipate and evaluate translocation activities.

Its second document, titled "Guidelines for re-introductions" (IUCN 1998), recognized that the health of released stock and any disease threats they may pose to native stocks were important in the pre-planning of a re-introduction. The guidelines emphasized that disease risk management is an important aspect of conservation programs that rely upon the re-introduction of animals to their native wild habitats. Recommendations were developed to ensure that "re-introductions achieve their intended conservation benefit, and do not cause adverse side-effects of greater impact" (p. 5 in IUCN 1998). The guidelines were general in nature and encouraged a multi-disciplinary approach for wildlife re-introductions; however, the guidelines also specifically recognized the importance of a veterinary screening process, health (disease) monitoring, and adequate provisions for quarantine if necessary (IUCN 1998).

The IUCN prepared a document entitled "Quarantine and Health Screening Protocols for Wildlife Prior to Translocation and Release" (Woodford 2001) that discusses some of the general and specific actions that need to be taken to minimize disease risks to released and native animals. These protocols outline a systematic approach (Figure 1) and provide an epidemiological basis for health screening and quarantine protocols. Five key elements were identified as important components of a risk reduction plan:

1) consideration of the health status of the source animals and animals at the translocation destination;

2) a period of quarantine;

3) appropriate health screening of captive animals;

4) consideration of legal and veterinary restrictions on the translocation of animals to and from certain geographic areas or populations; and

5) pre-release treatment or immunization when necessary.

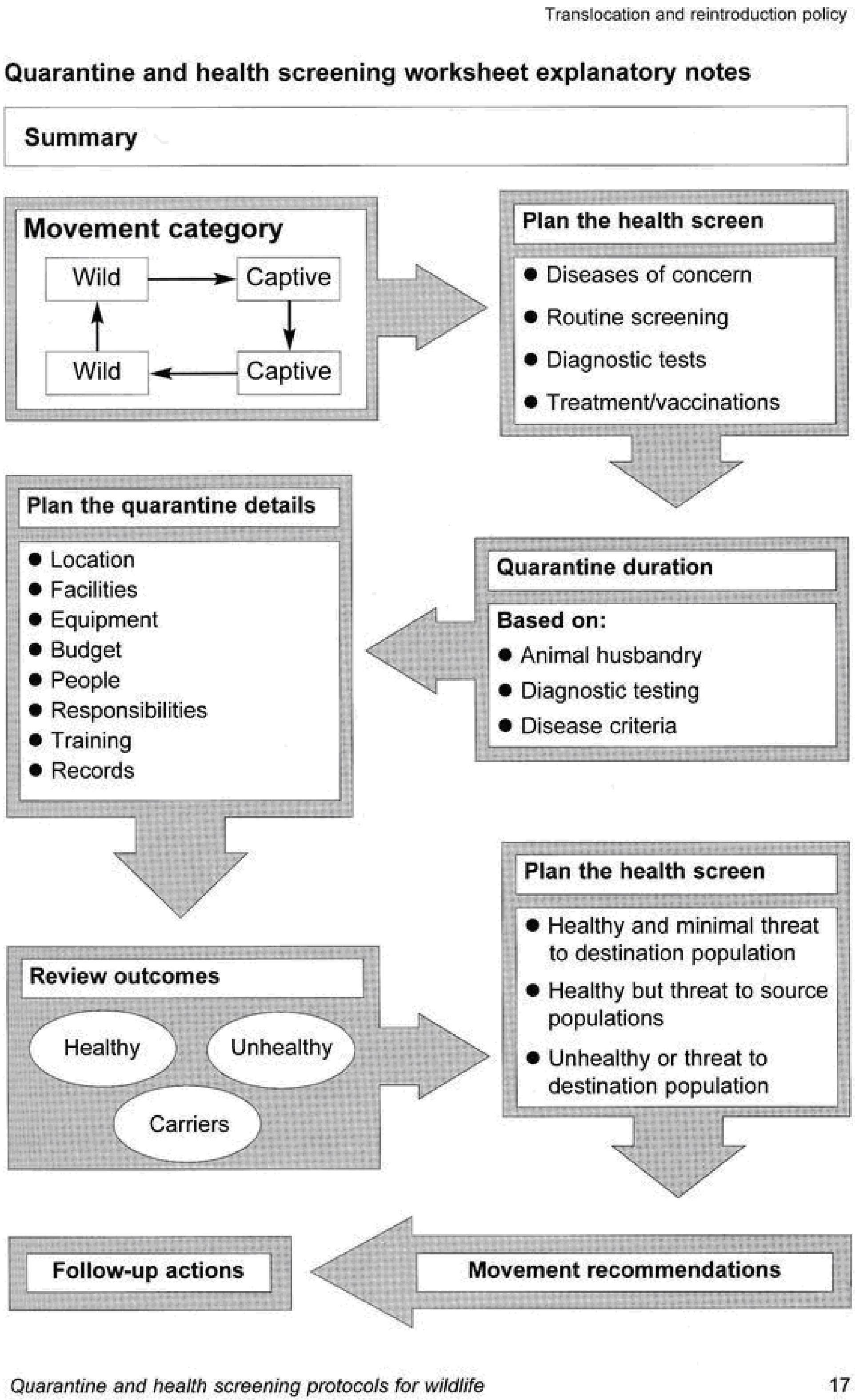

Figure 1. Summary of general quarantine and health screening protocols for wildlife prior to translocation or release to the wild (p. 17 in Woodford 2001).

Two elements were essential to effective quarantine. First, the duration of quarantine must be sufficiently long to allow for the detection of the disease. In the cases of diseases with lengthy or variable incubation periods, such as tuberculosis, it was recommended that quarantine be longer than the minimum recommendation of 30 days. For cervids in the United States, 93 days was suggested as a minimum for quarantine to allow for two tuberculosis tests 90 days apart. Recommendations for disease screening of Artiodactyla (even-toed ungulates) in quarantine include repeating negative tests several times in order to reduce the likelihood of false negative results. Second, quarantine methods and facilities must effectively isolate animals to prevent the transfer of disease-causing agents to or from quarantined animals. Proper housing and hygiene are essential to prevent disease transmission. Animals not from the same consignment or exposure cohort are isolated prior to release. Quarantine should be run as an "all in, all out" operation where any group that has an individual test positive begins quarantine anew after the removal of the positive animal.

The IUCN recognized limitations in the detection of a variety of wildlife diseases due to the lack of validated tests. However, they still emphasized clinical examination and disease screening as important components of quarantine (Figure 1). The IUCN suggested that prophylactic treatments and immunization be considered when captive animals present a possible disease risk or when domestic livestock at the release site could transmit important diseases to re-introduced animals. Only vaccines that were shown to be safe and effective in wildlife were recommended; as such, no vaccines for tuberculosis or brucellosis were recommended for Artiodactyla. The IUCN suggested that, where diseases of domestic animals were the primary concern, it may be preferable to immunize the domestic animals in the re-introduction area. There were specific instances where re-introductions were discouraged or restricted. For instance, it was recommended that Artiodactyla should not be moved to or from geographic areas or populations with BTB.

In a recent report, “Guidelines for the Placement of Confiscated Animals” (IUCN 2002b), the IUCN outlined wildlife disease considerations using a decision tree analysis that provides guidance to managers in selecting among three conservation-based options for confiscated wildlife:

1) return to the wild;
2) maintain in captivity; or
3) euthanasia.

They stated that “because of the risk of introducing disease to wild populations, confiscated animals that may be released must have a clean bill of health” and that “the animals must be placed in quarantine to determine if they are disease-free before being considered for release” (p. 22 in IUCN 2002b). A critical question posed in the decision tree is: “Have animals been found to be disease-free by comprehensive veterinary screening and quarantine, or can they be treated for any infection discovered?” Despite the fundamental importance of this question and the value of the decision tree approach, the guidelines do not provide specific criteria to address this question nor did they explicitly define how different factors would be considered at the decision point(s).

Disease Freedom, Disease-free Status, Health Status

The demonstration of disease-free status has become increasingly important because of trade requirements for international livestock markets (Dufour et al. 2001) and due to the greater awareness and need for managing disease risks in wildlife conservation (Woodford 2001). However, the recent development of new epidemiological methods and approaches to declare disease freedom has largely been driven by the need for objective evaluations of disease status in the livestock industry (Martin et al. 1992, Cameron and Baldock 1998a and 1998b, Audigé et al. 2001, Martin and Cameron 2002, Cameron et al. 2003, Stärk et al. 2002, Salman et al. 2003). The move towards science-based and transparent means for defining and establishing disease status in livestock (and wildlife) is based on a clear definition of disease freedom and the use of formal risk analysis approaches as an internationally accepted decision tool for managers.

Freedom from infection

Although "disease freedom" and "disease-free status" are often used interchangeably to mean the absence of a disease-causing pathogen(s) in an animal(s), herd(s), or population(s), it is important to distinguish between "disease" and "infection" and clearly define these tacit expressions of health status. The term disease[4] is generally used to describe a condition of illness or abnormal functioning in animals that is recognizable by a suite of clinical symptoms. Infection[5] refers to the condition where a

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[4] Disease: any deviation from or interruption of the normal structure or function of any part, organ or system of the body that is manifested by a characteristic set of symptoms and signs and whose etiology, pathology, and prognosis may be known or unknown. (Dorland's Medical Dictionary, 28th Edition)

[5] Infection: 1. invasion and multiplication of microorganisms in body tissues, which may not be clinically apparent or results in local cellular injury due to competitive metabolism, toxins, intracellular replication, or antigen-antibody responses. 2. an infectious disease. Cf. infestation. (Dorland's Medical Dictionary, 28th Edition)

susceptible animal has been exposed to and successfully invaded by a pathogen but may or may not show clinical signs. Although infections of animals may follow a typical course of pathogenesis, which includes incubation, prodromal presentation of early symptoms, manifestation of classic clinical disease symptoms, and a chronic or unapparent latent stage (Figure 2), the occurrence of infected non-diseased individuals (i.e. asymptomatic infections) presents a challenge because they cannot be identified clinically and yet their presence is unacceptable if the herd is claimed to be disease-free.

This is especially true for pathogens such as bovine tuberculosis (Pollock and Neill 2002) and brucellosis (Thorne 2001, Oliveira et al. 2002) where pathogenesis is not fully understood. The occurrence of unapparent latent infections combined with less than perfect sensitivity and specificity of diagnostic tests for live animals, which can vary unpredictably with stage and severity of disease, adds uncertainty to claims of disease-free status.

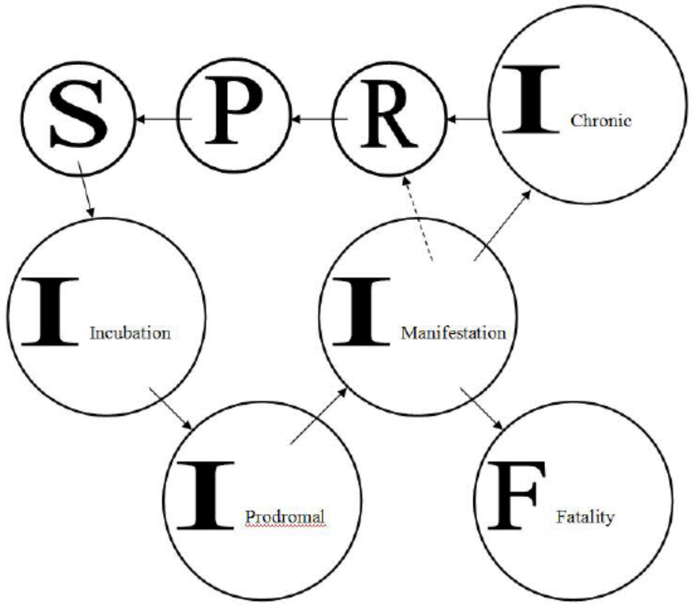

Figure 2. Extended SIRP disease model of Epiflex (taken from Hanley 2006). S: susceptible, I: Infected, R: recovered, P: partially immune, F: fatality. Extended SIRP breaks the infected stage (I) into 4: $I_{Incubation}$, $I_{Prodromal}$, $I_{Manifestation}$, $I_{Chronic}$, and adds a fatality terminating stage.

Therefore in the context of infectious diseases and animal health policies, the term "free from disease" or "disease-free" should be translated as "free from infection", and the inherent limitations of the detection principles for the infection must be considered (Martin et al. 2006). The different statuses of animals relevant to the problem of disease freedom are shown in Figure 3.

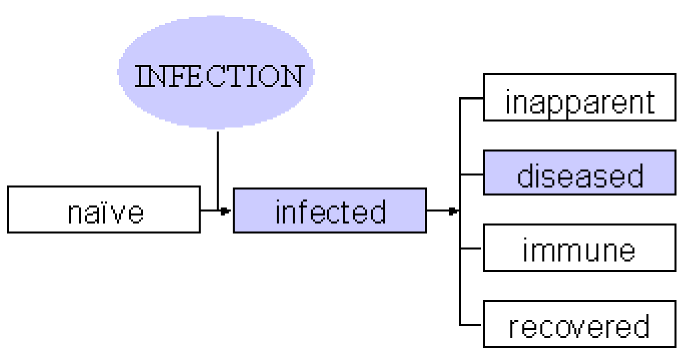

Figure 3. Statuses of a (live) animal relevant for the "disease-freedom" problem (from Martin et al. 2006).

The Risk Analysis Approach

The WTO's SPS agreement outlines international guidelines and provisions for member countries to facilitate trade, while taking measures to protect the health of humans, animals and plants (Thiermann 2000, Zepeda et al. 2005). In cases where there are no specific international standards, or where a member country chooses to apply more restrictive measures than those outlined by the OIE, it has to justify its position through a formal risk analysis. Thus, risk analysis principles have been accepted by the OIE, as a scientifically valid and defendable means of establishing policy on animal health (Zepeda et al. 2001, Murray et al. 2004a and 2004b).

Risk is defined as the probability of occurrence of an undesirable event, and the magnitude of the consequences (Ahl et al. 1993). With respect to animal health issues, risk analysis is a science-based, transparent framework that links an appraisal of an animal health hazard(s) to management decisions regarding the health status and movement of the animals. The complete risk analysis process (see Vose 2000, Leighton 2002, Murray et al. 2004a and 2004b) comprises four distinct steps, which include:

1) hazard identification;

2) risk assessment;

- release assessment

- exposure assessment

- consequence assessment

- risk estimations

3) risk management; and

4) risk communication.

An important risk-based principle is that, from a scientific perspective, it is impossible to prove the complete absence of infection in a population and disease freedom cannot be conclusively demonstrated (Zepeda et al. 2005). A risk assessment based on modern statistical approaches in veterinary epidemiology can provide a technical means of incorporating the uncertainty and variability of available data, and estimate the probability of occurrence and consequences of an animal health hazard. The determination, however, of whether the risk is acceptable is influenced by political and economic considerations (Hueston 2003). Therefore, in order to ensure the process is credible and transparent, it is important to undertake a risk analysis using a collaborative, multi-stakeholder approach (Zepeda et al. 2005).

Current approaches to demonstrating that a population is free from disease have been based on a formal or informal probabilistic approach (see Cameron et al. 2003). Formal probabilistic approaches are based on a statistical evaluation of surveillance data collected from a sample of the population. The evidence for disease freedom is based on the probability of obtaining the results observed if the population were infected (Cameron and Baldock 1998a and 1998b). This approach provides an estimate of confidence for a minimum detectable prevalence in the population, which is subsequently used as evidence for disease freedom (Dufour et al. 2001, Cameron et al. 2003). In this approach, however, as the prevalence in a population approaches zero, required sample sizes for a reasonable level of confidence can become extremely large. The indirect probabilistic approach incorporates multiple sources of data (in addition to data from structured population surveys), and was used by the OIE to establish the initial list of countries that were considered to be free from FMD (Cameron et al. 2003). This subjective approach, however, lacks quantitative rigor and is therefore less transparent and repeatable.

Recent advances in demonstrating disease freedom (Martin and Cameron 2002, Cameron et al. 2003, Salman et al. 2003) have emphasized the need to incorporate both risk analysis and analysis of surveillance systems to provide evidence that disease has not entered a population/country, and that disease is not present in a population/country (see Figure 1. in Cameron et al. 2003). To date, most of the analyses conducted on surveillance systems have been based on population-based sampling surveys (see Cameron and Baldock 1998a and 1998b, Cannon 2002). Recently, the use of decision trees (Smith and Slenning 2000) and scenario trees has been developed to provide a powerful and useful modeling framework for quantitative analysis of complex, non-survey based data sources (Cameron et al. 2003, Martin et al. 2006, Martin et al. 2007a and 2007b).

Policies, Practices and Experience in Canada and Abroad

Canada

British Columbia (BC)

Dr. Helen Schwantje, the province's wildlife veterinarian, (pers. comm. – Appendix B) stated that there are no set policies that are used in BC to govern the translocation of wildlife or for establishing a herd as being disease-free. An "in-house" transplant committee judges intraprovincial translocation of wildlife on a case-by-case basis. Dr. Schwantje will review the results of previous disease surveys in the capture and receiving area when those data are available as well as require serological testing and anthelmintic treatment of animals being moved.

The BC *Game Farm Act* does include bison in their definition of game (Government of BC 1996). Dr. Merv Wetzstein (pers. comm. – Appendix B) reported that the Act has a provision that says the province will work in conjunction with the CFIA to develop disease-free status for game farms, but no action has ever been taken on this. Disease control has largely been on a test and exclusion model in that animals being added to game farms require a veterinary certificate stating that the animals are free of clinical disease and because additions cannot come from zoos or wildlife parks.

Alberta (AB)

The document "Requirements for Movement of Farmed Cervids from Canada and the United States into the Province of Alberta" defines a captive animal as one that was "born and raised in captivity and, so far as can be determined, must not have had exposure to wild animals other than fence line contact" (Government of AB 2000). One of the conditions for importing a cervid into Alberta is that a written statement accompanies the animal verifying that the herd of origin was free from contagious and infectious diseases. Details of testing, treatment and source requirements are provided for a number of parasites and for chronic wasting disease, but none are specified for tuberculosis or brucellosis. The conditions for chronic wasting disease are based upon the transplanted animal originating from a premises on which the disease had not been diagnosed in the past five years, the animal and herd of origin not having contacted any animal diagnosed with the disease, the animal not being the progeny of an affected dam or sire, and the region of origin having an acceptable surveillance program in place for the disease.

The Deer and Elk Procedures Manual for Alberta (Alberta Agriculture, Food and Rural Development n.d.) does state that a herd must have a “negative health status” before any production animal can leave the game farm. Disease tests conducted by the CFIA for tuberculosis based on the mid-cervical skin test and brucellosis based on serum agglutination tests comprise a fundamental component of negative health status. All mature elk *(Cervus elaphus)* or deer *(Odecoileus spp)* in the herd must be tested to get a negative herd status. If a new farm originates from a negative farm, it may assume that negative status, but must have a whole herd test within one year. The AB Animal Health Division requires re-testing of the herd every three years.

Due to a 1988 moratorium affecting the importation of ungulates into Alberta, only one import protocol is allowed – the AB-Yukon Elk Importation Protocol. Under this protocol, importations are only allowed if the elk originate from a herd with a current CFIA rating of negative for tuberculosis and brucellosis, or in the case of a calf, the cow has tested negative for these diseases. In addition, all animals, excluding calves, must test negative for both diseases.

Elk Island National Park

Bison in Elk Island National Park have been the subject of two disease eradication campaigns. The first targeted brucellosis in plains bison. The disease was first detected in the winter of 1947. Park personnel fenced off a separate area of the park to use as an “isolation area.” The goal was to salvage a group of *Brucella*-free bison, maintain them in isolation and depopulate the infected wild herd (Nishi et al. 2002b). After 12 years of this approach, over half of the tested animals remained positive. A more intensive disease eradication program, which included vaccinating bison and a massive population reduction, was undertaken. Slaughter later progressed to include elimination of non-vaccinated and reactor animals. Twice-yearly herd testing was initiated. Those animals that evaded capture for testing were killed in the field. By 1969, only two reactor animals were detected. After two more years of testing and not finding any reactors, the herd was declared free of brucellosis.

The second disease eradication campaign occurred following a shipment of 21 wood bison in 1965 from WBNP. As part of an effort to salvage wood bison from WBNP, 47 free ranging bison were captured and those that tested negative for brucellosis and tuberculosis were shipped to the isolation area at Elk Island. In 1968, both diseases were detected in the herd. The herd was divided in two with the originally imported animals sent to one location and those animals born at Elk Island plus some calves added to the herd sent to another. Male reactors were slaughtered, as were pregnant females after calving. Calves were hand-reared. After removal of the original members of the herd, all tuberculosis and brucellosis tests in 1970 on the remaining animals were negative. The herd was declared free of the disease in 1971 (Nishi et al. 2002b).

The bison at Elk Island National Park are currently subjected to the same testing requirements imposed on game farmed bison in Canada (N. Cool pers. comm. – Appendix B). There are a number of unique elements that support this approach. First, the herd is considered to be a “fenced herd”. Second, infection from outside sources is unlikely because the herd is self-supporting and there is no nidus of infection near these animals. Finally, there are several decades of test results from which the disease status of the herd can be determined. These features may support the current classification of the herd as negative, but it is interesting to note that declaration of these herds as being free of brucellosis originally occurred after a very short period of negative herd results despite the presence of “spill-over” cases in local wildlife and the possibility of false negative results.

Saskatchewan (SK)

The *Diseases of Domestic Game Farm Animals Regulations* (Chapter D-30 REG 1, part 9) (Government of SK 1999) states these regulations do not apply to preventing the spread of diseases named under the *Health of Animals Act* or for quarantining animals affected by those diseases. Thus, these regulations would not apply to the control of CA and BTB. Moreover, bison are not included in either the definition of a big game animal or domestic game farm animal (Meschishnick et al. 2003).

Manitoba (MB)

The *Livestock Industry Diversification Act* was passed in 1997 to facilitate game farming in MB (Government of MB 2006). The Act allowed for the one-time capture of wild elk to provide foundation stock to the commercial elk farming industry. This initiative was taken despite the occurrence of BTB in a wild elk near an infected cattle farm in the RMNP area in 1992, and the subsequent discovery of an infected cattle herd in 1997 in the same vicinity (Lees et al. 2003, Lees 2004). Between 1992 and 2002, 10 (0.68%) of 1,463 shot or found-dead elk in the RMNP area were positive for BTB (Lees et al. 2003).

In an effort to support the developing elk farming industry, the Government of MB (1997a, 1997b) announced that it would capture wild elk over a five-year period (the

Elk Seedstock Program) to provide high quality, genetically diverse founder stock for the new industry. Through an equal opportunity draw system, the Elk Seedstock Program would make wild-caught elk available to residents of MB to assist them in diversifying into elk farming. All captured elk and captive born offspring would be assigned to the Province of MB, contractors and the MB First Nations Elk and Bison Council (MFNEBC). The Province of MB had also contracted with the MB Assembly of Chiefs on behalf of the MFNEBC to supply up to 250 wild captured elk for an aboriginal elk farming program (MB Department of Agriculture, Food and Rural Initiatives 2001).

MB discontinued the capture of wild elk in 1999 and the Elk Seedstock Program ended. In the capture program's four years, staff, First Nations Elk and Bison Council, and landowner contractors captured 730 elk, 438 of which were captured in 1999 (MB Conservation 2000, p. 95). However, some elk captured in 1999 tested positive to BTB tests. Consequently, there was concern that the captured elk had been derived from an infected wild herd and could not be classified as negative for BTB. No elk would be dispersed to producers until additional testing was done to ensure the animals were disease-free. As of March 31, 2001 the Province of MB was holding 497 elk at three licensed elk farms (MB Department of Agriculture, Food and Rural Initiatives 2001).

At the time that the Elk Seedstock Program was running, provincial and federal authorities had differing views on the acceptability of the wild-caught elk as sources for a commercial game farm industry. Provincial authorities (Dr. T. Whiting pers. comm. – Appendix B) turned to the USDA’s recent rules on tuberculosis in cattle, bison and captive cervids to support their claim that these captive elk can be considered disease-free (USDA 2000). These rules define an “accredited herd” as “a herd of captive cervids that has tested negative to at least three consecutive official tuberculosis tests of all eligible captive cervids... The tests must be conducted at 9-15 month intervals" (p. 63,523 in USDA 2000). An "accredited-free zone" is defined as a "part of a State that… has zero percent prevalence of affected captive cervid herds, and has no findings of tuberculosis in any captive cervid herds in the… zone for the previous five years" (p. 63,524 in USDA 2000). Captive cervids that originate in an accredited-free zone can be moved interstate without restriction. These rules define an "affected herd" as "a herd of captive cervids that contains or has contained one or more captive cervids infected with *Mycobacterium bovis* and that has not tested negative to three whole herd tests" (p. 63,524 in USDA 2000). A newly assembled herd will be classified as having the status of the herd from which they originated. The rules do not discuss how they are to be applied when the herd of origin is an infected wild herd. Although the prevalence of infection in the MB wild herd could not be accurately estimated due to the sampling methodology used, it was reasonable to conclude that the herd of origin in this case was infected, thus the captive herd originating from the RMNP area could not be classified as negative when first assembled. But, if all animals in a herd had three negative tests 9-15 months apart, they would meet the definition of a disease-free herd if the status of infection in the wild herd was not considered.

However, because the federal *Health of Animals Regulations* required a federal movement permit for cervids in Canada, the CFIA had to determine and be satisfied that the movement of these elk would not be likely to result in the spread of bovine tuberculosis before it could issue the permit. Given the occurrence of nine confirmed cases of BTB in free-ranging elk between September 1998 and January 2001 (see

Copeland 2006), the CFIA considered there to be significant risk of finding at least one infected elk in the captured herds. The CFIA undertook a thorough review of all the evidence to date and issued a permit for approximately half of the captured group of elk in 2001 (the Northern Capture Zone (NCZ) group) because it considered the group not to have been exposed to bovine tuberculosis in the wild. The CFIA then recommended a series of additional testing and slaughter requirements for the Southern Capture Zone (SCZ) group that it deemed at "significant risk" of being infected with bovine tuberculosis (Table 2). The premise of the CFIA's recommendation was that all wild-caught elk in the SCZ – despite an initial series of negative skin tests – would have to be humanely destroyed and shown to be negative for BTB on post mortem and laboratory examination of representative tissues (and lesions) to demonstrate that there were no infected and/or infectious animals. The CFIA also required that all captive-born progeny be subjected to additional skin tests (with negative results) and that all skin test reactors be euthanized and subjected to a full necropsy and laboratory examination of tissues (Table 2). Following completion of the protocol and a final comprehensive assessment of skin test results, laboratory results and epidemiological investigation for all wild-caught and captive-born elk, a movement permit was issued for all remaining captive-born progeny (Table 2). Although the CFIA movement permit was not a declaration of official disease-free status, it essentially provided an equivalent level of confidence in the health status of the elk so that the animals could be integrated in to the commercial elk farming industry.

The initial debate associated with criteria for disease freedom of the captured wild elk in Manitoba is a good illustration of the uncertainty and difficulty in applying rules that were developed for commercial livestock to wildlife. Although the USDA rules and policies developed for commercial livestock (including captive bison and cervids) are very specific with respect to disease testing requirements, they do not necessarily apply to wild cervids (or bison) that are captured from a known infected free-ranging herd. The main reason for this is that the USDA rules (and rules for commercial livestock in general) do not consider the situation where the herd of origin is a wild free-ranging herd with either unknown or known infection status.

In general, health regulations for livestock are based on the circular logic of two tacit assumptions:

1) the herd of origin is disease-free, or at the very least there is a known history of the herd with a record of adequate disease-testing data; and

2) any known infected captive livestock or commercial ungulate herd(s) would have been eradicated as part of a national tuberculosis eradication program.

In effect, therefore, livestock rules are generally designed to maintain confidence in the current situation where a disease has been eradicated or where the prevalence is approaching zero. The rules do not provide the criteria or a mechanism for applying disease-free status to herds originating from a wild, infected herd of origin, because they are not designed to prescribe the basis for disease eradication.

Table 2. Summary and criteria for Canadian Inspection Agency (CFIA) movement permits for wild elk captured in MB.*

In 1999, *ca*. 250 wild elk were captured in MB from two general areas – Northern Capture Zone (NCZ) and Southern Capture Zone (SCZ) – and were being held in isolation at four and two game farm facilities, respectively.

- In July 2001, the CFIA reviewed all available information pertaining to the various groups of captured wild elk and their origins including:

    1) results of tests and examinations conducted since capture in 1999;

    2) the location of capture sites in relation to recent findings of *M. bovis*-infected elk in and around RMNP;

    3) the nature and scope of disease surveillance activities in free-roaming elk in and around RMNP, Duck Mountain and the Interlake area

NCZ – Swan Valley and Interlake areas

- The CFIA concluded that elk captured in the NCZ and their progeny were eligible for movement permits because they believed that moving those elk would not likely result in the spread of BTB. Considerations for this determination included:

    1) capture locations, which were located ≥100 km from the northwest corner of RMNP, and approximately ≥110 km from the nearest case of *M. bovis* in either wildlife or livestock (at the time);

    2) no evidence of *M. bovis* in the free-ranging elk population in the Duck Mountain area (population estimated at 1,600-2,200 animals) – surveillance data based on 50 samples from the most recent hunting season and samples from previous years (*ca*. 20-25 samples/yr) were negative for *M. bovis;* and

    3) all tests and examinations conducted on these elk were negative for *M. bovis.*

SCZ – Riding Mountain and Duck Mountain South areas

- Because of recent findings of nine confirmed cases of *M. bovis* in wild elk in and around RMNP since 1998, CFIA concluded that there was a significant risk that one or more animals captured in the SCZ could be infected with *M. bovis.*

- A risk management plan was developed based on additional surveillance measures required by the CFIA.

1) All captured elk would be euthanized humanely and subject to a full post-mortem examination under the supervision of a CFIA veterinary inspector.

2) No captive-born elk in SCZ group would be eligible for a movement permit until all captured elk had been euthanized and determined to be negative for *M. bovis* based on the following criteria:

   - A complete and representative selection of tissues (specific lymph nodes of the head, neck, and thorax) was to be submitted for histological examination to the CFIA Centre of Expertise for Mycobacteriology from every captured elk that was destroyed, together with any visible lesions that were found during the post mortem examination of each elk.

   - Any tissues with a histological diagnosis of mycobacteriosis or mycobacteriosis-suspect were to be submitted for culture examination with negative results.

     - mycobacteriosis – presence of granulomatous inflammation characterized by caseous necrosis with or without calcification, surrounded by variable number of inflammatory cells, such as neutrophils, macrophages, epithelioid cells and multi-nucleated giant cells, and with or without a fibrotic capsule in affected tissue and the presence of acid-fast staining bacilli in lesions; or

     - mycobacteriosis-suspect – presence of a tubercle or granulomatous but no acid-fast organism detected in the lesion, or presence of a tubercle or granuloma-like lesion resembling that of tuberculosis and mycobacterium-like organism is detected in the lesion).

All captive-born elk (progeny born in 1999 or 2000) would be tested at least two times with mid-cervical tuberculin (MCT) tests conducted at least six months apart, and the first test was to be done at no less than six months of age.

- Captive-born progeny born in 2001 required one MCT test conducted when the youngest animal born was at least six months of age.

4) Any elk with a positive MCT test (as outlined in item 3 above) would be euthanized humanely and subject to a complete post-mortem examination as outlined in items 1 and 2 above, with negative results.

5) The CFIA would carry out all required tuberculin tests, laboratory examinations and epidemiological investigations at its cost. The owners of the elk would be

responsible for all costs associated with presenting and holding the animals for testing.

- Of 175 elk captured in the SCZ during 1999, 166 animals comprised the founding group (four animals were released at time of capture because they were unsuitable and five animals were euthanized following capture- and transport-related injuries)

- Captured elk were tested three times in March 1999, November 1999, and December 2000. A total of 27 MCT reactors were identified, euthanized and necropsied. All samples were submitted for laboratory examination with negative results for *M. bovis*. Two additional animals were destroyed and euthanized (one animal was injured during testing and the other was diseased) with negative results for *M. bovis*. Capture group comprised 137 elk.

- Two animals died of apparently natural causes. Carcasses were not available for post-mortem examination, but both animals had negative results on preceding three MCT tests. Capture group comprised 135 elk.

- From August to October 2001, 134 of 135 elk were euthanized and subject to full necropsy and laboratory examination of tissues; one animal died and the carcass was unavailable for examination and sampling.

    - No visible lesions were observed in 90 of 134 elk at post mortem examination. Follow-up laboratory examination confirmed absence of *M. bovis.*

    - One or more visible lesions were found in 44 of 134 elk, most commonly involving the tonsils, lungs or liver.

        - Mycobacteriosis (including acid-fast bacilli detected) was observed in tissues of two elk. One of these elk tested positive for *M. tuberculosis* complex (of which *M. bovis* is a member) on a PCR assay**.

        - *Mycobacteria* species (*M. avium* complex) were cultured from tissues of 4 of 44 elk, but no acid-fast bacilli were observed.

    - Between December 1999 and spring 2002, all captive-born elk were subject to the required MCT tests as required by CFIA's risk management protocol. Eleven of these captive-born animals were MCT reactors and euthanized. Follow-up post-mortem and laboratory examination of submitted tissues were negative for *M. bovis.*

- On 9 October 2001, based on all available information (i.e. MCT tests, epidemiological investigations, post-mortem examinations and laboratory results), the CFIA concluded that moving any of the remaining captive-born elk in the SCZ group would not likely result in the spread of bovine BTB and that those animals were eligible for movement permits.

***** This summary table is based on correspondence from CFIA (Dr. M. Koller-Jones, Senior Program Specialist, Disease Control, Animal Health and Production Division) to MB Agriculture (Dr. A. Preston, Director, Veterinary Services Branch, Agricultural Development and Marketing) dated 30 July 2001, 10 August 2001, 24 August 2001, and 9 October 2002.

** Although the PCR assay results suggested that this one animal may have been infected with *M. bovis*, the evidence suggested that if this elk were infected, it posed a negligible risk of exposing the captive-born progeny to the infection.

United States

Yellowstone National Park

A final environmental impact statement on bison management for the State of Montana and Yellowstone National Park was published by the United States Department of Interior in August 2000 (US National Park Service 2000). An interagency team defined the purpose of this program as follows: “to maintain a wild, free-ranging population of bison and address the risk of brucellosis transmission to protect the economic interest and viability of the livestock industry in the state of Montana.” The team agreed that the elimination of brucellosis was not within the scope of this management plan, but that it was a long-term objective. A series of ten alternate courses of action are described in the environmental impact statement. Each contains one or a combination of the following methods to work towards brucellosis elimination: vaccination of bison once a safe and effective vaccine is developed, capture and slaughter of seropositive bison, control of bison population size and movement; and/or removal of seronegative animals to a quarantine facility.

Appendix B in Volume 1 of the Impact Statement described quarantine protocols that would allow brucellosis-exposed bison originating in Yellowstone or Grand Teton National Parks to qualify as brucellosis-free. Native American tribes, parks, preserves and "other appropriate recipients" are identified as likely recipients of bison released from quarantine. The plan makes the initial assumption that all animals entering an approved quarantine facility are brucellosis-exposed even though all must test negative on an official brucellosis serological test before entering the facility. All animals captured in a single season are managed as a group and subdivided into smaller separate individual test groups. Holding pens and individual test group pens are to be double fenced and at least 10 ft. apart. Once captured, the animals are subjected to a varying number of tests for brucellosis depending on their age and reproductive status (Table 3). It was recommended that each animal in a group be tested every 30-45 days until all reactors are removed and the remaining animals test negative. All culture-positive animals or reactors are removed from the herd within 15 days.

Additional requirements must be met before and after release from quarantine. Calves from females that are pregnant when entering quarantine or born into an individual test group with a reactor cannot be released as calves. Calves from a negative individual test group can be released from quarantine at six months of age if: (1) there have been no reactors in the tests group one month before and immediately after their birth; (2) all calves are seronegative; (3) each adult in the test group is seronegative at least 30 days post calving and culture negative five days post calving; and (4) the adults in the test group have tested negative on three tests over the past 12 months. Neutered animals can be released from quarantine without restrictions. Pregnant females must complete two calvings within the quarantine facility. Non-pregnant females not born in the facility must be bred to a test-negative male within an individual test group, complete gestation, calve, and then pass three tests. An entire individual test group must qualify for quarantine before an individual within that group can be released. All animals released from quarantine are required to be tested at six months and one year after release and maintain their negative status. If any culture or serologically positive animals are found within a test group, the entire group restarts its test requirements by testing every 30-45 days until all reactors are removed from the group. Bred females that are not pregnant are sacrificed and necropsied and a complete epidemiological assessment is conducted.

Establishing quarantine and release programs would require modifications to existing regulations for interstate movement. In particular, the US Animal and Plant Health Inspection Service (APHIS – USDA) would have to allow bison to be moved to the facility and the state animal health authorities would have to approve the operation of the facility and allow movement of bison into that state.

Table 3. Testing and quarantine requirements described in the final environmental impact statement on bison management for the State of Montana and Yellowstone National Park, US Department of Interior in August 2000 (US National Park Service 2000).

	Minimum number of negative tests required to release	Minimum test intervals	Minimum quarantine period
Sexually mature males	3	**1st**: start of quarantine **2nd**: at least 180 days after 1st test **3rd**: at least 12 months after 1st test	1 yr
Pregnant females	5	**1st**: before calving **2nd**: 30-90 days after calving during the 1st and 2nd calving **Last**: 6 months after the last animal in the group has calved during the 1st and 2nd calvings	1 ½ yrs
Non-pregnant sexually mature females	3	**1st**: before breed **2nd**: 30-90 days after each animal has calved **3rd**: 6 months after the last animal has calved	1 ½ yrs
Immature males	3	**1st**: start of quarantine **2nd**: at least 180 days after 1st test **3rd**: at least 12 months after the 1st test and at least 3 years of age	1 yr
Immature females	3	**1st**: before bred **2nd**: 30-90 days after each animal has calved **3rd**: 6 months after the last animal has calved	2 ½ yrs
Calves	1	6 months of age	½ yr

Recently, Aune and Rhyan (2004) developed a feasibility study for bison quarantine procedures for the Montana portion of the Greater Yellowstone Area (GYA), and designed a capture program to achieve three general goals:

1) Develop quarantine procedures, using the best available science and adaptive management strategies, which will allow bison from Yellowstone National

Park to be accepted for translocation and utilized for the establishment of new pubic and Native American bison herds or to augment existing populations in North America.

2) Conserve the genetics of free-ranging Yellowstone bison through creation of additional bison herds in other habitats in North America without transmitting brucellosis onto these landscapes.

3) Examine the feasibility of quarantine protocols and the reintroduction of bison to large grassland systems as a conservation strategy that may benefit the management of bison in the GYA where populations are expanding beyond social tolerance limits.

The quarantine protocol developed by Aune and Rhyan (2004) is summarized in Table 4, and is largely based on the testing and quarantine requirements outlined in the environmental impact statement on Yellowstone bison (US National Park Service 2000). The overall approach is consistent with the broad framework outlined by Woodford (2001) (see Figure 1).

The feasibility study has been initiated with Phase I implemented in January 2005. Of 103 bison transported into the Phase I research facility, 95 animals qualified for participation in Phase II of the study after being screened for brucellosis in the field and subject to subsequent testing (Aune 2006). Following extensive public consultation on an environmental analysis of Phase II and III (Montana Fish, Wildlife and Parks and APHIS 2005), construction of the Phase II facility was undertaken in fall 2006 (Aune 2006).

Table 4. Summary of proposed quarantine procedures to develop brucellosis-free stock through the capture and treatment of Yellowstone bison (Aune and Rhyan 2004).

1) The quarantine feasibility study procedures will include processing two groups of bison calves through the quarantine facilities in 4-5 years.
   a. Initial capture and testing operations will select 50 negative calves (40 females and 10 males) out of Duck Creek and/or Gardiner capture facilities during winter operations.
   b. All calves will be tested and held in field capture facilities until the FP and Card serologic tests are completed (1day).

2) Test negative bison will be assembled in a holding pasture at the Brogan Bison Facility-Phase I.
   a. After the final assembly, bison will be retested using all known test methodologies to sort them into two groups of 20-25 bison and all suspect or positive animals will be removed.
      i. The test panel may include - Card, BAPA, Standard Plate, Standard Tube, CF, Rivanol, PCFIA, and FP. Additional tests, such as PCR, may be added as they become available.
      ii. Blood samples will be collected and sent to the National Veterinary Services Laboratories for culture.
   b. Animals negative on this full panel testing protocol will be placed into two large upper pastures in test groups through the spring and early summer.
   c. Mid-summer the groups will be brought to two lower pastures (after pastures are irrigated through the growing period) and upper pastures will be rested.
   d. Bison will be retested and blood cultured prior to transfer to Phase II.
   e. The Phase I facility will be cleared in late summer, rested and then prepared for set number two of 50 calves. The grazing process outlined above is repeated again in year two.

3) Following the final testing in Phase I and sorting of any positives, the test groups will be moved into Phase II in November-early December. Hay fields at Dome Mountain will be harvested prior to introductions to the new facility.
   a. Bison will be sorted into test groups of 10-20 and fed through winter and summer.
   b. Group separation would be maintained through Phase II.
   c. Breeding will be allowed in late summer-early fall using the young bulls or introduced Yellowstone bulls retained by USDA from the Idaho facility.
   d. Bison will be retested in late November and sorted by pregnancy status.
      i. Non-pregnant cows will be retained in Phase II and as well as a few bulls from set number one. These females will be bred the next year. These animals will provide benefits in herd management during the second year because they will be familiar with the settings.
      ii. Pregnant cows and some bulls will advance to Phase III.
   e. The Phase II facility will be prepared for set number two.

4) Pregnant bison will be introduced into the Phase III calving facility at Lens Lake in November-Early December.
   a. Pregnant bison will be initially sorted into groups of 10-20 in large open pens in the company of a few bulls.
   b. Bison will acclimate through the winter – December-March.
   c. As calving approaches bison will again be sorted into smaller test groups of 5-8.
      i. Bulls will be separated from cows during calving.
      ii. Pregnant cows groups will be sorted into calving paddocks.
         - Pregnancy and calving progress will be monitored with vaginal implants and intense observation.
         - Calves will be allowed to mature in paddocks until they are ready for open pastures at about 2 months of age.
   d. All test negative cows with viable calves will be sorted back into field pastures until late fall or early winter.
      i. Bulls will be with these pasture groups to breed cows that cycle back in summer-late fall.
      ii. Bison test groups negative for all tests and demonstrating one successful calving are finally sorted and grouped for soft release.

5) Failed test groups (groups in which one or more animals become positive by serologic test or culture) will be sorted back to recycle.
   a. Negatives in that exposed group will be placed back through another breeding/testing cycle and will remain an isolated test group to avoid exposure to incoming test groups.
   b. Positive animals will be sent to slaughter or research.
   c. Selected animals and calves meeting quarantine standards will be reintroduced no later than December to allow acclimation at the release site. Many if not all of these may be pregnant.
   d. Phase III facility will be prepared for set number two.

6) A final review panel will evaluate protocols, then streamline procedures using data from the pilot study.
   a. A study report will be completed and a peer review of quarantine procedures will be conducted.
   b. If successful, new or improved strategies for quarantine procedures will be adapted in any future implementation.
   c. In the event of failure, further investments in capital will be reconsidered.
      i. Complete failure may result in a decision to remove facilities and abandon quarantine efforts.
      ii. Partial failure may result in modification of procedures and a second study effort.
   d. A cost/benefit analysis of the procedures will be conducted and the funding needs for future programs or a complete de-mobilization of the program will be estimated.

Montana

The Montana Department of Livestock (n.d.) has specific requirements for importing bison that include negative official brucellosis testing within 30 days of entry and negative tuberculosis tests within 60 days. This is waived if the animals originate from an officially certified disease-free herd or if they are progressing directly to slaughter. There is a provision to add on extra quarantine time for a 45-120 day brucellosis retest after arrival in Montana. Brucellosis testing is waived for steers, spayed heifers and calves younger than 12 months of age and for Official Calfhood Vaccinates. The Montana Department of Livestock makes no legal distinction between publicly and privately owned bison and will remove, from within the state boundaries by the safest and most expeditious means possible, bison originating from an infected herd (US National Park Service 2000).

Idaho – BTB in cervids

In the Idaho Health Requirements Governing the Admission of Animals (USDA APHIS Veterinary Services 2005), bison are included in the definition of cattle and it is implied that this refers to bison raised for meat. Typical of most jurisdictions, Idaho does not allow the importation of animals “affected with or which have been recently exposed to infectious, contagious or communicable diseases, or which originate in a quarantine area.” Additional brucellosis and tuberculosis entry requirements are imposed on cattle/bison. Brucellosis requirements focus on calfhood vaccination and testing. The nature and frequency of testing depends on the brucellosis classification of the state of origin. As for tuberculosis, entry is dependent on the classification of the herd of origin and the results of individual animal testing for tuberculosis.

Idaho Statute Title 25 Chapter 6 (Bang's disease) deals specifically with the risk to humans or livestock from infectious diseases that may be carried by bison moving from adjacent Yellowstone National Park into Idaho. It provides the legislative support to physically move the animal back into the park, destroy the animal with firearms, and/or properly dispose of any animals killed or found dead (State of Idaho n.d.).

Idaho Statute Title 25 Chapter 3 (Tuberculosis Free Areas) provides an unsatisfying definition of a disease-free area as one where "all cattle, other bovidae, captive cervids, captive antilocapridae or camelidae in such county, counties, areas or the entire state have been tested and the records of such test show the disease has been eradicated to a minimum percent that the state division and federal department of agriculture in their regulations designate as free from the disease…" (State of Idaho n.d.). It is assumed, therefore, that United States Department of Agriculture recent rules on tuberculosis in cattle, bison and captive cervids (USDA 2000) would serve as the guideline.

Wyoming – Brucellosis in free-ranging elk

Brucellosis was first diagnosed in free-ranging elk from the National Elk Refuge in Wyoming in 1930. There is a brucellosis management plan in place for elk in Wyoming that consists of surveillance and vaccination of elk. Vaccination efforts are targeting animals captured on state and federal feed grounds. Here, elk congregate in higher concentrations than normal, thus facilitating the transmission of disease. The goal of this program is to prevent the transmission of brucellosis to cattle. In recent years, emphasis has shifted from eradication of the organisms to risk reduction to a point where transmission between elk and to cattle is eliminated. However, there is no defined end-point for the program at which the wild herd will be declared free of the diseases.

Wyoming's State regulations (Livestock Board Import Rules) (Wyoming Secretary of State 2004) do not allow any animal with or recently exposed to infectious, communicable or contagious diseases or that originate from a quarantine area to be moved into the state. With respect to bison, the state requires that animals coming from a brucellosis-free or Class A or B state must test negative to two or more different USDA approved official tests. The tests must be confirmed at a state or federally approved laboratory within 30 days prior to entry (unless meeting exceptions below). Animals will be held under quarantine for 45-120 days pending a retest. The state requires that interstate movement meet the current Code of Federal regulations and Uniform Methods and Rules. No tests are required for animals going straight to slaughter, steer and spayed heifers, calves under four months of age from a negative herd, bison from brucellosis-free herds in class Free or A states and those heifers with official calfhood vaccination. All bison entering Wyoming must test negative for tuberculosis within 30 days prior to entry.

Michigan – BTB in white-tailed deer

BTB was first detected in free-ranging white-tailed deer in Michigan in 1994 and has since been established in the wild herd (Schmitt et al. 1997, McCarty and Miller 1998, O'Brien et al. 2002, O'Brien et al. 2006). A strategy was developed to eliminate the risk of transmission of BTB from free-ranging deer to livestock in Michigan. Deer densities had been maintained above carrying capacity by supplemental feeding practices. The combination of a high density of susceptible and infectious animals coupled with increased likelihood of transmission at feeding stations were implicated as the reason for this outbreak and were targeted for control efforts (Chaddock 1998). These included bans on feeding wild deer, especially the use of feeding stations, and relaxed hunting restrictions. The "Health Requirements Governing the Admission of Animals into the State of Michigan" requires that all animals being imported into Michigan originate from a herd or flock that is apparently free from and has not been exposed to infectious diseases nor be under quarantine for any reason. Specific testing requirements are given for tuberculosis and brucellosis for cattle and bison that include negative tests within a specified time prior to entry to the state and they need to originate from tuberculosis and brucellosis free herds. While the stated goal of the program is to eliminate the disease from Michigan's wild deer and other wildlife (Chaddock 1998) it is not clear how that endpoint is defined. Although Chaddock (1998) defines positive animals as those whose tissues reveal genetic and biochemical evidence that the bacteria grown from hunter submitted tissues are *M. bovis*, no definition of a negative herd is provided. Ongoing hunter surveillance of deer, elk and other wildlife along with continued livestock surveillance appears to be the primary mechanism for determining when this endpoint will be met, however, no endpoint has been declared. Early in the program, when the USDA was considering a split-state status for Michigan, a minimal prevalence was discussed. Once cases were found throughout the state, this option was dropped and Michigan officials are again discussing an acceptable prevalence and sampling plan with which to declare that there is no risk from wildlife.

Illinois

The *Illinois Bovidae and Cervidae Tuberculosis Eradication Act* requires that all owners of bison, cervids and other domestic ruminants submit their animal for tuberculosis testing upon demand, test positive animals are destroyed and, if confirmed by examination of tissues, the entire herd may be depopulated. As was seen in many jurisdictions, animals in quarantine may not be moved until the quarantine is removed, except to an approved slaughter facility. Quarantine may be imposed if there is suspicion of exposure to positive animals and would not be not lifted until official tests or necropsy failed to show evidence of tuberculosis. Their Act allows the state to certify a herd as being free of tuberculosis, although the precise requirements for attaining this status are not specified.

Oregon

The Oregon Revised Statues (Chapter 621, 2005 Edition) defines a disease-free herd as “a herd of cows, sheep or goats that is not an infected herd”. They define an infected herd as “a herd of cows, sheep or goats in which one or more reactor animals have been discovered by any test authorized by law and that has not regained its disease-free status following the slaughter of the reactor animals and retesting of the herd by the department” (Oregon State 2005).

Non-domestic Hoofstock

The National Tuberculosis Working Group for Zoo and Wildlife Species (2001) in the United States developed standardized and specific guidelines for the detection and control of tuberculosis in non-domestic hoofstock[6] from zoological collections. This work on exotic animal collections was done to support the goal of the Cooperative State/Federal Tuberculosis Eradication Program to eradicate bovine tuberculosis (see USDA 2005).

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[6] Livestock (cattle), bison, and deer were excluded because they are subject to federal regulations for control of tuberculosis.

Other Species/Situations

Sub-Saharan Africa – BTB and brucellosis in African Buffalo

Free-ranging African buffalo (*Syncerus caffer)* populations in the Kruger National Park and Hluhlwe/Umfolozi Park are endemically infected with a number of significant livestock diseases including bovine brucellosis and BTB (see de Vos et al. 2001, Caron et al. 2003, Jolles et al. 2005, Rodwell et al. 2001a, 2001b). In addition to brucellosis and BTB, the buffalo can also maintain and transmit FMD, and Corridor disease (CD) – approximately 85% of buffalo in South Africa are permanent carriers of one or both diseases (Anononymous 2002).

Due to the commercial value of buffalo as a big-game species for hunting and ecotourism, and the importance of salvaging genetic diversity from the diseased herds, a disease control protocol for buffalo has been developed. Dr. Roy Bengis (pers. comm. – Appendix B) outlined an approach that is being applied to create disease-free herds of wild buffalo (see Anononymous 2002). The protocol allows government agencies, commercial game capture operators and registered game farms to propagate disease-free buffalo within a defined quarantine and testing protocol subject to strict veterinary control and oversight. The motivation for this program is to enhance the capacity to translocate buffalo to other National Parks, game reserves or game ranches outside the traditional buffalo distribution area. Buffalo are highly desired in such areas due to their ecological and eco-tourism value, but concerns regarding transmission of disease to livestock limit their movements by man.

The protocol (Anonymous 2002) establishes rules for disease-testing that are applied to the adult buffalo parent stock and foster cattle (i.e. dairy cows) (see summary in Table 5), and then specifies the three approved systems for creating disease-free calves.

Testing of Adult Buffalo and Foster Dairy Cow (adapted from Anonymous 2002)

1) Buffalo Parent Stock

Tuberculosis (BTB) Testing

• If pregnant cows and breeding bulls are initially sourced from BTB negative projects[7], these adult animals must all be subjected to a negative comparative tuberculin skin test, and gamma interferon test at capture and a repeat test on release from the facility.

• For animals sourced from herds of unknown BTB status, two further BTB tests must be done at the first opportunity after the required three-month window following initial and secondary tuberculin exposure. Only then can they be considered BTB-free, and join a BTB-free herd. No calves with dams sourced from herds of unknown BTB status, may leave a project (stage 3) until their dams have undergone three negative tests for BTB.

• BTB negative adult buffalo sourced from known BTB-infected herds must be kept under intensive conditions throughout the year for breeding and calving and must undergo five negative BTB tests over a 15-month period. The first qualifying BTB test must be done a minimum of three months post capture. If all buffalo in that group remain negative on BTB testing over this period, then the group will qualify for BTB negative status.

• BTB negative buffalo may only be sourced from known BTB-infected herds with a BTB prevalence of less than 20%.

• Under exceptional circumstances, BTB positive pregnant cows may be held for experimental purposes, to harvest BTB- and CD-free calves. Their calves must undergo five negative BTB tests three months apart, in a separate isolation unit, before they can be considered negative. The whole facility must be dedicated to these positive animals.

[7] A BTB-free project is a project where buffalo sourced from BTB-infected herds are progressively cleaned up by a "test and slaughter" process (e.g. Phinda project in Kwa-Zulu Natal).

Brucellosis (CA) testing

• All adult buffalo must also be tested for bovine brucellosis (Complement Fixation Test - CFT), and must have a totally negative titre, since they are unvaccinated. The brucellosis screening must be expedited as soon as possible after capture, to avoid the potential animal and veterinary public health consequences of having positive cows aborting or giving birth within the intensive conditions of the quarantine boma.

• All new cows must have undergone at least three negative CA tests, including a (2) 12-week post calving test, before their calves will be released and the adult cows obtain CA negative status. During this period the adults must be quarantined away from the rest of the herd.

• Adult cows that have already qualified for CA negative status must still be tested at least once a year, (2) 12-weeks post calving.

• Adult bulls must also be tested once a year.

• No brucellosis positive animals may be introduced into BTB positive quarantine facilities where BTB-infected pregnant cows are held.

• In the event that a *Brucella* negative herd suddenly becomes positive, the control of this herd will be the same as for a cattle herd in a similar situation. This will include testing every 2-3 months with slaughtering of any positive animals, and monitoring of heifer calves from positive dams until after first parturition.

• The use of *Brucella* vaccines may be authorized by Provincial Veterinary Services.

• All results must be forwarded to the Buffalo Advisory Committee.

2) Foster Mother Dairy Cows

Foster mother dairy cows must be sourced from tuberculosis- and brucellosis-free dairy herds. Before entry into the facility, all foster cows must be tested for both BTB and CA to confirm their negative status. Foster cows may also not be vaccinated for FMD, so that they fulfill the additional role of FMD ”sentinels”. They will also function as CD sentinels. These foster mother dairy cows must be retested for FMD, CD, BTB and CA on an annual basis.

Table 5. BTB and the introduction of new African buffalo (*Syncerus caffer*) breeding stock into "clean herds" (adapted from Anonymous 2002).

Source Status	Definition	Tests required before introduction	Examples
BTB-free herd	This is a herd of buffalo in which all of the buffalo on the farm have already undergone three consecutive negative herd tests (if sourced from a herd of unknown status) or five consecutive negative herd tests (from an infected herd).	One negative gamma interferon	Established disease-free breeding projects that are run as a closed herd, have had three consecutive negative tests, and to which introductions are handled as per specification.
Unknown status herd	An "unknown status" herd is a buffalo herd that has never been tested, or in which a proportion of the herd may have been sampled or examined either through testing or necropsies and no positive cases have been identified.	Three consecutive negative tests, three months apart. The first and last test must be a comparative skin test.	Buffalo herds in conservation areas where sample surveys have been negative, or fenced registered buffalo farms where no BTB has ever been diagnosed.
Infected herd	An infected herd is a herd in which BTB has been detected and confirmed by culture of an animal from the herd.	Five consecutive negative comparative skin tests, three months apart.	Herds in conservation areas or registered buffalo farms where there has been a confirmed case of BTB.

Breeding Systems for Disease-free Buffalo Calves

There are three systems for creating disease-free calves outlined in the South African buffalo protocol.

- Type 1: newborn calves are removed from their biological mothers at birth prior to drinking buffalo colostrum. Calves are fed cattle colostrum and then bottle- or bucket-fed, or fed by a foster-mother dairy cow.

- Type 2: calves are removed from their biological mothers within 48 hrs of birth after drinking buffalo colostrum. They are bucket- or bottle-fed, or may be raised by a foster-mother dairy cow.

- Type 3: calves are raised by their biological mothers, achieve normal buffalo colostral immunity, and are removed from their mothers as a group at five to seven months of age.

Diagnostic Testing and Quarantine Protocols for Buffalo Calves (adapted from Anonymous 2002) (Tables 6 and 7)

1) First Stage Quarantine

• This stage begins immediately and must be carried out in the "clean" facility, which is a minimum of 100 m (as far as possible) from the adult buffalo and involves either newborn or weaned buffalo calves depending on the breeding system used. This first stage quarantine does not apply to Corridor disease projects in vector-free areas. All foster cattle must be tested in parallel with the calves for all diseases. All the calves in a group are to be tested simultaneously.

Type 1: Newborn Calves

• All the newborn buffalo calves should optionally be bled (commercial operators responsibility) for baseline FMD titres within their first month. These results will confirm whether any of the calves have drunk buffalo colostrum. Thereafter, the first stage quarantine testing may begin. If the calves are in a group, then the minimum age of the group must be one month.

• Once all these calves in the quarantine facility are confirmed as negative for FMD, CD and BTB (gamma interferon) by the local State Veterinarian, then Stage 1 quarantine is completed.

Type 2 and 3: Buffalo calves that have drunk buffalo colostrum

• At weaning, these 5-7-month-old calves – whether hand- raised or raised by surrogate dairy cattle or their biological mothers – must be bled to determine their residual FMD titres. Once they test negative for FMD, they must also be tested for CD and BTB (gamma interferon). Once all calves in this group have negative titres for FMD and a single negative test for CD and BTB, then Stage 1 is completed.

• Calves from vector free projects undergo their first tests for CD and BTB (blood test) a minimum of 30 days after separation from CD carrier breeding stock.

• Sampling for all negative qualifying tests must be done a minimum of thirty days after separation of the calves from the adult buffalo. After completion of Stage 1, the calves from all of the above systems must be moved to a suitable quarantine facility in the FMD surveillance zone of Limpopo Province or Mpumalanga. This movement will be controlled by the local State Veterinarian.

2) Second Stage Quarantine

• Calves and foster dairy cattle that have successfully completed Stage 1 now qualify to enter Stage 2 of the quarantine process in a facility within the FMD surveillance zone. Calves and foster cattle from all three of the above breeding systems now have the same status. This second stage quarantine process will take place at a facility where no adult buffalo are present. This quarantine facility should not be located in a game or cattle camp, or should have a surrounding 100 m wide exclusion zone that is free of susceptible animals.

• After a minimum period of 30 days in this second quarantine facility, all calves must be re-bled for FMD, BTB (gamma) and CD. If the test results are all negative, then these calves qualify to enter the third stage of quarantine, which takes place in the same facility. For Corridor disease projects in the vector-free area, calves must be re-tested for CD and BTB (blood test) at least 30 days later.

3) Third Stage Quarantine

• After a minimum period of a further 30 days at the same facility, all the calves and foster cattle must be re-bled for FMD and BTB. If the test results are all negative, then this stage of the quarantine process is completed. These animals can now be moved as a group, with PVS approval (Annexure 4), to a suitable quarantine facility that is registered for FMD-/CD-free buffalo outside the FMD/CD control areas to complete their fourth stage quarantine. This movement must take place within 30 days of the last test sampling in the surveillance zone, otherwise the FMD results may no longer be valid and the calves will lose their disease-free status and need to be retested for FMD.

• For CD projects, the calves must be re-bled for CD plus their first FMD and CA test. A skin test for BTB is also required before they qualify for movement to another property.

4) Fourth Stage Quarantine

• After a minimum of 30 days quarantine and not before all buffalo calves are a minimum age of nine months, a full panel of tests for FMD, CD, BTB (skin test) and CFT (Complement Fixation Test) must be repeated for all calves and foster cattle in a facility outside the FMD control area.

• This quarantine period is also necessary in case of any significant disease event occurring at origin shortly after a buffalo consignment has been moved out of the control area. Following receipt of negative test results, the calves must be released into a free range camp for a retention period of 12 months.

5) Fifth Stage Retention

• This retention period should take place in the presence of brown-ear tick exposure and preferably in the presence of sentinel cattle. After this 12 month retention period, these calves must be retested for all four diseases, and if negative, they may be released by the local State Veterinarian with approval from the Provincial Director of Veterinary Services.

6) Other Considerations

• Calves testing positive for bovine tuberculosis must be slaughtered, and if BTB is confirmed, then all other calves from that original group must undergo a further five consecutive negative intradermal tests over a period of 15 months before they can be considered BTB-free. Hand-reared calves from BTB-positive mothers must undergo five negative intradermal tests for BTB three months apart before they can qualify for BTB negative status.

• Calves testing positive for brucellosis must be slaughtered or may be sent to the State Veterinarian in Skukuza for research purposes. All other calves in the group will be handled at the discretion of the Provincial Director of Veterinary Services. It must be remembered that the brucellosis status of heifer calves is related to the brucellosis status of their dams.

Table 6. Disease test summary for buffalo calves originating from a FMD infected zone testing and movement protocols for disease-free African buffalo (*Syncerus caffer*) calves in South Africa (adapted from Anonymous 2002).

Quarantine stage	Location of facility	Duration of Quarantine	Tests required				Other requirements
			FMD[a]	CD[b]	TB[c]	BRUC[d]	
Stage 1[e]	FMD infected zone	Variable – until first negative test results	Yes	Yes	Yes (blood test)	No	Effective tick control
Stage 2	FMD surveillance zone	Minimum of 30 days after arrival at facility	Yes	Yes	Yes (blood test)	No	Effective tick control
Stage 3	FMD surveillance zone	Minimum of 30 days after Stage 2 tests	Yes	No	Yes (blood test)	No	Effective tick control
Stage 4	Outside the FMD and CD control areas	Minimum of 30 days after entering Stage 4 facility	Yes	Yes	Yes (skin test)	Yes	Minimum age of 9 months; no tick control
Stage 5	Free range on same property as Stage 4 facility	12 months	Yes	Yes	Yes (skin test)	Yes	Unprotected – full brown-ear tick exposure

[a] FMD
[b] CD – tick-born infection by the protozoal organism (*Theileria parva lawrencei*)
[c] bovine tuberculosis – tests are not conducted on calves under three months of age
[d] bovine brucellosis
[e] Start with either newborn or weaned calves; “all-in/all-out” quarantine system where no additional animals may be brought into or leave the quarantined group during the required quarantine period

Table 7. Test summary for buffalo calves from CD vector-free projects (adapted from Anonymous 2002).

Quarantine stage	Location of facility	Commencement and Duration of Quarantine or Retention	Tests required				Other requirements
			FMD[a]	CD[b]	TB[c]	BRUC[d]	
Stage 1[e]	Vector-free area	30 days after separation from infected breeding stock	No	Yes	Yes (blood test)	No	No movement
Stage 2	Vector-free area	Minimum of 30 days after primary test	No	Yes	Yes (blood test)	No	No movement
Stage 3	Vector-free area	Minimum of 30 days after secondary test; calves must be at least 9 months old	Yes	Yes	Yes (skin)	Yes	Other properties in the vector-free area
Stages 4 and 5	CD vector area	Same as for lowveld calves	Yes	Yes	Yes (skin test)	Yes	CD vector area

[a] FMD
[b] CD – tick-born infection by the protozoal organism (*Theileria parva lawrencei*)
[c] bovine tuberculosis – tests are not conducted on calves under 3 months of age
[d] bovine brucellosis
[e] Start with either newborn or weaned calves; “all-in/all-out” quarantine system where no additional animals may be brought into or leave the quarantined group during the required quarantine period

In summary, the South African buffalo protocol prescribes a specific methodology for acquiring and testing breeding stock, breeding systems for producing calves, and then an explicit testing and quarantine protocol that addresses and defines testing requirement to achieve disease freedom with respect to FMD, CD, bovine tuberculosis and brucellosis. The protocol evolved through a comprehensive and adaptive process that was based on design input and approval from provincial and national veterinary experts, provincial conservation agencies, commercial game farm operators, an expert "Buffalo Advisory Committee," animal welfare organizations, organized agriculture, and an approved diagnostic laboratory.

Non-Human Primates

The IUCN's "Guidelines for Nonhuman Primate Re-introductions" (IUCN 2002a) outlines specific requirements for quarantine and disease screening. With respect to tuberculosis in nonhuman primates, the IUCN (2002a) outlined the following:

> "To test for tuberculosis (TB), three intradermal Tuberculin skin tests, using the eyelid or abdominal wall area, should be administered. For a 60- or 90-day quarantine period, the tests should be conducted at no less than 1-month intervals (for a 60-day period, conduct the first test on day 1, the day of arrival). For a 31-day quarantine period, the tests should be administered on days 1, 14, and 28. Mammalian Old Tuberculin is recommended. Animals with three negative test readings are considered free of tuberculosis and can be introduced into a resident group after all other quarantine procedures have been carried out" (p. 13 in IUCN 2002a).

The IUCN (2002a) emphasized the importance of proper test interpretation and the likelihood for false positive test results in non-human primates. They also recommend additional ancillary testing protocols for TB to increase the sensitivity of the screening protocol.

Interstate and International Requirements for Moving Bighorn Sheep – Western North America

A memo obtained from a meeting of committee members of the Western Wildlife Health Cooperative and Western States Livestock Health Association discusses a consensus option on requirements for moving bighorn sheep. A health certificate from an accredited veterinarian at the point of origin and brucellosis testing were the two most common requirements. The release of animals before brucellosis tests were complete was deemed to be satisfactory if the source population history was adequate. The disease histories of the source and resident populations were important concerns. Testing for other diseases varied and was at the discretion of the state regulatory veterinarian. The memo concluded by stating that strict importation policies applied in western states and provinces to the movement of captive wild and exotic animals had been imposed on free-ranging wildlife movements between states.

In a document, attached to the memo, entitled, “Protocol for Movement of Bighorn Sheep from Canada to the United States”, the herd of origin was to be screened for tuberculosis (BTB test). Animals being moved to the United States required negative brucellosis tests. Any animals with positive results would be barred from entry and any herd with 10% or more positive would be rejected.

Gray Wolf Introductions to Yellowstone National Park

The first step in addressing concerns regarding disease risks in this case was the selection of wolves from areas free of significant diseases, such as rabies and tuberculosis. Captured animals would be screened for diseases by physical examination, haematology, serology, serum chemistry and fecal examinations. Despite the lack of drugs and vaccines approved for wolves, prophylactic use of vaccinations and anthelmintics was recommended as a means to minimize the transmission of selected viruses and parasites. Wolves would be released into the new location only if they had no clinical signs of illness or infection. Serological results were not considered a criterion for non-release, except for rabies.

Fish Translocations

The intent of the HLWBRP is similar to the long-established practice of fish enhancement programs. In both cases, animals are reared in captivity with the intention of releasing them to the wild to re-stock depleted wild populations. In both cases, the prevention and management of disease is a key consideration; both in terms of disease implications for wild stocks and for trade issues. An overview of fish disease management regulations highlight some of the shared problems and can provide some models for shared solutions to the issue of moving animals from a captive to wild situation.

There are a number of provincial, national and international regulations designed to try to prevent the movement of diseases with fish movement. Many fish health protocols aim to minimize the risk of introduction and spread of infectious disease agents in order to minimize the risks to wild stocks that are inherent in the movement of managed stocks by: (1) preventing the introduction of specific pathogens or parasites into a region; (2) preventing the spread of specific pathogens or parasites within a region; and (3) eliminating selected pathogens or parasites from a region. Typically, a precautionary approach is required as there is evidence that undesirable health effects can be associated with translocations, but there is insufficient capacity to predict the nature or frequency of their occurrence. Our inability to accurately predict the impacts of changing distributions of disease causing agents coupled with case histories of severe negative effects of disease in aquaculture or enhancement situations have lead regulators to err on the side of caution and manage fish resources to avoid changes in disease status whenever possible. This has resulted in management strategies that revolve around preventing the introduction or extension of the distribution of disease causing agents.

The precautionary approach is increasingly being applied to animal translocations. This approach, in general, advocates that measures should be taken to reduce the threat of serious or irreversible harm to the environment even if certain cause-effect relationships have not been scientifically established. Difficulties in determining what constitutes serious or irreversible harm, the thresholds for action, and who determines when to act have led to inconsistent applications of this principle and difficulties in operationalizing this concept (Hrudey and Leiss, 2003, Crawford-Brown et al. 2004, Dorman 2005).

Increasingly, policies and practices are being based upon risk assessment and management principles in hopes of addressing the needs of a precautionary approach (Stephen 2001, Crawford-Brown et al. 2004). In Canada, there are a variety of mechanisms for considering how to approach risk assessments for fish movements. For diseases of international significance, the OIE has outlined general considerations and guidelines for risk assessment in their international Aquatic Animal Health Code (Chapter 1.4.1) (OIE 2006a). The Federal Department of Fisheries and Oceans (DFO) draft policy on fish introductions and transfers also outlines the elements of a risk assessment. Unfortunately, many risk assessments rely largely on estimates of the probability of occurrence of adverse events. By defining risk only as the probability of occurrence of the hazard event, the risk assessor assumes that the outcome is significant (Ahl et al. 1993).

Protocols rely heavily upon exclusion as a primary means of avoiding disease hazards. Federal and regional regulations are generally prohibitive – restricting or excluding the importation of specific life-stages or species. In general, international import policies outline sanitary conditions for travel, list specific diseases that would preclude movement, and often refer only to political rather than ecological boundaries (Welcomme 1988).

Many fish disease policies use sampling and diagnostic testing of fish populations prior to movement across political boundaries as a means to ensure fish move from "like-to-like" areas. Most often, the goal is to classify the infection status of a group, culture facility or geographic or political region as being free of certain diseases. Because fish can be infectious but not ill (sub-clinical cases) most fish inspections are based not on signs of illness alone, but instead rely heavily on the detection of infectious agents in or on the fish. The current *Canadian Fish Health Protection Regulations* (FHPR) (Department of Justice Canada 1985) assumes that tests are 100% sensitive and specific. Sampling strategies do not take into consideration changes in the predictive value of diagnostic tests that occur with changes in the prevalence of the disease nor do they consider the statistical implications of using samples from populations as a means to determine the disease status of fish or zones. In addition, sampling protocols do not consider issues of non-uniform distribution of fish and diseases. Standardized risk assessment protocols are not available in the FHPR or its manual of compliance. At the group or culture facility level, typically, importation decisions are based upon sampling strategies which give a certain level of confidence of detecting at least one positive fish from populations with the disease at or above the prevalence of disease that is considered acceptable. A recent survey of fish diagnostic laboratories in Canada revealed that a homogenous approach to screening fish is unlikely to achieve the same results for all species and all pathogens (Thorburn 1996). As there has been little work done on the performance of many of these tests, the true level of confidence we can have in classifying a group as free of disease is likely much lower than assumed for current regulations. An effective eradication program requires increased research on the performance characteristics of diagnostic tests because it is not unusual to find that the characteristics of diseased individuals near the end of the program are dramatically different than when the disease was more prevalent (Martin et al. 1987). Given the deficits and difficulties in conducting surveillance on wild stocks, identification of this end point would be difficult. This is further complicated by a lack of knowledge of the changing diagnostic performance of tests as the prevalence of the disease decreases.

Local decisions regarding fish movements are currently based upon the recommendations of Federal-Provincial Fish Transplant Committees. The Transplant Committees evaluate outcomes – focusing on genetic, ecological and disease impacts – associated with proposed fish transplants which could adversely affect the ability of existing wild or cultivated fish to maintain healthy, productive populations. Although they all operate under similar principles, each provincial committee assesses requests for transfers somewhat differently. Currently, only draft federal policies exist that specify the basis for risk assessments for fish translocation. Despite these mechanisms for assessing risk, there are still deficits with respect to repeatability and degree of confidence that can be assigned to fish health risk assessments. A common element of all international aquatic introductions is the lack of monitoring of the real impacts on society and ecosystems (Bartley and Subasinghe 1996). When coupled with our lack of knowledge of the epidemiology of disease in wild fishes (Bakke and Harris 1998), it is virtually impossible to make definitive statements on the risks of introduced pathogens or parasites. While a risk assessment framework is advocated by many jurisdictions, the lack of specific guidelines for applying risk assessment methods coupled with the lack of data on significant impacts often result in decisions about fish transfers being made on a political or short-term economic basis (Bartley and Subasinghe 1996).

Summary and Management Implications

We identified four main issues relevant to the recognition of the HLWBRP as being free from brucellosis and tuberculosis:

1) Policies that address disease management and eradication in wildlife, and specifically the salvage of healthy animals from diseased populations are lacking.

2) The health status of the source herd has a strong influence on the health status of a newly created or salvaged herd, and there has been little attention paid to development of epidemiological-based salvage and disease (i.e. infection) eradication protocols.

3) Given the absence of explicit policies and guidelines for wildlife disease management and salvage protocols, risk analysis and risk assessment approaches may provide a transparent and science-based decision-making framework with which to consider the health status and movement of salvaged animals (i.e. wild-caught and/or captive-born) for conservation purposes.

4) Lack of meaningful interdisciplinary and inter-agency collaboration from the outset has hindered policy development and agreement on specific epidemiological-based criteria and approaches that would define project success, i.e. disease-free and infection-free status. Since the criteria for defining disease-free status of the HLWBRP were not explicitly defined and agreed upon at project initiation, the health status of the captive herd became a moving target.

Lack of Policies

The technique of salvaging animals from wild populations, by rearing founding stock and captive-born offspring in captivity for later release, is a relatively new practice for wildlife management. The added dimension of salvaging animals from a known infected population adds a layer of complexity and uncertainty to this practice. Presently, there is an associated lack of well-developed and specific policies dedicated to the eradication, prevention, and management of disease risks in wildlife conservation projects that incorporate salvage and captive breeding. Notifiable (reportable) diseases affecting wild ungulates are typically considered under agricultural rather than wildlife policies. Many jurisdictions do not consider the disease status of wild bison or other wildlife when declaring an area free from brucellosis or BTB, relying instead on the prevalence of disease in cattle or other captive species as their main criterion. Policies uncovered in this review that did apply to bison were intended for captive, specifically gamed farmed, animals. The goal of all tuberculosis or brucellosis control policies that we reviewed was to prevent cases of these diseases in domestic cattle or captive bison and cervids. Wildlife, when mentioned, was considered more as sources of infection whose contact with cattle must be prevented, rather than as cases that required management or eradication of disease to protect and conserve the diversity of wild herds. The Yellowstone bison and South African buffalo projects were two notable exceptions that have influenced disease eradication protocols as well as wildlife disease policies.

Protocols used to establish disease-free herds or minimize the risk of disease during wildlife translocations appear to be *ad hoc* and were developed on a case-by-case basis rather than on specific existing policies. Some of the protocols established to reduce disease risks with wildlife translocations were limited in their ability to adequately characterize the infection status of animals moved due to the relatively short time the animals were held before the translocation. Often, there is a strong reliance on the results of a clinical examination and an acute serological test. In the absence of data on the clinical performance characteristics of various tests in wildlife species, diagnostic errors could not be reliably quantified and thus risk estimates would have some inherent imprecision. In addition, limitations in our capacity to systematically sample wild populations reduced the capacity to compare disease status of animals in the capture versus receiving ecosystems. In other cases, such as Yellowstone and Kruger National Parks, disease management plans allowed for animals to be held for a prolonged period of time so that multiple tests could be conducted, adding confidence in the capacity to detect infected animals. However, as was seen in Yellowstone, these plans were contingent on successful changes in existing agricultural policies at the state and federal levels and required federal approval to establish a level of testing and quarantine that fulfilled the requirements needed for a salvaged herd to be classified as disease-free.

Despite the lack of an official predetermined protocol for establishing disease-free status for captured elk in MB, the CFIA’s review and *ad hoc* risk management protocol for approving movement permits sets an important standard for disease eradication protocols for wild elk taken from a known BTB-infected wild herd. The CFIA’s protocol provides two general epidemiological-based criteria that have application to the broader issue of salvaging disease-free animals from infected wild herds:

1) Although it did not specifically define it as such, the CFIA applied the concept of zoning (Kellar et al. 2001) and compartmentalization[8], developed for disease surveillance and management in livestock production systems, to the wild herd of origin by defining a NCZ and SCZ and assigned the captured elk herds to one capture zone or the other. The CFIA used existing surveillance data to define the NCZ as negligible risk and the SCZ with significant risk of having one or more infected animals.

2) Based on its assertion that the SCZ posed a significant risk, the CFIA developed a risk management protocol, which required the euthanization of all wild-caught founders followed by complete post-mortems and laboratory testing of tissues to confirm presence or absence of infection. The CFIA granted the movement permit (which was an unofficial surrogate for disease-free status) to captive-born progeny once follow-up testing was completed. Using this approach, the health status of the herd of origin – the captured founders – could be determined and an epidemiological break in potential

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[8] Zoning and compartmentalisation are procedures implemented by a country under the provisions of Chapter 1.3.5 of the Terrestrial Animal Health Code (OIE 2006b) with a view to defining subpopulations of different animal health status within its territory for the purpose of disease control and/or international trade. Compartmentalisation applies to a subpopulation when management systems related to biosecurity are applied, while zoning applies when a subpopulation is defined on a geographical basis.

"A new concept for the management of animal health is compartmentalization, which is a procedure to define ecologically distinct animal populations of different animal health status" (Zepeda et al. 2005).

The OIE (2006a) defines a compartment as "one or more establishments under a common biosecurity management system containing an animal subpopulation with a distinct health status with respect to a specific disease or specific diseases for which required surveillance, control and biosecurity measures have been applied for the purpose of international trade." The main criterion for a compartment is that the animals contained in it are clearly recognisable as part of a unique subpopulation with limited or no epidemiological links to other populations of risk.

disease transmission was established between the founders and captive-born progeny.

Consequently, the CFIA's approach for BTB in the MB elk situation provides an important *ad hoc* standard and a benchmark from which to improve epidemiological and risk-based approaches in wildlife disease eradication. Some areas for further development may include:

1) a definition of ecological zoning/compartmentalization of wildlife disease risk that incorporates radio telemetry data for a more accurate and defendable spatial delineation of seasonal movements and herd range(s);

2) disease risk categories for wildlife populations that are linked to collection and assessment of surveillance data based on epidemiological sampling requirements and a standardized approach for defining a minimum detectable level of prevalence; and

3) a transparent and collaborative approach for designing an *a priori*, quantitative epidemiological risk assessment approach that integrates inter-agency expertise, and facilitates continued improvement and adaptation of new techniques for salvaging and translocating healthy wildlife from infected populations.

Options:

- Over the short term, and in the absence of existing policy, an inter-jurisdictional working committee should be established to assess movements of captive bison (or other species involved in other wildlife conservation programs) on a case-by-case basis. The committee – comprising federal, provincial and territorial representatives as well as outside expertise as required, i.e. the National Wood Bison Recovery Team – would advise the CFIA and territorial/provincial government on the risks associated with a translocation, consider the effects of mitigating factors to reduce that risk, and advise recipient agencies on the safety and advisability of a specific release. This committee would not be able to exceed existing regulatory powers, but would speed the process of formally reviewing bison salvage and translocation projects and would better allow local factors to be integrated into decision-making.

- To facilitate discussion, the GNWT should forecast potential uses or translocations that will be required for the herd in the future (for genetic conservation and management framework that provides rationale for translocations of HLWBRP bison, see McFarlane et al. 2006). Two issues that will affect risk assessments and policy application are: 1) whether translocations require the animals to cross political boundaries, and/or 2) whether animals may enter the commercial bison industry. By anticipating how these various scenarios affect risk assessments now, the team can examine their management practices to see if potential concerns can be mitigated.

Source Herd

A variety of national and international animal health policies – designed primarily around the livestock industry – base the disease status of a herd on the status of its source herd or the history of contact with potentially infected animals. This matter complicates the classification of a newly formed herd originating from a known infected source, as was the case with the HLWBRP. However, there are also policy options for making the transition from a known infected source herd to a newly-formed disease-free herd through repeated herd and individual quarantine, testing and selective slaughter. Both the South African buffalo protocol and the Yellowstone bison quarantine study provide models. The probability of transferring infected individuals from the wild source herd to the captive herd is reduced by: 1) maintaining for captivity only animals that test negative for the disease in the field; 2) reducing the likelihood of false negatives by making any one positive test in a series of repeated tests conducted over a prolonged time sufficient evidence to classify an individual as positive; 3) reducing the risk of missing intra-group transmission in quarantine by making the finding of any one reactor animal in a group sufficient evidence to classify the group as suspect and, therefore, start all testing protocols over.

The OIE and the testing and quarantine protocols for Yellowstone National Park and South African buffalo provide mechanisms through which a herd can be granted the status of freedom from disease (i.e. infection) without being derived from a herd of similar status. In each case, a series of tests over time must yield negative results. Similar repeated testing is required by USDA regulations to move regions or States through the various stages from infected to accredited free of disease. Therefore, establishing a disease-free herd from an infected herd is not without policy support or precedence.

As described above, the experience with the capture of wild elk in Manitoba and the CFIA's risk management protocol and criteria for approval of federal movement permits for what it deemed a high-risk group also provides an important development in the practice and policy for salvaging animals from a known BTB infected wild population. In this case, the epidemiological links between the wild free-ranging herd, the founding herd of wild-caught animals, and the captive-born progeny were considered. The source herd in this case was considered to be the wild-caught founders. By depopulating the founder herd of wild-caught elk and using post-mortem examination and laboratory diagnostic tests as a means of defining the health status, true prevalence of infection in the source herd could be determined and a break was inserted in the potential disease transmission link between the wild-caught founders and the captive-born progeny. Follow-up ante-mortem testing of captive-born progeny followed by euthanization and post-mortem diagnostics on skin test reactors increased the confidence of health (i.e. infection-free) status in the remaining captive-born animals.

Given that surveillance capacity in free-ranging wildlife is limited by resources and technical/logistical challenges, it is virtually impossible to define disease-free or infection-free wild herds with full confidence or to rule out which pathogens may produce adverse consequences. None of the case studies reviewed had definitive endpoints at which time they would classify a disease as eradicated in a wild herd. Imperfect surveillance methods, such as hunter surveys, were typically used in conjunction with imperfect diagnostic tests. Explicitly or implicitly, often the target was the reduction of disease in the wildlife to a point that no cases were detected in cattle. Therefore, the goal was not true eradication but the elimination of the disease to a level at which transmission was reduced to a point that the prevalence of infections was below the limits of detection of the surveillance system and did not result in trans-specific transmission. This can be considered a non-zero, but unspecified level of acceptable risk.

Options:

- Generate additional information to demonstrate that the HLWBRP founders originally captured were free from infection in order to grant their progeny disease-free status. This may require slaughter and necropsy for histopathological and microbiological examination. Whether this is part of a routine cull for food animals or a specific project for the purposes of herd accreditation will depend on management plans for the herd, the capacity to isolate founding stock from progeny, and the timeline for establishing the herd's disease status.

- Establish a second captive herd that is comprising captive-born animals only that is physically and functionally isolated from the first. Setting up a second location for breeding and holding the captive herd would provide a new layer of isolation between the two groups of bison. The first herd would serve as the source herd for the second, while the wild herd would supply animals into the first. On-site surveillance of the first herd coupled with a test and slaughter program and eventual euthanization and post-mortem examination of all founders would be used to demonstrate the low risk of infected animals being present in the first herd. Therefore, the second herd would be derived from a low-risk herd and could then progress to the next level of being classified as a disease-free herd. This approach would have the additional advantage of avoiding "putting all your eggs in one basket." In other words, if a catastrophic event affected the first herd, not all of the effort and genetic heritage would be lost as there would remain a viable second herd elsewhere. However, the cost implications of this recommendation may be a limiting factor.

- An evidence-based argument could be presented to the CFIA to demonstrate that the current management plans not only meet other international models for classifying the herd as disease-free, but also provide an extra-level of confidence because of additional steps taken to reduce the likelihood of infected animals being part of the herd. Immediate steps should be taken to validate this claim by ensuring that all past data from animals – including test results, necropsies, reproductive success and slaughter check results – are available to evaluate the herd’s health status using risk assessment techniques.

Risk Assessment

The CFIA advised that in cases such as the HLWBRP translocation, requests would be assessed on a case-by-case basis using risk assessment methods if their movement was to private facilities or could affect the disease status of the nation’s cattle herd. The CFIA has previously assessed the risk of bovine brucellosis and BTB in WBNP and area (APFRAN 1998). This assessment focused on three at-risk groups (cattle, captive bison, and disease-free wild bison) and quantified risk simplistically by estimating the probability of annual transmission from infected wild bison to at-risk groups and the subsequent monetary impacts of transmission. Gates et al. (2001a) and Gates and Wierzchowski (2003) conducted follow-up research to incorporate biophysical landscape characteristics and spatially define bison movement corridors within the landscape. The CFIA has also conducted a risk assessment specific to the HLWBRP (APFRAN 2003, Nishi et al. 2004), which was designed to address the risk of at least one true positive founder animal given the repeated testing of the founder cohorts for bovine tuberculosis and brucellosis. Although the risk model suggested that the risk estimates for the animal health hazards, *B. abortus* and *M. bovis*, were very low and negligible, respectively, the confirmation of BTB in the HLWBRP (Lutze-Wallace 2006) highlights the difficulties and challenges of disease eradication, and the limitations of risk assessment methods.

A common limitation of traditional risk assessment methods for wildlife conservation projects is the difficulty in estimating the parameters needed to quantify risk. Specifically, deficits in our understanding of the wild population's ecology, the epidemiology of their diseases, and the often-undefined performance of diagnostic tests limit the precision and accuracy of probability estimates in risk assessment. Using risk assessment to address animal health issues is challenging because it typically requires a hazard to be dichotomously classified as a hazard or not. Determining whether or not a particular agent will be a hazard will depend on the location and species being considered, diagnostic test performance, whether individual, population, ecosystem or market effects are of concern, and on the outcomes assessed. From a broader perspective, there are significant difficulties in estimating or placing a value on ecological and social impacts of disease management options in wildlife.

Based on the policies reviewed and interviews conducted, we conclude that risk reduction rather than zero-risk or disease eradication is the practical, achievable short-term goal of brucellosis and tuberculosis management in wildlife. Unfortunately, none of the management strategies reviewed adequately defined what level of risk would be acceptable. It was not possible to guarantee zero-risk of an animal health hazard, even if approved diagnostic tests yield only negative results. This is well illustrated by the confirmation of tuberculosis in the HLWBRP (Lutze-Wallace 2006) despite an objective evaluation of disease testing results suggesting that the likelihood was negligible (APFRAN 2003).

Realistically, eradication programs that rely only on diagnostic tests and population sampling can only expect to reduce the prevalence of infection below the level of detection, and program success will be greatly influenced by performance characteristics of the diagnostic tests. Variability in performance characteristics of the tests combined with a prevalence rate that approaches zero precludes the declaration of 100% certainty that a herd is free of a specific pathogen. However, additional information can be used to classify a herd as negative or to identify end-points for eradication programs. For example, USDA, OIE and CFIA regulations require repeated negative values by requiring individuals to be tested in series over a period of time and by requiring a region to not yield positive results over a period of years. In other cases, such as for brucellosis in elk in Wyoming, more practical outcomes, such as the lack of cases in cattle, are used to judge the risk presented by wildlife. This approach is consistent with Thrusfield's (1995) definition of elimination as the "reduction in incidence of infectious diseases below the level achieved by control, so that either very few or no cases occur, although the infectious agent may be allowed to persist".

Given that we cannot ensure zero-risk, that there are deficits in our ability to quantify risk and that there is no defined minimum acceptable risk, risk assessments associated with the HLWBRP will, in the end, be subjective and at the discretion of risk managers. If only disease issues are considered, the risk managers will be part of the CFIA. The single focus of the CFIA on disease management may be unsatisfactory for stakeholders in this project who may wish for a more comprehensive consideration of the risks and benefits of further actions to reduce disease risks. It will be difficult for the HLWBRP to identify targets for their risk reduction programs and for their planning for future uses of the captive herd given these limitations and uncertainties.

Options:

- Establish a HLWBRP risk assessment/translocation committee that can advise on the costs and benefits of translocation risks or further disease control measures. The committee would help to: identify parameters required to be assessed; estimate values for these parameters; and judge the acceptability of the final risk estimate. There would need to be memoranda of understanding between various concerned agencies so that key stakeholders recognize the judgments of the committee.

- Establish a non-zero endpoint for risk assessment to serve as a target for the HLWBRP. Targets may vary for different scenarios (e.g. risk when moved to another captive facility versus risk associated with release to the wild). Risk assessors will have to either support research to fill in information deficits that limit the current risk assessments or hold regular consensus meetings that provide estimates of these uncertain parameters based on best scientific knowledge.

- Consultations should be undertaken between the CFIA and the GNWT to establish a more comprehensive approach to risk assessment that is able to evaluate social and ecological values that are not directly related to impacts on cattle agriculture. This mechanism may provide a means to manage the herd's movements and uses in the absence of policy that provides for risk assessment for animals not affecting commercial interests.

- Improve upon the risk assessment model developed by APFRAN to evaluate the risk of BTB and brucellosis in the HLWBRP. Future risk assessments must estimate the effects of measures taken by the HLWBRP. Specifically, they need to consider how disease control and prevention measures that are applied beyond what is required by existing guidelines (such as antibiotic treatments of new arrivals) modify risk estimates. The epidemiological investigation of the HLBWRP (Elkin and Nishi, unpublished data) will improve the basic knowledge of pathogenesis and transmission of bovine tuberculosis in bison, which would improve risk assessment based techniques.

Interdisciplinary and inter-agency collaboration

The HLWBRP was initially implemented without an approved *a priori* protocol based on specific epidemiological criteria, developed through consensus from both wildlife and livestock health perspectives, for defining disease-free status. Since the HLWBRP extends into the interests and policy arenas of both wildlife conservation and livestock health interests, the challenge of defining and establishing health status for the HLWBRP could only be realistically met through meaningful inter-agency and interdisciplinary collaboration. The collaborative approach between the GNWT, CFIA, and the Wood Bison Recovery Team, has been developed through the evolution of the HLWBRP. The success of this approach is well illustrated by the progress made to date by the South African buffalo project and the Yellowstone bison salvage initiative.

There has been an emerging recognition that wildlife populations can act as disease reservoirs for livestock. For example, the USDA has determined that US trading partners will eventually need to demonstrate that appropriate management strategies are implemented to mitigate the potential for disease transmission from wildlife to livestock (USDA 2005). Recently in Canada, development of the National Wildlife Disease Strategy (Environment Canada 2004) has emphasized the need for national scale, interdisciplinary and inter-agency collaboration when it comes to developing management strategies for diseases in wildlife. Currently, the National Wildlife Disease Strategy has been implemented to address the issues arising from the detection and spread of chronic wasting disease in SK and AB, and will also incorporate BTB and brucellosis (H. Reynolds pers. comm. – Appendix B). The National Wildlife Disease Strategy provides a potentially useful vehicle by which to improve the way wildlife disease issues are recognized, defined, and resolved. But the essential requirement for the strategy to be useful will be a new way of engaging and broadening the involvement of local stakeholders, academics and other disease experts, and government agencies that reflects the complex and interwoven aspects of issues that have the potential to affect wildlife human, livestock health.

Options:

- Engage a wider group of technical experts in both academia and government agencies that may contribute to development of: 1) an approved science-based salvage protocol that integrates recent advances in epidemiology and risk assessment techniques, and 2) an integrated animal health policy (i.e. for livestock and wildlife).

General Recommendations

1) By focusing on northern bison, government departments responsible for wildlife management and livestock health (i.e. the CFIA) should work collaboratively to develop salvage and translocation guidelines for wildlife that are specifically designed for captive breeding and release programs. These guidelines should also explicitly consider programs that propose to salvage wildlife from known infected populations (with diseases of regulatory or management significance) and should emphasize 'what needs to be shown' rather than 'how to do it' (Cannon 2002, Zepeda et al. 2005, Martin et al. 2006). A fundamental principle on which to base future development and implementation of wildlife salvage and disease eradication projects is to develop the protocol through collaboration and consensus of stakeholders and base it on an *a priori* epidemiological-based quantitative risk assessment framework. The use of scenarios tree analyses as an assessment framework would likely be very powerful. This transparent approach would engender trust among stakeholders and provide confidence in the health status of the salvaged herd because it is based on specific epidemiological mechanisms and probabilities. Ideally, the risk analysis and risk assessment framework are established *a priori* so that there is prior agreement on the epidemiological criteria needed to successfully achieve disease-free status before the project is undertaken.

2) Wildlife management agencies (federal, provincial, and territorial) should increase their capacity to evaluate and manage wildlife disease risks that are part of species management plans. This can be done by: (1) training wildlife managers in risk assessment, with an emphasis on disease; (2) training risk assessors in basic

ecological and wildlife management concepts; (3) providing sufficient funds to collect data required to generate risks assessments specific to local conditions and issues; and (4) integrating wildlife health concerns into wildlife management programs, specifically those involving translocations or other population manipulations. A key part of such efforts will include improving our understanding of the clinical performance of diagnostic tests used in bison and other captive ungulates and improved knowledge of the natural history and epidemiology of diseases of concern. From a broader perspective, risk-based approaches for developing wildlife disease policies would facilitate: (1) the incorporation of ecological and social parameters; and (2) the definition of an acceptable level of risk that must be reached in order to move forward.

3) It is paradoxical that existing livestock disease policy does not apply to wild or publicly owned bison, yet these policies are important drivers underlying the desire to eradicate disease from these herds (Nishi et al. 2002b). It is worth considering that the National Wildlife Disease Strategy (Environment Canada 2004) may provide a useful mechanism for developing new wildlife policies dealing specifically with disease management of infected wild bison and development of salvage protocols from known-infected populations. It will be important to ensure that contradictions in policy are not created between the health management objectives for livestock and conservation objectives for wood bison. Collaboration would be needed with the CFIA, and other provincial/territorial wildlife and agricultural departments before policy changes are implemented in order to avoid such conflicts. In consideration of the iterative and extensive process that leads and continues to influence the

Yellowstone bison initiative and the South African buffalo protocol, it will be necessary to consult with northern aboriginal communities and commercial bison producers as these stakeholders have a strong vested interest in the northern diseased bison issue.

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Appendix A. Relevant Documents Reviewed

World Trade Organization (WTO)

- *Sanitary and Phyto-Sanitary Regulations*.

International Union for the Conservation of Nature (IUCN)

- Quarantine and Health Screening Protocols.

Office International des Epizooties (OIE)

- Animal Health Code

Food and Agricultural Organization of the United Nations (FAO), World Health Organization (WHO), OIE

- Guidelines for a Regional Brucellosis Control Programme for the Middle East

Provincial and State Regulations/Guidelines

- Health Requirements Governing the Admission of Animals into the State of Michigan
- Health Requirements Governing the Admission of Animals into the State of Montana
- *Illinois Bovidae and Cervidae Tuberculosis Eradication Act*
- *British Columbia Game Farm Act*
- *The Diseases of Domestic Game Farm Regulations* – Saskatchewan
- *The Deer and Elk Procedures Manual*. Alberta
- Requirements for Movement of Farmed Cervids from Canada and the United States into the Province of Alberta.

Canadian Federal Acts, Regulations and Policy

- *Health of Animals Act* and Regulations.
- *Captive Ungulate Policy*
- Regulations Respecting the Movement by Permit of Certain Ungulate
- Animals to and from any Place in Canada

US Federal Regulations

- Tuberculosis in Cattle, Bison and Captive Cervids; State and Zone Designations; Final Rule. 9 CFR Part 77

United States Animal Health Association (USAHA)

- 2000 Committee Report – Report of the Committee on captive Wildlife and Alternative Livestock
- 1999 Proceedings – Greater Yellowstone Interagency Brucellosis Committee
- 1998 Proceedings – USAHA Comments on Draft Yellowstone Bison Environmental Impact Statement (EIS)
- 1998 Proceedings – Northeast Michigan Surveillance Activities for Bovine Tuberculosis in the Livestock and Free-Ranging Deer Population
- 1997 Committee Reports – Cervidae Brucellosis Eradication

Appendix B. Professional Contacts and Interviewees

Aune, Keith, Research and Technical Services Section Chief. Montana Fish, Wildlife and Parks. Helena, MT.

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Palmer, Mitchell, Veterinarian. National Animal Disease Centre, United States Department of Agriculture. Ames, IA.

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Reynolds, Hal, Wildlife Biologist. Canadian Wildlife Service. Edmonton, AB.

Salman, Mo, Professor Veterinary Epidemiology. Animal Population Health Institute, College of Veterinary Medicine, Colorado State University. Fort Collins, CO.

Schwantje, Helen, Wildlife Veterinarian. BC Ministry of Environment, Lands and Parks. Victoria, BC.

Soorae, Pritpal, Senior Conservation Officer. IUCN Reintroduction Specialist Group. United Arab Emirates.

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Wetzstein, Merv, Manager/Assistant Chief Veterinarian. Animal Health Management and Regulatory Services, BC Ministry of Agriculture, Fisheries and Food. Abbotsford, BC.

Whiting, Terry, Epidemiologist. Veterinary Services Branch, Agricultural Services Veterinarian, Manitoba Department of Agriculture. Winnipeg, MB.

Ziccardi, Mike, Chair. US Working Group for Zoo and Wildlife Species and Wildlife Disease Centre. University of California (Davis), CA.