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THE EPIDEMIOLOGY OF *MYCOBACTERIUM BOVIS* IN CANADIAN CATTLE AND CERVIDAE BETWEEN 1985-1994

A Thesis

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ABSTRACT

Microorganisms of the genus *Mycobacterium* cause tuberculosis in many animal species including humans. Generally, *Mycobacterium bovis* (*M.bovis*) infects cattle and cervidae, but it has the potential to infect virtually all species of mammals including humans. Currently in many developed countries, including Canada, its major impact is as a barrier to international trade. In 1923 the Canadian tuberculosis control and eradication program was established. By 1961 a prevalence of 0.11% was reached. In 1978 the main program thrust changed from testing and slaughter to slaughter surveillance with depopulation of infected herds. A program for the eradication of bovine tuberculosis in captive ungulates was implemented in 1989.

This study examined and analysed the data from the 9 outbreaks of tuberculosis in Canadian cattle and cervidae from 1985-1994. Descriptions and diagrams were given for each outbreak. For the purposes of this study, a positive animal was one which was culture positive. A reactor animal was one which was positive or suspicious on a mid-cervical, comparative cervical, or gross or microscopic test for tuberculosis. Reactor or positive herds were farms with one or more reactor or positive animals, respectively. Herd data were collected from every farm while individual animal data were extracted only from farms which were classified as positive or reactor farms. Herd classification was either positive/reactor or negative. Data for the study were collected from the outbreak records in the Regional or District offices of Agriculture and Agrifood Canada's Animal and Plant Health Directorate. Index animals in 8 out of 9 outbreaks were identified either at routine slaughter surveillance or post mortem examination. One index herd was identified through routine skin testing at the owner's request.

logistic regression was used to study between herd spread of tuberculosis. Two risk factors were identified - increasing herd size and the reason why a herd was investigated as part of the outbreak. Increasing herd size was associated with an increased risk of being positive for tuberculosis with herds of 16-35, 36-80, and >80 animals having odds ratios of 2.94, 5.76, and 9.32 respectively when compared to a herd size of up to 15 animals ($p<0.00$). When compared to perimeter testing, all reasons for investigation had a higher odds ratio. These odds ratios were 57.84 for traceout, 31.8 for pasture or fence line contact, and 14.94 for traceback investigations.

The individual animal data were evaluated for risk factors associated with within herd spread of *M. bovis* using a negative binomial regression. Increasing age of the animal was a statistically significant risk factor with the incidence rate ratios of 12-24 month old animals and those greater than 24 months being 7.65 and 10.42 respectively when compared to the base line group of animals less than 12 months of age ($p=0.009$).

Observed incidence rates (IR), measured in the number of new cases of reactor/positive animals per 100 animal years, were calculated for all the outbreaks. The Ontario cervid outbreak was the only one where comparisons could be made between cervids (IR=9.3), dairy (IR=5.0), and beef (IR=3.1). Cervid IR's were consistently higher when compared to bovine IR's. The highest IR was in the Alberta/Saskatchewan elk outbreak (IR= 18.6) - considerably higher than the next highest which was for mature beef (IR=10.1) in the Quebec bovine outbreak.

Factors which would significantly enhance all disease control and eradication programs repeatedly emerged in the study. These included a universally applied animal identification system and formal animal movement records.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Microorganisms of the genus *Mycobacterium* cause tuberculosis in many animal species, including humans. Tuberculosis is a contagious disease which primarily affects the respiratory system. Other body systems may be affected, however, with spread via the lymph system and the blood vessels (1). Generally *Mycobacterium bovis (M. bovis)*, *Mycobacterium tuberculosis (M. tuberculosis)*, and *Mycobacterium avium (M. avium)* infect cattle and cervidae, humans, and birds respectively. However these Mycobacteria species are capable of infecting a variety of animal species. *M. bovis* can infect virtually all species of mammals, including domestic animals and wildlife species, with cattle, goats and pigs being most susceptible. Horses and sheep show a high natural resistance (1).

The tubercle bacillus was first isolated by Robert Koch, a German physician and scientist, in 1882 (2). The original isolations were from human and animal sources and Koch named the organism *Mycobacterium tuberculosis* in 1896. Two years later, subtle differences were noted in organisms recovered from humans and cattle and Smith was able to distinguish them as *M. tuberculosis* and *M. bovis* respectively. It is an acid fast bacillus and is difficult to culture for a variety of reasons. It may be present in numbers too low for efficient isolation or may be in sites which are inaccessible to sampling. Several processing steps are required to remove contaminants and this may decrease the number of viable *M. bovis* organisms. It takes several weeks to isolate the organism. Identification to the species level is possible but takes additional

time and specialized biochemical tests (2,3).

Although globally distributed, the impact of *Mycobacterium bovis* as an animal and human pathogen varies significantly from continent to continent and even country to country. Before large scale control and eradication programs began in many developed countries, the major impacts of tuberculosis were as a human health risk and an animal pathogen causing production losses. Currently in many developed countries its major impact is as a barrier to both domestic and international trade.

The objective of this thesis, in the broadest sense, was to increase the knowledge and improve the understanding of the epidemiology of tuberculosis caused by *M. bovis* in Canadian cattle and cervidae. The purpose is to help Canada to improve national tuberculosis surveillance and eradication programs.

1.2 Tuberculosis in developed countries

Europe

In Europe, by 1991, the status of tuberculosis in cattle varied between countries (4). At that time, eradication had still not been achieved in Italy, Ireland and Spain which had herd prevalences of 3.71%, 8.8% and 10.8% respectively. France and Greece still had sporadic occurances and the prevalence in these countries was 0.37% and 0.31% respectively. When the Treaty of Rome was signed in 1957, creating the European Community, provisions were made to facilitate trade and movement between Member States without undue risk to countries which had achieved greater success in their control and eradication programs. These rules applied to Member States and provided an opportunity for trade between Members based on a number of conditions, the most important, in relation to tuberculosis, being the designation of a herd as

officially tuberculosis free (OTF). Application of the rules, in fact, hindered trade between Member States and a voluntary plan was instituted which provided financial and technical support to aid in control and eradication programs. In 1994, Spain for example, had a herd prevalence of approximately 10% and had recently initiated an eradication program (4).

In both the United Kingdom and Ireland, infection with *M. bovis* is endemic in badger populations and transmission to cattle is an important aspect in the epidemiology of tuberculosis (5).

Australia and New Zealand

Australia and New Zealand had both established national TB eradication campaigns for cattle by 1970 (6). Both countries have strong scientific/technical bases and well trained veterinary services. Research priorities vary in the two countries due to the underlying differences in the problem. In New Zealand, possum/TB related research has priority due to the fact that *M. bovis* is endemic in possums and other feral/wild animals including pigs, cats, ferrets, stoat, weasels, goats, rabbits, hare and hedgehogs (7).

Tuberculosis in farmed deer in New Zealand was first diagnosed in 1978 and at that point a group of concerned farmers implemented their own eradication program. This program is administered by The Ministry of Agriculture and Fisheries although deer farmers pay for testing and receive no compensation for slaughtered reactors, other than carcass value. The level of tuberculosis in New Zealand is measured by the number of herds on movement control and by the end of June, 1995, 2.4% of the 59,796 cattle herds in New Zealand were under movement control. Although only 12% of cattle herds are located within endemic areas, these herds comprised 77% of the movement control herds (8). At the same time 4% of the 5245 farmed deer

herds were on movement control.

In December 1992 all areas of Australia were declared Impending Free, with no known tuberculosis although there were 93 premises still under quarantine (6). The mainstay of the control program was slaughter surveillance with epidemiological follow-up procedures such as traceback investigation.

Canada and the United States

A national meat inspection program was implemented in Canada in 1907 and statistics from this program gave the federal government the information and impetus to develop tuberculosis control programs (9). The Supervised Herd Plan was introduced in 1908. This was a voluntary plan and although reactors were identified and had to be removed from the herd, they were not ordered slaughtered. In 1914 the Municipal Tuberculosis Order was passed and municipalities could pass bylaws that required that the supply of milk to a municipality was from herds free of tuberculosis. Under this plan the federal Department of Agriculture paid compensation for test reactors. In 1919 the Accredited Herd Plan was introduced which was designed primarily for purebred herds so that they could provide tuberculosis negative status breeding animals, particularly bulls, to other producers.

In 1923 the Canadian tuberculosis control and eradication program was established. This plan was known as the Restricted Area Plan and was mandatory. Tuberculin testing of cattle in designated areas was the main surveillance method. Reactor animals were slaughtered. Testing commenced in 1923 and was completed in June, 1961. During this time a total of 50,000,000 tuberculin tests were performed; 400,000 reactors were slaughtered; and, approximately $15,000,000 in compensation was paid to livestock owners. In 1961 a prevalence of 0.11% was

reached.

In 1978 the main program thrust changed as a result of a major program review and there was a switch from testing and slaughter to slaughter surveillance with depopulation of infected herds (9).

Slaughter surveillance continues to be the major national tuberculosis monitoring program for cattle. When a suspicious lesion is identified at slaughter, specimens are submitted to a government diagnostic laboratory for histological examination and culture. When a culture is positive for the growth of *M. bovis*, the herd of origin is considered a positive herd. An epidemiological investigation is then initiated. All animals leaving the farm (traceouts) must be located and tested. In order to identify the source of the infection all farms of origin for animals in the positive herd (tracebacks) must be tested. Perimeter herds and contact herds must also be tested.

A program for the eradication of bovine tuberculosis in captive ungulates was implemented in 1989 (9). The ungulate program includes Cervidae and the North American bison and provides for on-the-farm testing annually in areas experiencing tuberculosis and at 3-year intervals in non-problem areas. Ungulate herds in which *M. bovis* has been confirmed are depopulated. Epidemiological investigations of ungulate herds are the same as for bovine herds.

Canada is fortunate in that there is no disseminated wildlife reservoir of tuberculosis infection. However there is one problem area. The free ranging bison (*Bison bison*) found in the area of Wood Buffalo National Park are the only known wildlife reservoir of tuberculosis in Canada (10). An opportunistic survey on the remains of 72 bison found dead in and around the park was performed between June, 1983 and October, 1985. Brucellosis was found in 18 (25%),

tuberculosis in 15 (21%), and a combined prevalence of 42%. The many concerns with this nidus of infection include health risks to native hunters, transmission to other wild animal species, and contact with and transmission to domestic cattle and other livestock. Agriculture is expanding in a northerly direction and bison have been sighted near agricultural zones such as one located close to Fort Vermilion, Alberta (10,11). In addition bison have been sighted up to 75 kilometres outside the southwest corner of Wood Buffalo National Park. Privately owned bison populations and the introduction of elk and bison game farms in Western Canada have increased the potential for spread of the disease to domestic cattle and other susceptible wildlife.

Between 1986 and 1988 post mortem examinations were performed on 51 wood bison (*Bison bison athabascae*) killed as part of a multidisciplinary research project in the Mackenzie Bison Sanctuary (12).The results of this study indicated that tuberculosis and brucellosis were not endemic in the wood bison in and around the Mackenzie Bison Sanctuary. However, this sanctuary is a mere 100 km northwest of the Wood Buffalo National Park where tuberculosis is a well established disease. Thus there is concern that the disease will move into the population of wood bison in the Mackenzie Bison Sanctuary.

The United States' and Canada's early histories regarding tuberculosis were very similar. Prevalence, management strategies and political philosophies all followed the same lines (9,13,14). Now however there are significant differences in the situations of the two countries. The most important factors in the United States and how they vary from Canada are as follows.

(1) Whereas both countries have *M. bovis* in their captive ungulate herds, the United States does not have the regulations and authorities in place to deal with the problems in this livestock sector (9).

(2) The United States has an ongoing problem of importation of Mexican steers which have been exposed to tuberculosis (9,14). Approximately 1 million steers enter the United States from Mexico, annually, of which approximately 100 (0.01%) are found with tuberculosis at slaughter (9). Canada does not have this constant threat of exposure to tuberculous animals through importation.

(3) There is an area in the United States where bovine tuberculosis is concentrated and eradication does not look imminent. This area is in Texas and New Mexico near the Mexican border (El Paso Milkshed) where there are 10 large (more than 2000 cows each) infected dairies. The test and slaughter program has not been successful in eliminating the infection in these herds. Total depopulation would be economically overwhelming and not necessarily a long term successful solution (14).

1.3 *Mycobacterium bovis* control and eradication programs

There are three basic reasons why countries decide to eradicate or control tuberculosis caused by *M. bovis* in their domestic livestock. These reasons have been the same since the first control or eradication programs were initiated. The relative importance of each varies both with the individual country situation and the point that country has reached in its control or eradication program. The reasons countries enter and maintain tuberculosis control or eradication programs are as follows.

(1) Public health and occupational hazards associated with livestock infection with *M. bovis*

Many countries decided to embark on tuberculosis control and eradication programs because of *M. bovis* ' zoonotic potential (4,6,15). Before pasteurization of milk and depopulation of infected cattle herds *M. bovis* accounted for 6-30% of the cases of human

tuberculosis in the United States (16). Collins and Grange (1986) concluded that *M. bovis* was still a threat to human health in spite of the decline of tuberculosis in cattle and that infection could occur through the aerogenous route as well as the alimentary route (2). In 1991, a veterinarian in Alberta, Canada became sputum culture positive after surgically treating a sick elk which was later diagnosed with tuberculosis due to *M. bovis* (16). This case initiated follow-up testing of 446 human contacts with an overall initial reactor prevalence of 21%. This was estimated to be twice as high as the normal prevalence in Western Canada in 20-40-year old people although the normal prevalence was difficult to assess because of the lack of baseline testing and relatively high proportion of immigrants in the group (17). In the United States, *M. bovis* tuberculosis in humans has not been known to exist, for many years, except in those recently immigrated to the United States from high-prevalence countries (9).

(2) Decreased production due to the disease

Public health considerations are generally the initial driving force behind eradication programs however when the prevalence of the disease in livestock is high, economic factors related to production can be significant. These have been quantified in some situations. In Europe, early in the century, it was determined that tuberculosis was not only a public health threat but that it was causing massive losses due to animal mortalities and carcass and offal condemnations (4). In Argentina, in 1988, loss of milk production from tuberculous cows was found to be 18% (15). This was related to a delay in first lactation and a decrease in the number and duration of lactations compared to healthy cows. During a ten year period between 1984-1994, in the United States, the average loss

per year due to tuberculosis in cows was estimated at $130,000. This was considered trivial when compared to the positive impact of bovine tuberculosis eradication on the slaughter industry and also its impact on public health (9).

(3) Trade considerations

As prevalence of tuberculosis decreases the relative importance of trade considerations increases compared to public health and losses due to decreased production. Where importing countries have been successful in their eradication and control programs they will increase sanitary requirements for importation of cattle and markets may be closed to exporting countries where tuberculosis has not been controlled or eradicated (15). By 1970, Australia and New Zealand had launched national tuberculosis eradication campaigns with maintenance to markets as the primary driving force (6). The European Community had established programs to aid Member States in achieving tuberculosis control and eradication so that trade between the Members would be enhanced (4).

The specific management approach that each country takes to its control or eradication program also varies according to specific conditions or problems which exist in that country at a particular time. One of the most important of these conditions is the underlying reason that tuberculosis persists or exists in that country. For example, when a wildlife reservoir of the organism exists, the management program to control or eradicate *M. bovis* is considerably different than when a problem exists due to importation of infected animals. Tweddle compared the Australian and New Zealand tuberculosis control and eradication programs and concluded that the differences in the Australian and New Zealand *M. bovis* eradication campaigns were primarily due to the introduction into New Zealand of the Australian Brushtail Possum

(*Trichosurus vulpecula*) (6). The possum has become a wildlife reservoir of *M. bovis* in New Zealand but not in Australia. The United States and Canada had parallel histories of tuberculosis eradication until quite recently (9). Importation of steers with lesions of tuberculosis from Mexico into the United States and pockets of tuberculosis in the El Paso area, differentiate the situations in the two countries. Other important country conditions interact with or coexist with these underlying problems and can be grouped as follows.

(1) Availability of public resources, specifically human resources and money

Compensation costs to producers from governments may be prohibitive, especially for large depopulations of rare and exotic species. In general, funds are less likely to be available in these economic times. Some countries subsidize control and eradication programs, as for example the European Community for Member States (4); while in others the producers pay for the program (testing and technology development) but the program is administered by the government, as in New Zealand (6).

(2) Whether the infrastructure exists in the private or public sector, to administer the program

For example, by 1975 in the United States, only four States had achieved "Free" status. The greatest difficulty in achieving this status was not the required 5 years of freedom from tuberculosis, but rather the requirement to record the individual identification of all adult cattle purchased or sold (9).

(3) Technical and scientific capabilities to successfully implement a program

(4) Support by producers and industry for a control or eradication program

In New Zealand deer farmers are responsible for the costs and arrangements for their own TB control programs (8).

(5) The existence of a wildlife or feral reservoir of *M. bovis*

Wildlife or feral reservoirs of *M. bovis* may have a profound impact on control and eradication programs. The brush tailed possum in New Zealand and the badger in the Republic of Ireland and the United Kingdom are two examples of situations where eradication is hampered if not made impossible by the presence of the wildlife reservoir (5). In Canada there is concern that tuberculosis could spread from bison in the Wood Bison National Park (the only known wildlife reservoir of tuberculosis) into farmed elk and bison as well as more traditional domestic livestock (11).

(6) Specific diagnostic technologies that are chosen and the specific problems that may arise associated with these technologies

In deer, tuberculosis is most often transmitted from infected hinds to fawns. These young animals may harbour high levels of infective organisms without any pathological evidence of disease or diagnostic reactivity typical of infection with tuberculosis (18).

1.4 The present situation

The Office International des Epizooties (OIE) reports yearly on the number of herds and the number of animals identified as tuberculosis positive in different member countries. Table I provides the information relating to the 1996 prevalence of tuberculosis in several countries (including Canada) which would be of interest to Canada from a trading perspective (19). Several countries are reported free of bovine tuberculosis. These include Denmark, Finland, Greenland, Iceland, Luxembourg, Norway, and Sweden.

Table I
Herd and population prevalence of tuberculosis in cattle and buffalo (Australia) for 1996 from the Office International des Epizooties's World Animal Health Report

COUNTRY	HERD PREVALENCE	POPULATION PREVALENCE
Australia	bovine - 0.007% buffalo - 8.3%	N.A[a]
Austria	0.004%	0.0002%
Belgium	0.09%	0.01%
Canada	0.0008%	N.A.
France	0.07%	N.A.
Greece	0.4%	0.8%
Hungary	0.002%	0.0001%
Ireland	5.8%	N.A.
Italy	N.A	0.06%
The Netherlands	0.002%	0.00002%
New Zealand	1.8%	0.03%
Switzerland	0.003	0.0001%
United Kingdom/Great Britain	0.01%	0.01%
United States of America	0.0008%	0.00002%

[a] Information not available

1.5 Objectives and chapter overviews

There were two main objectives for this project. The first objective was to review the literature on the epidemiology of *Mycobacterium bovis*, specifically in cattle and cervidae. Chapter Two contains information on the source of infection, the mode of transmission, clinical signs, gross and microscopic lesions, and diagnostic technologies for detection of tuberculosis.

The second objective was to analyse all of the tuberculosis outbreaks in Canadian cattle and cervidae over the last ten years and to develop models for between and within herd spread of *M. bovis*. Data were collected at the herd and individual animal level and included demographic information and testing results. Risk factors associated with different management styles, for example housing and feeding facilities, were not a major component of the study as these factors could not be deduced from the available outbreak files. Chapter Three outlines the general information, which was collected from the outbreak files, on each of the outbreaks of tuberculosis in Canadian cattle of cervidae between 1985-1994. Chapter Four contains a more detailed written narrative of each outbreak which is accompanied by outbreak diagrams showing the assumed transmission of the organism from farm to farm and an overview of the evolution of the outbreak from the perspective of the veterinary inspector. Chapter Five is an analysis, utilizing a logistic regression, and discussion of the risk factors associated with being a potentially infected or culture positive farm. Chapter Six is an analysis of the risk factors for spread of *M.bovis* between animals. A negative binomial regression analysis was used. Chapter Seven is a summary of the conclusions drawn from the analyses of the data in this study.

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CHAPTER 2

TUBERCULOSIS IN CATTLE AND CERVIDAE AND DIAGNOSTIC TECHNOLOGIES

2.1 Introduction

The objective of this chapter was to present a general review of tuberculosis in cattle and cervidae with emphasis on infection and detection of infection. Areas covered include:

(1) sources of infection and methods of transmission;

(2) pathogenesis;

(3) clinical signs;

(4) pathology; and,

(5) diagnosis of *M. bovis* infection.

2.2 Sources of infection and modes of transmission

Mycobacterium bovis is the most important and common etiologic agent of bovine and cervid tuberculosis. This organism has the potential to infect primates including humans, and a wide range of animal species including domesticated, wild, and exotic species (1). These species may serve as a source or reservoir of infection for cattle and cervids. For example, in Argentina and Brazil, where bovine tuberculosis remains a problem, pigs are relatively commonly infected with *M.* bovis (2). Wildlife reservoirs are an important source of infection in several countries (2,3). The brush-tailed possum (*Trichosurus vulpecula*) in New Zealand and the badger (*Meles meles*) in Great Britain and the Republic of Ireland are significant examples. In Northern Ireland the cattle population itself is considered responsible for the maintenance of the disease in this population (2). There are examples of countries which have a wildlife reservoir of tuberculosis

but transmission to domesticated livestock has not occurred. Canada, for instance, has tuberculous bison in Wood Buffalo National Park. However there has been no evidence of spread to other wildlife species or to domesticated species (4). Exotic species in zoos and wildlife parks may harbour *M. bovis* and represent a public health hazard to workers and visitors as well as domesticated livestock (5).

An analysis of data from a series of experiments on the excretion of *M. bovis* from infected cattle indicated that there was an inverse exponential relationship between the "dose" of the organism and the delay before excretion began (6). Field data supported the findings and suggested that in natural bovine tuberculosis, excretion of *M. bovis* begins approximately 87 days after infection occurs.

An interesting observation concerning reservoirs of infection was that humans infected with bovine strains of Mycobacterium posed a considerable risk to cattle populations (1,3,7). This realization may require a paradigm shift in eradication and control programs and certainly will require increased communication between the human and veterinary health communities.

Contaminated environmental sites may maintain *M. bovis* organisms for considerable periods of time with the availability of organic nutrients being the most important factor to their survival (8). Other important factors include temperature, sunlight, moisture, oxygen concentration, pH, and competition with other organisms. It has been shown experimentally that the organism can survive up to 2 years outside an animal host in the Northern European environment and up to 7 weeks in the North Queensland, Australia environment (9). The considerable difference in survival time was thought to be due to the difference in temperature which was higher in Australia. A New Zealand study showed that there was a relatively short

survival time for *M. bovis* outside the host (10). The authors suggested that this showed the relative unimportance of contaminated pasture, especially in summer, in the epidemiology of tuberculosis in cattle, deer, and possums. *M. bovis* organisms which persist in carcasses of infected animals may pose a threat to scavengers and to livestock which may graze the contaminated area (8).

The main route of transmission of *M. bovis* in cattle and cervids is aerogenous (1,3,11-13). Infection via ingestion of contaminated milk also occurs but less commonly than infection via the respiratory route (1). Ingestion is a more important route in farmed deer than in cattle (8). Other routes of infection include: cutaneous, via infection of another primary lesion; congenital, with infection of the fetus occurring via the umbilical vessels from the infected maternal uterus; genital, when the male or female reproductive organs are infected or contaminated; and, intra mammary, from contaminated infusions (2).

The distribution of lesions is felt to be associated with the mode of transmission. Lepper and Pearson studied the distribution of tuberculous lesions in the thoracic and abdominal cavities of beef cattle raised under improved pasture and range conditions (14). They concluded that infection via the alimentary route occurs under temperate conditions which support survival of organisms on pasture and in the environment. A different opinion is expressed by Morris (8). He states that the size of the minimum infective dose for oral infection is high and that survival of organisms on fomites as a source of infection is rare.

Langmuir differentiates infection via "droplets" and infection via "droplet nuclei" (15). The former he calls contact infection, and the latter, airborne infection. He defines droplets as particles which are generated from the mouth or nose during talking, coughing, and sneezing.

They do not extend more than 1 meter from the mouth and generally fall to the ground and dry to form a residue. Droplet nuclei, on the other hand, arise from the dried droplets and remain suspended in the air or move with air currents to areas distant from the source. Dust, larger particles than the first two, exist on floors and bedding and may be suspended or resuspended by various activities such as sweeping. The resuspended particles may be droplet nuclei. The requirements for airborne transmission of *M. bovis* between animals are met by droplet nuclei. That is, droplet nuclei are: (1) capable of carrying *M. bovis*; (2) can persist in the air long enough to be inhaled; and, (3) can penetrate into the lung and initiate infection (8).

The dominant theory of the mechanism of spread of tuberculosis was based on the idea of close contact however airborne infection via droplet nuclei is a much better explanation of the evidence of spread (15). There are instances, in humans, where household contacts and marital partners of sputum-positive patients often do not become infected. There have been epidemics in humans however where most people in a group became infected at the same time.

The post-aerosolization environment is also critical to the droplet nuclei and thus to the host-parasite relationship (16). This includes environmental factors such as temperature, light, availability of organic material, and pH. The host factors which are important include genetic susceptibility, stress level, and disease/health status. Animal behaviour was also suggested as a host factor (17). Sauter and Morris showed that 86% of the tuberculin test-positive cattle were among the 20% most dominant animals in their herds.

2.3 Pathogenesis and immunology

The route of infection has a significant impact on the expression of the disease. The route of infection is in turn influenced by age, environment, and the management practices to which the animal is exposed. As previously stated, inhalation of *M. bovis* is the most common route of infection. A primary lesion or focus of infection follows interaction of the host and the organism and the primary lesion together with the associated regional lymph nodes is called the "primary complex" (2).

The expression of disease in other less common routes of infection are significantly different. The cutaneous route usually results in a localized infection with possible local lymph node involvement while the congenital route results in a primary lesion in the liver and portal lymph nodes with death of the calf usually within a few weeks of birth. The more common ingestion route primarily results in lesions in the mesenteric lymph nodes. Mesenteric lesions may be secondary to primary lung lesions and result from the animal swallowing heavily contaminated sputum (2).

The immune response in tuberculosis infection varies between and within species (1,18). It is unwise to make assumptions regarding the immune response of bovines and cervids to *M. bovis* infection from studies of the immune response of humans to *M. tuberculosis* infection. There are, however, several generalizations which can be stated for tuberculosis infection and the immune response to it. These are as follows.

(1) Both antibody and cell mediated immune responses can be induced following mycobacterial infection but it is generally accepted that the cell mediated immune system has the most significant role (2,18).

(2) In the cell mediated immune response, T-cells recognize processed mycobacterial antigens which are associated with major histocompatibility complexes (MHC) on antigen presenting cells. The anti-mycobacterial capacities of macrophages are activated via cytokines, through this interaction with the T-cells. Cytokines are produced by T-cells. Activated macrophages have the ability to inhibit and possibly destroy the organism. Similar cellular interactions may cause delayed type hypersensitivity (DTH). In this situation activated macrophages containing organisms are destroyed, leading to possible release of organisms and material which is toxic to tissue. Thus the organism may be spread and tissue damage (necrosis) may occur (2,18-23).

(3) Immune reactions to *M. bovis* infection are modified by host factors such as genetics, concurrent disease, health status and age (5).

2.4 Clinical signs

Clinical signs of tuberculosis in cattle and deer are non-specific and generally occur only in the advanced stages of the disease (24,25). Cattle may be infected for years yet appear clinically normal. The disease may be evident within six months of infection in cervids or only after several years. Death will usually follow within 1-2 weeks after clinical signs appear in deer (5). The signs of the disease in both species include emaciation, fever, coughing, laboured breathing, respiratory rales, and occasionally, diarrhea. In both deer and cattle, superficial lymph nodes may enlarge due to abscessation and these abscesses may break through the skin surface and discharge a thick creamy pus. Antler growth may be retarded in animals that are in poor condition and the antlers may moult. Reproductive performance may be affected with stags being sexually indifferent and females failing to come into estrus.

There is a sharp contrast in clinical pictures between experimental and natural infections with *M. bovis*. The findings in three separate studies on experimental inoculation were similar (26-28). Three categories of infection were observed - peracute, acute, and chronic. Clinical findings and the distribution and severity of post mortem lesions were similar and decreased from the peracute to the chronic cases. Thus in experimental studies the clinical picture is very similar and yet natural infections are extremely variable in onset, duration, and outcome. It is rare to find natural cases of tuberculosis that present as acute fulminating disease. The only real similarities between natural and experimental infections are respiratory signs. The organism does not seem to kill acutely unless received in very high doses. The severity of the clinical signs in these experimental studies is probably related to the size of the infective dose and to the route of infection - that is, intravenous injection or intra nasal infection.

2.5 Gross pathology and histopathology

Table II describes and compares the gross and histopathological lesions of tuberculosis in cattle and cervids. Cattle lesions are more frequently caseous granulomas whereas cervid lesions are more frequently pyogranulomas. The distribution of lesions is very similar. Corner notes that since tuberculosis is a disease of the reticuloendothelial system, lesions may occur in any anatomical site (29). This is not to suggest that the distribution is random. There are differences in the microscopic features of cattle and cervid lesions as well as considerable differences between cervid species (30). It should be noted that the frequency of lesions and the sensitivity of detection of lesions is related to the detection procedure used to identify infected animals (31). The more detailed the procedure, the greater the percentage of animals found with more than one lesion.

Table II
Comparison between cattle and cervids of gross pathological and histopathological lesions resulting from infection with *Mycobacterium bovis*

Cattle	Cervid
Location of Primary Complex	
Depending on route of transmission:(1) (1) aerogenous: subpleural location in the dorsal-caudal portions of the diaphragmatic lobes of the lung and associated lymph nodes (2) haematogenous: may lead to lesions in lungs, spleen, bone marrow liver, kidney, adrenals, testes, uterus, udder, meninges, or serous cavities (3) alimentary: primary complex may develop in the intestine	Similar to cattle with some variation:(25,30) (1) apical and cardiac lobes of the lung (in addition to caudal lobes as in cattle) (2) splenic abscesses may occur in deer other than through the congenital route of transmission
Morphology	
(1) pulmonary granulomas that progress to tubercles (2) large firm lymph node granulomas (3) lesions with centers of caseous necrosis with mineralization (4) encapsulated by well organized connective tissue (32)	(1) primarily suppurative lymph node lesion (2) primarily pyogranulomas and abscesses that are thin-walled as compared to the well encapsulated lesions found in cattle (25,30,33,34)
Evidence of Spread	
Infection may be generalized by: (1) Local spread which manifests as a generalized bronchopneumonia (cavity formation is not common as in humans) (2) extra-thoracic disease (relatively uncommon) with affected organs in decreasing frequency being liver, kidney, spleen, uterus and udder (3)	(1) may be spread from large abscesses or may develop on the diaphragm and on adjacent pleura and extend to subcutaneous swellings (25)

Cattle	Cervid
Distribution of Lesions	
1) 70% - 90% of lesions in either lymph nodes of head or in the thoracic cavity [a] (2) 83.4% of lesions in lungs and mediastinum, bronchial and medial retropharyngeal lymph nodes (3)11.5% of lesions in mandibular, parotid, mesenteric and hepatic lymph nodes, liver (1,29)	(1) primarily suppurative lymph node lesions (retropharyngeal) and lung lesions (2) 60.5% of elk with lesions had thoracic involvement (3) purulent tonsillitis (25,33-35)
Size of Lesions	
(1) microscopic foci to large readily identifiable tubercles (24)	(1) lymph node lesions from 1 - 30 cm. (2) very large abscesses in the mesenteric lymph nodes (25,34)
Frequency of Lesions	
(1) may vary from single primary lesion to multiple secondary (2) 66% of cattle had single lesions [b] (24,29)	No information
Microanatomy	
(1) focal granuloma (tubercle) (2) some central caseous necrosis (3) lesions encircled by zone of epithelioid cells, lymphocytes, granulocytes, and multi-nucleated giant cells (4) mineralization may be present in necrotic centres (5) outer boundary of fibrous connective tissue is usually present between lesion and normal tissue (6) lesions have few if any *M. bovis* organisms but occasionally are numerous and randomly scattered in the caseous necrotic tissue and inflammatory cell matter (7) lung lesions are similar to lymph node lesions (30,32)	(1) granuloma or pyogranuloma with M. bovis often present (2) suppurative versus caseous (3) many organisms (4) fewer giant cells than cattle (5) fallow deer are very similar to elk and red deer in both lung and lymph node lesions (6) Sika Deer lymph node and lung lesions are very different from other cervids, having abundant bizarre giant cells (25,33)

Cattle	Cervid
Mineralization	
(1) common but usually does not extend into the peripheral inflammatory cell mantle (30)	(1) relatively less common but extends into the peripheral inflammatory cell mantle (30,33)

[a] Using different techniques, the frequency of single lesions was:
(1) Detailed necropsy: 65.5% (of 374 cattle)
(2) Abattoir necropsy: 49.7% (of 167 cattle)
(3) Export Abattoir: 66.6% (of 455 cattle)
[b] 66% of 374 tuberculous cattle had single lesions

2.6 Diagnosis of *M. bovis* infection

This section is devoted to a description of clinical diagnostic techniques, routine diagnostic tests, and some of the more advanced molecular biological technologies for identification of animals exposed to or infected with *M. bovis*. These diagnostic methods are briefly described and the advantages and disadvantages discussed. A comparison of the sensitivity and specificity of the various technologies is given in Table III. Care must be taken in the interpretation of these estimates of sensitivity and specificity. In most cases, studies used the culture of *M. bovis* as the gold standard for diagnosis of tuberculosis. However this was not always the case and if alternative standards were used, these are identified in the footnotes of the table.

Table III
A comparison of the sensitivity and specificity of different diagnostic technologies in bovine and cervid tuberculosis

Type of Test	Cattle			Cervids		
	Sensitivity	Specificity	Reference	Sensitivity	Specificity	Reference
Caudal Fold	81.8%	96.3%	(36)	N.R.[a]	N.R	(5)
	72%	96-98.8%	(36)			
Single Cervical	91.2%	75.5%	(37)	81%	80%	(38)
				45%-86% [b]		(39)
				81.3%		(5)
					73.5% - 100% [c]	(40)
Comp. Cerv.	77-95%		(36)	31-80%	61-88%	(5)
Slaughter Inspection	33-67%		(31)			
Post Mortem				90%[d]	95%	(33)
				93%	89%	(38)
Histology				88%	89%	(38)
Gamma Interferon	76.8% - 93.6%	96.3 - 98.1%[e]	(41)			
Gamma Interferon +Skin Test	95.2%		(41)			

Type of Test	Cattle			Cervids		
	Sensitivity	Specificity	Reference	Sensitivity	Specificity	Reference
ELISA (antibody)	35.9%	92.3% [f]	(42)	83%		(5)
		98.1%	(42)			
Gamma Interferon Sandwich ELISA	53.8%					
		98%				
MPB70 ELISA	12.5% - 49.5% [g]		(43)	74%	81%	(38)
		96.4%				
	18.1%		(43)			
ELISA (antigen)	55%-100%	75%	(44)			
Blood TB Test				59%	84%	(38)
				95.8	98%	(5)

[a] The caudal fold test is not recommended for use with deer and elk because of its low sensitivity.
[b] The sensitivity was 86% when the presence of a visible or palpable skin reaction was taken as a positive test. The sensitivity fell to 45% when an increase in skin thickness of greater than or equal to 2.5 mm was used to denote reactor status.
[c] The single cervical intradermal test was evaluated on Tasmania deer, a population which is purported to be free of bovine tuberculosis. The specificity went from 73.5% to 100% when a positive test was evaluated as a skin thickness raised from 1 mm or more to 2 mm or more.
[d] In this study the sensitivity and specificity were determined using histological results as a baseline. The study was on elk from a *M. bovis* positive farm under abattoir conditions. There was an overall herd prevalence of gross lesions of 39.8%
[e] Specificity was determined by testing more than 6000 cattle from tuberculosis-free herds. The range in specificity resulted from different cut-off points chosen to define a positive reactor. Sensitivity was determined from cattle herds being depopulated because of bovine tuberculosis.
[f] A sandwich ELISA for the detection of gamma interferon and an indirect ELISA for detection of mycobacterial antibodies were compared. Sera and blood from 39 culture positive animals and 52 negative status animals were used. Post mortem and culture were not performed on the 52 negative status animals.
[g] sera from 109 culture positive cattle which had been previously skin tested; and, 229 tuberculosis free cattle, were used. There were 32 sera from cattle that had not been previously skin tested. Only 4 of these were positive to the MPB70 ELISA, yielding a sensitivity of 12.5%.

2.6.1. History and clinical signs

Diagnosis of tuberculosis based on clinical findings is difficult for many reasons. The clinical signs are very nonspecific even in the advanced stages of the disease and the onset of clinical signs over time is very variable. Most cattle in Canada are slaughtered or culled before clinical signs would be evident. Tuberculosis in cervids may progress to the stage where clinical signs are apparent and animals displaying such signs should be handled carefully because of the zoonotic potential of this organism. However, the signs are still non-specific and given the very low prevalence of tuberculosis in the Canadian livestock population, practitioners and clinicians are not likely to have experience with the clinical picture of tuberculosis. A herd history may be useful in some cases. Animal movement, species and breeds on the farm, production type, number of animals of each species on the farm, management factors, and past skin test history are all relevant. Thus, the suspicion of tuberculosis based on clinical signs and history may be possible, but other tests are required for a more definitive diagnosis.

2.6.2 Skin tests

The most commonly performed skin tests for tuberculosis in cattle and cervids consist of an intradermal injection of a purified protein derivative (PPD) of *M. bovis* (also called tuberculin). The caudal fold test and the mid-cervical test each consist of a single intradermal injection of bovine PPD. The tuberculin is injected intradermally either in the caudal fold skin of the tail or in the cervical area of the neck. Swelling, with or without heat, 72 hours after injection is considered a positive reaction to the injection and thus evidence of previous exposure to mycobacterial antigens. A variation of the skin test is the comparative test where PPD of *M. bovis* and *M. avium* are injected separately in two sites in the cervical area of the neck and a

comparison is made of the reactions at the different sites. The reactions to the two different antigens are graphed and compared to assure that the reaction to the bovine tuberculin is significantly greater than the reaction to the avian tuberculin. This allows for the assessment of cross reactivity due to sensitization with other mycobacteria such as avian mycobacteria. Skin tests are usually read at 72 hours after injection although other intervals can be used. The basis of the test is a cell mediated immune reaction which when it occurs in skin is termed delayed type hypersensitivity. The caudal fold site is not considered useful in cervids because of its extremely low sensitivity in these species (5).

The intradermal test in cattle using the caudal fold was first employed in 1908 by Moussu and Mantoux (36). However the history of intradermal testing, in general, began eight years after Robert Koch announced the identification and culture of the tubercle bacilli. The basis of the intradermal skin test is an immunological reaction which is now known as the Koch Phenomenon (19). The reaction is seen in animals that have been previously exposed/infected with *M. bovis*. The site of injection becomes hard and darkened within 72 hours of injection. Over the next few days the skin at the site may become necrotic, slough, and finally, heal.

The reaction to the inoculation occurs regardless of whether a culture of the bacteria or a concentrated culture filtrate is used. The concentrated filtrate was termed "Old Tuberculin". The process for the production of tuberculin has been improved and standardized, resulting in the production of PPD (36). The usefulness of the Koch phenomenon for the diagnosis of tuberculosis was advanced by the studies of the Austrian physician Clemens von Pirquel (19). He postulated that "allergic" reactors to tuberculin indicated an infection with the tubercle bacillus.

Different countries have adopted different protocols for using intradermal skin tests. The

single intradermal test using the caudal fold site was most popular in the United States and was adopted as the official test in 1920. This test and site is also used in New Zealand and Australia. In Canada, the caudal fold test is the most commonly employed skin test for preliminary testing. The comparative cervical test is used as a follow-up test to eliminate animals that are false positives due to cross-reactivity from infection with other mycobacteria. The skin on the neck is considered more sensitive and this site is more popular for the single intradermal tuberculin test in Europe.

Skin testing using A60, a thermo-stable macromolecular antigen complex of M. bovis strain BCG, has also been evaluated (45). It was compared, using the same animals, to results from testing with PPD. Similar results were attained. This antigen has also been used in an ELISA and an immunoblot technique.

When sensitivity of the skin test is low, there is a problem with false negative reactions. False negatives occur for a number of reasons including the following (36,46):

1. reactivity to the intradermal test using PPD does not occur until 30-50 days following infection so recently infected animals may have negative test results;

2. anergic reactions in which infected cattle fail to react to the test due to an immune mediated or stress mediated mechanism;

3. PPD or antigen of low potency;

4. multiple dose syringes may pose a problem especially with the first and last dose are not a full aliquot;

5. variable operator skill in performing and reading the test;

7. desensitization to tuberculin for approximately 42-60 days after an intradermal skin

test (causes a problem if animals are repeat tested);

8. early post-partum immunosuppression (skin reactivity returns within 4-6 weeks after calving);

9. malnutrition, which may suppress cell mediated immunity;

10. combined effect of malnutrition and pregnancy;

11. treatment with steroids;

12. and, purposeful deceit.

Likewise, there may be problems with false positive reactions. These problems may arise for the following reasons.

1. The specificity of the test in the population is low as a result of:

a. cross reaction with infection by *M. paratuberculosis;*

b. infection or exposure to *M. avium;*

c. skin tuberculosis, with lesions resembling those of tuberculosis histologically and in which acid-fast organisms can be demonstrated but from which *M. bovis* cannot be isolated;

d. or, exposure to environmental Mycobacteria or related organisms.

2. The proportion of positives which are false will also increase as the prevalence of the disease in the population decreases. This is true for all screening tests. As the prevalence of disease becomes low, the proportion of positive test results which are indicative of true infection (i.e. positive predictive value) also becomes low.

3. There may also be apparent false positive animals. This may occur when the sensitivity of the screening test is higher than the reference test. For example, there are NVL (no

visible lesion) animals which react to the tuberculin skin test but do not have lesions at slaughter or on histological examination. These animals may be true false positive or apparent false positive. It is essential for the investigators to determine the true status of all test reactors as the eradication program enters the final stages. NVL animals represent a dilemma for investigators. The proportion of these animals increases as an eradication program progresses (assuming that the program is successful and that the prevalence does decrease) . They may be true false positive animals or apparent false positive animals. One method to address this problem is through a more thorough post mortem examination of tuberculin test reactors - in other words, increase the sensitivity of the reference test.

Problems with false negative and positive reactions do occur with tuberculin skin tests. However these tests still represent the best system available for screening large numbers of animals. It is likely that they will continue to be used in control and eradication programs for many years to come.

2.6.3 Slaughter inspection and post mortem diagnosis

Routine slaughter inspection is the main screening test in Canada and the United States for detection of bovine tuberculosis. It is a convenient method for monitoring large numbers of animals but the sensitivity is low for detection of lesions in individual animals, especially when compared to more detailed post mortem examination. A tentative diagnosis of tuberculosis may be made on the basis of macroscopic lesions especially during the early stages of an eradication program when the prevalence of disease is high. However when the prevalence is low or if a definitive diagnosis is required, culture and isolation of the organism is required to determine the

true status of the reactor or lesioned animal. A detailed necropsy procedure is more sensitive than routine abattoir inspection (31). Corner et al. found that in a comparison of routine abattoir screening and detailed necropsy procedures, the former failed to detect an estimated 47% of cattle with lesions.

Corner determined the distribution of lesions in 374 tuberculous cattle (29). In this study 66% of tuberculous cattle had only one lesion and 86% of the lesioned animals were detected if only the medial retropharyngeal lymph nodes (left and right), the mediastinal lymph nodes (anterior and posterior), bronchial (right and left) and the lung were examined. When all 8 sites (above four plus the mesenteric lymph nodes, parotid lymph nodes (left and right), caudal cervical lymph nodes (left and right) and the superficial inguinal lymph nodes (left and right) were examined, 95% of the tuberculous animals would have been detected.

Whiting and Tessaro examined, under abattoir conditions, the gross lesions of a tuberculosis positive herd of farmed elk (47). The prevalence of lesions was 39.8% in the 337 elk. Examination of only the lymph nodes of the head and thorax would have detected 118 (88.1%) of the 134 elk with lesions. Griffin states that in farmed deer tuberculosis lesions are usually present in the lymph nodes of Waldeyer's ring, especially in the retropharyngeal lymph nodes (5). However, the predominant location in the head, thorax, or abdomen may be related to the route of transmission - that is respiratory versus oral.

Detection of positive herds and individual animals is much simpler when the prevalence of disease is high and a broad spectrum of disease manifestations exist. At the end of an eradication program or in herds with no history of tuberculosis, a definitive diagnosis is extremely important to determine the true status of the animal and the herd. In these cases it is

very important to make every effort to culture the organism as this is the most conclusive method to establish that the animal is infected with *M.bovis*.

2.6.4 Histology

Diagnosis of tuberculosis is based on identification of typical lesions with or without the presence of acid fast bacilli. A comparison of the typical histological lesions seen in cattle and cervids is found in Table IV (5,25,27,31,33).

Table IV
Comparison of histological characteristics of tuberculosis lesions found in cattle and cervids

CHARACTERISTIC	CATTLE	CERVIDS
Distribution	similar	similar
Type of Lesion	granuloma	pyogranuloma
Capsule	connective tissue	connective tissue
Sub-capsular cells	epitheliod cells multi nucleated giant cells few to numerous lymphocytes and neutrophils	lymphocytes epitheliod macrophages Langhans-type giant cells
Character of central area	caseous necrosis with mineralization	caseous or liquefactive necrosis
Cells in central area	calcified and usually with caseous necrosis when lesions are more mature	substantial populations of neutrophils and widely scattered foci of mineralized debris

2.6.5 Culture

Culture of *Mycobacterium bovis* is required for a definitive designation of an animal or herd as tuberculosis positive. In Canada it is the gold standard test for the detection of *M. bovis* and is generally required for the depopulation of a herd. There have been exceptions in Canada where herds were depopulated when isolation of the organism did not occur. This has been the case when there was a high risk of transmission or very strong evidence that transmission did occur but isolation was not successful.

M. bovis is usually isolated by direct culture of affected tissues, with the exception of milk, which is usually isolated through procedures involving animal inoculation. The review by Collins and Grange provides a brief but succinct review of the microbiology of the bovine tubercle bacillus and it will not be discussed further here (3).

There are several disadvantages of culture as a diagnostic technology. The sensitivity of the test is low. It may be difficult to grow the organism due to contamination of the specimen by other organisms. Decontamination of the clinical material is therefore required but has a detrimental effect on the mycobacteria as well. The organism requires a prolonged period of incubation for isolation - up to 12 weeks. A fairly high level of knowledge and technical skill are required as identification is not simple and requires a combination of tests to identify human or bovine strains and to differentiate them from other mycobacteria. Specialized facilities with a high bio-security clearance are also required.

2.6.6 *In vitro* immunodiagnostic assays

In vitro cellular assays for the diagnosis of bovine tuberculosis measure the reactivity of T cells from *M. bovis* infected cattle. Two diagnostic tests have been described - the lymphocyte

transformation test (LTT) and the gamma interferon (IFN) test. The LTT measures antigen specific responses to PPD antigen but has several disadvantages. (43) It takes 3-5 days to complete and therefore, is lengthier than the tuberculin tests. It is a technically difficult test to perform and requires radioactive nucleotides. This latter requirement means that only a relatively small number of laboratories are equipped to perform the test.

The IFN test is based on the fact that a cytokine, interferon gamma, is released from sensitized lymphocytes when they are exposed to *M. bovis* antigens (bovine PPD). Monoclonal antibodies to gamma interferon were developed for use in enzyme immunoassay for bovine interferon gamma (43). The problem of cross-reactivity to *M. avium* can be eliminated by performing a comparable assay using avian PPD. The IFN test is the first in-vitro cellular assay to be used in routine veterinary diagnostic testing (48). Certain conditions must be met to optimize the test including procedures for collection and handling of blood and type of anticoagulant used. However it is a rapid, sensitive, specific and inexpensive alternative to the LLT. A study by Neill et al. demonstrated a significant number of animals which were in the early stages of infection, but were tuberculin test negative, reacted to the IFN test (21). They conclude that this test may be useful for early detection of *M. bovis* infection.

2.6.7 Serological tests

Various serological techniques have been studied for the diagnosis of bovine tuberculosis (43). These included the following.

1. the bentonite flocculation test
2. the kaolin agglutination test
3. the indirect fluorescent antibody test (IFA)

4. the complement fixation test

5. the ELISA test for detection of antibody in serum

6. the ELISA test for the detection of mycobacterial antigen in tissue (44)

The first four tests lack sensitivity and specificity (27,43). The bentonite flocculation and IFA tests detect antimycobacterial antibody but the level of antibody fluctuates markedly during the course of the disease (27). Consequently these tests have not been useful for screening for *M. bovis*.

The ELISA test has been studied extensively in an attempt to find a fast, reliable, and inexpensive alternative or adjunct to the skin test. Several interesting observations have emerged from these studies.

(1) There is evidence to suggest an inverse relationship between cellular and humoral immune responses to *M. bovis* in cattle with natural infection (49,50).

(2) In one study, a high serum antibody titre was highly positively correlated with the ability to culture *M. bovis* from respiratory swabs from that animal. Thus high serum antibody titres may suggest infectivity (42).

(3) Plackett et al. reported that the ELISA would be a useful diagnostic tool for use with infected cattle that fail to react to the caudal fold tuberculin test (51). They also concluded that the low sensitivity and specificity of this test precluded its use as a replacement for the tuberculin test.

(4) Cross reactivity between mycobacterial antigens is the main reason for the low specificity of the ELISA (26,43,51,52). Cattle experimentally infected with *M. bovis* were tested for their antibody response to the *M. bovis* specific protein MPB70 (43). The

antibody response began to increase at 19-28 weeks post infection and varied significantly between individual animals. Tuberculin testing of infected animals induced a strong anamnestic antibody response to the MPB70 antigen. This response has an impact on the sensitivity of this ELISA.

(5) Sagerman *et al.* performed an ELISA test using the A60 thermostable macromolecule antigen complex of *M. bovis* BCG. This antigen had been used for diagnosis in human tuberculosis (45). It was found to elicit both a cellular and a humeral immune response. Its immunodominance (at least in humans) is illustrated by the fact that most anti-mycobacterial immunoglobulins in the cerebrospinal fluid of patients with tuberculous meningitis are directed against A60. At a cut-off value of 400 - 800 EU (elisa units) as suspect and >800 EU as positive, this serological test detected more positive plus suspect animals in a group of supposedly infected animals than the PPD cutaneous test or the A60 cutaneous test (95% versus 80%).

An ELISA test to detect antigen has also been studied (32). Sputum would be the only practical clinical sample for an *in vitro* test. The variability of shedding and the fact that the necessary level of organisms would probably only occur in advanced disease suggest that this is not a very useful diagnostic tool . Thoen *et al.* investigated the development of an ELISA to detect mycobacterial antigens in tuberculous lesions of cattle from which *M. bovis* had been isolated (44). This ELISA would be useful to replace or augment histology and eliminate the very lengthy wait required for culture results. This outcome of this test is based on a reaction measured from 0-4+, with 0 being negative, suspect 1+ and positive 2+ or greater. Four types of tissue were evaluated in the test. The sensitivity of the test was 100%, 75% and 55% when the

interpretation of a positive reaction increased from 2+, 3+ and 4+ respectively. Positive tissue consisted of granulomas from which *M. bovis* was isolated. The specificity was 100% for the negative controls (tissues from thoracic lymph nodes in which granulomatous lesions were not observed and mycobacteria were not isolated) . There was cross-reactivity in the suspect range in animals that had granulomas from which mycobacteria were not isolated. Animals which had granulomas from which *M. avium* was isolated, showed cross-reactivity in the suspect and in the 2+ category.

2.6.8 Blood tuberculosis test (BTB)

The BTB assay system consists of three components which are interpreted together in order to evaluate the status of an animal. The first component is a modified lymphocyte transformation assay in which mononuclear lymphocytes are co-cultured with PPD. This component of the assay is used to define specific immunological function. The second component consists of a non-specific inflammatory profile which includes determination of white blood cell counts, haematological values, plasma viscosity, and fibrinogen. The third component of the assay is an ELISA test which measures the humoral antibody response of the animal. Thus the assay measures different pathways of immune reactivity. This assay system can be used in combination with the skin test and is generally performed 10 days after the single intradermal skin test. In a study by Griffin et al. involving 96 *M. bovis* culture positive deer, the sensitivity of the BTB was 95.8%.The specificity was determined to be 98% (5). This assay system was compared to the LTT, ELISA, single intradermal tuberculin test and a combination of single intradermal tuberculin test and ELISA. The respective sensitivities of these tests was 88.5%, 86.5%, 81.3% and 94.8%.

The main advantage of this assay system would seem to be the fact that various pathways of the immune response are measured at the same time. Thus, animals in different phases of infection may be detected. Griffin suggests also that animals which have a low LT reactivity on the BTB or convert from a high to a low reactivity, may actually be animals which have acquired protective immunity against tuberculosis. These animals seldom have tuberculous lesions at necropsy.

There are two significant disadvantages of the BTB. The first is that the laboratory analysis is technically demanding and labour intensive, and, the second is that conditions under which sampling is carried out may cause abnormal haematological profiles if animals are stressed significantly during mustering and sampling.

2.6.9 Molecular biological techniques

Restriction fragment analysis (RFA) is a technique which cleaves bacterial DNA into specific characteristic patterns or "finger prints". Thus different organisms and strains of species can be identified by their particular pattern. This technique is particularly useful for epidemiological studies where the source of infection is to be determined or compared. It is not however a technique that is applied to routine diagnostic testing for tuberculosis (26).

Another technique for comparing bacterial isolates is the Southern Blot technique (26). The restriction fragments are exposed to DNA probes which must be able to identify very specific DNA polymorphisms. This technique does not seem to have the potential to replace the RFA technique. However these two techniques are useful for epidemiological studies as they can detect a possible common source of infection (53).

The polymerase chain reaction test (PCR) is capable of detecting DNA from a single

organism of a pre-determined species in a few days (26). There are two key steps in this test: (1) finding a suitable method to remove the organism from tissue lesions so that the DNA can be exposed in a form to be amplified by the PCR; and, (2) amplifying a specific segment of the *M. bovis* DNA. Liebana et al. compared the results of a PCR test to culture for *M. bovis* and found that the PCR identified as positive 35 of 49 (74%) culture positive animals (54). All of the 19 animals that were from tuberculosis free herds and were skin test negative, interferon gamma negative and culture negative were also negative with the PCR. The sensitivity of the PCR is not particularly high when one considers that the sensitivity of culture is low and the sensitivity of PCR is only 74% when compared to culture. The major advantages of the PCR over culture are that it is much faster, can detect *M. bovis* when rapidly growing *Mycobacterium* spp. are present, and may be able to detect non-viable organisms in the sample.

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CHAPTER 3

TUBERCULOSIS OUTBREAKS IN CANADIAN CATTLE AND CERVIDAE BETWEEN 1985-1994

3.1 Introduction

The assumed principle agent causing tuberculosis in cattle and cervids, in Canada at this time, is *Mycobacterium bovis*. The major route of transmission is aerogenous in both cattle and cervids but the oral route is significant in cervids as well (1). Location of lesions is associated with the route of transmission but since this is an organism that spreads via the lymphatic system, lesions can be found in any body system.

Tuberculosis is important as a public health issue, as an occupational hazard for people working in animal agriculture especially in slaughter plants, and because of its implications for trade.

Canada embarked on a tuberculosis eradication program in 1923 with the Restricted Area Plan. A test and slaughter program reduced the prevalence to 0.11% by 1961 (2). Slaughter surveillance has been the principle method of screening for tuberculosis since 1978, although there are other methods of detection such as export testing or herd accreditation.

The objective of this chapter was to summarize the tuberculosis outbreaks in Canadian cattle and cervidae from 1985-1994. Summary data are presented from all of the outbreaks.

3.2 Materials and methods

A questionnaire was sent in the Fall of 1994 to people within Agriculture and Agri-food Canada who knew the history of tuberculosis outbreaks in Canadian cattle and cervidae between 1985-1994. The purpose of the questionnaire was to determine what data were available concerning outbreaks and where the data were located. The files for a tuberculosis outbreak in Prince Edward Island (P.E.I.) were examined briefly and it was decided, based on the response to the survey and what was found in the P.E.I. files, to do a more in-depth analysis of the Manitoba bovine outbreak files. This was a fairly recent (1990) outbreak which was well documented and all the files were readily accessible. These files were amenable to analysis and if they represented the other outbreak files, it was felt that the study could be completed. Therefore a decision was made to do a full study of tuberculosis outbreaks in Canadian cattle and cervidae between 1985 - 1994.

There were 9 outbreak investigations during this time period, consisting of the following:

1. The Prince Edward Island bovine outbreak, 1987
2. The New Brunswick bison outbreak, 1985
3. The Quebec bovine outbreak, 1986 - 1987
4. The Quebec cervid outbreak, 1993 - 1994
5. The Ontario cervid outbreak, 1990 - 1994 (Testing ongoing in 1994, with no new positive farms identified)
6. The Manitoba bovine outbreak, 1990 - 1991
7. The Alberta bovine outbreak, 1985 - 1986
8. The Alberta/Saskatchewan cervid outbreak, 1990 - 1993

9. The British Columbia cervid outbreak, 1989 - 1990

3.2.1 The outbreak files

Data for the study were extracted from files which were kept at the Regional or District offices of Agriculture and Agri-food Canada. Collection of data in these offices started with the initial identification of a tuberculosis suspect animal. Generally the index animal was identified at slaughter or post mortem although positive herds (in British Columbia and Ontario) were identified after skin testing and subsequent follow-up performed at the request of the owner. Tissues from the initial suspect animal in each outbreak were submitted to a federal laboratory for histology and culture. Skin testing of the herd was initiated if the histology was suggestive of tuberculosis. The herd was usually depopulated only if the culture was positive for *M. bovis*. An investigation was conducted to determine the source and spread of the disease. All of the following herd categories were identified and tested for tuberculosis as part of an investigation:

(1) source herds to the positive herd (tracebacks);

(2) herds which received animals from the positive herd (traceouts);

(3) herds which had contact with animals from the positive herd (co-pasture or fence line);

(4) herds within a certain (variable to a certain extent, by province) radius of the positive herd (buffer or perimeter or zone).

(5) and, herds which were tested as part of an Area test outside of the defined buffer zone.

Data that were collected during the outbreak investigation were stored in files for the individual farm.

3.2.2 Data collection process

Physical retrieval of the outbreak files was accomplished through personal visits to Regional and District Offices and photocopied files sent by mail. The collection process took place from April, 1995 - March, 1996.

The data used in this study were generally found in one of two formats in the outbreak files. The first was from test result sheets. This included skin test reports, post mortem reports, histology and culture reports. The second format was an Inspector's Report. This included: a history of the herd as it related to the outbreak; the herds which had been identified for further testing as a result of their relationship with the farm under investigation; epidemiological information such as subsequent testing information and animal history; and, miscellaneous information unique to each situation. There were two exceptions to this process. The first was the Saskatchewan/Alberta cervid outbreak where data were stored in a computer database (Epi-Info) and the second was the New Brunswick (N.B.) bison outbreak where extensive outbreak records were not available. Overview material was collected for the N.B. outbreak primarily for the index farm and one perimeter premise. The data were not available to generate computer records as for the other outbreaks.

Both herd and individual animal information were extracted from the files. Herd data were extracted for every farm that was identified in the outbreak and for which the test results were available. Individual animal data were extracted only from farms which were classified as positive or "reactor" farms. A positive farm was one where *Mycobacterium bovis* had been cultured. A reactor farm was one which had one or more animals that were positive on a mid-cervical tuberculin test, a comparative cervical tuberculin test, or gross or histopathologic examination for tuberculosis. A farm which had positive animals on the caudal fold tuberculin

skin test but negative follow-up tests was considered negative. Test results from the last test were recorded when an animal or a herd was tested more than once with the same test.

The data were extracted from the files and entered on data entry forms (Appendix A). The data were then entered into a database program (Microsoft Access) and subsequently transferred to a general purpose statistical package (Stata) using a database manipulation program (DBMScopy). The Saskatchewan/Alberta elk files which were obtained as Epi-Info files were transferred directly to Stata and then reorganized to be in the same format as the data from the other outbreaks.

Data validation was performed by checking the accuracy of the data after it was entered into the database and after it was transformed to Stata format. Every record in the Prince Edward Island outbreak was validated from the data entry forms and after it was transformed to the Stata format. This assured that the data entry and transformation were accurate without exception. The remainder of the outbreak files were validated by checking 10 to 20 per cent of the records randomly chosen from the electronic form (Stata) against the original paper records.

3.2.3 Herd data collected

The paper data collection forms and the code list and computer acronym legend for the data collected are in Appendix A and C respectively. Table V gives the name and a brief description for each of the herd level variables in the study. Data were collected for every farm for which there were records. Not all records were complete as there were missing data in the outbreak files. Where variables were the same in the herd and individual animal data, they were defined only once .

Each OUTBREAK had a unique number. The FARMID, farm identification number, was

a unique number for each farm in the study. The farms in each outbreak were numbered consecutively with a four digit number having as the first digit the outbreak number.

The PRINCIPLE FARM TYPE identified the main species/breed in a herd based on numbers and production. There was only 1 principle farm type per farm. These were numerically coded into 9 categories consisting of dairy, beef, elk, bison, deer, sheep or goats, pigs, zoo animals, and other. A herd with 100 Holsteins, 50 Angus, and 50 Fallow Deer had a principle farm type of dairy. However if these Holsteins were steers or Holstein crossbreds used for beef production, the principle farm type was beef. These subtleties were not always clear in the records and often the principle farm type was determined by the traditional use of the predominant species. This variable was recoded to 6 categories for the statistical analysis by combining the last four categories into the group called other.

BREED was used to distinguish different breeds/species for test results when there was more than one animal type on a particular farm. The database allowed for up to 4 different breed test records per farm. In the example above, there could be (if they were available) test records for three groups of animals - dairy, beef, and cervids. However for each of these records the principle farm type remained dairy. The breed categories were recoded into 5 categories as follows: dairy, beef, cervids (deer and elk), bison, other.

The INVESTIGATION CODE (study start code in the data entry forms) was the reason why the investigation of a particular farm was initiated from the viewpoint of the inspector in charge of the outbreak. In the original data there were 9 categories which consisted of the following: a tuberculosis suspect animal discovered at slaughter inspection or on post mortem; a herd investigated because it was the herd of origin of a tuberculous or reactor animal (traceback);

a herd investigated because it received animals from an infected or reactor herd (traceout); a herd that had pasture contact with an infected or reactor herd; a herd that had fence line contact with an infected or a reactor herd; a herd tested because it was in a perimeter zone around an infected or a reactor farm; a herd tested as part of an area test other than perimeter testing; and, a herd tested for other reasons such as at the owner's request. The farm which initiated the investigation into another farm was called the REFERENCE FARM (source herd for the study start code on the data collection forms).

The HERD END CODE (study end code on the data entry forms) was the status of the herd at the end of the herd investigation This was coded into four groups. Negative herds were those which, regardless of their testing history, were not *M. bovis* culture positive. Farms which no longer had animals were coded as having sold or slaughtered all their stock. Farms that were culture positive or deemed to be extremely high risk were depopulated. Some farms were not tested because the risk of infection was considered too slight. These were coded with a herd end code of "other". An example of a farm in this category would be a very large feedlot where animals were shipped directly to slaughter and thus were considered very low risk.

HERD CLASSIFICATION (farm classification code on the data collection form) was the status of the farm at the end of the study based on the results of testing. It was coded into 4 groups. Negative herds were tested and all animals were either negative to all tests or if positive to a caudal fold tuberculin skin test were subsequently negative on all follow-up tests. Reactor meant that the farm had animals which were positive to tests other than the caudal fold test. Positive was used for farms where *M. bovis* was cultured. If no animals were tested the farm was coded as such and the status of the farm could not be determined.

The EARLIEST EXPOSURE DATE was related to the INVESTIGATION CODE and the REFERENCE FARM. The earliest exposure date was the earliest possible date that the individual animals on a specific farm may have been exposed to a potentially tuberculous animal(s) from some reference farm. There was no earliest exposure date for farms where the source of infection could not be determined. Farms which were investigated because they were traceback farms would have an earliest exposure date only if the source and date of potential contact with infectious animals was known. In the case of sales of animals (traceout farms), the earliest exposure date was the date that the potentially infectious animal(s) entered the traceout farm from the reference farm. Earliest exposure dates for farms that co-pastured were the dates when co-pasturing first started after it was known that the reference farm had been exposed to potentially infectious animals. In the case of fence line contact, the earliest exposure date for the farm was the date that the reactor/positive reference farm was first exposed to potentially infectious animals. If this date was unknown, then the earliest exposure date was the date when the reference farm was known to be a reactor or a positive farm. Farms investigated for other reasons were given an earliest exposure date if the farm had received animals from or been in contact with animals that were from a reactor/positive farm. Otherwise these farms were not given an earliest exposure date.

The LATEST EXPOSURE DATE was the date when contact ended between potentially infectious animals from a reference farm and animals in another farm. This date was not always available. In traceout farms for example, it was the date when the purchased animals were removed from the farm. In farms that co-pastured, the latest exposure date was the date that co-pasturing ended. In fence line contact situations it was either the date that the status of the

reference farm was known to be negative or the depopulation date of the reference farm.

The LAST TEST DATE was the date when the status of the herd was last assessed. It may have been a herd test, the post mortem date on an individual suspect animal or the slaughter inspection of a depopulated herd.

HERDSIZE (TAOF on the data collection forms) was the sum of all species and age groups of animals, except poultry, which were kept on the premise.

Table V
Names of variables and descriptions of data collected for herds involved in cattle or cervid tuberculosis outbreaks in Canada between 1985 - 1994

Variable Name	Description	Frequency Distribution (%) or Range, Mean and Std. Dev.	
Farm ID	A unique farm identification number	Not Applicable	
Outbreak	The outbreak identifiers	PEI Bovine	164 (13.4%)
		Que. Bovine	291 (23.8%)
		Que. Cervid	39 (3.2%)
		Ont. Cervid	118 (9.6%)
		Man. Bovine	221 (18.1%)
		Alberta Bovine	125 (10.2%)
		Alb/Sask Cervid	184 (15.0%)
		BC Cervid	81 (6.6%)
Principle Farm Type	The primary type of farm in terms of species and product	Dairy	178 (17.8%)
		Beef	589 (59.0%)
		Elk	162 (16.2%)
		Bison	2 (0.2%)
		Deer	26 (2.6%)
		Other	42 (4.2%)
Breed	The breed/species on which tests were carried out (more than 1/farm in some cases)[a]	Dairy	164 (17.8%)
		Beef	528 (57.3%)
		Cervids	195 (21.2%)
		Bison	8 (0.9%)
		Other	26 (2.8%)
Investigation Code	The reason why a farm was investigated as part of an outbreak. This was determined from the point of view of the Inspector.	Slaughter or Post Mortem	17 (1.5%)
		Traceback	90 (8.1%)
		Traceout	346 (31.3%)
		Pasture contact	61 (5.5%)
		Fence line	86 (7.8%)
		Perimeter	446 (40.3%)
		Area	47 (4.2%)
		Other	14 (1.3%)
Herd End Code	The status of the farm at the end of the study	Negative	949 (86.8%)
		Sold/Slaughter	40 (3.7%)
		Depopulated	53 (4.8%)
		Other	52 (4.7%)

Variable Name	Description	Frequency Distribution (%) or Range, Mean and Std. Dev.	
Herd Classification	The status of the farm at the end of the study based on the results of herd testing	Negative Reactor Positive Other	877 (79.8%) 81 (7.5%) 54 (4.9%) 86 (7.8%)
Reference Farm	The farm that prompted investigation in the current farm.	Not Applicable	
Earliest Exposure Date	The first possible date that animals on a farm were exposed to potentially tuberculous animal(s) from another, reference farm.	Not Applicable	
Latest Exposure Date	The last date that animals were exposed to potentially tuberculous animals from a reference farm	Not Applicable	
Last Test Date	The date of the last recorded test on animals on that farm	Not Applicable	
Herdsize	The total number of dairy, beef, cervids, sheep and goats on the farm	Range Mean SD	0 - 9998 91 363

[a] It was theoretically possible to have more breed than principle farm type categories. However, this was not the case because the files contained more data regarding principle farm type than data on the different breeds that were tested.

Herd and individual animal data for the New Brunswick bison outbreak were not available. It was possible to determine a limited amount of data from the overview material which is presented in Section 3.3, Results and also in Chapter 4.

3.2.4 Individual animal data collected

Table VI gives the name and a brief description for each of the individual animal level variables in the study. Individual animal information was collected only on animals from positive or reactor farms. Paper data collection forms and the code list and acronym legend for the data are in Appendix B and C respectively.

ENTRY STATUS was the status of the individual animal in a study herd at the beginning of the investigation. It was coded into 6 categories. These were: animals present in the study herd at the first test on the herd; animals born into the study herd after the investigation began; animals which were in the study herd at the first herd test but which were known to have originated in a negative herd; animals which were in the study herd at the first herd test but which were known to have originated in a herd with reactor animals; animals which were in the study herd at the first herd test but which were known to have originated from a positive herd; and, a final category for animals which were not present at the first test of the herd but were subsequently identified at a later herd test.

ENTRY DATE is the date of the first test on an individual animal. For most animals present at the beginning of the investigation, this is the first herd test. Animals born after the investigation began were given an entry date of their birth date.

AGE was the age in months of the animal at the entry date. Animals that were present at the beginning of the study had age recorded as at the first test. Animals that were born after the investigation began were recorded as 0 months of age and their birth date was the entry date. When an animal was not recorded on the first herd test, the entry date was the date of the first test on that animal. It was assumed that animals whose entry date was not the first herd test were in

fact present at the beginning of the investigation because in an outbreak investigation situation, herds which were not immediately deemed to be negative on the first test were not permitted to move animals in or out of the herd until the final status was determined. Individual animal information was gathered only on herds where there were reactor animals on tests other than the caudal fold. Thus, movement in and out of these herds would be restricted until a final status of negative was achieved.

SEX was recorded as female, male, or neutered.

REACTOR represented the status of the animal at the end of the study. For the final analysis the individual animal was considered either negative or reactor/positive. Negative status meant that the animal was negative on every test it was submitted to, other than the caudal fold test. Animals that were caudal fold test suspicious or positive were submitted to other tests. If an animal was suspect or positive on any test other than the caudal fold test, it was considered as a reactor/positive animal. Therefore reactor/positive status meant that an animal was suspect or positive on a comparative cervical, mid-cervical, gross pathology, histopathology, or culture.

Table VI
Names of variables and descriptions of data collected for individual animals on positive and reactor farms involved in cattle or cervid tuberculosis outbreaks in Canada between 1985 - 1994

Variable Name	Description	Frequency Distribution (%) or Range, Mean and Std. Dev.	
Ear Tag Number	The farm or Agriculture Canada Ear Tag identification number	Not Applicable	
Barn Name	Animal's familiar name if given	Not Applicable	
Entry Status	The status of an animal in the herd at the beginning of the investigation of a farm	Present	5196 (74.8%)
		Born	402 (5.8%)
		Bought (N)*	5 (0.07%)
		Bought (R)	44 (.6%)
		Bought (P)	116 (1.8%)
		Other	1180 (17.0%)
Entry Date	The entry date depends on the entry code and is related to whether the animal was present at the start of the investigation	Not Applicable	
Age	Age of the animal at entry into the study (in months)	Range	0 - 216
		Mean	30
		SD	31
Sex	The sex of the animal - female, male, neutered	Female	4780 (77.1%)
		Male	1246 (20.1%)
		Neutered	176 (2.8%)
Breed	The breed or species of the animal	Dairy	784 (11.4%)
		Beef	2610 (38%)
		Cervid	3238 (47.1%)
		Bison	54 (0.8%)
		Other	185 (2.7%)
Reactor	Animal classification	Negative	1534 (88.7%)
		Reactor/Pos	195 (11.3%)

*N,R,P were the status of the source herd and represented negative, reactor, and positive, respectively.

3.2.5 Statistical analysis

Data were analysed and tabulated using a computer statistical program (Stata). The mean, range and standard deviation were generated for all continuous variables. Frequency distributions were calculated for categorical variables. The percent of the total of each group within a categorical variable was also calculated.

Cross tabulations of categorical variables were used to generate tables which presented different perspectives of the data. The data could be presented separately for each outbreak. For example the farm type in each outbreak was tabulated against the count of the number of herds in each of the negative, reactor, positive and other categories of the farm classifications variable.

The total number of each of the individual tests performed in reactor/positive herds from each outbreak was calculated. The sum of the herds tested with a particular technology and the percent of the herds tested that were positive was also determined.

Chi square (chi^2) tests of association to test the difference in proportions positive/suspicious were performed across different breed/species within each test. Each test was evaluated with chi square analyses of the complete table and sub-tables to look at specific breed comparisons (using the method of Fisher and Belle). When there were less than 55 tests in a specific breed/species in a particular test, the results were not analysed with the chi square tests. The other category was also not included as it consisted of many different breed/species and thus it was not biologically logical to include them as a group.

3.3 Results

3.3.1 The New Brunswick bison outbreak

The data obtained from the New Brunswick outbreak were primarily from overview

material related to the index herd. The index farm had both beef and bison. The index animal was a female bison identified as a tuberculosis suspect during a post mortem examination. The beef animals were tested and because there were no animals positive on the caudal fold test and the two species were isolated from one another, the beef herd was not depopulated with the bison herd. One beef farm in the area was also tested and all animals were negative. There is additional information on this outbreak in Chapter 4 but no further analyses of these data were carried out.

3.3.2 The herd data results

Table VII gives a summary of the outbreaks (including the NB outbreak). The dates of the outbreak, the farm type of the index herd, and the manner in which the index farm was identified are presented. The earliest outbreaks were bison and beef outbreaks (1985) and the most recent was a cervid outbreak (1993) with a bison as the index animal. The table also gives a breakdown of the final classification of herds according to their principle farm type.

The data in Table VII indicate that 4 out of the five largest outbreaks in terms of the number of herds investigated were bovine and the three smallest were primarily cervid outbreaks. There were four bovine and four cervid outbreaks.

Almost 60% of the farms investigated in the outbreaks were beef farms with dairy and elk being approximately equal with 17.8% and 16.2% respectively.

The results of the breed classification are slightly different from the results of the principle farm type. The database was designed to have up to four breed records per farm. The principle farm type would be the same in all of the records but the breed would vary. The master file had to be reduced to 1 breed per farm for some of the computations and tabulations. When one breed only was to appear, the breed was chosen in the following manner. The breed was

chosen which had the largest number of animals that were positive or suspicious on a test that determined the final classification of that farm. The order of tests that was chosen as highest to lowest significance was culture, histology, gross pathology, comparative cervical and mid-cervical. Farms which did not have any positive or suspicious animals on any of these tests had breed selected based on which breed had the largest number of any test. In the herd data the proportions of dairy breed remained virtually identical to the principle farm type proportion. Beef declined slightly and there was a slight increase in the proportion of cervid (deer plus elk) in the breed breakdown. There was a four fold increase in the proportion of bison, relative to the principle farm type breakdown. There was a decrease in the proportion of breeds classified as other.

The two largest categories of INVESTIGATION CODE were perimeter testing (40.3%) and traceout (31.3%). The other contact classifications, pasture, fence line, and traceback were roughly equivalent to one another and ranged from 5.5% to 8%.

HERDSIZE had limitations as an estimate of the number of animals at risk. For example, if there were multiple breeds (for example beef and infected bison) but they were quite separate, the total animals on the farm would have included both species whereas in fact there may have been little risk to the beef animals (for example, in the NB bison outbreak).

3.3.3 Individual animal data - all herds investigated

There were 87 herds with 7090 individual animal records in these data. Sixty-five per cent of the animals with individual records were depopulated because they were on positive farms. The number of depopulated herds was 53 which represented 61% of the herds with individual animal records and 4.8% of the total herds in the study.

ENTRY STATUS indicated that animals were predominantly present at the beginning of the investigation (74.8%) and tested at the first herd test. Seventeen per cent were not tested at the first herd test possibly because they were not considered high risk animals.

The mean AGE of animals in the individual animal data was 30 months, but the standard deviation was quite high (SD = 31 months).

This population of animals in positive/reactor herds were predominantly female (77.1%). Cervids (47.1%) were the most common breed/species followed by beef (38%) and dairy (11.4%).

Table VII
General Information on tuberculosis outbreaks in Canadian Cattle and Cervids between 1985-1994

Outbreak	PEI Bovine				NB Bison				Quebec Bovine				Quebec Cervid				Ontario Cervid			
Start Date (m/y)	02/87				03/85				09/86				02/93				01/90			
End Date (m/y)	07/87				10/85				06/87				01/94				*			
Index Farm Type	Dairy				Bison				Beef				Zoo				Elk			
Initial Detection	Slaughter				Post Mortem				Slaughter				Post Mortem				Post Mortem			
HC(a) / Type of Herd	N	R	P	O	N	R	P	O	N	R	P	O	N	R	P	O	N	R	P	O
Dairy Herds	46	0	1	0	-	-	-	-	50	2	2	2	1	0	0	0	6	0	1	1
Beef Herds	116	1	0	0	2	0	0	0	61	0	8	7	13	0	0	0	23	1	1	0
Elk Herds	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0	3	2	3	0
Bison	-	-	-	-	0	0	1	0	-	-	-	-	0	0	1	0	-	-	-	-
Deer Herds	-	-	-	-	-	-	-	-	-	-	-	-	6	2	2	0	2	0	6	0
Other Herds	-	-	-	-	-	-	-	-	1	0	0	2	4	0	1	0	2	0	0	1
Unknown Type	-	-	-	-	-	-	-	-	83	11	0	11	4	0	0	0	26	0	0	0
Total Classified	162	1	1	0	2	0	1	0	195	13	10	22	29	2	4	0	62	3	11	2
Not Classified	0				0				51 (17.5%)				4 (10.2%)				40 (33.9%)			
Total Herds	164				3				291				39				118			

* Testing on-going in 1995 for traceout herds
a. HC is HERD CLASSIFICATION: Negative (N), Reactor (R), Positive (P), Other (O)

Table VII cont'd
General Information on tuberculosis outbreaks in Canadian Cattle and Cervids between 1985-1994

Outbreak	Manitoba Bovine				Alberta Bovine				Alb/Sask Cervid				BC Cervid			
Start Date (m/y)	10/90				10/85				07/90				09/89			
End Date (m/y)	10/91				04/86				06/93				04/90			
Index Farm Type	Beef				Beef				Elk				Deer			
Initial Detection	Slaughter				Slaughter				Post Mortem				Herd Test			
HC(a) / Type of Herd	N	R	P	O	N	R	P	O	N	R	P	O	N	R	P	O
Dairy Herds	6	2	0	1	8	0	0	0	-	-	-	-	37	0	0	0
Beef Herds	146	16	5	10	81	3	4	19	9	0	0	0	35	0	0	0
Elk Herds	-	-	-	-	-	-	-	-	83	37	16	17	-	-	-	-
Bison	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0
Deer Herds	-	-	-	-	-	-	-	-	-	-	-	-	5	0	1	0
Other Herds	2	0	0	0	-	-	-	-	16	4	2	0	-	-	-	-
Unknown Type	0	0	0	7	0	-	-	7	-	-	-	-	0	0	0	1
Total Classified	154	18	5	18	89	3	4	26	108	41	18	17	78	0	1	1
Not Classified	26 (11.8%)				3 (2.4%)				0				1 (1.2%)			
Total Herds	221				125				184				81			

* Testing on-going in 1995 for traceout herds
a. HC is HERD CLASSIFICATION: Negative (N), Reactor (R), Positive (P), Other (O)

Table VIII illustrates the association between the INVESTIGATION CODE and the HERD CLASSIFICATION for all herds in each of the outbreaks. That is, the reason why an investigation was initiated on a herd and the final status of that herd. Herds were classified in the 8 categories of study start code as well as one for an unknown study start code and tabulated according to their final status as positive, reactor, negative and other. In the positive classification there were: 33 herds identified as a result of traceout investigations, 16 herds identified as a result of slaughter surveillance or post mortem; 2 herds identified from traceback investigations; and, 1 each from pasture, fence line , perimeter and other contact. The largest number of reactor herds (45/81) was also from the traceout investigation group. The largest number of negative herds was in the perimeter testing group (385/392).

The outbreak with the largest proportion of positive or reactor herds was the Alberta/Saskatchewan cervid outbreak (32.1%). The Quebec cervid and the Ontario cervid were the next highest with approximately 17% each. The PEI and BC outbreaks had the smallest proportion of reactor/positive herds and were both under 1.55%.

Table VIII
Reasons for investigation of herds in tuberculosis outbreaks in Canadian cattle and cervidae from 1984-1994 in relation to their final status (Herd Classification) at the end of the investigation

Outbk	P.E.I. Bovine	N.B. Bison	QUE. Bovine	QUE. Cervid	ONT. Cervid	MAN. Bovine	ALB. Bovine	ALB. Cervid	B.C. Cervid	Total (%of category)
1. Slaughter Screening or Post Mortem										
positive	1	1	2	1	6	1	1	3	-	16(88.9)
reactor	0	0	0	0	0	0	0	0	-	0
negative	0	0	1	1	0	0	0	0	-	2(11.1)
other	0	0	0	0	0	0	0	0	-	0
2. Traceback										
positive	0	-	0	-	1	0	1	0	0	2(2.5)
reactor	1	-	2	-	1	1	1	0	0	6(7.6)
negative	7	-	44	-	0	2	7	2	1	63(79.8)
other	0	-	3	-	0	1	3	0	1	8(10.1)
3. Traceout										
positive	0	-	7	3	4	3	2	14	0	33(10)
reactor	0	-	1	2	1	6	0	35	0	45(13.6)
negative	5	-	47	20	6	47	5	67	4	201(60.7)
other	0	-	14	0	0	15	19	4	0	52(15.7)
4. Pasture Contact										
positive	0	-	0	-	0	0	0	1	0	1(2)
reactor	0	-	2	-	0	6	0	-	0	8(16)
negative	7	-	11	-	1	14	3	-	1	37(74)
other	0	-	4	-	0	0	0	-	0	4(8)
5. Fence Line Contact										
positive	0	-	0	-	0	1	0	-	-	1(1.2)
reactor	0	-	1	-	0	5	1	-	-	7(8.3)
negative	1	-	4	-	3	66	0	-	-	74(88.1)
other	0	-	0	-	0	2	0	-	-	2(2.4)
6. Perimeter (Buffer Zone)										
positive	0	0	1	0	0	0	0	0	0	1(.25)
reactor	0	0	0	0	0	0	1	0	0	1(.25)
negative	141	2	79	8	24	14	36	9	72	385(98.2)
other	0	0	1	0	1	0	3	0	0	5(1.3)

Outbk	P.E.I. Bovine	N.B. Bison	QUE. Bovine	QUE. Cervid	ONT. Cervid	MAN. Bovine	ALB. Bovine	ALB. Cervid	B.C. Cervid	Total (%of category)
7. Area Testing										
positive	-	-	0	-	-	0	0	-	-	0
reactor	-	-	0	-	-	0	0	-	-	0
negative	-	-	2	-	-	10	35	-	-	47(100)
other	-	-	0	-	-	0	0	-	-	0
8. Testing for Other Reasons										
positive	0	-	0	-	0	0	0	0	1	1(7.1)
reactor	0	-	0	-	1	0	0	0	0	1(7.1)
negative	1	-	7	-	0	1	2	0	0	11(78.7)
other	0	-	0	-	1	0	0	0	0	1(7.1)
9. Unknown Investigation Code										
positive	-	-	0	-	0	-	0	0	-	0
reactor	-	-	7	-	0	-	0	6	-	13(15.1)
negative	-	-	0	-	28	-	1	30	-	59(68.6)
other	-	-	0	-	0	-	1	13	-	14(16.3)
Total										
positive	1	1	10	4	11	5	4	18	1	55(4.5)
reactor	1	0	13	2	3	18	3	41	0	81(6.6)
negative	162	2	195	29	62	154	89	108	78	879(71.7)
other	0	0	22	0	2	18	26	17	1	86(7)
no rec.	-	-	51	4	40	26	3	-	1	125(10.2)
Grand Total	164	3	291	39	118	221	125	184	81	1226

Table IX and Table X examine the different diagnostic technologies employed in the outbreaks. The total tests performed for each test represented the total number of times that test was employed in each outbreak. The percent of herds with a positive or suspect animal in each test was an estimate of the percent of all the herds in the outbreak that were tested with that technology and had positive or suspect animals on that test. It does not mean that every herd in the investigation was submitted to the test. The number of herds represented the total number of herds which had at least one animal in a suspect or positive category in the particular test being considered. Again, it does not mean that all herds in the investigation were tested with this technology.

Suspect and positive reactions were grouped for several reasons. The first was due to the variation in recording or reporting of some tests. For example, some inspectors recorded caudal fold reactions as "suspect", others as "positive", and still others as "reactors". Histology reports may have recorded results as "mycobacteriosis", "suspect mycobacteriosis", or "granuloma". All these designations were suspect for or suggestive of tuberculosis.

The most frequently employed test was the caudal fold tuberculin test (27814, 62.1%). As a group, skin tests accounted for 87% of the total tests applied during the outbreaks. The large number of post mortems can be explained by the fact that in many cases, farms that were depopulated had post mortems performed on every animal slaughtered.

Table IX
Results of skin testing for tuberculosis in Canadian cattle and cervid herds involved in tuberculosis outbreaks in Canada between 1985-1994

Outbreak	Total Tests (#Herds)	# Susp or Pos Herds (%)	Total Tests (# Herds)	# Susp or Pos Herds (%)	Total Tests (# Herds)	# Susp or Pos herds (%)
	Caudal fold		Mid-cervical		Comparative Cervical	
P.E.I. Bovine	9138 (163)	7 (4.3%)	0	0	54 (1)	1 (100%)
N.B. Bison	133 (3)	1 (33.3%)	55 (1)	1 (100%)	0	0
Quebec Bovine	7372 (191)	47 (24.6%)	83 (1)	1 (100%)	157 (42)	20 (47.6%)
Quebec. Cervid	708 (16)	1 (6.2%)	368 (10)	3 (30%)	4 (2)	2 (100%)
Ontario. Cervid	2280 (68)	9 (13.2%)	136 (6)	3 (50%)	18 (4)	2 (50%)
Manitoba Bovine	9089 (180)	20 (11.1%)	867 (33)	16 (48.5%)	264 (20)	10 (50%)
Alberta Bovine	8265 (97)	36 (37.1%)	285 (2)	2 (100%)	140 (39)	8 (20.5%)
Alb/Sask Cervid	168 (10)	10 (100%)	5762 (123)	30 (24.4%)	71 (29)	5 (17.2%)
BC Cervid	1799 (73)	1 (1.4%)	741 (4)	3 (75.0%)	27 (4)	1 (25%)
Totals	29,814 (801)	132 (16.5%)	8297 (180)	59 (32.8%)	735 (141)	49 (34.8%)

Table X
Results of gross pathology, histopathology and culture testing for tuberculosis in Canadian cattle and cervid herds involved in tuberculosis outbreaks in Canada between 1985-1994

Outbreak	Total Tests (# Herds)	# Susp or Pos Herds (%)	Total Tests (# Herds)	# Susp or Pos Herds (%)	Total Tests (# Herds)	# Susp or Pos Herds (%)
	Gross pathology		Histopathology		Culture	
P.E.I. Bovine	111 (2)	2 (100%)	3 (2)	2 (100%)	3 (1)	1 (100%)
N.B. Bison	55 (3)	1 (33.3%)	4 (1)	1 (100%)	4 (1)	1 (100%)
Quebec Bovine	638 (13)	10 (76.9%)	21 (10)	9 (90%)	20 (10)	10 (100%)
Quebec. Cervid	301 (30)	4 (13.3%)	147 (23)	4 (17%)	63 (21)	3 (14%)
Ontario. Cervid	526 (25)	12 (48%)	52 (14)	6 (43%)	73 (16)	7* (44%)
Manitoba Bovine	846 (40)	10 (25%)	146 (34)	10 (29%)	109 (31)	5* (16%)
Alberta Bovine	678 (7)	3 (42.8%)	9 (4)	4 (100%)	9 (4)	4 (100%)
Alb/Sask Cervid	2092 (52)	48 (92.3%)	326 (71)	**	**	**
BC Cervid	421 (6)	1 (16.7%)	12 (1)	1 (100%)	29 (5)	1 (20%)
Totals	5668 (178)	91 (51.1%)	721 (160)	37 (23.1%)	310 (89)	36 (40.4%)

* A herd was depopulated which was not culture positive but was considered very high risk.
** Data from Epi-info files incomplete

3.3.4 Individual animal data - reactor/positive herds

Table XI and Table XII present the results of tests on individual animals in the study. Only animals in positive or reactor herds were included in these data and not all animals were tested with all technologies. Also, it cannot be assumed that the population tested by one technology is the same population tested with another technology across a breed or species. That is, the dairy animals subjected to a caudal fold test are not necessarily the same ones in the gross pathology results.

The results of the caudal fold testing indicated a considerable difference in the proportion positive between dairy (0.1) and beef (0.04). A chi square test indicated that there was a statistically significant difference between the two groups ($chi^2_1 = 43.7$; p=0.000).

There was a fairly consistent proportion positive on the mid-cervical between the dairy (0.14) and beef (0.15). The proportion positive in the cervid group was lower (.09) and there were more animals tested. There was a statistically significant difference between these breed/species ($chi^2_2 = 20.4$; p=0.00). The bovine groups had significantly more positive/suspicious reactors than the cervid group (p=0.00) but there was no significant difference between the dairy and the beef groups (p=0.76).

The proportion positive for the comparative cervical test was very high ranging from 0.34 (dairy) to 1 (bison). There was a statistically significant difference between the dairy, beef and cervid groups ($chi^2_2 = 7.8$; p= .02). The dairy group had a significantly smaller proportion positive than the beef and cervids (p=0.011) but there was no difference between the beef and the cervids (p=0.24)

The variation in proportion positive in the gross pathology examinations was marked.

The highest proportion was in the bison group (0.45) and the lowest proportions were in the bovine groups (dairy 0.06 and beef 0.10). There was a statistically significant difference between the dairy, beef and cervid groups ($chi^2_2 = 43.9$; p=0.000). The bovine group was significantly less than the cervid group (p=0.00) and the dairy were significantly lower than the beef group (p=0.011)

There was considerable similarity in the proportion positive between breeds in the histology results. There was no statistically significant difference between the beef and cervid groups ($chi^2_1 = 1.3$; $p = 0.26$).

The variation in proportion positive for culture was slight except for the bison group (0.64). There was no statistically significant difference between the beef and cervid groups ($chi^2_1 = 0.0134$; $p = 0.9$).

Table XI
Results of skin testing for tuberculosis in Reactor/Positive herds involved in tuberculosis outbreaks in Canada between 1985-1994

Breed/Species	Caudal Fold	Mid-Cervical	Comparative Cervical
Dairy			
# positive/suspicious	72	49	29
# tested	709	349	86
Proportion pos/susp	0.10[a]	0.14[a]	0.34[a]
Beef			
# positive/suspicious	65	137	77
# tested	1832	931	165
Proportion pos/susp	0.04[b]	0.15[a]	0.47[b]
Cervid			
# positive/suspicious	1	204	51
# tested	3	2150	94
Proportion pos/susp	0.33[nt]	0.09[b]	0.54[b]
Bison			
# positive/suspicious	7	0	2
# tested	37	1	2
Proportion pos/susp	0.19[nt]	0[nt]	1.0[nt]
Other			
# positive/suspicious	0	5	1
# tested	17	43	2
Proportion pos/susp	0[nt]	0.12[nt]	0.50[nt]

[a,b,c] breeds/species with different superscripts had significantly different proportions positive/suspicious
[nt] Breed effect not tested statistically

Table XII
Results of gross pathology, histopathology and culture testing in Reactor/Positive herds involved in tuberculosis outbreaks in Canada between 1985-1994

Breed	Gross Pathology	Histopathology	Culture
Dairy			
# positive/suspicious	20	8	6
# tested	320	29	31
Proportion pos/susp	0.06[a]	0.28[nt]	0.19[nt]
Beef			
# positive/suspicious	138	42	35
# tested	1448	128	122
Proportion pos/susp	0.10[b]	0.33[a]	0.29[a]
Cervid			
# positive/suspicious	289	54	48
# tested	1799	200	171
Proportion pos/susp	0.16[c]	0.27[a]	0.28[a]
Bison			
# positive/suspicious	24	8	23
# tested	53	18	36
Proportion pos/susp	0.45[nt]	0.44[nt]	0.64[nt]
Other			
# positive/suspicious	30	16	9
# tested	131	45	48
Proportion pos/susp	0.23[nt]	0.36[nt]	0.19[nt]

[a,b,c] breeds/species with different superscripts had significantly different proportions positive/suspicious
[nt] Breed effect not tested statistically

3.4 Discussion

There were several difficulties encountered with the data collection process. The files were in different regions and within some regions they were in different district offices. This, plus the fact that the supervisors often did not want files to leave their offices meant that personal visits were necessary. Files were photocopied and the data were then extracted at a later time. It was impossible to do data extraction directly from the original files as there was considerable reading, interpreting and collating to be done. Where files were kept together in an organized fashion, the process was much simpler. For example in the Manitoba outbreak, the files had already been numbered, colour coded based on farm testing history, and were kept in one location together. Although this outbreak had a large number of farms and individual animal records to be collected, it was probably the easiest one from which to extract data. The British Columbia cervid outbreak was organized in much the same manner but there were fewer farms in total and only 1 positive/reactor farm. Every effort was made to complete the data set. In spite of this there were data which existed but which could not be collected for various reasons. For example, in the Quebec outbreak the animals were simply labelled as bovine and thus it was not possible to determine if they were beef or dairy. Table VI indicates the outbreaks with the most missing data in terms of herd classification as it related to principle farm type. In the Ontario cervid and Quebec bovine outbreaks, 34% and 17.5%, respectively, of the farms were not classified according to farm type and final herd classification. It is not possible to determine all the biases associated with these and other missing data. Certainly, records for positive and reactor herds were more likely to have complete data than negative herds. It is unfortunate that it was so time consuming to retrieve the data as this implies that other retrospective studies may

not be done simply because the resources to perform them are not available. In this way, the useful information which emerges from an outbreak and the follow-up investigation is lost.

The results indicated that the relative importance of tuberculosis was greater in cervids and bison than in cattle during the period 1985-1994 in Canada. The findings which supported this view were as follows.

1. There were four primarily bovine and four primarily cervid outbreaks during this time period in spite of the fact that the number of bovine herds in Canada was far greater than cervid herds. Dairy and beef herds dominated (76.8%) in terms of the number of herds investigated but this was probably due to the fact that perimeter testing was the leading reason for investigating herds (40.3%) and there were many bovine herds investigated during the cervid outbreaks.
2. Cervids were responsible for establishing the positive/reactor status of the farm in some cases even if the principle type of animal on the farm was bovine. The manner in which breed was chosen was such that if a predominantly beef herd kept cervids and the reactors or positive animals were in the cervid portion of the herd, cervid would be listed as breed. There were instances where this happened in beef herds. The predominant farm type in the data was beef but the proportion of breed listed as beef was smaller than the proportion of farm type listed as beef. There was a 4 times increase in the bison breed category relative to the principle farm type and a decrease in the other category in breed relative to principle farm type. This suggests that bison often resulted in the mixed herds being classified as positive/reactor herds.

3. In the individual animal records (derived from positive/reactor herds) the predominant breed was cervid (47.1%) which was roughly equal to the number of dairy (38%) and beef (11.4%) animals combined. This was noteworthy again because the proportion of cervid herds and individual animals in Canada was far less than the number of beef and dairy herds and animals.

In the outbreak investigations, the greatest proportion of herds were tested because of proximity to a positive or reactor herd (40.3%). However 60% (33/55) of the positive farms and 56% (45/81) of the reactor farms were discovered from traceout investigations. Clearly traceout investigations were extremely important in identifying positive and reactor herds. There are numerous examples in the literature of transmission of tuberculosis from one herd to another via sale of animals and introduction into another herd (3,4). A significant proportion of the resources invested in an outbreak are directed toward testing of perimeter herds (40%). These herds are the lowest risk group in terms of potential exposure and transmission. This also means that there was a large investment in testing bovine herds in cervid outbreaks and visa versa based on perimeter association. If no exchange of animals or direct contact occurred, the wisdom of using resources in this manner is questionable and the return on investment is likely to be small.

Examination of the herd end code together with the herd classification reflected the difference in classification between a more traditional method (the former) and that employed for this study (the latter). The percent of herds depopulated in the herd end code classification (4.8%) was almost identical to the percent positive in the farm classification system. The farm classification system included a group termed "reactor" (7.5% of herds) which was considered to include herds which had been exposed to *M.bovis* and which were at higher risk for infection

although the organism had not been cultured. Thus, according to the hypothesis of the study there were at least 7% more herds in the study population that were at higher risk of being infected with *M. bovis* than the traditional classification system would have recognized.

It may be argued that the classification system is a matter of semantics and that in fact the protocols and procedures exist to continue to test these herds with reactors regardless of the classification system. There are scenarios however where these herds may not be subjected to as thorough a follow-up as is warranted. For example, the following procedures could have been followed on a traceout investigation of a farm that received three animals from a positive farm 30 days before the source farm was declared potentially positive. The three animals would be slaughtered and examined histologically and by culture if there were gross pathological lesions. The rest of the herd would be given an initial screening test. If the post mortem results were negative and the screening test was negative, the herd would be declared negative and not subjected to another compulsory screening test at a later date. Studies of experimental and natural infections with *M.bovis* have shown that the period of time between exposure and excretion is approximately 87 days in natural infections and that there is an inverse exponential relationship between the number of organisms in the exposure and the delay to excretion (5). It is reported that animals which react to the tuberculin test should be considered potentially infected and infectious (1). It is reported in cattle that it takes four weeks from exposure to *M.bovis* to sensitization to tuberculin (6). Young cervids may be heavily infected yet remain negative to a tuberculin test (7). It is possible that the introduced animals were recent infections but were shedding organisms during some period on the new farm. They would not necessarily have gross visible lesions but would be potentially infectious. It would take at least four weeks for the herd

mates which were exposed to the organism to become tuberculin test positive. Theoretically it would be possible to miss a herd that was in the very earliest stages of infection with *M.bovis* and there would be no protocols in place to recheck the status of this farm at a later date. At the end stages of an eradication program, as is the case with tuberculosis in Canada, it is imperative to ferret out each possible herd that may be infected and to design individual farm investigation plans. These custom made plans need not be extremely resource intensive but rather biologically sound in terms of the situation on each farm.

Table VI provides, at a glance, the breakdown of the farm types in each outbreak and the number of herds that fall into each of the herd classifications of negative, reactor, positive and other. For example it is immediately clear that although the index farm in PEI was a dairy farm, the largest number of herds investigated were beef farms (116/164). The one reactor herd was a beef herd. A more complex example was the Manitoba bovine outbreak. Clearly the predominant farm type is beef (177/210). All 5 of the positive herds and 16 out of 18 of the reactor farms were beef herds.

The Ontario outbreak was the only outbreak in which there were both positive cervid and bovine farms. The exact mechanism of infection of the bovine herd was not determined but the owner of the beef herd was the herdsman for two positive cervid herds. This farm did receive bovine animals from one of these herds but when depopulated all were negative on histology and post mortem. Two years later when the owner of the bovine herd requested retesting of his beef cattle, they were found to be positive and were depopulated. It was impossible to determine the source of infection on the beef farm but it was postulated that the infection was due to the owner's association with the positive cervid herds and that he acted as a mechanical vector or

that somehow residual infection remained on his farm (environmental contamination perhaps) from the previously depopulated (and assumed negative) cattle received from the cervid farm. Transmission from cattle to deer via a mechanical vector (a water trough) was investigated in an outbreak of tuberculosis in deer in Australia but a direct connection was never established (8). Transmission of tuberculosis via a contaminated environment is not considered significant in tuberculosis (1).

The outbreak with the highest proportion of positive and reactor herds was the Alberta/Saskatchewan cervid outbreak (32.1%). This number could have been decreased simply by extending the perimeter testing and hence including more negative herds in the outbreak investigation. It is not possible to interpret the proportion of positive herds in the investigation as implying the prevalence of herd positive or reactor status in the whole population of the outbreak location.

The predominant investigation code for each outbreak does have possible interpretations and could be used in retrospect to analyse the control and eradication program and the epidemiological procedures used in follow-up. The number of herds discovered by slaughter inspection or post mortem are not compared to the number of animals that are examined in these ways and thus this investigation code cannot be interpreted by itself. However in association with the traceout code it can be interpreted. If positive and reactor herds are not related to one another through animal contact then a relatively larger number of positive/reactor animals discovered at slaughter indicates that the slaughter surveillance program is identifying at least some if not all of the infected herds. However, if the positive/reactor herds are related to one another (through contact) and a majority of the herds are being detected by slaughter surveillance or post mortem,

then the epidemiological procedures to track down tuberculosis infected herds are not working very well. The possible reasons for the breakdown in these techniques include: (1) a long time interval between dissemination of infection from a source herd and thus poor records of animal movement; (2) purposeful deceit on the part of the involved owners or managers; (3) inadequate epidemiological follow-up; and, (4) adequate follow-up but an inability to detect infection as a result of insensitive screening tests. For a more detailed discussion on the investigations and source and spread of infection in these outbreaks, see Chapter 4, The Outbreak Investigations.

There was considerable variation between species in the proportion positive to different tests in the individual animal results. This was not surprising as this was not a random sample nor was it expected that the prevalence of tuberculosis reactors was the same in these different groups. Histology and culture were the two tests where there were no statistically significant differences in the proportion of positive tests in different breed/species. This could mean that the same proportion of samples that were submitted for histology and culture were positive in the different breed/species and not that the prevalence of histology and culture positive animals is the same in the different breed/species. The proportion positive to all tests, except the caudal fold, was high in the bison group. It was not possible to determine if the bison: were simply a small population of animals with a very high prevalence of infection; were more susceptible and the rate of transmission was higher; or, were a group with higher test sensitivity and specificity. Certainly there was a clustering effect as these animals came from a relatively small number of farms.

The difference in the proportion of positives in the caudal fold test between the dairy (0.1) and the beef (0.04) is supported by popular belief that dairy animals have a higher rate of

transmission because they are housed more densely than beef cattle (9).

It is possible to draw some conclusions about the comparative cervical results as it is very rare for an animal to be given this test without first having been positive on another screening test. Thus the animals that were tested with the comparative cervical were probably at a higher risk of being positive than the animals submitted to the caudal fold and mid-cervical tests. The proportion positive on the comparative cervical test was quite similar for the cervids (0.54) and the beef (0.47) and slightly lower in the dairy animals (0.34). Considering that these animals were positive on another test, the proportion positive on the comparative cervical was not as high as it first appeared. In a study of tuberculosis in cattle in a specific area of England, the highest proportion of positive animals, using a comparative cervical test, over a 24 year period, was 29.8% (6). This proportion was calculated using all animals at risk and not simply the animals from herds that had positive or reactor animals or were positive on a screening test. The comparative cervical test is used because of its relatively higher specificity. It is important to be sure of the true status of an animal when the repercussions of a positive test are slaughter and the threat of lengthy quarantine and possible depopulation of a herd.

3.5 Conclusions

The results of the investigations into the nine tuberculosis outbreaks in Canada between 1985-1994 were presented. Summary statistics were given for both herd and individual animal information.

The relative importance of tuberculosis was greater in cervid herds in this time period than in bovine herds. Bovine herds represented the largest proportion of herds investigated but cervids were the primary breed of animal classified as positive/reactor.

Traceout investigations were extremely important in identifying positive and reactor herds after an index herd was discovered at slaughter or post mortem. It was possible to critically evaluate some of the outbreak investigations based on the proportion of positive and reactor herds identified and investigated for different reasons.

A system of herd classification which incorporates a group based on the presence of reactors was compared to a more traditional classification system. It was suggested that procedures that are consistent with the biology and epidemiology of *M.bovis* be developed to investigate reactor herds which under present procedures may be classified as negative and not subjected to further follow-up.

Variation between breeds of the proportion positive to different diagnostic tests was analysed. There was statistically significant variation in the caudal fold, mid-cervical, comparative cervical and gross pathology, but not in histopathology or culture.

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CHAPTER 4

SUMMARIES AND OVERVIEW DIAGRAMS OF TUBERCULOSIS OUTBREAKS IN CANADIAN BOVINES AND CERVIDS FROM 1985-1994

4.1 Introduction

The objective of this chapter was to present a summary of each of the tuberculosis outbreaks in Canadian cattle and cervids from 1985-1994. Each summary describes the outbreak investigation and the final resolution of the outbreak. The written summary is accompanied, in most outbreaks, by diagrams which aid in understanding the outbreak summary. A discussion of the qualitative aspects of this study is given. Changes in the tuberculosis control program which resulted from these outbreaks are discussed.

4.2 Materials and Methods

There were four sources of information for these summaries and outbreak diagrams.

(1) Data were collected from outbreak files and records which were located in Agriculture and Agrifood Canada's Regional and District offices. The data were extracted from the files, entered on data entry forms and then transferred to a database program (Microsoft Access). A complete description of the original data in the study and of the data collection procedures can be found in Chapter 3. These data represent what was generally available to different extents, for every outbreak as per Directorate documentation requirements.

(2) Data were also extracted from other written sources of information at Regional and District offices, for certain outbreaks - specifically the Quebec bovine outbreak (1) and the Quebec cervid and Alberta/Saskatchewan cervid outbreaks. Sources of information from the latter two

outbreaks were written by Regional Office staff to summarize specific aspects concerning the outbreaks. These summaries were in the outbreak files but were not standard in every file in every Regional office.

(3) The Alberta/Saskatchewan cervid outbreak data were stored in a database program. (Epi Info)

(4) Oral communications with Agriculture and Agri-food Canada staff who were involved in the outbreaks and had first-hand insights and information on these outbreaks.

Two overview diagrams were prepared for most outbreaks. There is only a proposed transmission overview for the Alberta/Saskatchewan cervid outbreak as it was impossible to construct a comprehensive outbreak overview from the data base. This overview presents one possible scenario that emerged from the database and other written material. There are no diagrams for the New Brunswick and British Columbia outbreaks because there was only one positive farm in each and no reactor farms. There are no diagrams for the Prince Edward Island outbreak as there was only one positive and one reactor herd and the written summary was sufficient to describe the outbreak.

The first of the two diagrams (labelled Transmission Overview) for each outbreak shows the relationship of the culture positive herds to one another and depicts the assumed movement of *M. bovis* from one farm to another. Farms were identified with capitol letters starting at 'A' (the assumed source herd) to show the movement of the organism. Therefore the index farm was not necessarily 'A'.

The second of the two diagrams (labelled Investigation Overview) is an overview which shows the relationship of the herds investigated in the outbreak from the point of view of the investigating inspector. Herds which sold or received animals from other herds were identified as

traceback or traceout herds respectively. Herds which shared pasture or had fence line contact with one another were identified, as were those which were in a perimeter or buffer (1.5 or 10 kilometre) of a positive or depopulated herd.

Not all herds investigated in the outbreak were represented in the second diagram. A herd must have been either (1) culture positive, (2) depopulated, (3) "reactor", or (4) a source herd for one of the first three categories, in order to be included in the diagram. A "reactor" farm was one where one or more animals: reacted to a mid-cervical or comparative cervical test; had tuberculosis suspect lesions on post mortem; or, had histologically suspect or positive lesions. The diagram represents an evolution of the outbreak investigation in time by numbering the herds consecutively as they were identified. The index farm is always "1". Numerical designations became less exact as they got larger as several herds were identified at approximately the same time.

The investigation overview also shows the physical relationship of each herd to the others in terms of type of contact. Types of contact included, co-pasture, fence line and contact through animal movement as traceouts or tracebacks. Animals were also tested if they were on a farm which was within a certain radius of a positive farm. The radius varied between outbreaks from 1.5 - 10 kilometres. Thus, a farm which was identified as A3 was the third herd identified in the outbreak but was the proposed source herd for the infection going to the other farms.

The farm classification in both diagrams (as defined above) is shown by the colour of the box. Red is culture positive; yellow is reactor; green is negative; and, grey is no tests performed.

Outbreak investigation overviews contain the same information as the transmission overviews plus some additional information. Solid lines with arrows indicate that the

investigation was a traceout; interrupted lines indicate that the investigation was a traceback. Fence line contact between farms is indicated by a double line joining the farms. An undulating dotted line indicates undefined contact between two farms. Overlapping boxes indicates that the two farms co-pastured. Herds which were identified at abattoir inspection, on post mortem or at the owner's request, are also indicated. Farms which were unconnected to others were found on perimeter or buffer zone testing.

4.3 Results

The outbreak summaries are presented followed by the outbreak transmission overviews and the outbreak investigation overviews.

4.3.1 The outbreak summaries

The Prince Edward Island Bovine Outbreak

On February 27, 1987 a mature Holstein cow was detected, during routine abattoir slaughter, with lesions consistent with tuberculosis. The primarily dairy index farm was tested first with the caudal fold test and then with the comparative cervical. Eighteen of thirty-seven animals were positive to the caudal fold test. The complete dairy herd was tested with the comparative cervical test and seventeen animals were positive. Fifteen out of 37 were positive to both; 2 out of 37 were positive to just the caudal fold; 3 out of 37 were positive to just the comparative cervical; and 17 out of 37 were negative to both. Histology and culture tests were subsequently positive for the index animal and the herd was depopulated. At depopulation, 9 out of 35 animals examined for gross lesions of tuberculosis were positive, 2 out of 2 examined for histological lesions were suspect or positive, and 2 out of 2 cultured for *M. bovis* were positive. Investigation into the source and spread of the disease was initiated.

There were 164 farms investigated during this outbreak. Eleven sources of animals for this herd were identified going back to the early 1980's. Eight of these source herds were either non-existent at the time of the trace back or were negative on a caudal fold test. One of the source herds had animals (2.8%) which were reactors on the caudal fold test. The index animal had originated on a farm outside of the jurisdiction of the Prince Edward Island investigators. The farm was tested however and was negative. The index animal was sold to another farm in May of 1982. She remained on this farm until October of 1982 and then was sold to the index farm. Prior to this outbreak, an animal from the farm which had the index animal before it moved to the index farm, was detected at routine abattoir slaughter with lesions suggestive of tuberculosis. The lesions were suspect on histology but follow-up culture was negative. This herd had 72 out of 72 animals negative on the caudal fold test when it was tested as a traceback herd in this outbreak.

Traceout and buffer zone investigations revealed five farms with reactor animals to the caudal fold test. The herd reactor prevalences were .5%, 1.3%, .9%, 2.1%, and .9%. Specific information on the comparative cervical test results from these herds was not available. Of these five farms, two had community pasture contact with animals from the index farm, and three were tested because they were within a ten kilometre radius of the index farm. Animals that were traced from the index farm to other premises and slaughtered (as per Animal Health policy) were also negative on post mortem. Lacking gross lesions, histology and culture were not performed in these traceout animals. The remaining 149 farms which were investigated in this outbreak were either negative to the caudal fold test or did not have any animals on the farm at the time of the investigation. The source of tuberculosis on the index farm was not identified.

The New Brunswick Bison Outbreak

In March, 1985, tissues from a female bison which had died were histologically positive for tuberculosis. The index animal, a three year old female buffalo had been found dead. The carcass was sent to the provincial veterinary laboratory where findings of emaciation and numerous calcified abscesses in the mediastinal and bronchial lymph nodes were reported. The tissues were reported *Mycobacterium bovis* culture positive on July 29, 1985.

At the time that the animal was found dead, the farm consisted of 56 bison and 40 beef cattle. There was no contact between the cattle and the buffalo on this farm and in fact the cattle herd was not depopulated with the buffalo herd. Therefore the cattle herd was considered a separate herd.

The bison on the index farm originated from 3 farms in Ontario. The index animal was thought to have been one of 20 animals purchased from one premise in March, 1984. It was not possible to definitively identify animals from the three different sources as they were not tagged on arrival at the index farm. The beef cattle originated from several sources although they were purchased by the index farm from primarily one local dealer. There were 40 cattle at the beginning of the bison quarantine but the herd increased through births and purchase to approximately 140 animals by the time the cattle herd was tested in September, 1985.

On September 26, 1985, 55 buffalo were tested using the single cervical intradermal tuberculin test. Eleven of the animals were reactors. The buffalo herd was ordered slaughtered on October 16, 1985. Six animals with single lesions were found. All six were skin test reactors. Three of the six animals had caseous mediastinal lymph nodes; two had mineralized liver abscesses; and, one had a calcified mediastinal lymph node. Tissues from 4 of the 6 animals with

lesions were sent for histology and culture. One animal was histologically positive for mycobacteriosis and culture positive for Mycobacterium bovis. Three animals were negative on culture and the histology reports were not available.

On September 30, 1985, 133 cattle were tested using the caudal fold intradermal tuberculin test. There were no reactors. The cattle herd was not ordered slaughtered because: (1) there had been no contact between the buffalo and the cattle and therefore the risk of transmission was considered to be no greater than a perimeter property; and, (2) there were no reactors in the cattle herd. One neighbouring property was tested and all 11 cattle on this farm were caudal fold negative.

The Quebec Bovine Outbreak

Figures 1 and 2 show the outbreak transmission and investigation overviews.

A beef cow was detected at slaughter with gross visible lesions (GVL) of tuberculosis in September, 1986 from farm B1. The herd was tested and 9 out of 20 animals were suspect on the caudal fold test. The herd was depopulated and 12 out of 31 cattle had GVL on post mortem examination. This farm had received 3 animals from A2 in May of 1985. However A2 was not identified at the time of the investigation as a possible source of animals and in fact no longer had animals.

In November, 1986, another GVL animal was detected at slaughter. It was from a small herd, C3, of 4 cattle which when tested had no reactors to the caudal fold test. Farm C3 was depopulated and 1 out of 4 animals had GVL consistent with tuberculosis. Farm C3 had purchased 25 animals in October, 1985 from A2. Several of these animals were sold to other premises in Quebec and Ontario. These herds were identified and tested using the caudal fold test. All 25 animals were subsequently tuberculin test positive and had GVL on post mortem examination. Farm F4 received 5 animals in November, 1985 from C3. The caudal fold test of this herd showed 6 out of 27 animals as suspect reactors and 5 out of 27 had GVL. Farm G5 received 1 animal from C3 in November, 1985 and 1 in October, 1986. There was 1 suspect out of 29 animals tested on the caudal fold test and 1 reactor out of 29 tested on the comparative cervical test. There were 2 animals with GVL on post mortem examination. Farm H6 received 1 animal from C3 in October, 1986. One out of 16 was suspect on a caudal fold and a reactor on the comparative cervical test. This animal had GVL on post mortem examination. Farm I7 received 1 animal from C3 which was the only animal on this premise. It was suspect on the

caudal fold test, positive on the comparative cervical, and had GVL on post mortem examination. Farm J8 received 1 animal from C3 in October, 1986. This animal was suspect on the caudal fold and comparative cervical tests and had GVL on post mortem examination. Farm K9 received 3 animals from C3 in October, 1986. There were 3 out of 50 comparative cervical reactors and two with GVL. Farm L10 received 1 animal in October, 1986. Subsequent mid-cervical testing revealed 17 out of 178 mid-cervical reactors. Three animals had GVL on post mortem examination.

Farm D11 was discovered as a result of 10 kilometre perimeter testing. However it was subsequently determined that in October, 1985 this farm had purchased 2 animals from A2. There were 2 comparative cervical reactors and 1 animal with GVL on post mortem examination. Another positive herd, E12 was identified during the perimeter zone testing but no contact or source of infection was determined. Farm E12 had 1 comparative cervical reactor which had GVL on post mortem examination.

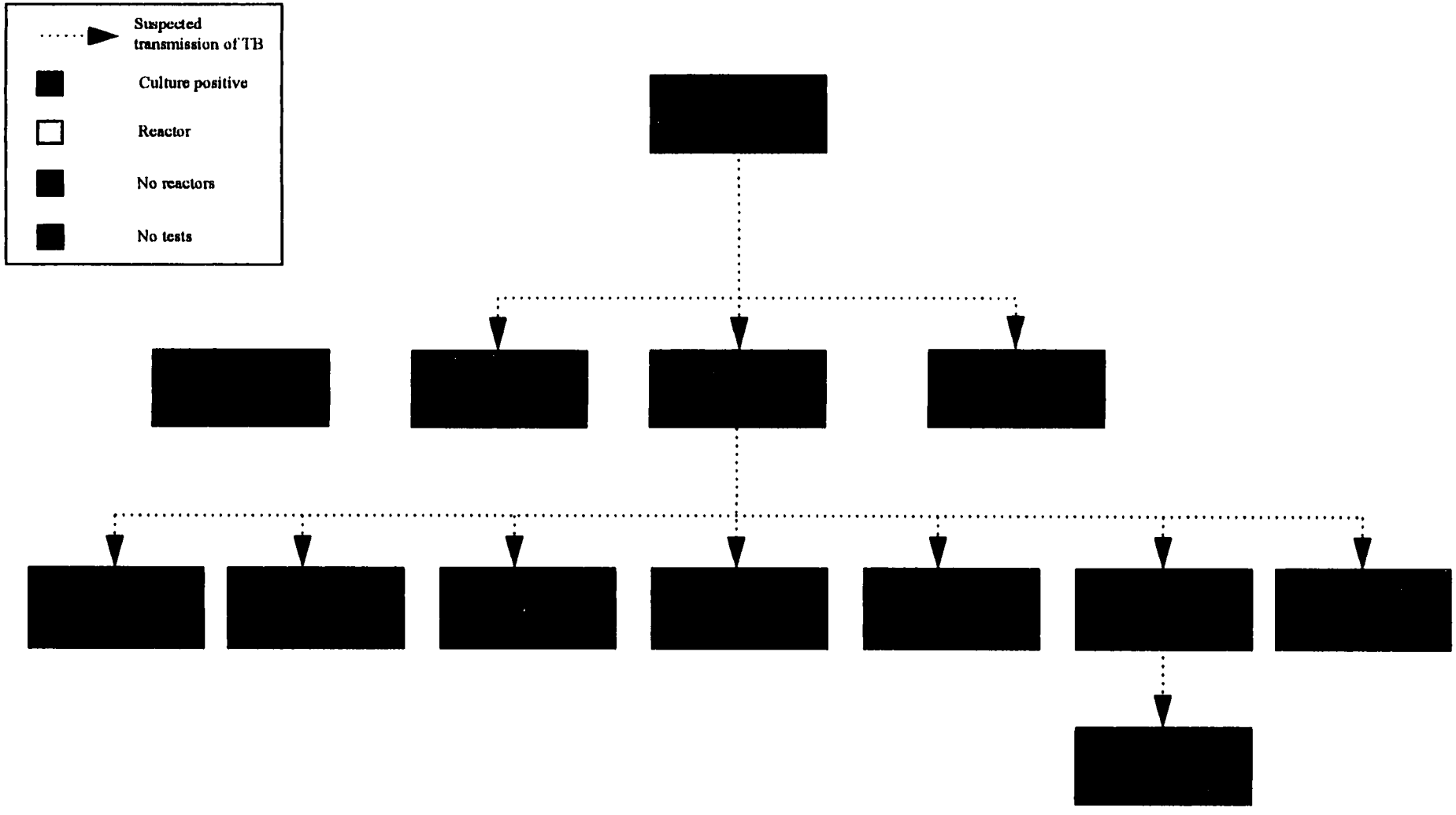
Farm M13 was identified because an animal with GVL was found at slaughter in March, 1987. No source of *M.bovis* was identified after epidemiological investigation. However, 1 animal from M13 was reported to have been in contact with 6 cattle from K9, on a separate premise, during one winter. These 7 animals were negative when tested as part of the other premise (not shown). Farm M13 had 9 out of 48 caudal fold suspects and 6 comparative cervical reactors all of which had GVL on post mortem examination. *M.bovis* was isolated and the herd was depopulated.

During this investigation there were 13 herds identified with comparative cervical positive reactors. All but one were directly linked to B1. The other, AA27, was a traceout farm

from a negative farm, Z26 which was investigated because it received animals from a farm which had also furnished animals to B1. This intervening farm was negative on caudal fold testing. Following are the number of reactor herds and the reason for the initiation of the investigation: 2 farms (N14 and O15) co-pastured with B1; 1 (P16) had fence line contact with B1; 2 (Q17 and R18) had supplied animals to B1; 7 (S19-Y25) were linked to B1 but the nature of the contact could not be determined from the overview data.

Figure 1

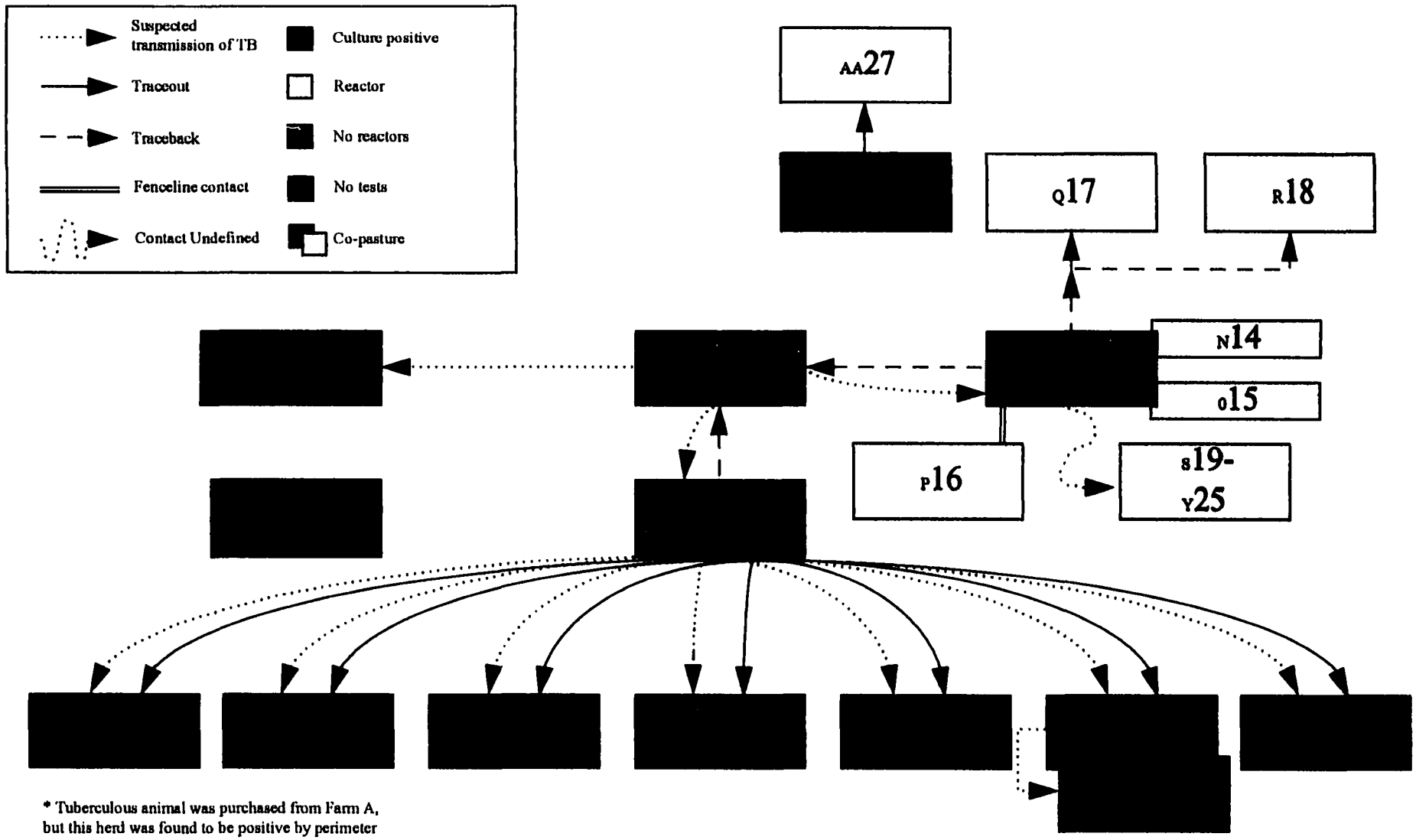
Quebec Bovine Outbreak Transmission Overview



* Tuberculous animal was purchased from Farm A, but this herd was found to be positive by perimeter testing.

Figure 2

Quebec Bovine Outbreak Investigation Overview



* Tuberculous animal was purchased from Farm A, but this herd was found to be positive by perimeter testing.

The Quebec Cervid Outbreak

Figures 3 and 4 show the outbreak transmission and investigation overviews.

In February, 1993, two female African antelopes, between 8-13 years of age, died at a zoological park (A1) . These animals were autopsied and had multiple tuberculosis lesions. A positive culture was reported in May, 1993. There were 825 animals from many different species, both domestic and exotic, housed at this facility. Depopulation was carried out and 695 animals were slaughtered. One hundred and twenty-two animals in nine different species were left in permanent quarantine at the zoo. Nineteen bison from this facility were histology and culture positive for *M. bovis*. There were eleven other species which had histology positive animals. Five out of eleven of these were also culture positive. The culture positive species included African Antelope, Watusi, Chianina and Chianina cross-bred cattle, Cerf de Virginie, and Yak.

Traceouts from A1 revealed two positive farms, B2 and C3. Farm B2 had purchased 10 bison from A1 in November, 1992. Farm B2 was placed under quarantine with 7 of the original bison still on the property. One animal died during the quarantine. The remaining 6 were slaughtered in October, 1993. Of the seven animals, there were 5, 4, and 2 positive on post mortem examination, histology, and culture, respectively. The whole herd was ordered slaughtered and 42 animals were destroyed. Traceout and traceback investigations of 102 animals on 5 premises revealed one reactor farm, F6.

Farm C3 bought 3 bison from A1 in December, 1992. These animals were 6 months old at the time of the purchase. They were slaughtered in December, 1993 and 3 out of 3 had gross visible lesions and histological lesions; and, 1 out of 3 was culture positive. There were no other animals on this farm at the time of the purchase or during the time that the three animals were on

the farm.

Farm B2 sold one bison, which had originated at A1, to D4 in January, 1993. It was slaughtered in December, 1993 and had gross and histology lesions, and was culture positive. The bison had been in contact with beef cattle on D4. There were 15 cattle tested and all were negative to the mid-cervical skin test. The herd was depopulated in November-December, 1994, and at that time 5 of the previously skin test negative cattle were condemned with tuberculosis lesions. Histology and culture were not performed on these animals. The cervids (39 animals) on this farm were free of lesions at the time of depopulation.

Two farms in this investigation were identified as reactor herds through traceout. Farm E5 was a traceout from A1. There were three deer on this farm which had originated on A1 and one was positive on histology but negative on culture. The second reactor farm was F6, a traceout from B2. Farm F6 received 5 bison from B2 in 1990. These bison originated from A1 (date unknown). One of these animals was suspicious on histology and negative on culture.

Figure 3

Quebec Cervid Outbreak Transmission Overview

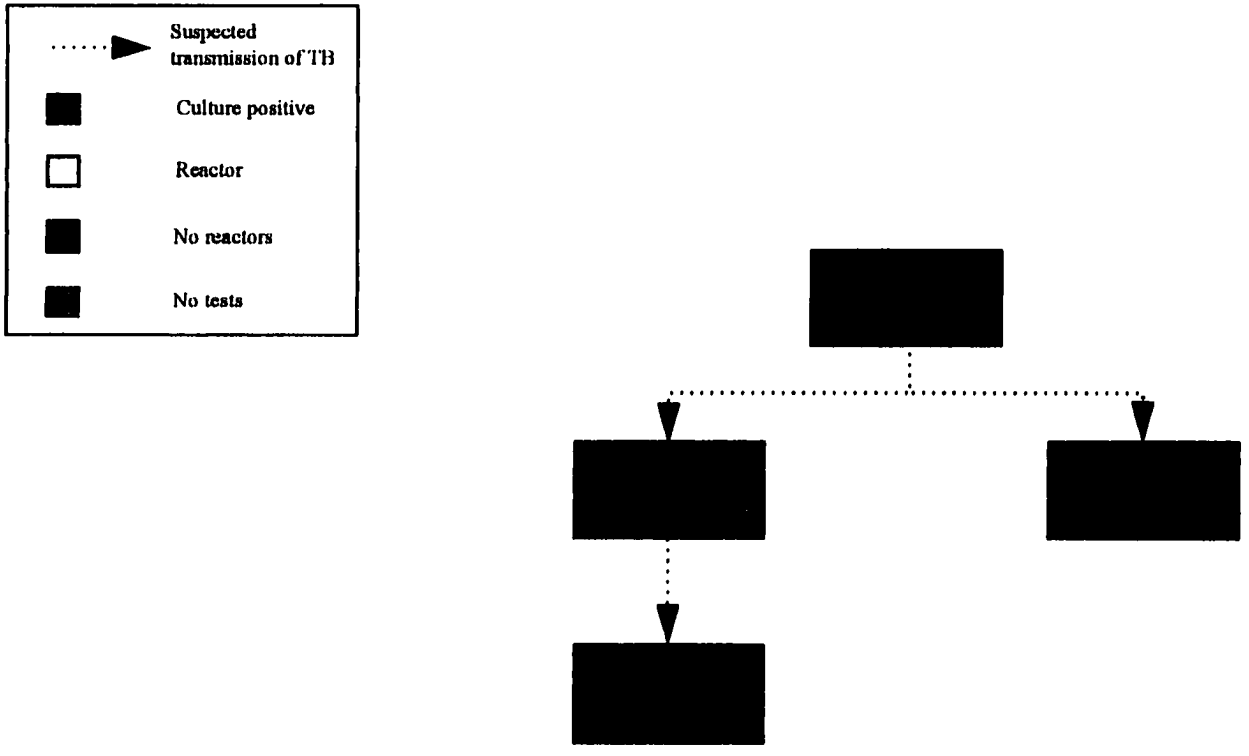
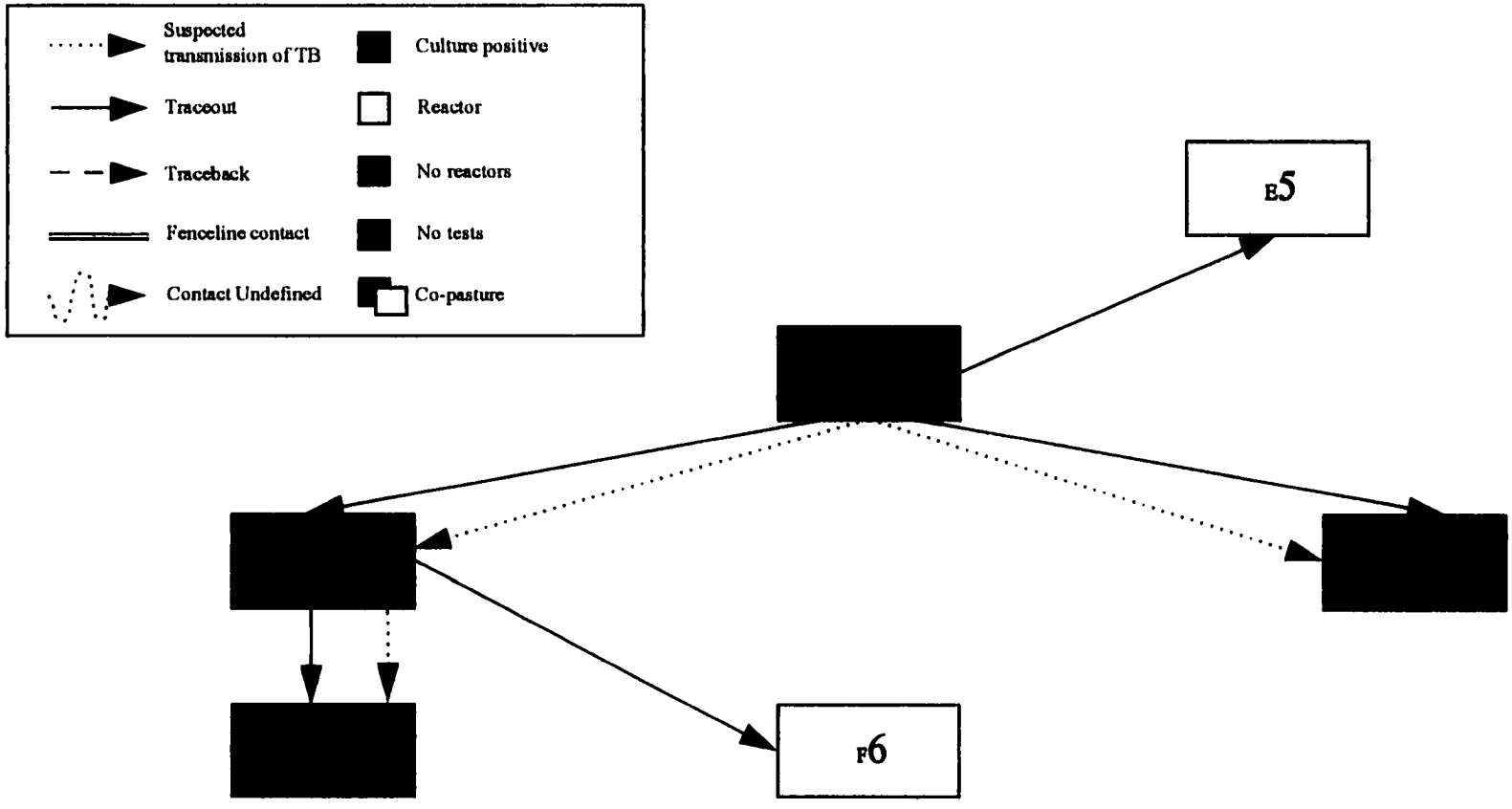


Figure 4

Quebec Cervid Outbreak Investigation Overview



The Ontario Cervid Outbreak

Figures 5 and 6 show the outbreak transmission and investigation overviews.

In January, 1990 a mature male elk, on loan to the index farm, B1, for the breeding season, died and was autopsied. The animal was found to have gross lesions consistent with tuberculosis and was subsequently culture positive. This animal originated from and was still owned by A2. Farm B1, consisting of 23 Pere David deer and 5 elk was depopulated in October, 1990. There were 3 out of 23 deer suspect on gross pathology; 1 out of 6 suspect on histology; and, 1 out of 23 positive on culture. There were 1 out of 5 elk positive on gross pathology; 2 out of 3 positive on histology; and, 3 out of 5 positive on culture.

This same farm (B1) had received 231 deer and elk from New Zealand in December, 1989. These animals were tested in December, 1989 and April, 1990. There were several suspicious reactors but in a retest 10 days later they were classified as negative. These imported deer were considered geographically separate from the animals on B1 which were depopulated in October, 1990, and thus were not depopulated. This premise was depopulated again in 1992 and is labelled on the diagram as L9.

Farm A2 was depopulated and although there were animals with suspect lesions on post mortem the results of histology and culture on these animals were not available for analysis.

Farm D3 was identified because an elk which had been chronically ill died in January, 1991 and was submitted for postmortem examination. It had lesions consistent with tuberculosis and was subsequently culture positive. The index animal on this farm had originated in western Canada 5 years previously and had an American tag. The animal died 3 days after a negative skin test. This farm had also received animals from A2 in 1987, and elk from a Zoological Park in

1986. Farm D3 was depopulated in September, 1991 and there were 38 out of 73 elk condemned with lesions of tuberculosis. On previous mid-cervical skin testing, 0 out of 6 were positive.

Farm E4 had received a majority of its animals from A2 starting in 1981. This farm was identified because the index animal, a 12 year old Fallow Deer was identified with lesions at slaughter in November, 1990. The herd of 147 animals was depopulated in September, 1991. Testing results were not available for this herd.

Farm G5 received an elk from A2 in mid-December, 1990. This elk died in February and was subsequently positive for *M. bovis* on culture. The herd was depopulated in November, 1991. There were 3 of 22 elk positive on gross pathology; 2 out of 3 positive on histology, and 7 out of 7 positive on culture.

Farm F10 was discovered as a result of a traceout from A2. There were 10 out of 58 cervids positive on gross pathology. Histology and culture results were not available.

Farm C12 was identified because a cull male elk was found with tuberculous lesions at slaughter in June, 1994. Eight elk had been purchased from A2 between October, 1989 and February, 1990. These 8 animals, including the index animal had been skin test negative on a December 20, 1990 test. On testing of this herd, there were 9 out of 44 elk and 0 out of 13 beef cattle positive on gross pathology; 1 out of 1 elk positive on histology; and 1 out of one positive on culture. Three of these eight original elk had been purchased from C12 in November, 1992 by K13. This farm was depopulated in February, 1995. All 50 cattle were negative on post mortem. One of the 3 elk purchased from C12 was condemned for tuberculosis lesions at slaughter. It was also suspect on histology and positive on culture.

Farm H7 was depopulated because there were 14 cattle on the farm which had contact

with the animals on B1 which was depopulated in October, 1990. All the animals on H7 were depopulated. The original 14 animals and their calves were NVL at slaughter. The remaining 8 cows and 7 calves were skin tested and all were negative. The owner of H7 was the animal caretaker at B1 and J6. He requested that his own farm be retested in February, 1992. He was herdsman at L9 when it was depopulated and wanted to assure himself that his cattle were still free of tuberculosis. Five cattle out of 56 were positive on the caudal fold tuberculin test; 1 was positive on a comparative cervical test; and 1 out of 113 was suspect on gross pathology. There were no culture positive animals but the herd was depopulated in October, 1992. Subsequent culture testing from this herd resulted in culture positive animals. This second depopulation on this premise is denoted as I11.

Farm J6 bought 50 female deer from L9 in July, 1991 and two stags in August, 1990. The index animal, discovered at slaughter, on this farm was one of the suspect reactors from the herd test carried out just following importation to B1. One hundred animals were depopulated from J6. There were 36 out of 100 with gross lesions on post mortem; 1 out of 6 with lesions on histology; and 1 deer positive on culture. The diagrams show transmission from H7 and L9 to J6. The more likely route is from L9 as there were infected animals transferred in this case whereas the caretaker of J6 was the owner of H7.

Farm L9 was the same farm as B1 (only 2 years later). B1 was depopulated and a cleaning and disinfection had been carried out. It had received 231 deer and elk from New Zealand at about the same time as the first depopulation. The imported deer and elk were skin tested after their arrival and although several were suspect, a retest after 10 days was negative. Thus these animals were considered negative status animals. When this farm was eventually

depopulated in March, 1992 as a result of a traceback from J6, there were 6 out of 140 deer with visible lesions of tuberculosis. All of these animals were from the 1990 importation.

Farm M8 bought 15 animals from L9 in January, 1991. The cattle on this farm were skin tested and 21 out of 70 were positive on the caudal fold; three out of 15 were positive on the comparative cervical; 1 out of 3 was positive on gross pathology. An 18 year old deer was culture positive.

There were two reactor farms identified as a result of this investigation. Farm O15 was a Trace out farm from C12. There was 1 out of 40 elk suspicious on a mid-cervical tuberculin skin test; 4 out of 8 elk were suspicious on gross pathology but all 8 were negative on histology and culture. Farm N14 was investigated as a traceback to C12. There were 1 out of 7 elk positive on the mid-cervical test. The results for follow-up tests were not available in the files.

Figure 5

Ontario Cervid Outbreak Transmission Overview

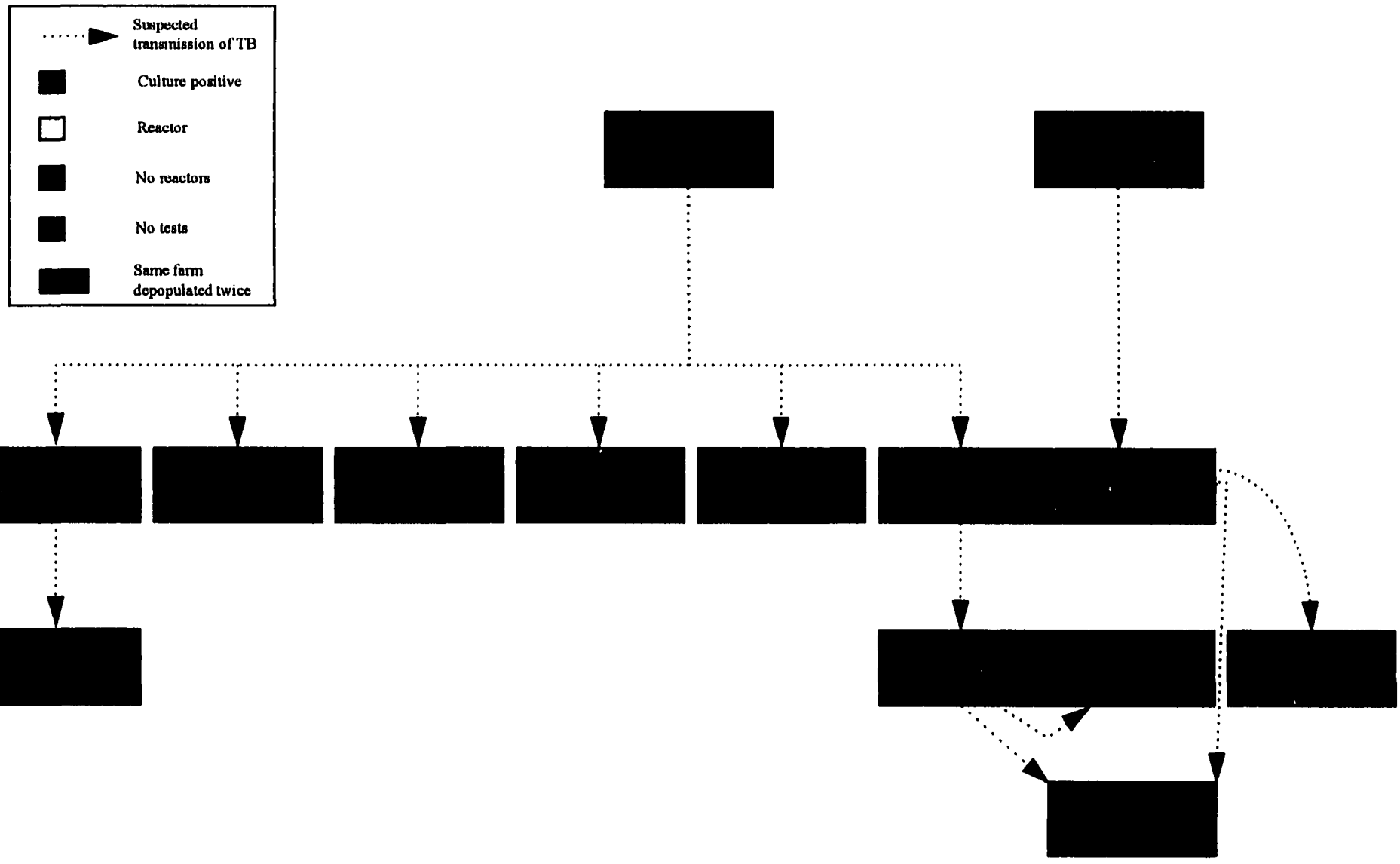
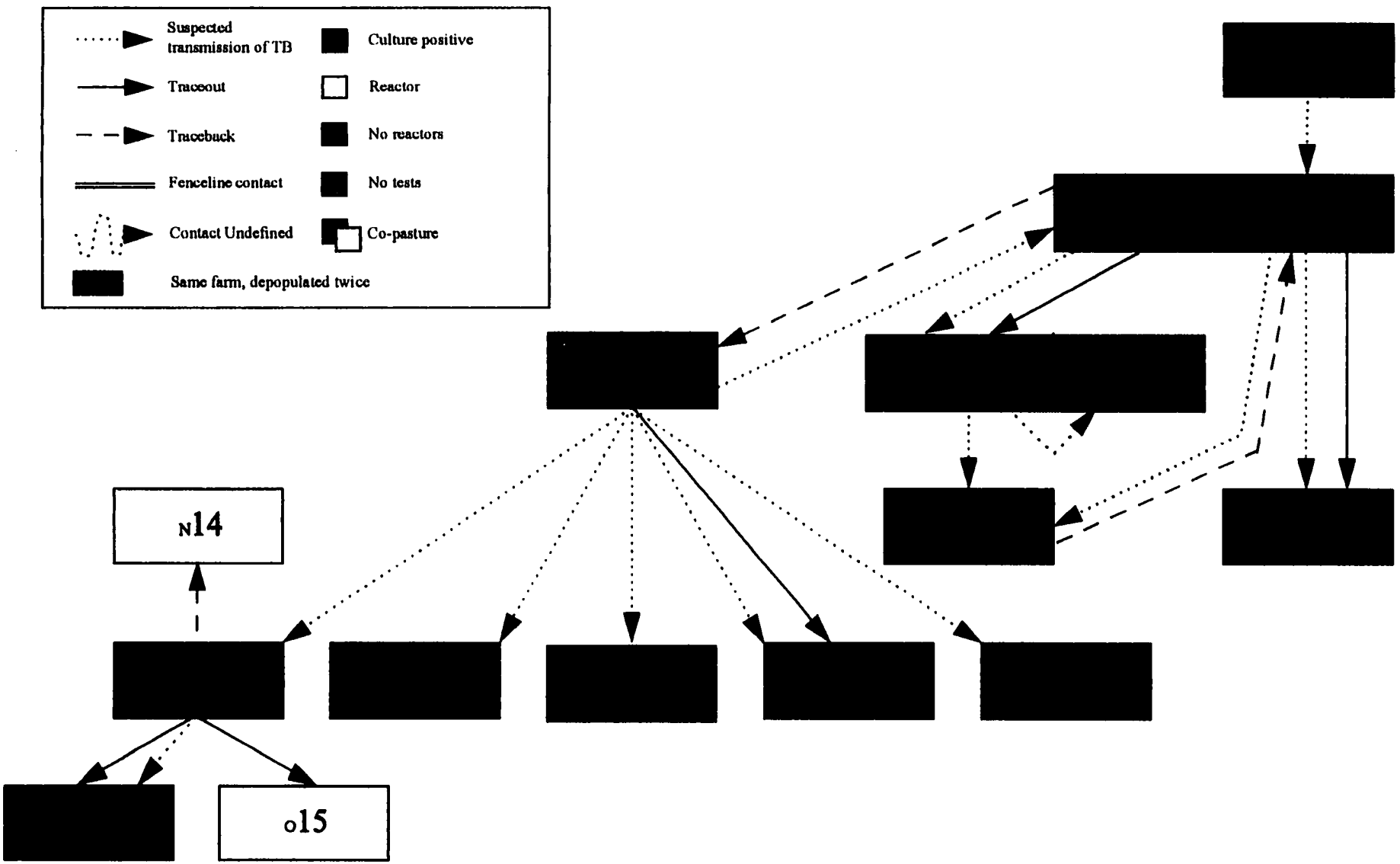


Figure 6

Ontario Cervid Outbreak Investigation Overview



The Manitoba Bovine Outbreak

Figures 7 and 8 show the outbreak transmission and investigation overviews.

On October 30, 1990 a three year old Charolais grade heifer was detected with lesions of tuberculosis at a federally inspected slaughter house in Manitoba. This index case originated at a beef herd, B1, which had been established in January of 1990. All the animals from this herd except a male Holstein calf had originated from one source. This source herd, A2, was investigated. Between January and October 1990, the farm had changed from primarily a beef operation to a dairy operation. The transition had been gradual and the beef cattle had been on the farm with at least some of the dairy animals. However, the overlap was short; there was no direct contact; and, only a portion of the dairy animals were actually present during the overlap time. Testing of the source herd, A2 in August, 1991 revealed fifteen out of 55 animals positive to the mid cervical test. All reactor animals were slaughtered, one had GVL on post mortem, but all were negative on culture. Farm A2 was not depopulated. Farm B1 was depopulated and all 36 animals were NVL on post mortem. All 36 beef animals on B1 were also caudal fold test negative. A third beef farm, C8, tested as a result of prolonged fence line contact to the source herd, A2 (when it had beef animals), revealed 2 of 91 cattle suspect on a caudal fold test. These two animals were subsequently positive on the comparative cervical test they were culture positive as well. C8 was depopulated. In the final analysis of this herd there was a total of three of 174 animals that had lesions on post mortem and were culture positive also. At depopulation one animal was found with extensive lesions of tuberculosis but had been skin test negative. All other lesioned animals from this herd had small lesions and no dissemination of lesions. Three primarily beef farms, D17, E18, and F19, tested as traceouts to C8 had 1 of 7, 1 of 46, and 3 of

100 animals, respectively, positive on the mid-cervical test. These farms had 2 of 2, 1 of 1, and 1 of 4 culture positive respectively. Farm G13 was tested as a result of co-pasturing with C8. There were 8 of 47 mid-cervical reactors and 3 of 83 with post mortem lesions of tuberculosis. There were no histology or culture positive animals. Farm G13 was depopulated however because of the yearly close contact (co-pasturing) with C8.

Four farms, H4, I3, J5, and K16, which shared pasture with B1 were identified as reactor farms. Farm H4 had also received animals from Farm C8. Five reactor farms were identified from association with Farm A2. Farm L10, Farm M9, and X24 had fence line contact; and, Farm N6 and Farm O7 had received animals from Farm A2. Farm M9 also had fence line contact with Farm C8 and another reactor farm, Farm P14. Two reactor farms were identified from association with Farm C8. Farm P14 had fence line contact; and Farm Q15 received animals from Farm C8. Two reactor farms were identified from association with Farm E18. Farm S20 had fence line contact and had received animals from E18; and, Farm R21 co-pastured with Farm E18. Reactor farm T22 co-pastured with another reactor farm, P14. Reactor Farm U23 had fence line contact with reactor Farm T22. Farm V11, a negative farm was investigated because it had provided animals to Farm A2. Farm W12 also received animals from V11. Farm W12 had 1 animal which had lesions on post mortem and histology but was culture negative.

It was impossible to definitively determine in this outbreak if A2 or C8 was infected first. This overview follows the investigation in a chronological sense. C8 and A2 had fence line contact for many years. It is possible that the transmission pattern was from C8 to A2.

Figure 7

Manitoba Bovine Outbreak Transmission Overview

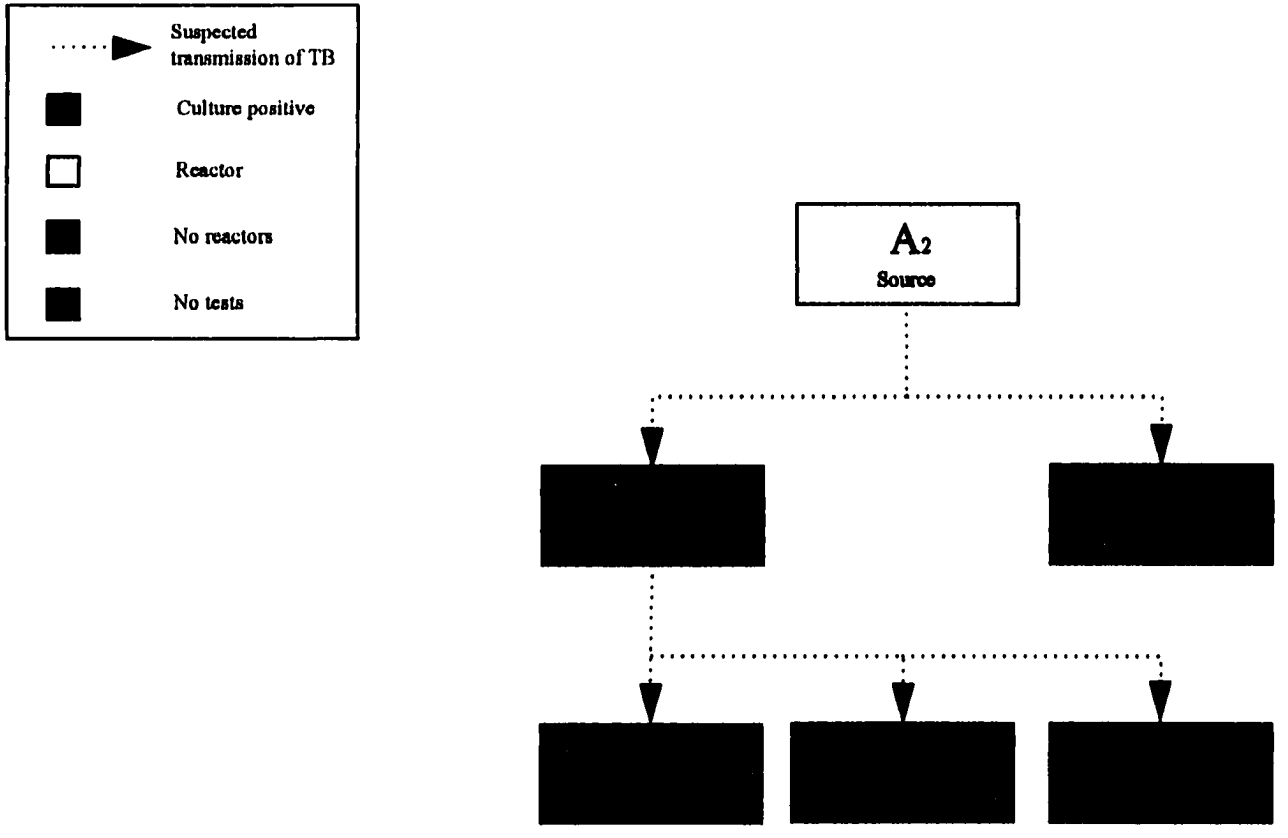
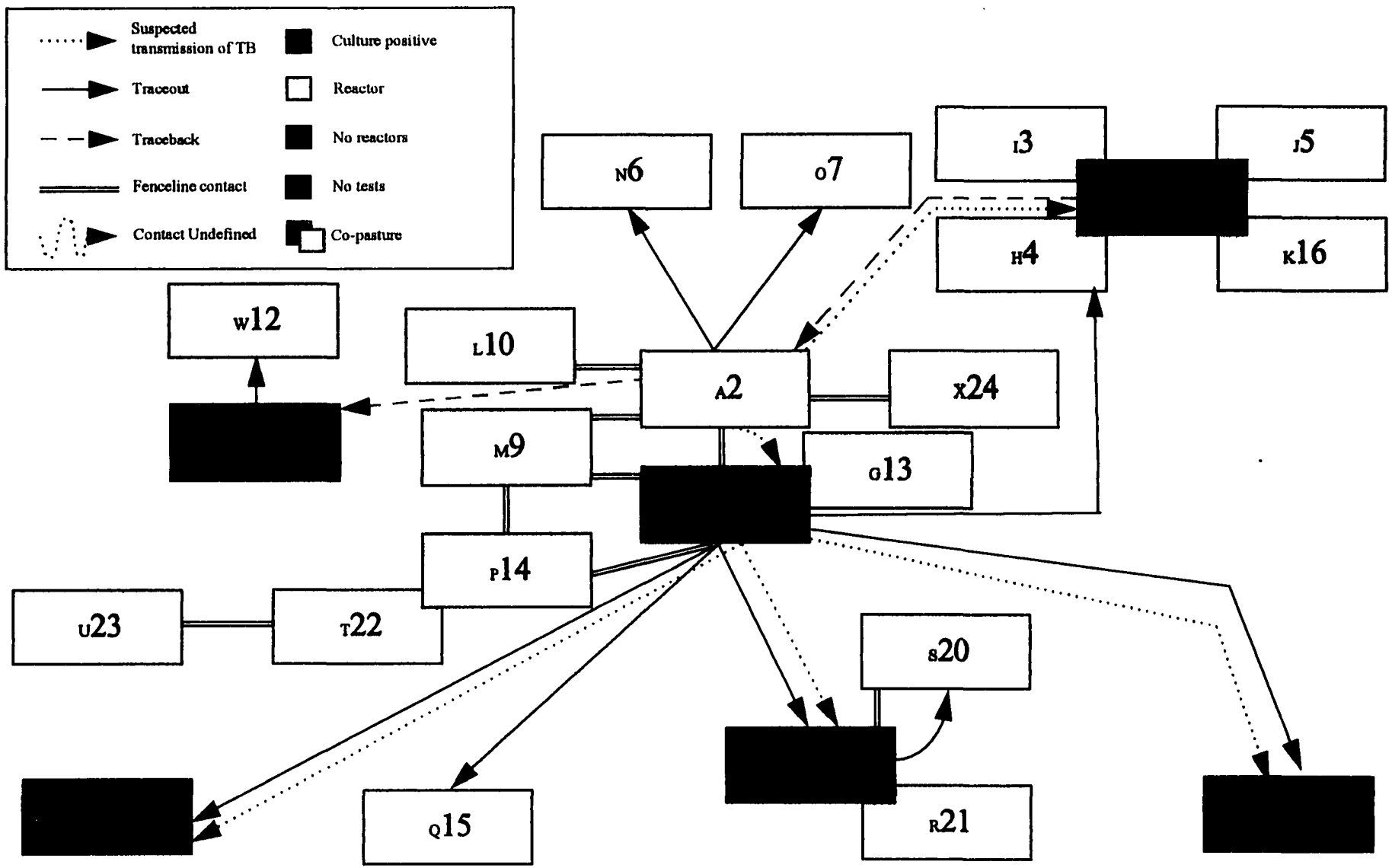


Figure 8

Manitoba Bovine Outbreak Investigation Overview



The Alberta Bovine Outbreak

Figures 9 and 10 show the outbreak transmission and investigation overviews.

In October, 1985 a three year old Charolais bull was identified at slaughter in the United States with extensive lesions consistent with tuberculosis. This animal was one of a group of 31 bulls that had been gathered by a livestock dealer, D2, and sold to E1 for slaughter in the United States. A traceback was initiated and it was determined that the animal had originated from A3. Testing of animals in A3 revealed that 48 out of 84 animals were positive on the caudal fold test and 5 out of 84 were suspect; 46 out of 53 were positive on the comparative cervical and 1 out of 53 was suspect. The herd was depopulated and gross visible lesions were evident on 41 out of the 166 animals slaughtered. This farm had not purchased animals except for bulls since 1973. Tracebacks were carried out but the source of infection was not determined. However, links were found to a possibly infected herd. It was said that A3 had purchased 4 cows in 1972 from a dealer who had purchased 18 cow-calf pairs from a farm that was known to have lesioned reactors. It is possible that 1 or more of the 4 animals may have been infected with *M.bovis* and that this was the source of infection on A3.

Farm C4 both co-pastured with and purchased animals from A3. Five animals were purchased in 1985 and two others sometime after that date. Five out of 57 animals on C4 were reactors on the first caudal fold test and 20 out of 51 were reactors on the second caudal fold test. One out of one animal was positive on the comparative cervical test. The herd was depopulated in March, 1986 and eight animals out of 56 had GVL on post mortem examination. Culture results were not available.

Farm B5 purchased a cow from A3 in 1977. There were 11 reactors (1 positive and 10

suspect) out of 233 animals tested with the caudal fold test. The follow-up comparative cervical revealed 9, 1, and 1 out of 11 animals as negative, suspect, and positive respectively. The mid-cervical test showed 26 positive, 0 suspect and 207 negative animals. Three animals out of 436 had lesions on post mortem examination. Two out of 2 animals tested with histology and culture were positive on both. The complete herd of 444 animals was depopulated in April, 1986.

Three reactor herds were identified in this outbreak. Farm H8 was a traceback to B5. There were 1 out of 11 positive on the caudal fold test; 1 out of 1 positive on the comparative cervical test; and 1 out of 1 negative on post mortem. Histology and culture results were not found in the outbreak files.

Farm F7 was a reactor farm which had fence line contact with A3. Of the 17 caudal fold reactors in this herd one was positive on a comparative cervical test. There were no gross lesions on post mortem and histology and culture results were not found in the outbreak files.

Farm G6 was identified as a reactor farm from perimeter testing associated with A3. One out of three animals were positive on the comparative cervical test and 3 out of 3 were negative on post mortem. Histology and culture reports were not found in the outbreak records.

Figure 9

Alberta Bovine Outbreak Transmission Overview

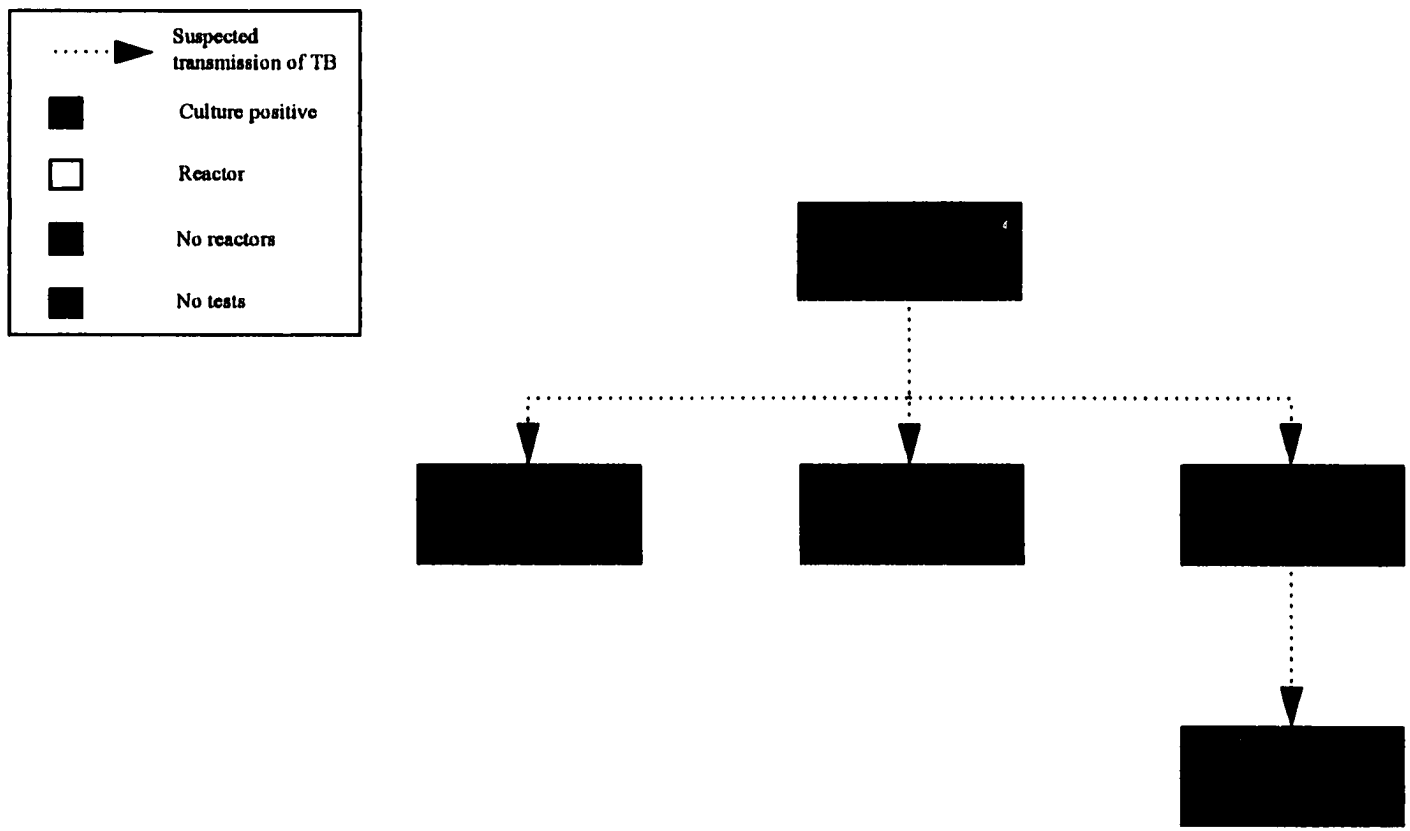
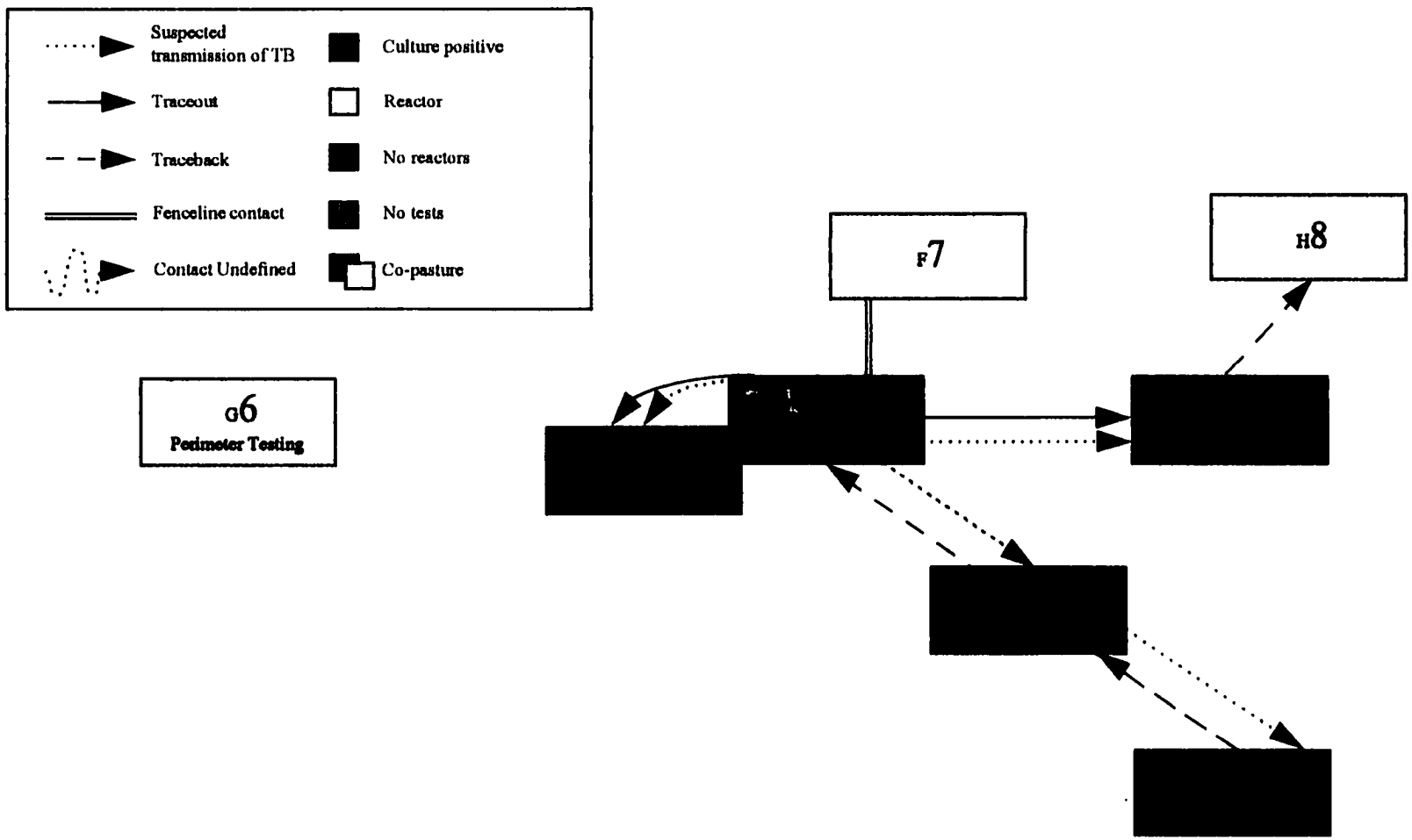


Figure 10

Alberta Bovine Outbreak Investigation Overview



Alberta and Saskatchewan Cervid Outbreak

Animal movement patterns between farms in this outbreak were extremely complex. A transmission diagram which shows all the possible routes of transmission would be hopelessly complex. Therefore only one diagram, which represents a generalized scenario of transmission of the organism during this outbreak is presented in Figure 11. This diagram does not represent all of the possible contacts between these farms. Four groups of reactor farms, G1 - G4 are shown. Group 1 contains farms that were known to have had contact with the 3 first identified farms. Group 2 contains farms which were known to have had contact with at least 1 of A1 - C3 and with at least 1 of the 13 other positive farms. Group 3 had farms which were only known to have had contact with the 13 positive farms of D4 - P16. There were 16 reactor herds which could not be linked to a specific source herd based on information in the data base and these are in Group 4. They are AP42, AQ43, AR44, AS45, AT46, AU47, AV48, AW49, AX50, AY51, AZ52, BA53, BB54, BC55, BD56, BE57. The nature of the contact (sale, purchase, contact) was also unavailable. The chronological development of the outbreak is accurate for A1 - B3 only. Other farms were labelled for convenience and for their relationship to the source herds.

Between July and December, 1990, three Alberta elk herds were identified as either tuberculosis positive or highly suspect. All three had imported elk from the same herd(s) in the United States between 1986-1988. The first Canadian herd, A1 was identified after a female elk was euthanised due to an untreatable retropharyngeal abscess.[1] This female was one of a group that had been imported from Montana in 1988. The post mortem revealed multi-focal abcessation

[1]Notes from the Alberta Regional Office of Agriculture And Agri-Food Canada, concerning the Alberta elk tuberculosis outbreak

of the lymph nodes of the head, lungs, and mesentery. Thirty-one out of 109 elk that were tested with the single cervical test were reactors (28.5%). The herd was depopulated in December, 1990 and 51 out of the 150 animals examined at slaughter had lymph node pathology (34%). There were 35 herds tested or investigated as a result of sales or contact with A1 and 7 were classified as reactor herds (Q17, R18, S19, T20, U21, V22, W23).

The second herd, B2 had a slaughter pig which was *M.bovis* positive in June, 1990. This was the second tuberculous pig discovered from this farm. The first was identified at slaughter in November, 1988. Farm B2 had imported a large number of elk from three different farms in the United States between 1986 - 1988. It was believed initially that there was no contact between the elk and the swine but eventually it was revealed that the pigs had been fed several elk carcasses in past years. Three hundred and thirty-three elk were tested on this farm, using the single-cervical test, between December, 1990 and March, 1991. Two hundred and ten were reactors (64%). Sixteen herds were tested or investigated as a result of sale or contact with B2 and three were classified as reactor herds (X24, Y25, Z26).

The third herd, C3 was skin tested by a private practitioner in November, 1990. This farm had imported 231 elk in 1987 from a single United States source. There were 3 single cervical positive animals out of 172 tested (1.7%). All three showed gross lesions at slaughter and at least one of these animals was reported culture positive in April of 1991. In March, 1991, federal veterinarians and inspection staff tested 252 elk in this herd. All tests were negative. In July, 1991, a private practitioner euthanised 1 animal and the post mortem revealed gross lesions and subsequent infection with *M. bovis*. This animal had been negative on the March herd test. The herd was depopulated in September, 1991. There were 331 animals slaughtered and 154 had

gross visible lesions (46.5%). Eighteen herds were tested or investigated as a result of sale or contact with C3 and 9 were classified as reactor herds (AA27, AB28 AC29, AD30 AE31, AF32, AG33, AH34, AI35).

As stated earlier, A1, B2, and C3 were identified within 6 months of each other and all had imported animals from the United States. Investigations of herds based on sales or contact to these three primary herds, identified a total of thirteen positive herds (D4, E5, F6, G7, H8, I9, J10, K11, L12, M13, N14, O15, and P16). Information on these positive herds is contained in Table XIII. The table lists: the number of cervids in the herd; the results of the last recorded herd test; the number of animals that had gross visible lesions and/or how many were culture positive (often there is no information on how many animals were examined); and, the depopulation date. Six herds with reactor status were identified from the investigation of contact or sales with these positive herds (AN40, AO41, AJ36, AK37, AL38, AM39).

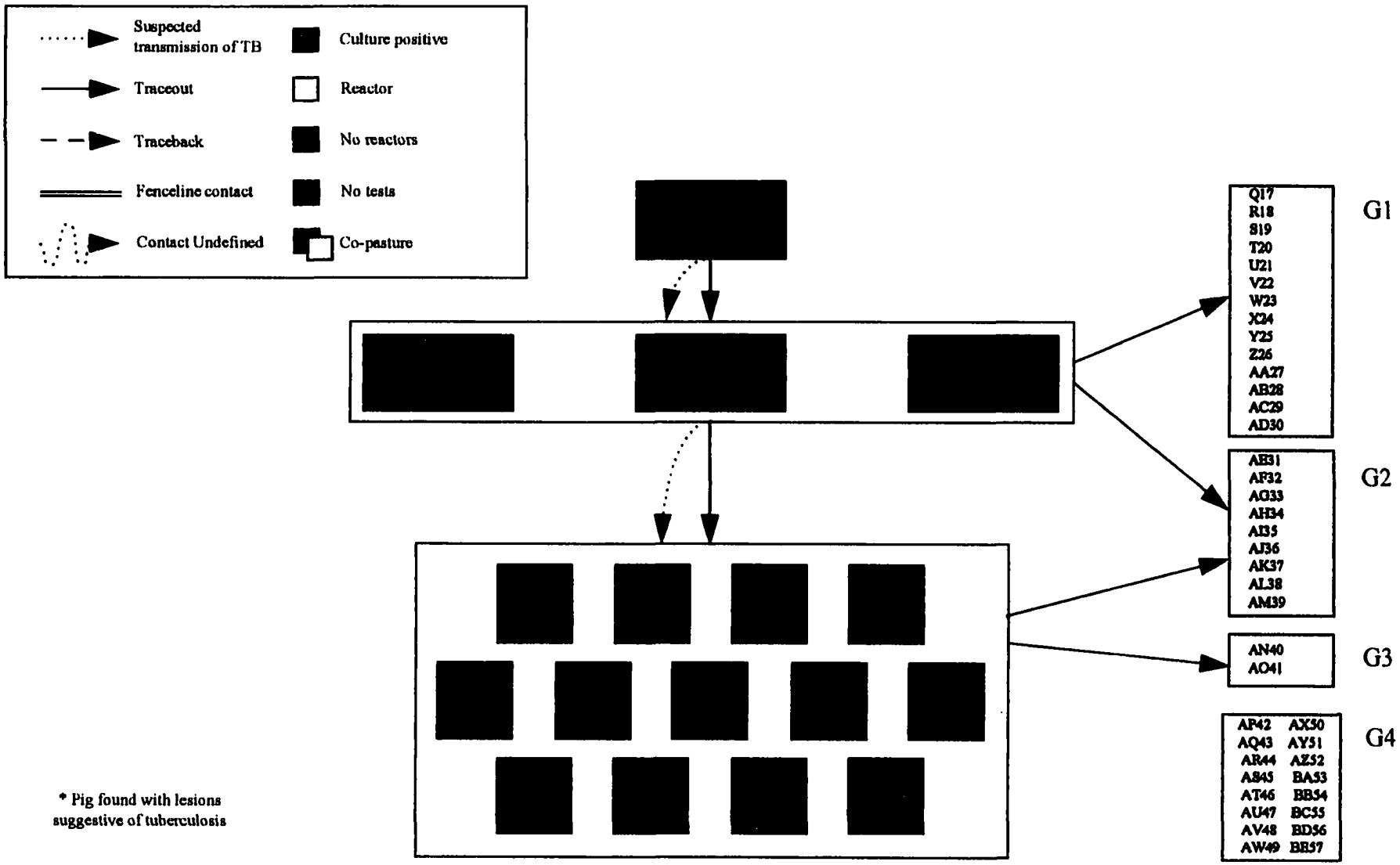
Table XIII
Summary information on thirteen of the positive herds in the Alberta/Saskatchewan cervid tuberculosis outbreak

FARM	NUMBER OF ANIMALS	SINGLE CERVICAL REACTOR RATIO	NUMBER OF ANIMALS WITH GVLs	NUMBER OF CULTURE POSITIVE ANIMALS	DEPOP. DATE (MON/YR)
D4	78	4/68	5	1	8/91
E5	164	3/119	1	1	10/91
F6	55	1/46	2	1	11/91
G7	14	0/14	-	-	1/92
H8[a]	192	19/192	1	-	2/92
I9	20	3/11	2	3	3/92
J10	173	0/99	1	1	3/92
K11	156	3/79	11	3	5/92
L12	50	0/25	3	0	8/92
M13	20	1/16	1	-	2/93
N14[a]	106	0/106	-	-	6/93
O15	327	25/191	11	3	8/92[b]
P16[a]	389	86/540	108	1	5/91

[a] The data are from written overview information not Epi-info as for the others
[b] All animals depopulated except calves born in 1992

Figure 11

Alberta/Saskatchewan Cervid Outbreak Transmission Overview



* Pig found with lesions suggestive of tuberculosis

The British Columbia Deer Outbreak (There are no diagrams associated with this outbreak)

In September of 1989 a herd test was carried out on 288 deer on a farm, Farm A1, in British Columbia. There were 14 suspect animals on the mid-cervical test. Twelve of these animals were negative on the comparative cervical test, one was suspect, and one was not located. A retest of the suspect animals was carried out in early December, 1989. The animal was positive to the comparative cervical test and was ordered slaughtered. The deer had macroscopic lesions of tuberculosis, was histology positive, and subsequently was culture positive. The whole herd was depopulated during late March and early April of 1990. Post mortem examination of the herd at this time showed 12 more animals with lesions suggestive of tuberculosis.

A trace back of farms of origin of the lesioned animals was initiated at A1. There were 2 Canadian source farms and one from the United States. The first Canadian source was a farm in Saskatchewan. Farm A1 purchased 97 deer from this farm on April 17, 1989. Two of these animals had lesions when A1 was depopulated. Both of these animals had originated in the United States prior to going to the Saskatchewan farm. The Saskatchewan farm had started operation in April-October, 1988 with the importation of 663 adult deer from the United States. In June, 1990 this herd was tested and 4 animals were subsequently determined to be mid-cervical reactors. These 4 were negative on comparative cervical testing and one of the 4 was slaughtered and found to be a NVL reactor. The records do not record the final culture status of this animal.

The second Canadian source farm was in Quebec. The index animal was bought from this premise sometime in 1988 as one of a group of 130 deer. The owner of A1 purchased all of the deer from this farm. This Quebec farm was involved in an investigation of a tuberculosis

outbreak in Quebec involving bison but was not depopulated.

There were no reactor farms identified as a result of traceout or perimeter testing.

4.4 Discussion

The outbreak summaries and overviews were included to provide a more qualitative and visual understanding of the tuberculosis outbreaks in Canadian cattle and cervids over the last ten years. The quantitative aspects of these outbreaks are found in the preceding and in the 2 following chapters. There were several insights or observations which surfaced while researching these outbreaks which cannot be substantiated with quantitative data from the study as these data were not available or were not collected. Therefore these insights do not appear elsewhere in the discussions of these outbreaks and are presented here. In some cases the insights support the quantitative data and results that emerged from Chapters 5 and 6.

Age was a recurring factor in these outbreaks both at an individual animal and herd level. Older animals, particularly cervids, were often found on postmortem or at slaughter with marked lesions of tuberculosis. This was illustrated in the Quebec cervid and Ontario cervid outbreaks. It is understandable in the Quebec cervid outbreak where the source herd was a zoological park where animals were kept to old age and not slaughtered as young animals. Index animals in the Ontario cervid outbreak included an older breeding bull, a cull male elk, and an 18 year old deer. Several positive herds were identified because the index animal had died after extensive treatment. This industry is relatively new compared to dairy and beef production. Perhaps animals are kept to an older age because they are more valuable. In this case, infected animals would be more likely to progress to clinical cases with extensive lesions. The other possibility is that cervids are more susceptible to tuberculosis and therefore a higher proportion will progress to full blown disease and in a shorter time period.

The average age of animals in a herd was also significant in several source herds. The

Quebec bovine, Quebec cervid, and Alberta bovine source herds all had an average age of individuals greater than the industry norm. Infected animals remained in the herd for a longer time period allowing disease to develop and providing increased opportunities for transmission.

Bison appeared to be a very high risk species. In the Quebec cervid outbreak, 19 animals in the source herd were histology and culture positive. Very young animals, 1-1.5 years of age, which were investigated as traceouts had well developed lesions of tuberculosis. These observations are based only on the herds which were studied and do not represent a random sample. However, it appears that when present in bison, *M bovis* progresses rapidly to overt clinical disease with well established lesions. This hypothesis, if correct, has implications concerning the one wildlife reservoir of tuberculosis in Canada - the bison in Wood Buffalo National Park. Carcasses of infected animals have been found as far as 75 miles outside the boundary of the park (2). Traditional livestock agriculture continues to move closer to the park also.

These overviews showed the importance of traceout and traceback investigations. Two limiting factors to the success of these epidemiological procedures were the quality of the farm records and the attitude and compliance of the farm owners. When written records on sales and purchases do not exist, the traceout and traceback investigations will be only as complete as the memory of the owner. It is difficult to quantify the second factor but it was very clear in the overview material that many difficulties were encountered when the owner was not cooperative. These difficulties included: delays in (and in some cases lack of) traceout and traceback investigations; delays in depopulations; extra costs due to need for increased manpower and legal counsel; reduced effectiveness of the program as infected herds continued in business for long

periods of time until they were identified on post mortem or on slaughter surveillance; and, tremendous personal stress to the investigating officers including death threats.

A hypothesis of this study was that farms which were reactor status (as defined in this project) had an increased risk of transmitting tuberculosis. In the Manitoba outbreak, a farm which was classified as "reactor", A2, was never culture positive and was not depopulated but was implicated as the source of animals for two positive farms.

Although qualitative in nature, these insights may be considered when planning a strategy for tuberculosis control and eradication programs, and in outbreak situations.

Several steps were taken as a result of these outbreaks to improve the tuberculosis control program in Canada.[2] These included the following.

1. For cervidae

    a. A ban on any imports of cervids from the United States was initiated in January, 1990 and extended to January ,1997.

    b. A captive ungulate program was initiated which required a whole herd test for tuberculosis every 3 years and movement permits for movement between qualified herds.

    c. The comparative cervical test was to be applied at least 60 days after the most recent tuberculin test. Thus the 10 day window (after another tuberculin test) no longer applied to cervidae.

    d. A newly defined "suspect zone" for cervidae was defined on the comparative

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[2]Information from Animal Health Division memos and personal communications with Program staff.

cervical reaction chart. This new zone for cervidae included a portion of the graph that fell in the negative zone for bovine. Essentially the sensitivity of the test for cervidae was increased.

e. All cervidae 1 year of age and older became eligible for testing.

2. For Bison

a. All bison six months of age and older became eligible for testing.

3. For all species

a. When the sample size of test animals was less than 50 animals, than the age restriction was removed and all animals would be tested.

The ban for import of cervids from the United States has expired but Canada will not resume import of cervids until a risk assessment has been completed.[3]

[3]Personal communication with Program Staff

4.5 Conclusion

The written descriptions and the overview diagrams of the tuberculosis outbreaks in Canadian cattle and cervidae were presented. Qualitative insights into these outbreaks were discussed. Agriculture Canada and Agri-food Canada responded to these outbreaks by introducing new policies to improve the control of tuberculosis in cattle and cervidae in Canada. These new policies were presented.

Chapter 4 References

1. Girard, H. A Tuberculosis Outbreak in Quebec Cattle 1986 - 1987? [Science]. 1988; University of Guelph;

2. Tessaro SV, Forbes LB, Turcotte C. A survey of brucellosis and tuberculosis in bison in and around Wood Buffalo National Park, Canada. Can Vet J 1990;31:174-80.

CHAPTER 5

BETWEEN HERD SPREAD OF *MYCOBACTERIUM BOVIS*

5.1 Introduction

Bovine and cervid tuberculosis is primarily caused by the bacterium *Mycobacterium bovis*. This organism is infective to all warm-blooded animals including humans (1). Cattle and cervids may become maintenance hosts, unlike other species, which generally are spillover hosts only (2). The main route of transmission of tuberculosis in cattle and cervids, in Canada, is assumed to be aerogenous. There are other potential routes of transmission but other than the alimentary route in cervids they have little significance as they have been eliminated by modern management practices. It has been suggested that cervids have a greater susceptibility to tuberculosis than cattle and that the rate of transmission is faster within herds (2,3).

The prevalence of tuberculosis in Canadian cattle herds declined dramatically after the initiation of the Tuberculosis Eradication Plan in 1923. The plan consisted of testing all Canadian cattle with the caudal fold test and slaughtering the reactors. The national prevalence fell to 0.11% by 1961 (4). In 1978 the test and slaughter system was replaced by slaughter surveillance with associated epidemiological investigation as the main screening test and procedure for tuberculosis control. A tuberculosis eradication program for ungulates was initiated in 1989. There are several examples of countries that have eliminated tuberculosis using a test and slaughter and slaughter surveillance system. A test and slaughter program in Sweden, which lasted over a period of 40 years, was successful in eradicating *M.bovis* from the cattle population (5). Sweden was declared free of tuberculosis in 1958 and there have been no reported cases since 1978. Canada's and Sweden's programs illustrate the success which can be achieved if the

principles of these control programs are followed and if there is no wildlife reservoir of the disease. A wildlife reservoir which interacts with domestic cattle or deer compromises a test and slaughter program. For example, New Zealand instituted a similar tuberculosis control program but the presence of tuberculosis in the brush tailed possum has hampered the success of the program (6). At the end of a control/eradication program it is imperative to identify all possibly infected herds and to definitively determine their status. Thus it is important to identify risk factors for herds which are in the positive and the reactor classifications.

The objective of this chapter was to present the analysis of factors affecting the between herd spread of tuberculosis in Canadian cattle and cervid herds that were investigated as part of specific outbreaks. Brief descriptions of the data collection procedures used, the data collected and the statistical analysis are given.

5.2 Materials and methods

Data for the study were collected from outbreak files and records which were located in Regional and District offices of Agriculture and Agri-food Canada. The data were extracted from the files, entered on data entry forms and then transferred to a database program (Microsoft Access). A complete description of the original data in the study and of the data collection procedures can be found in Chapter 3.

5.2.1 Data

The variables used in these analyses are presented in Table XIV. These variables are HERD CLASSIFICATION, OUTBREAK, BREED, HERDSIZE, and INVESTIGATION CODE. A description, classification and breakdown of each variable in terms of the dependent

variable is given along with the significance (p-value) of the unconditional association between the independent variable and the dependent variable (HERD CLASSIFICATION).

HERD CLASSIFICATION was the dependent variable used in the logistic regression. It was the herd status at the end of the outbreak investigation. In the original study, herds were classified as positive, reactor, negative or other. These classifications were based on the results of herd testing. A positive herd had animals that were culture positive for *Mycobacterium bovis*; a reactor herd was one in which 1 or more animals were positive to a comparative cervical, mid-cervical, postmortem, or histologic examination but in which no animals were culture positive. A negative herd was one where all animals were negative to the tuberculin skin tests, or other tests with the exception of the caudal fold test. If a herd had caudal fold reactors but no other positive tests it was still considered negative. A herd classified as other usually was not tested or did not have any animals to test. Examples of herds in this category were farms which no longer had animals at the time of the investigation or, large feedlots where animals went directly to slaughter. Tests on animals in these large feedlots were often not done, other than slaughter surveillance, and thus the herd status at one point in time could not be determined. For these analyses, the positive and reactor herds were combined into a simple positive/reactor classification. The rationale for this is that reactor status, as defined in this study, impies a greater risk of having been exposed to *M.bovis*. Thus there is a risk of latent infection in herds which are classified as reactor herds.

OUTBREAK was a categorical variable which represented the location of the outbreak. Generally each outbreak was limited to one province with the Alberta/Saskatchewan outbreak being the one exception. The New Brunswick bison outbreak and British Columbia cervid

outbreaks were excluded as the index herd was the only positive/reactor herd in each.

BREED represented the predominant species or breed in each herd that was involved in the outbreak. The database was designed to accommodate test results for up to 4 different breeds/species per herd. For the between herd analyses of these farms it was necessary to chose 1 breed only. The breed chosen could be different from the PRINCIPLE FARM TYPE (see Chapter 3). In herds with more than 1 breed or species, BREED was determined in the following manner: (1) In positive/reactor herds, this was the species or breed which had the largest number of animals responsible for the positive/reactor status of the herd; (2) In negative herds, the species or breed which had the largest number of animals tested was chosen to represent BREED.

HERDSIZE represented the total animals on the farm. It included all breeds and species except poultry. For example a herd with 70 dairy, 30 beef, and 10 deer would have a HERDSIZE of 110. This variable was categorized into 4 levels, namely farms with 0-15, 16-35, 36-80, and greater than 80 animals, respectively.

INVESTIGATION CODE represented 5 reasons why the herd was investigated as part of the outbreak. The INVESTIGATION CODE was assigned from the perspective of the investigating officer. The 17 positive farms discovered as a result of slaughter or post mortem were eliminated from the analyses as they represented a perfect correlation (by definition in this data set) with a positive/reactor farm designation (the dependent variable). If every farm which had been investigated as a result of slaughter surveillance or post mortem had been included in the data than it would not have been necessary to remove the 17 positive farms. However, this information was not available in the records. The first category (perimeter), represented herds

which were investigated because they were within a specific radius of a positive or reactor farm or were part of routine area testing. The second category (tracebacks) contained herds which were the herds of origin of animals in reactor or positive herds. The third category (traceouts) contained herds which received animals from herds which had positive or reactor animals. The fourth category contained herds with animals that had been in contact with positive or reactor animals while on pasture or due to fence line contact. The fifth category contained herds which were investigated for other reasons than the above, for example, the owner's request.

Table XIV
The names , descriptions, and categories of variables used in the evaluation of risk factors affecting between herd transmission of tuberculosis in Canadian cattle and cervid tuberculosis outbreaks between 1985 and 1994

VARIABLE	DESCRIPTION	CLASS.	NEGATIVE HERDS NUMBER AND % (b)	POSITIVE HERDS NUMBER AND %(b)
FINAL HERD CLASSIFICATION	Herd Status (a)	0 = Neg 1 = Pos/Reac	876	119
OUTBREAK	Outbreak Number	PEI Bovine	162 (99.4%)	1 (0.6%)
		Que. Bovine	194 (90.2%)	21 (9.8%)
		Que. Cervid	28 (84.5%)	5 (15.5%)
		Ont. Cervid	62 (88.6%)	8 (11.4%)
		Man. Bovine	154 (87.5%)	22 (12.5%)
		Alb/Sask Cer	109 (66.1%)	56 (33.9%)
		Alb. Bovine	89 (93.7%)	6 (6.3%)
			p < 0.001	(c)
BREED	Breed/Species that is the predominant animal involved in the outbreak	Dairy	151 (95.6%)	7 (4.4%)
		Beef	477 (93.3%)	34 (6.7%)
		Cervid	110 (66.7%)	55 (33.3%)
		Bison	3 (42.9%)	4 (57.1%)
		Other	18 (69.2%)	8 (30.8%)
			p < 0.001	
HERDSIZE	Total animals on the farm	1=0-15	167 (95.4%)	8 (4.6%)
		2=16-35	142 (87.7%)	20 (12.3%)
		3=36-80	216 (84%)	41 (16%)
		4= >80	209 (83.3%)	42 (16.7%)
			p = 0.001	
INVESTIGATION CODE	Reason for the initiation of the investigation	Perimeter	430 (99.5%)	2 (0.5%)
		Traceback	63 (88.7%)	8 (11.3%)
		Traceout	201 (72%)	78 (28%)
		Pasture/ Fence line	111 (86.7%)	17 (13.3%)
		Other	11 (91.7%)	1 (8.3%)
			p < 0.001	

a. See text for a complete description of the variables.
b. The index herds have been eliminated from the data for these analyses.
c. The p-values presented represent the unconditional association between the independent variable and the dependent variable (FINAL HERD CLASSIFICATION)

5.2.2 Statistical analysis

All statistical analyses were carried out using a statistical analysis computer program (Stata). Each of the categorical risk factors was tested using a chi-square test to determine if there was a statistically significant unconditional difference between the negative and the positive/reactor herds. The results of this analysis are given in Table XIV.

All factors presented in Table XIV were evaluated using multiple logistic regression with FINAL HERD CLASSIFICATION as the dependent variable. OUTBREAK was considered to be a confounder and was thus forced into each of the models. Three models were analysed: (1) a model based on both the cattle and cervid data, called the full model; (2) a model which used only cattle outbreak data. This included the Prince Edward Island bovine, Quebec bovine, Manitoba bovine, and Alberta bovine outbreaks; and, (3) a cervid model which used the Quebec cervid, Ontario cervid and the Alberta/Saskatchewan cervid outbreaks. The full model was developed first. Two-way interaction terms among all risk factors were investigated but severe multicollinearity problems made the coefficients of these terms meaningless. Thus the interaction terms were not incorporated into any of the models.

Likelihood ratio tests were performed on all the variables in the models to determine their significance level. The fit of the model was evaluated with the Hosmer-Lemeshow chi-square goodness of fit test with the data partitioned into 10 groups. The diagnostic statistic, delta beta was generated and used to evaluate the impact of specific covariate patterns on the model coefficients. Delta beta measures the impact, on the model coefficients, of removing a covariate pattern. All covariate patterns which had a delta beta of one or greater were removed from the model and the resulting models were assessed.

5.3 Results

There were 876 negative (88%) and 119 (12%) positive/reactor herds used in the univariable analysis. All the risk factors were unconditionally associated with the dependent variable FINAL HERD CLASSIFICATION ($p < .05$). In every outbreak except the Alberta/Saskatchewan cervid, negative herds represented 85% or more of the herds investigated in the outbreak. In the Alberta/Saskatchewan outbreak 66.1% of the herds investigated were negative. The percent positive herds per outbreak ranged from a low of 0.6% in the PEI outbreak to a high of 33.9% in the Alberta/ Saskatchewan outbreak. Within BREED, dairy and beef had the lowest percent positive (<10%); and, cervid, bison and other had the highest percent positive (>30%). Within the four categories of the HERDSIZE variable, the percent positive herds increased as the herd size increased, from 4.6% in herds having 0-15 animals to 16.7% for herds with more than 80 animals. The percent of herds investigated that were positive within the HERD CLASSIFICATION variable was highest for the traceout category (28%). The lowest percent positive within this variable was for herds investigated because they were within a perimeter zone of a positive/reactor herd (0.5%).

All risk factors were entered in the full model as their p-values in the univariable analysis were < 0.05. The results of the logistic regression for the full model are in Table XV. The chi-square for the model was 178.20 (16 df) with a p-value of 0.00. OUTBREAK was included a priori as a confounding variable. BREED was also assessed as a possible confounder. Omitting BREED moderately to markedly changed the odds ratios of both OUTBREAK and INVESTIGATION CODE but not HERDSIZE. Due to its effect on the coefficient for the INVESTIGATION CODE it was considered a confounder and was left in the model in spite of

the fact that the p-value was well beyond the cutoff value of 0.05. INVESTIGATION CODE was also assessed as a confounder and it was found to cause mild to moderate changes in the OUTBREAK and BREED. Its omission did not change the odds ratio for HERDSIZE however. Thus in the full model INVESTIGATION CODE may be a confounder as well as a risk factor. HERDSIZE, when omitted from the model did not cause a change in the odds ratios of the remaining variables. Thus HERDSIZE would appear to be a true risk factor but not a confounder in the full model.

For all outbreaks except the cervid outbreak in Quebec the risk of a herd being positive/reactor was greater than the risk in the PEI outbreak. The influence of BREED on being a positive/reactor herd was not significant in this model. The INVESTIGATION CODE for a farm in a tuberculosis outbreak was a significant risk factor for being a positive/reactor farm, in the full model. Compared to herds tested because they were a perimeter farm, the risk was greatest for traceout farms with an odds ratio of 57.8 , followed in descending order by farms investigated for other reasons (example: at the owner's request), pasture/fence line contact, and traceback contact. The odds ratios for these investigation codes were 46.1, 31.8 and 14.9 respectively. Thus a farm that was a traceout farm was 57.8 times more likely to be a reactor/positive farm compared to a farm tested as a perimeter farm. INVESTIGATION CODE was assigned from the viewpoint of the investigating officer. A traceout farm could have been investigated as follow-up to its reference farm that was discovered due to traceout, traceback, or pasture/fenceline contact. For example: Farm A is a positive farm found at slaughter surveillance. Farm B is tested as a traceback from Farm A and it (B) is positive. Farm C received animals from Farm B. Farm C is called a traceout farm. However, it was tested as a

traceout farm to a farm that was tested as a traceback farm. In these data there were 20 farms that were reference farms to the 78 positive/reactor farms discovered due to traceout investigations. Of these 20 reference farms: 13 were initially discovered as a result of traceout from a positive/reactor farm; 5 were initially discovered due to traceback investigations; and, two were initially discovered as a result of fenceline or pasture contact with a positive/reactor farm.

The size of the farm was also a significant risk factor in the full model. The baseline herd size was 0 - 15 animals. The risk of a herd being a positive/reactor herd generally increased as the herd size increased. However, the confidence interval for farms containing 16 - 35 animals contained an odds ratio of 1. Thus it cannot be firmly concluded that the risk in this category was significantly higher than for the base HERD SIZE of 0 - 15 animals. However, the risk for the next two categories, 36-80 and over 80 animals was 5.8 and 9.3 times greater respectively, compared to the risk in a farm containing 0-15 animals.

Table XV
Results of the logistic regression analysis of herd classification for tuberculosis in Canadian cattle and cervid tuberculosis outbreaks between 1985-1994

Variable	Odds Ratio	SE(OR)	p-value	95% CI(OR)
Outbreak				
PEI Bovine	1	-	0.010	-
Quebec Bovine	6.45	7.13		0.74 - 56.37
Ontario Cervid	5.15	8.40		0.21 - 125.95
Manitoba Bovine	2.61	2.99		0.28 - 24.58
Alberta Cervid	2.20	3.84		0.07 - 67.16
Alberta Bovine	9.05	10.90		0.85 - 95.93
Quebec Cervid	0.26	0.51		0.006 - 11.81
Breed				
Beef	1	-	0.605	-
Dairy	0.79	0.47		0.24 - 2.54
Cervid	5.00	6.83		0.34 - 72.67
Other	4.42	6.60		0.24 - 82.26
Investigation Code				
Perimeter	1	-	0.000	-
Traceback	14.94	13.42		2.57 - 86.87
Traceout	57.84	48.76		11.08 - 301.87
Pasture/Fence	31.80	27.71		5.76 - 175.47
Other	46.12	66.16		2.77 - 767.15
Herdsize				
0 - 15	1	-	0.000	-
16 - 35	2.94	1.68		0.96 - 9.03
36 - 80	5.76	3.20		1.94 - 17.09
> 80	9.32	5.16		3.15 - 27.58

All variables were entered in the second model for the primarily cattle outbreaks. The results of the logistic regression model for the primarily cattle outbreaks are in Table XVI. OUTBREAK and BREED were considered to be confounding variables as in the full model and were forced into the logistic regression. In the cattle model, HERDSIZE was not significant ($p>0.05$) and was eliminated. The final cattle model had a chi-square of 63.08 (7 df) and a p-value of 0.000. The baseline location for OUTBREAK was PEI. The greatest risk of being a positive/reactor farm, in comparison to the PEI outbreak, was Alberta (OR=11.14), followed by Quebec (OR=6.09) and Manitoba (OR=3.40) respectively. BREED was left in the model as a confounder but was above the cutoff significance level. INVESTIGATION CODE was a significant risk factor. The risk of being a positive/reactor farm was greatest for traceout herds when compared to perimeter herds. The risk was 48.8 time higher for the former over the latter. A pasture or fence line contact was 29.6 times more likely to be a positive/reactor herd in comparison to a herd investigated as a perimeter herd to a positive/reactor herd. The odds ratio for a traceback herd was 13.4.

Table XVI
Results of the logistic regression analysis of herd classification for tuberculosis in Canadian cattle tuberculosis outbreaks between 1985-1994

Variable	Odds Ratio	SE(OR)	p-value	95% CI(OR)
Outbreak				
PEI Bovine	1	-	0.056	
Quebec Bovine	6.09	6.72		0.70 - 52.91
Manitoba Bovine	3.40	3.86		0.37 - 31.39
Alberta Bovine	11.14	13.21		1.09 - 113.90
Breed				
Beef	1	-	0.885	-
Dairy	0.92	0.53		0.30 - 2.84
Investigation Code				
Perimeter	1	-	0.000	
Traceback	13.40	12.25		2.24 - 80.33
Traceout	48.76	40.18		9.70 - 245.24
Pasture/Fence	29.59	25.40		5.50 - 159.14

All risk factors were entered into the third or cervid model and submitted to logistic regression. Results of the regression are in Table XVII. OUTBREAK AND BREED were left in the model as confounders. INVESTIGATION CODE was above the cutoff significance level and was dropped. In the three categories of HERDSIZE the odds ratio increased in comparison to the baseline herd size of 0-15 animals. The odds ratios for the three categories of this variable against the baseline were 2.88, 8.93, and 11.53 respectively. Thus the greatest risk of being a positive/reactor farm was in the >80 herdsize category which was 11.5 times more likely to be a reactor/positive herd than a herd of 0-15 animals.

Table XVII
Results of the logistic regression analysis of herd classification for tuberculosis in Canadian cervids tuberculosis outbreaks between 1985-1994

Variable	Odds Ratio	SE(OR)	p-value	95% CI(OR)
Outbreak				
Quebec Cervid	1	-	0.038	
Ontario Cervid	13.4	16.78		1.15 -155.35
Alberta Cervid	7.68	8.50		0.88 - 67.11
Breed				
Other	1	.	0.004	.
Beef	0.05	0.08		0.004 - 0.82
Cervid	1.22	0.84		0.30 - 4.67
Herdsize				
0 - 15	1	-	0.0001	
16 - 35	2.88	2.08		0.70 - 11.87
36 - 80	8.93	6.39		2.20 - 36.30
> 80	11.53	8.31		2.80 - 47.36

The results of the Hosmer-Lemeshow goodness of fit test for all three models are in Table XVIII. The p-value for the full model was 0.995. Thus we cannot reject the hypothesis that the data fit the model. Similar Hosmer-Lemeshow test results were obtained for the cattle and cervid models (Table XVIII). The validity of each model was further assessed by computing the delta betas for each covariate pattern. These statistics indicate the impact of a particular covariate pattern on the odds ratios or coefficients in the model. All delta betas greater than 1 were eliminated from the models and the regressions were run without them. In each case, removal of observations with high delta betas resulted in substantial changes in the odds ratios for OUTBREAK and BREED. There were only small changes in the odds ratios for INVESTIGATION CODE and HERDSIZE. However there was no plausible reason for eliminating these observations from the data and consequently the results based on the full data sets for each of the models have been presented.

Table XVIII
The Hosmer-Lemeshow Goodness of Fit Test and percent of correctly classified herds in the three logistic models of between herd spread of tuberculosis in Canadian cattle and cervids from 1985- 1994

Model	No. Of Observations	Hosmer-Lemeshow chi-square	Degrees of Freedom	p-value	% Correctly classified at cutoff p=0.5
Full	679	1.33	8	0.995	88.2%
Cattle	505	1.87	6	0.931	92.7%
Cervid	167	2.99	5	0.701	74.7%

5.4 Discussion

The three models had significant chi-square statistics indicating some predictive ability. They all had non-significant Hosmer-Lemeshow goodness-of- fit test statistics indicating that the model fit the data reasonably well. The best model in these respects was the full model, followed by the cattle and cervid models, respectively. Predictive ability was not the primary purpose of these models, however, the per cent of herds correctly classified was quite high for all three models. The ranking of the models is supported by the model diagnostics. The full, cattle, and cervid models had 4.3%, 14.2%, and 65.3% respectively of their observations with a delta beta greater than 1. This means that these observations had a substantial impact on the standard errors and thus the stability of the coefficients and the odds ratios of the models. The tests of the models indicate however, that the conclusions concerning risk factors for tuberculosis spread between herds were supportable by these data.

The objective of the study was to identify risk factors for being a tuberculosis reactor or positive herd. This relies on the assumption that most if not all of the *M.bovis* reactor and positive herds were found. It was assumed that the investigations in these outbreaks identified all these herds. This assumption is supported by the fact that there have been no new positive herds in these areas associated with any of the previously identified herds. It is possible that there were reactor or positive herds where infection did not persist. Therefore the risk factors which were identified represent risk factors associated with being a positive/reactor herd and not simply risk factors for being found to be a positive/reactor herd.

The coefficients for breed/species and outbreak were very unstable when covariate patterns with high delta betas were removed. This instability was due to multicollinearity

between the two variables. Most outbreaks dealt with primarily one breed/species and it was impossible to separate the effects of the two variables as in fact they are both essentially measuring the same thing.

The apparent risk, measured by the odds ratio, due to the outbreak location was the relative proportion of herds that were positive/reactor in the outbreak investigation. The odds ratio for any location could be decreased simply by increasing the number of negative herds that were investigated. These numbers did not measure a risk of being positive that was inherent in the location. However, there were some subtle and indirect components of this risk factor which may have contributed to the risk of being discovered as a positive/reactor herd. The location was a proxy for the predominant breed/species in the outbreak that had occurred. All the management factors and species risk factors which were governed by the type of breed/species (cervids vs bovine) involved in an outbreak were contained in the location variable. For example cervid herds were more likely to keep older animals than traditional dairy or beef herds because the industry was newer and animals were more valuable and more difficult to replace. If age was a risk factor (as is discussed in Chapter 6), then the risk of being a positive/reactor herd in the Ontario cervid outbreak was greater, because the average age was greater, than for example a beef feeder operations in Manitoba where animals were shipped to slaughter at a younger average age. The location risk factor encompassed a whole set of intangibles such as how long the source herd was infected before it was discovered. For example, the source herd was infected for a long time in the Quebec bovine outbreak and a very long time as in the Alberta bovine outbreak. The longer a source herd was infected the more opportunities there were for the infection to be transmitted to other herds. Thus the more positive/reactor herds there were likely to be. There

were also differences in investigative techniques and investigators in different locations. These differences may have had an impact on the risk of a herd being discovered as a positive/ reactor herd and included such things as how a tuberculin test is administered and interpreted.

The risk due to breed/species was statistically significant only in the cervid model. When compared to the category called other, beef cattle were significantly less likely to be positive/reactor herds, and cervids were 1.22 times more likely to be positive. The other category included swine, sheep, goats, and zoo animals and any miscellaneous breeds or species not covered by the specific classifications. The dairy category was dropped from this model by the statistical software as the few observations that were in the data set predicted failure perfectly. Thus there were not enough dairy and beef herds in the cervid models to determine if there was a difference in risk between these two. Breed was not significant in the bovine models suggesting that there was no difference in risk due to breed in the dairy and beef herds.

Tuberculosis is a disease which is spread primarily through aerosol transmission of droplet nuclei. Ingestion is a possible route of infection which is more important in deer than in cattle but the infective dose has been shown to be considerably larger than for the aerosol route. Environmental spread via contaminated pastures is considered insignificant (2). This study showed that herds tested in outbreak situations were at greater risk of being reactor/positive herds if they had purchased animals from reactor/positive farms, had requested testing because the owner knew the animals were possibly in contact with reactor/positive animals, or were herds which had provided animals to a reactor/positive farm. This increased risk was measured against the risk of being a reactor/positive farm if the herd was in a certain perimeter of a positive/reactor farm. It is also interesting to note that a farm that was tested because it received animals from a

positive/reactor farm or co-pastured or had fence line contact with one, had a greater risk of containing positive/reactor animals than a farm that was tested because a reactor/positive animal may have originated there. This makes intuitive sense as traceback investigations look at every farm that provided animals to a positive/reactor farm. Considering the low prevalence of tuberculosis it is unlikely that more than one farm was the source of positive/reactor animals. However the apparent risk associated with traceback investigations is biased downward because it does not take into consideration that the investigation of the reference farm, which initiated the next generation of investigation, may have been initiated because of traceback. In reality, traceback investigations will continue to be an important component of outbreak investigations as will any situation where there is the possibility of animal to animal contact. All these increased risk situations imply increased contact with potentially infected animals. In the United States in 1990, similar results were found (7). Six tuberculous animals/herds were found as a result of traceback of regular kill slaughter animals - ie suspicious animals at slaughter. Six exposed and six infected herds were identified as a result of tracing animals previously associated (ie traceouts) with these six index herds. Routine testing in 1990 did not reveal any infected/exposed herds. "High risk testing" (undefined but possible perimeter testing) revealed one tuberculous herd. The greatest proportion of herds tested in an outbreak are perimeter herds. Thus, the largest proportion of resources is being used on the lowest risk group. There were 123 *M. bovis* infected herds found in the United States during the period 1982-1993 (8). Five (4%) of these were detected through skin testing. The remainder, 118 (96%) were detected at slaughter and through follow-up epidemiological procedures. Furthermore, for States with at least one reactor (positive to caudal fold and follow-up comparative cervical), in fiscal year 1990, no

statistically significant relationship was found between the number of animals tested and the number of reactors detected (8).

A case control study in Ireland showed that herds which purchased animals in a six month period after being derestricted following an outbreak, were twice as likely to fail the six month check test than herds which did not purchase animals (the status of the source herd was not stated) (9). In another study in the Republic of Ireland, purchase was not found to be a risk factor for becoming a positive herd (10). There are numerous reports in the literature concerning transmission of tuberculosis to previously free herds and countries via purchase of infected animals (5,11).

Herd size was a significant risk factor in the study although the factor was only significant in the cervid model. In a study in the Republic of Ireland increasing herd size was also found to be a risk factor for being a positive tuberculosis herd (10). Larger herds may be associated with management practices that increase the risk of transmission of the organism. There is greater potential for movement of animals from a larger number of different sources, into larger herds than into smaller herds. The greater movement would be necessitated by need for replacement animals and breeding stock. The density of animals may be higher in larger herds and thus the probability of transmission may be greater because of more opportunities for interactions between infected and susceptible animals. Given the same prevalence of disease and the same test with the same sensitivity and specificity, a larger herd is more likely to be identified as a positive/reactor herd than a smaller herd because it is more likely to identify one reactor/positive animal. The reason is that the disease is likely to be in different stages in different animals. The more animals (in absolute numbers) that are tested the more likely it is to

find one that is at a stage of the disease that is detectable by the test.

5.5 Conclusion

Three models for the between herd spread of tuberculosis were studied. The model which incorporated all the data from the cattle and cervid outbreaks was the best and most stable model.

There were no statistically significant inherent differences in breed susceptibility shown, except for a slight increase in risk for cervids and a decrease in risk for beef, versus other species, in the cervid model. Location of the outbreak incorporated risk due to the breed/species but it is impossible to assess risk due to breed/species separately in the location variable.

Herds which were investigated because they had received animals from a reactor/positive farm were at a significantly greater risk of being a reactor/positive herd than those investigated because they were tested as perimeter herd to a reactor/positive herd. There was increased risk of being a positive/reactor herd if investigated for other reasons (owner's request for example), fence line or pasture contact and traceback contact.

Increasing herd size was associated with increased risk of being found as a positive/reactor herd in an outbreak investigation situation particularly for cervid herds.

Chapter 5 References

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CHAPTER 6

WITHIN HERD SPREAD OF *MYCOBACTERIUM BOVIS*

6.1 Introduction

Mycobacterium bovis is the causative agent of tuberculosis in cattle and cervid and is capable of infecting a broad range of animals including humans. Cattle and cervids may become maintenance hosts while most other animals, although they become infected, act only as spillover hosts (ie. become infected but do not normally transmit the disease) (1). An understanding of the role of animal-to-animal transmission of *Mycobacterium bovis* is important for an understanding of the epidemiology of this disease. There are many facets of animal-to-animal transmission that have been investigated. One important element is the particle size requirement for aerosol transmission (1,2). Aerosol or aerogenous transmission of *M. bovis* is considered to be the most important route of infection (3). The alimentary route is considered important in cervids only (1). Factors other than frequency of shedding and route of transmission which are significant in animal-to-animal transmission include the infective dose, exposure time, level of animal to animal interaction, and host susceptibility (4). Behaviour may also play a role in transmission (5). For example, in New Zealand it was found that cattle and red deer which were on the higher end of the dominance hierarchy within herds were more likely than animals on the bottom end to investigate tuberculosis infected possums which were exhibiting unusual behaviour. It is possible that this behavioural dominance could be expressed in other situations in a herd leading to more frequent interactions between dominant animals and thus an increased probability of transmission between more dominant animals.

The objective of this chapter was to present the analysis of risk factors affecting the within herd spread of tuberculosis in Canadian cattle and cervids and to estimate the incidence rate of new infections. A description of the data collection procedures, the data, and the statistical analyses are given. Individual animal risk factors for between animal spread of tuberculosis were examined using a negative binomial regression analysis. The significance of these risk factors is discussed in light of some of the literature concerning animal to animal spread of tuberculosis in both experimental and natural situations.

6.2 Materials and methods

Data for the study were collected from outbreak files and records which were located in Agriculture and And Agri-food Canada's Regional and District offices. The data were extracted from the files, entered on data entry forms and then transferred to a database program (Microsoft Access). A complete description of the original data in the study and of the data collection procedures can be found in Chapter 3. Data used for these analyses were for individual animals and were collected only on animals that were in reactor/positive herds. A herd was classified as reactor/positive if one or more animals in the herd were positive or suspicious on a comparative cervical or mid-cervical tuberculin skin test, gross pathological or histopathological test for tuberculosis, or culture for *M. bovis*.

6.2.1 Data

The variables used in the analysis are described in Table XIX. Observations for the Prince Edward Island bovine, New Brunswick bison, and British Columbia cervid outbreaks were dropped as there were no data for reactor/positive animals other than the index farms. Data from

all purchased animals were dropped from the analysis to assure that if an animal was in the positive/reactor category it was due to exposure from an animal in its own herd and not from exposure in the herd of origin.

REACTOR was a dichotomous variable representing the status of the individual animal at the end of the investigation and was either negative or reactor/positive. Negative status meant that the animal was negative on every test it was submitted to, other than the caudal fold test. Animals that were caudal fold test suspicious or positive were submitted to other tests. If an animal was suspect or positive on any test other than the caudal fold test, it was considered as a reactor/positive animal. Therefore reactor/positive status meant that an animal was suspect or positive on a comparative cervical, mid-cervical, gross pathology, histopathology, or culture.

AGE was a categorical variable with animals assigned to one of three groups. These groups were animals 0-12 months old, 12-24 months old, or 24 or more months of age.

SEX was a dichotomous variable with females in one category and males and neutered males in the other.

BREED represented the species/breed of the individual animal. It was coded as dairy, beef, cervid, bison, or other. Other included swine, sheep, goats, and zoo animals and any miscellaneous breeds or species not covered by the specific classifications.

OUTBREAK was a categorical variable which represented the location of the outbreak. Generally each outbreak was limited to one province with the Alberta/Saskatchewan outbreak being the only exception.

Since the probability of transmission of *M.bovis* within a herd depends on the duration of the exposure to the organism, an exposure time was calculated for use in the negative binomial

regression analysis. To understand the exposure time it is necessary to define two terms used in its calculation - the earliest exposure date for the "study" herd and the departure date for each animal. The earliest exposure date was the earliest possible date that the study herd may have been exposed to a potentially tuberculous animal from some "reference farm". The reference farm was the farm which prompted the investigation of the study farm. In the case of sales of animals, this was the date that the potentially infectious animal entered the study farm from the reference farm. In the case of fence line contact or perimeter contact, the earliest exposure date for the study farm was the date that the reference farm was first exposed to potentially infectious animals. If this date was unknown, then the earliest exposure date for the study farm was the date when the reference farm was first known to be a reactor or a positive farm. Study farms which were identified as reactor/positive farms as a result of traceback investigations would have an earliest exposure date only if the likely source of infection (i.e. the reference farm) was known. The earliest exposure date for study farms that co-pastured with a potentially infectious animal was the date that the co-pasturing began after it was known that the reference farm was a reactor/positive farm. There was no earliest exposure date for index farms where the source of infection could not be determined. Farms investigated for other reasons were given an earliest exposure date only if the farm had received animals from or had been in contact with animals that were from a reactor/positive farm. Otherwise these farms were not given an earliest exposure date. The departure date was the last date that the status of an animal was known, i.e. the date of the last test on an individual animal.

The exposure time for most animals in the study was the elapsed time in days from this earliest exposure date for the herd to the departure date for the animal. The exposure time for

animals that were born after the earliest exposure date for their herd was the time from their birth until their departure date.

Table XIX
Description of variables in the univariable and negative binomial regression analyses of animal to animal transmission of *Mycobacterium bovis* in Canadian cattle and cervids from tuberculosis outbreaks between 1985-1994

Variable(a)	Description	Frequency Distribution	Negative Animals (%)	Positive/ Reactor Animals (%)
REACTOR	Animal status	0 = neg	1534 (88.7%)	
		1 = pos/react		195 (11.3%)
AGE	Age category in months	0 = 0-12	365 (99.5%)	2 (0.5%)
		1 = 12-24	301 (86.2%)	48 (13.8%)
		2 > 24	868 (85.7%)	145 (14.3%)
			p < 0.000	
SEX	Sex of animal	0 = female	1188 (88.2%)	159 (11.8%)
		1 = male or neutered	346 (90.6%)	36 (9.4%)
			p = 0.19	
BREED	Breed or species	dairy	284 (92%)	25 (8%)
		beef	666 (91.1%)	65 (8.9%)
		cervid	556 (84.5%)	102 (15.5%)
		other	28 (90.3%)	3 (9.7%)
			p < 0.000	
OUTBREAK	Location of outbreak	Quebec cattle	261 (91.9%)	23 (8.1%)
		Ontario cervid	673 (90.8%)	68 (9.2%)
		Manitoba cattle	47 (94%)	3 (6%)
		Alberta/Sask cer	194 (79.8%)	49 (20.2%)
		Alberta cattle	320 (86.5%)	50 (13.5%)
		Quebec cervid	39 (95.1%)	2 (4.9%)
			p <0.000	

6.2.2 Statistical analysis

Analyses were performed using a statistical software package (Stata). The variables used in this analysis are described in Table XIX. A chi square test was used to test the unconditional association of the independent variables age, sex, breed, and outbreak, with the dependent variable. A p-value of less than or equal to 0.2 was designated as the cut off to incorporate the variable into the final negative binomial regression models.

The data were organized into covariate patterns based on outbreak, age, sex, breed, and farm. For each covariate pattern , the number of REACTOR animals and the number of animal-days at risk were determined. The number of REACTOR animals was the dependent variable and the number of animal-days at risk was included in all regression models as an offset. The mean and standard deviation of the dependent variable were computed. The data were first incorporated into a Poisson regression model. The goodness of fit test for the Poisson regression ($chi^2_{123} = 319.26$; $p = 0.000$) showed that the data did not fit a Poisson distribution. Data fell into 134 distinct co-variate patterns with the mean and standard deviation of the number of reactors (REACTOR) being 1.46 and 4.01 respectively. Thus the assumption in a Poisson regression that the variance and mean of the dependent variable are equal was not supported. A negative binomial regression model was fit to the data using all variables from Table XIX. OUTBREAK was included a priori as a confounder. BREED was also evaluated as a confounder. Likelihood ratio tests were performed to determine the significance of the variables which were retained in the model. Outliers were assessed to determine their impact on the coefficients. A likelihood ration test was used to determine if the data used in the negative binomial regression followed a Poisson distribution.

Actual and predicted incidence rates were calculated based on outbreak location, breed or species involved, and age (greater than 24 months of age). These incidence rates indicate the number of new cases of tuberculosis per 100 animal years or in other words, the number of new cases of tuberculosis in a 100 animal herd in 1 year.

6.3 Results

This study included only animals from reactor/positive herds. A positive herd had animals that were culture positive for *Mycobacterium bovis*; a reactor herd was one in which 1 or more animals were suspicious or positive to a comparative cervical, mid-cervical, postmortem, or histologic examination but no animals were culture positive. Data used in these analyses were derived from 1534 negative animals from 30 herds and 195 reactor/positive animals from 23 of the 30 herds. (Reactor/positive herds may not have had any reactor/positive animals to these analyses since index animals and those brought in by sale were removed.) Thus the average herd size was 58 animals and the average number of reactor/positive animals per herd was 6.5. Prevalence of reactors varied in the herds and could be categorized in the following manner: 7 herds (0 prevalence), 14 herds (>0 - 10%), 2 herds (>10% - 20%), 2 herds (>20% - 30%), 5 herds (>30% - 50%). Table XIX gives a brief description of the variables used in the univariable analysis, a frequency distribution in terms of the outcome variable (REACTOR) and the p-value of the test for unconditional association of each independent variable with the dependent variable.

The percent positive animals increased as the age category increased with very few positive animals less than 12 months of age. The percent positive animals in the 12-24 month old and the greater than 24 month categories were very close (13.8% and 14.3% respectively). The p-

value for sex of the animal was just below the cutoff value required to be considered in the regression analyses. There were 11.8% and 9.4% positive females and males respectively. Dairy, beef and "other animals" had a similar percent positive at 8%, 8.9%, and 9.7% respectively. The cervids had a higher percent positive (15.5%). The Alberta/Saskatchewan cervid outbreak had the highest proportion of positive animals (20.2%) followed by the Alberta bovine outbreak (13.5%). The lowest proportion of test positive animals was in the Quebec cervid outbreak (4.9%).

All variables were entered into the negative binomial regression analysis. The p-value for SEX was greater than 0.05 and it was dropped from the model. OUTBREAK was left in the model as a confounder. When BREED was dropped from the model the coefficient of OUTBREAK changed moderately to markedly. Thus it was also left in the model as a potential confounder. The variables which remained in the full model were OUTBREAK, BREED and AGE. The results of the negative binomial regression analysis are in Table XX. In the age category the greatest risk of being a positive/reactor animal was in the > 24 month old category. The IRR (incidence rate ratio) for this group was 10.42 when compared to the baseline group of 0-12 months of age. The IRR in the 12- 24 month old category (IRR = 7.65) was also greater than the baseline category.

All outbreak locations had an IRR greater than the Quebec cervid outbreak. The largest IRR, after controlling for the differences in age and breed, was the Quebec bovine outbreak (IRR = 8.03) and the smallest was the Manitoba bovine outbreak (IRR = 1.15).

Table XX
Results of the negative binomial regression for the risk of transmission of tuberculosis between animals in Canadian cattle and cervids from 1985-1994, using an overestimated exposure time for all animals

Variable	Coef.	IRR	SE(IRR)	p-value	95% C.I (IRR)
MATURE					
0 - 12 months		1		0.009	
12 - 24 months	2.03	7.65	6.96		1.29 - 45.43
> 24 months	2.34	10.42	9.21		1.84 - 58.94
BREED					
Dairy		1		0.210	
Beef	0.73	2.08	1.34		0.59 - 7.34
Cervid	1.61	4.99	3.76		1.14 - 21.84
Other	1.76	5.80	6.54		0.64 - 52.90
OUTBREAK					
Quebec cervid		1		0.039	
Quebec bovine	2.08	8.03	8.41		1.03 - 62.52
Ontario cervid	0.50	1.65	1.58		0.25 - 10.82
Manitoba bovine	0.14	1.15	1.42		0.10 - 12.96
Alberta/Sask cervid	1.01	2.75	2.90		0.35 - 25.71
Alberta bovine	1.96	7.13	7.66		0.87 - 58.60
Constant	-12.64			0.000	(-15.40)-(-9.88)

The model chi square (10 df) was 22.45 ($p = 0.013$) which indicated that the model did have statistically significant predictive ability. Alpha, the variance inflation factor, was 1.37, indicating that the variance of the dependent variable was significantly greater than its mean. Alpha indicates whether the variance of the data is significantly greater than the mean and thus if the data fit a Poisson distribution or not. The likelihood ratio test to determine if these data followed a Poisson distribution indicated that they did not ($chi^2_1 = 144.43$; $p = 0.000$). Thus the choice of a negative binomial regression was more appropriate than a Poisson regression analysis.

Predicted outcome values and leverage values were calculated for all of the co-variate patterns. The predicted values were graphed against the actual REACTOR values. This graph showed three co-variate patterns that were significant outliers. These co-variate patterns are described in Table XXI. Two of these patterns had a lower predicted number of positive/reactor animals than the actual number and one of the patterns had a higher number of predicted positive/reactor animals than the actual number. The leverage values for all three co-variate patterns were low. When the outliers were removed, the overall model chi square was more highly significant ($p = 0.004$) than the full model ($p= 0.013$). The coefficient for AGE changed very little and this variable was still significant. There were marked changes in the coefficient for the two confounding variables, BREED and OUTBREAK. There were no biological or other reasons to remove these observations and therefore they were left in the model.

Table XXI
Values of variables associated with three co-variate patterns that were outliers in the negative binomial regression analysis for the risk of transmission of tuberculosis between animals in Canadian cattle and cervids from 1985-1994

Pattern Number	Actual Number of Reactors	Outbreak Location	Breed	Age in Months	Sex	Predicted Number of Reactors	Leverage
1	20	Alberta Bovine	beef	over 24	female	1.9	0.09
2	29	Ontario Cervid	cervid	over 24	female	7.0	0.11
3	11	Alberta Bovine	beef	over 24	female	30	0.09

Table XXII gives the actual and predicted incidence rates of new cases of tuberculosis reactor or positive animals in different groups of mature animals (greater than 24 months of age). The groups are characterized by breed and outbreak location. The rates are the number of new cases of reactor/positive animals per 100 animal years. For example, mature dairy cattle in the Quebec bovine outbreak had actual and predicted incidence rates of new cases of reactor or positive animals, of 2.8 and 9.8, respectively, per 100 cow years. There were insufficient cases in some breed/species classifications in all of the Outbreak locations, except Ontario, to calculate incidence rates for all breed/species categories. Breed/species classifications with insufficient cases are indicated.

Table XXII

Actual and predicted incidence rates for tuberculosis in mature (>24 months) Canadian cattle and cervids in tuberculosis outbreaks in Canada between 1985-1994 measured in the number of new cases of reactor/positive animals per 100 animal years

Outbreak Location and Breed	Actual and Predicted Incidence Rates in Different Breeds/Species					
	Mature Dairy		Mature Beef		Mature Cervid	
	Act.	Pred.	Act.	Pred.	Act.	Pred.
Quebec Cervid	N.C*	1.2	4.9	2.5	N.C[a]	6.1
Quebec Bovine	2.8	9.8	10.1	20.4	N.C	49.2
Ontario Cervid	5.0	2.0	3.1	4.2	9.3	10.1
Manitoba Bovine	N.C	1.4	2.0	2.9	N.C	7.1
Alb/Sask Cervid	N.C	3.4	N.C	7.0	18.6	16.9
Alberta Bovine	4.9	8.7	2	18.1	N.C	43.6

* N.C: Number of cases = 0

[a] There were no new reactor/positive animals in the study herd after the purchased animals were removed from the data.

6.4 Discussion

Every possible effort was made during data collection to assure completeness and correctness of the data. However there were several difficulties encountered in certain outbreaks. These problems were often due to the fact that the outbreaks dated back several years and the investigators who worked on the outbreak were not available to clarify questions. In other outbreaks, records were stored in so many different District Offices that it was not feasible to spend the manpower to retrieve the records. Two examples of the kind of problems encountered include the following.

1. In the Alberta/Saskatchewan cervid outbreak it was impossible to calculate an exposure time for many animals. The information to calculate an exposure variable was not in the electronic database, Epi-info, from which the rest of the data were collected. Where an exposure time was calculated, the information was retrieved from overview written material.

2. Missing data. In the Quebec bovine outbreak, it was often impossible to differentiate beef from dairy as the breed/species was listed as bovine only. If it was not possible to definitively determine the breed/species this information was excluded from analyses.

Only records which had complete data for the variables incorporated in the models were used in the analysis. Thus there was a reduction in the number of records which were used in the analyses compared to the total number of records.

It was impossible to know the exact exposure time for each animal and the method of estimation used has probably resulted in there being an upward bias in exposure time. Using the earliest exposure date assumes that the animal was at risk of becoming infected with *M. bovis* on

exactly that date. The departure date represented when the investigators knew the status of positive animals and assumes that all positive/reactor animals became infected immediately before testing. It does not give the exact date that the animal became positive. The overestimation of the exposure time would lead to an underestimation of the incidence rates.

Animals within a herd could not be considered independent. However, with the small number of reactor/positive animals per herd in most cases, the effects of clustering were considered minimal and were not considered in the analysis.

The validity of classifying reactor animals in a group with positive animals can be supported by the view that, unlike humans where pulmonary lesions of tuberculosis may heal, cattle, once they react to a tuberculin test should be considered potentially infected/infectious and in most cases proceed to full disease (1).

In this study, the age of the animal was found to be a risk factor for being a reactor/positive animal. Increasing age increased the risk. This was also found in a study in Northern Ireland where older animals (cows, heifers, and bullocks) were significantly more likely to fail a tuberculin test than calves (6). Young cervids however may be infected with *M.bovis* yet remain negative to a tuberculin test (7). This would contribute to an apparent increase in risk with age for cervids.

The p-value for BREED in the binomial regression analysis was 0.21 and thus this variable was not statistically significant. However the results indicated that all breed categories were at greater risk of being reactor/positive animals than the comparison dairy group. This is contrary to the view that dairy animals are at greater risk than beef animals (1,8).The confidence intervals for the IRRs in the other and beef categories (IRR= 5.80 and 2.08, respectively)

included 1 and thus were not statistically significant. Cervids had an IRR of 4.99 in comparison to the baseline dairy breeds and its confidence interval did not include 1. This supports the view that cervids are more susceptible to tuberculosis than bovines.

The incidence rates (IR) for tuberculosis in mature Canadian cattle and cervids in outbreaks from 1985-1994 are presented in Table XXI. The Ontario cervid outbreak is the only one where there are enough cases to perform a comparison between cervids, dairy, and beef animals. The actual incidence rate for cervids (IR = 9.3) was almost twice that of dairy cattle (IR = 5.0) and three times that of beef cattle (IR = 3.1). This supports the view that cervids are at greater risk and dairy animals are at greater risk than beef animals. In other outbreaks where IRs could be compared between cervids and bovine breeds, the cervid rates were consistently higher. Overall the highest IR was in the Alberta/Saskatchewan cervid outbreak (IR = 18.6). This was almost twice the next highest rate which was for mature beef cattle in the Quebec bovine outbreak. These data indicate that spread of tuberculoid in cervid herds is faster than in bovine herds. The data do not clearly indicate if the speed of spread in dairy herds is greater or less than in beef herds. There were three outbreaks where rates between dairy and beef animals could be compared and of these, 2 dairy had higher incidence rates than the beef. Thus no conclusions can be reached regarding the rate of spread in dairy versus beef herds.

The predicted incidence rates in the model were similar to the actual incidence rates for cervids. The correlation between actual and predicted rates in the dairy and beef breeds was much more variable and the predicted rates were higher in all but two outbreaks (beef cattle in the Quebec cervid outbreak and dairy cattle in the Ontario cervid outbreak). This model was not meant to be a predictive tool for within herd incidence rates of tuberculosis but was designed to

give some estimate of the IR of tuberculosis in infected herds.

There is of course an important distinction between the impact, on incidence rates, due to susceptibility to infection versus that due to the risk of transmission . If cervids are more susceptible, the incidence rate would be greater for them versus bovines under similar conditions. Beef animals are considered to be at less risk than dairy animals primarily because of management factors such as housing density not because of differences in inherent susceptibility to tuberculosis. This study does not assume that the conditions which have an impact on incidence rate were constant on all the farms and that the only variable was breed/species. However there is an indication that the incidence rate for cervids was greater and one of several possible explanations for this higher rate may be that they are inherently more susceptible than bovines.

Many factors in addition to inherent susceptibility and management practices may have influenced the IRR and the incidence rates in these data and included the following.

(1) The more potentially infectious animals that were introduced into a herd or encountered as a fence line or pasture contact the greater the probability of transmission as the number of possible interactions increased.

(2) The larger the herd that was exposed to potentially infectious animals the greater the risk that the herd would be identified as a positive/reactor herd. (See Chapter 5, Discussion of herd size as a risk factor)

(3) Studies of experimental and natural infections have shown that the time from infection to excretion of *M. bovis* is approximately 87 days. However the time from infection to infectiousness and the factors which govern this are not well known. Griffin

and Dolan (4) discuss examples of outbreaks where infected animals were in contact with other animals for long periods of time and transmission did not occur. They also give an example of transmission of tuberculous pneumonia to 46 of 56 animals from 42 attested (negative status) herds in Germany. These animals has all been at an agricultural fair together and when tested 80 days after the fair all reacted to the tuberculin test. The source of the original outbreak (ie the source of infection at the agricultural fair) was never determined.

(4) Management practices may have had a significant impact on the transmission rate of the disease. For example housing or stocking density may have been very important (9). In an epidemiological investigation of an outbreak of tuberculosis in Swedish deer farms, it was concluded that the large number of tuberculous animals originating directly from one importation of deer probably arose due to intense crowding of the deer during their transport to Sweden and subsequent quarantine (10).

The IRRs in the outbreak location were probably not due to factors inherent in the geographical location of the outbreak. In fact the effects measured by the location variable and the breed/species variable are essentially the same as each outbreak consisted of primarily one breed/species. An example of the sort of influence the location variable would have was if there was a wildlife reservoir of disease which caused transmission to occur from the reservoir to animals in herds in the specific location. Farming practices characteristic of certain areas, movement patterns and frequency in different geographical areas and the individuals and techniques involved in the outbreak investigation were all possible contributors to the IRRs of this variable. The confidence intervals for the IRR values for all of the locations, contained 1 and

were thus not statistically significantly different from the IRR for the baseline OUTBREAK (Quebec cervid).

6.5 Conclusions

A model for animal-to-animal transmission of *M. bovis* was studied using information on 1727 individual animals from 30 positive/reactor herds. A negative binomial regression analysis was used to evaluate the effects of age and breed/species on the incidence rate of new infections and to provide estimates of those incidence rates.

As age increased, the IR of tuberculosis infections also increased.

The incidence rate appeared to be greater among in cervids than in bovines. It was not possible to determine if incidence rates were higher in dairy or beef herds. It is possible that the larger incidence rates in the cervid herds is related to greater susceptibility but further research on other risk factors and management factors is necessary to determine if this is the case.

Chapter 6 References

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CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Introduction

Tuberculosis remains an important disease for Canadian cattle and cervid farms despite successful control and eradication programs and the low prevalence and incidence rate. The impact of the disease on animal production is negligible. The greatest economic impact to individual farms occurs when *M. bovis* is cultured and all animals on the farm must be depopulated. Tuberculosis remains an important disease primarily because of international trade considerations.

This study reviewed and analysed all outbreaks of tuberculosis in Canadian cattle and cervids between 1985-1994. Herd and individual animal data were extracted from all available outbreak files which were kept in Agriculture and Agrifood Canada's Regional and District offices. These data were analysed for risk factors for transmission of tuberculosis between herds and within herds.

There were two main objectives for the study. The first was to review the literature on tuberculosis in cattle and cervids. The second was to study the epidemiology of the disease in a Canadian context. The purpose of the project was to integrate the information gained in the study so that recommendations could be made, based on sound scientific principles, to help improve Canada's tuberculosis control and eradication programs. The results of the study could also be used as scientific evidence to support policies associated with national and international trade.

7.2 Project Conclusions and Recommendations

The main findings, conclusions and recommendations of the project can be summarized under several headings, as follows.

7.2.1 Outbreak investigations - initial discovery and epidemiological follow-up

In every outbreak, the strategy for the investigation must be suited to the circumstances and cannot be rigidly prescribed in advance. However, following are general principles developed through this study which may improve Canada's tuberculosis control, eradication and surveillance programs. These recommendations can be categorized and discussed in the following way:

1. initial discovery of tuberculous animals - index cases;

2. epidemiological follow-up; and,

3. testing and surveillance in reactor herds and high risk negative herds

Generally the first positive farm in each outbreak in this study was identified because of slaughter surveillance or post mortem examination of an animal that had been sick. The exception was the British Columbia deer outbreak where the one culture positive farm was identified as a result of herd testing requested by the owner. All cervid and the New Brunswick bison outbreaks were identified through post mortem examination. In contrast, all cattle outbreaks were identified through slaughter surveillance. Post mortem examination is a discretionary decision made by the owner of a herd. Slaughter surveillance is mandatory at all federally inspected abattoirs. A much smaller proportion of cervids pass through federally inspected abattoirs compared to cattle. It was also more common for cervids and bison to progress to a point where clinical signs and death due to tuberculosis occurred. Consideration of

these findings and possible mechanisms to assure better post mortem and slaughter surveillance of cervid herds should be undertaken. One mechanism would be to make post mortem examination mandatory, in cervid herds, for animals which died after clinical signs suggestive of tuberculosis. Compliance on the part of the owner would be a problem. The Captive Ungulate Program now in place in Canada requires whole herd testing for tuberculosis every three years. Given operational constraints this is a reasonable time frame. However if susceptibility to tuberculosis and incidence rate are higher in cervid herds than in bovine herds, a three year interval may be too long, especially in reactor herds (as defined in this paper). These herds could be handled in a different manner from the negative herds. This would not be as resource intensive as universally applying a shorter interval for herd testing. The mechanics of this proposal are discussed under testing and follow-up to reactor herds for both cervids and cattle.

Identification of the source herd for a tuberculosis outbreak is an important aspect of any investigation. Nevertheless, when all the outbreaks were viewed together, traceout investigations were an extremely important mechanism to identify infected (33/55) and reactor (45/81) farms. When combined with other investigations based on actual contact between animals (traceback, fence line contact and co-pasture) the proportion of positive and reactor farms detected was 0.7 (37/55) and 0.8 (66/81) respectively. The same type of observations have been recorded in other countries (1-3). On the other hand, perimeter testing accounted for 35.6% (392/1101) of the herds investigated but only 1 positive and 1 reactor herd were identified in this way. It is questionable, in the light of these findings, if resources should continue to be directed to intensive perimeter testing. Certainly in primarily cervid outbreaks the number of bovine herds which are screened because they are in a perimeter zone of a reactor or positive herd could be decreased if not

eliminated, and vice versa. The findings of this study can be used to justify this position to trading partners.

According to this thesis there were three possible outcomes, at both the herd and individual animal level, to testing for tuberculosis. The herd or the individual could be negative, positive (culture positive) or a reactor. In this study reactor meant an animal or farm that had any suspect or positive reaction to a tuberculosis test other than the caudal fold test. Caudal fold suspect or positive animals or herds which were negative to other follow-up tests were considered negative. The hypothesis of this study was that herds containing reactor animals were at much greater risk of contributing to the spread of *M. bovis* than herds which did not contain reactor animals. These herds should be investigated more intensively to determine their true status and should be considered as possible sources of *M. bovis*. There were instances in the outbreaks investigated (Manitoba for example) where a "reactor" herd did serve as the source of infection for other herds which were found to be either culture positive or reactor herds.

In positive/reactor herds, increasing herd size was identified in this study as a risk factor for spread of tuberculosis between herds. Increasing age of individual animals in a herd was found to be a risk factor for transmission within herds. These two factors, if known, could be taken into consideration, along with the contact category (traceout, co-pasture etc.) when investigators planned the strategy to investigate an outbreak. To summarize, epidemiological investigations should concentrate on identification of the source herd and traceout investigations followed by other instances of actual animal contact. Large herds with an older than average animal population are probably at greater risk of being infected or reactor herds than smaller, younger herds.

Another category of herd that is at higher risk, yet may be considered negative, is the herd that received animals from a positive herd. The testing that is applied may actually "miss" a potentially infected herd because there had been insufficient time for animals to mount a detectable immunological response to the tuberculin skin tests.

Given the very high risk associated with traceout herds and the increased risk of transmission proposed for the reactor category herd, it is proposed that these two categories of higher risk herd undergo increased surveillance in order to ascertain with greater certainty, their true status. There are two basic approaches to increased surveillance and the two can be applied separately or together depending on the history of the herd, resources available and results obtained. The two approaches are: (1) increase frequency and intensity of testing with the same technologies; and/or, (2) use a number of different technologies on the same animals in a short time frame.

A technology that is amenable to the first approach is slaughter surveillance with attendant histology and culture testing. Specific farms could be "targeted" for increased testing. Samples from a higher proportion of animals from these farms could be submitted for testing and the sample selection could concentrate on "high risk" tissue. These are the left and right medial retropharyngeal lymph nodes, anterior and posterior mediastinal lymph nodes, right and left bronchial lymph nodes, and the lungs (4). Sensitivity of culture is low but it could be increased at the herd level by pooling tissues from several animals. This could be done at slaughter without a great burden being placed on inspection and laboratory staff. This approach is particularly amenable to the reactor category of farm where repeated skin testing is not indicated or recommended and gross lesions may not be present. Often young animals are slaughtered before

lesions have a chance to develop but the animal may be infected with *M. bovis*. Culture would be the only test to definitively diagnose tuberculosis. Cervids are less likely to be sent to slaughter and therefore all animals that die in reactor herds (especially cervid herds) where clinical signs are suggestive of tuberculosis should be autopsied and a broad range of tissues sent for histology and culture.

It is known that different tests may detect infection at different times during the course of the disease. Thus it is important to apply different tests to all of the animals in a high risk herd to increase the probability of finding infected animals. Herds which are considered negative because they have not had time to mount an immune response to the tuberculin skin tests are particularly suited to this approach and can be tested with tests such as the gamma interferon test which is known to detect earlier infection than the tuberculin tests. Diagnostic technologies are discussed at length in Chapter 2 and the advantages and disadvantages to using these technologies at different times are reviewed.

7.2.2 The relative importance of bovine and cervid tuberculosis

The significance of tuberculosis in Canada appeared greater in cervids and bison than in cattle in the last ten years. Five of the nine outbreaks were primarily cervid or bison in spite of the fact that the number of cervid and bison herds in Canada was much smaller than the number of bovine herds. Analysis of the outbreaks in which some of the herds investigated were cervid herds (Chapter 5) indicated that cervid herds ($OR = 1.22$; $p = 0.004$) were at greater risk of tuberculosis. In addition, there was some evidence that the within herd spread was higher among cervids than cattle. The observed incidence rate in the Alberta/Saskatchewan cervid outbreak was 18.6 reactor/positive animals per 100 animal years. The highest incidence rate for cattle was for

beef cattle in the Quebec bovine outbreak (10.1 cases per 100 animal years) and was approximately half the Alberta/Saskatchewan cervid rate. Surveillance becomes a more important issue in cervid farms because of the potential for rapid spread of tuberculosis and the fact that cervids are likely to be kept to an older age than cattle.

It is suggested that cervids are more susceptible to tuberculosis than cattle (5). It is difficult to determine if the actual susceptibility is greater or if management factors on cervid farms enhance the transmission of the disease. The immune response in tuberculosis infection varies between and within species (6-8). Certainly there are significant differences in management of cattle and cervids enterprises and these differences may be linked to maintenance of infection in the cervid herds and to the risk of transmission both between and within herds. One difficulty which occurred in Canada during the early stages of the problem with tuberculosis in cervids was that the principles for control of tuberculosis in cattle (through skin testing) were applied to control of tuberculosis in cervids. Thus administration and interpretation of tuberculin skin tests for cervids were the same as for cattle. However it was later determined that it is not possible to extrapolate the bovine methodology directly to cervids and changes were made to accommodate the difference in the two.

7.2.3 Problems encountered and observed during the project which should be addressed.

Data collection was a long, tedious and labourious procedure. There were several areas which caused difficulties in the collection and analysis of the data. These included:

1. Incomplete and missing data
2. Difficulty in obtaining and extracting data because of the non-uniformity of files
3. Variation in the storage and organization of files

4. Lack of consideration of the data that were required for a sound epidemiological investigation and analysis, and data collection methods which were not statistically sound (random samples versus convenience samples for example). These were problems not because of lack of knowledge or expertise but rather because the objective of the investigation was to detect infected herds and depopulate them. However, a minimal amount of pre-planning would assure high quality data collection and analysis in the long term. The prevalence and incidence of tuberculosis is decreasing and every opportunity to learn more about this disease in a Canadian setting should be taken.

Data retrieval, collection, and recording for this project were tedious and time consuming. Even when data were stored electronically as in the Alberta/Saskatchewan elk outbreak, it was not possible to simply transfer the data from one electronic format (Epi Info) to another (Access) because the data were not in a standardized format nor defined in the same way in the two different systems. Perhaps one of the most valuable lessons learned in this project was that a core set of standardized data should be defined for each disease for which data are collected and that all Regions should collect the same data. It would be preferable for the data to be stored electronically so that transfer, retrieval and analysis would be facilitated. Transfer of data between different electronic formats is relatively simple and although it may be preferable for all regions to use the same software, it is not essential that this be so. The expertise gained in this project could be used to begin this process for tuberculosis. An outbreak is not the time to begin a study of the data which are needed. In fact the system should be designed and in use on an every day basis, prior to an outbreak, so that the users are familiar with its use and output.

Psychological stress to investigators because of legal problems and difficult interpersonal

relationships with owners, managers and other government staff was very obvious in several outbreaks even from the overview material alone. Veterinarians and inspectors were spending large proportions of their time on legal issues and communication with extremely irate clients. These kinds of work and interactions are probably unavoidable but there should be some mechanism to monitor the stress and impact of these elements on the individuals involved and also on the program as a whole. Counselling and training should be provided on a proactive basis.

It is assumed that the only wildlife reservoir of tuberculosis in Canada is the population of bison in the Wood Buffalo National Park. The danger of transmission of *M. bovis* from bison in this park to other wildlife species and domestic stock is worrisome. The probability of introducing tuberculosis to other wildlife species and domestic livestock is difficult to determine but the repercussions if it occurred would be significant and would have the potential to change the whole dynamic of Canada's tuberculosis control and eradication program as well as Canada's status with trading partners. It is, however, beyond the scope of this thesis to recommend actions which should be taken to address this situation, at this time.

7.2.4 Improvements which would have a positive impact on all control and eradication programs

Several factors, which would significantly enhance all disease control and eradication programs, repeatedly emerged in the study. These were:

1. The need for a reliable and universally applied animal identification system even at the farm level

A national system which required all animals to have a unique animal identification

number is the ideal but is not absolutely required. Even if every farm had its own system there would have been fewer instances of problems due to lack of identification or mis-identification. There were instances where it was impossible to do a reliable traceback to a source herd (even though there were only 3 possible sources) because animals were not identified in any manner. Presently, The Livestock Identification Working Group, an industry/government subcommittee reporting to the Canadian Animal Health Consultative Committee on livestock identification issues, is making considerable progress in livestock identification initiatives in Canada.

2. Formal animal movement records

Restrictions and regulations governing cervid movement are in place. Owners of bovine herds should be required to keep standard records of animal movement both into and out of their farms. In an outbreak situation, the completeness of an epidemiological investigation in traceouts and tracebacks is only as good as the individual owner's memory when records are not kept. The epidemiological investigation in Ontario was seriously hampered by the lack of animal movement records. Many farms were infected from one source farm but these farms were not identified as traceout farms. They were identified at post mortem or at slaughter often several years later. The potential for these farms to infect other farms was high. Industry must play a role in both animal identification and standardization of animal records, especially movement records.

7.2.5 Research needs for the future

The following are some tuberculosis research needs for Canada. Several of these areas could include study of diseases in addition to tuberculosis. This would increase the productivity

of these efforts.

1. Animal identification systems are being researched and implemented in some species and locations in Canada. The relevance of this work to tuberculosis can be evaluated and its usefulness maximized.

2. Field evaluation of other diagnostic technologies in a Canadian setting. The Tuberculosis in Elk Project (9) did this for cervids. However newer diagnostic technologies for cattle have not been evaluated in the same manner.

3. Surveys on targeted high risk populations - bison herds and animals in and around Wood Buffalo National Park for example. As brucellosis is another disease of note in this population it could be studied at the same time.

4. Knowledge of animal movement patterns even in broad terms would be useful not just for tuberculosis control and eradication programs but for all disease control programs.

5. Development of standardized outbreak management systems and epidemiology databases.

7.3 Conclusion

The main purpose for this study was to integrate the information gathered in a literature review and a study of the tuberculosis outbreaks in Canadian cattle and cervids in the last ten years, into a coherent series of conclusions and recommendations to improve tuberculosis eradication and control programs in Canada. The success of the project will be measured in terms of the improvement in these programs if the recommendations are implemented and the results measured.

Chapter 7 References

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APPENDIX A

Epidemiology of *Mycobacterium Bovis* in Canadian Cattle and Cervidae

HERD INFORMATION

Farm ID _____

PROV	FARMTYP	EED	LED	LTD	SSC	SEC	DIST	FCC	TAOF	FHT
Prov	Principle Farm Type	Earliest Exposure Date	Latest Exposure Date	Last Test Date	Study Start Code	Study End Code	Dist. to Infected Farm	Farm Classif. Code	Total Animals on Farm	First Herd Test	Source Herd for SSC

BREED	CF N	CF S	CF P	CC N	CC S	CC P	MC N	MC S	MC P	GP N	GP S	GP P	HT N	HT S	HT P	CT N	CT S	CT P

BREED	RA N	RA S	RA P	EL N	EL S	EL P	GI N	GI S	GI P	LS N	LS S	LS P	BT N	BT S	BT P	OT N	OT S	OT P

COMMENTS: _____

APPENDIX B

FARM ID_____ Page_____

Epidemiology of *Mycobacterium Bovis* in Canadian Cattle and Cervidae

INDIVIDUAL ANIMAL INFORMATION

Ear Tag No.	Barn Name	Age (Mo)	Sex	Breed	Entry Code	Entry Date	Depart Code	Depart Date	CF DATE	CF R	CC DATE	CC R
1		1				1			1		1	
2		2				2			2		2	
3		3				3			3		3	
4		4				4			4		4	
5		5				5			5		5	

MC DATE	MC R	GP DATE	GP R	HT DATE	HT R	CT DATE	CT R	RA DATE	RA R	EL DATE	EL R	GI DATE	GI R	LS DATE	LS R
1		1		1		1		1		1		1		1	
2		2		2		2		2		2		2		2	
3		3		3		3		3		3		3		3	
4		4		4		4		4		4		4		4	
5		5		5		5		5		5		5		5	

COMMENTS _____

APPENDIX C

EPIDEMIOLOGY OF *MYCOBACTERIUM BOVIS* IN CANADIAN CATTLE AND CERVIDAE

CODE LIST AND ACRONYM LEGEND

A. **HERD CODES**

1. LOCATION: PROVINCE = PROV; AND FARM ID = FARMID

1 = N.B
2 = P.E.I
3 = NFLD
4 = QUE
5 = ONT
6 = MAN
7 = SASK*
8 = ALB
9 = B.C

* The Saskatchewan/Albert elk outbreak files will be given a farm Identification number beginning with '7' but the province will be identified according to the province code.

The outbreak files will be named according to the location and to the type of data in the files. Herd information files will be named according to the following convention:

HRD1000 = N.B BISON
HRD2000 = P.E.I CATTLE
HRD4000 = QUE CATTLE
HRD4500 = QUE CERVID
HRD5000 = ONT. CERVID
HRD6000 = MAN. CATTLE
HRD7000 = SASK/ALB ELK
HRD8000 = ALB CATTLE
HRD9000 = B.C. DEER

Individual animal information files will be named according to the following convention:

HRD1000 = N.B BISON
IND2000 = P.E.I CATTLE
IND4000 = QUE CATTLE
IND4500 = QUE CERVID
IND5000 = ONT. CERVID
IND6000 = MAN. CATTLE
IND7000 = SASK/ALB ELK
IND8000 = ALB CATTLE
IND9000 = B.C. DEER

2. PRINCIPLE FARM TYPE: BASED ON SPECIES AND BREED = FARMTYP

10 = DAIRY CATTLE
- 11 = HOLSTEIN
- 12 = AYRSHIRE
- 13 = JERSEY

20 = BEEF CATTLE
- 21 = WHITE FACE
- 22 = CHAROLAIS
- 23 = LIMOUSIN
- 24 = SIMMENTAL
- 25 = HEREFORD
- 26 = ANGUS

30 = ELK
40 = BISON
50 = DEER
- 51 = FALLOW DEER (DAIM)
- 52 = SIKA DEER
- 53 = REINDEER
- 54 = PERE DAVID DEER
- 55 = RED DEER
- 56 = CERF DE VIRGINIE

60 = SHEEP/GOATS
70 = PIGS
80 = ZOO ANIMALS
90 = OTHER
- 91 = LLAMA
- 92 = YAK
- 93 = GIRAFFE

3. EARLIEST EXPOSURE DATE = EED (dd/mm/yy)

4. LATEST EXPOSURE DATE = LED (dd/mm/yy)
These dates represent when exposure to *M. bovis* began in a herd. There are cases when it is not possible to be certain when the initial exposure occurred. An example of this would be fenceline contact over a period of time. In this situation the earliest exposure would be the date that animals were in contact and it is KNOWN that one of the herds was infected with tuberculosis at that time. The latest exposure date would be the date that contact stopped between these two herds. In the case of a sale of an animal from an infected herd to another herd, the earliest and latest exposure dates will be the same. That is the date that the purchased animal entered the new herd. (Note: I have collected the data in a somewhat different manner however. When it is possible to track the length of time that an animal from an infected herd was in another herd, I have called these the EED and the LED. In these cases we may want to look at the transmission rate given these different length 'windows of opportunity'. It is important to look at the SSC (see # 6 below) however to determine which is the appropriate exposure date to use in various calculations.

5. LAST TEST DATE = LTD (dd/mm/yy)
This is essentially the study end date. It represents the last date that the status of the herd is known. It may be, for example, the last herd test or it may be the date of post mortem on a suspect animal.

6. INVESTIGATION (STUDY) START CODE = SSC

1 = TUBERCULOUS ANIMAL DISCOVERED AT SLAUGHTER
2 = HERD INVESTIGATED BECAUSE IT IS HERD OF ORIGIN OF A TUBERCULOUS OR REACTOR** ANIMAL (TRACEBACK)
3 = HERD INVESTIGATED BECAUSE ANIMALS FROM AN INFECTED OR REACTOR HERD WERE PURCHASED (TRACEOUT)
4 = PASTURE CONTACT WITH INFECTED HERD
5 = FENCELINE CONTACT WITH AN INFECTED HERD
6 = PERIMETER HERD TO AN INFECTED HERD
7 = AREA TESTING - MAY BE ROUTINE TESTING OR MAY BE IN THE SAME AREA AS A POSITIVE OR SUSPECT HERD BUT AT A GREATER DISTANCE THAN THE 10 KM. RADIUS
8 = OTHER
9 = OTHER UNSPECIFIED CONTACT (USED ORIGINALLY IN THE ELK FILES BECAUSE THE REASON FOR INVESTIGATION WAS DUE TO CONTACT BUT THE TYPE OF CONTACT WAS NOT GIVEN)

When more than one code is applicable, the code with the highest level of risk is chosen. The determination of traceback versus traceout is made from the investigator's point of view and does not make any assumptions about the true chronology or direction of transmission.

The term reactor must be defined for the purposes of this project. A REACTOR animal is one which had a **positive mid-cervical or single cervical test reaction A reactor farm is one which contains a reactor animal. This is not to be confused with a positive farm - one where *M. bovis*

has been isolated from an animal.

7. INVESTIGATION (STUDY) END CODE = SEC (yy/mm/dd)
    1 = NEGATIVE
    2 = SOLD/SLAUGHTER
    3 = DEPOPULATED
    4 = OTHER

8. DISTANCE FROM AN INFECTED FARM = DIST (KM)

9. FARM CLASSIFICATION CODE = FCC
    1 = NEGATIVE
    2 = NEGATIVE WITH REACTORS (GREEN)
    3 = POSITIVE (RED)
    4 = NO ANIMALS TESTED

These codes are for the use primarily of the project scientists and do not represent the classification that Agriculture Canada uses. Agriculture Canada would investigate a herd until it was determined to be either positive or negative. A positive herd is one deemed to be infected based on positive culture results. These herds must be depopulated. A negative herd is one where all the animals are negative to the tuberculosis skin test either initially or after removal of reactor animals on the comparative or single cervical test with no subsequent isolation of M.bovis from the reactor animals.
For purposes of illustration, these different classifications are colour coded.
Occasionally herds are investigated but there are no longer any animals on the farm. These farms are coded as # 4 as it is impossible to determine what their status was before sale or slaughter.

10. TOTAL ANIMALS ON FARM = TAOF
This number represents the total number of animals (beef, dairy, sheep, goats, cervidae) on the farm at the beginning of the study as closely as it can be determined. (poultry and swine not included)

11. LARGEST HERD TEST = LHT
This is the largest herd test and is the best indicator of the total number of animals at risk. Occasionally, at the beginning of an investigation, not all of the animals on a farm are tested. Later it may be deemed necessary to test all of the animals. The best indicator of the total animals at risk is this largest herd test.

12. SOURCE HERD FOR SCC = SOURCE
This is the herd that is the contact which makes investigation necessary. It is determined from the veterinary investigator's point of view.

B. INDIVIDUAL FARM CODES

1. FARM ID: SEE # 1 ABOVE = FARMID

2. EAR TAG NUMBER: EARTAG

3. BARN NAME: ANIMAL'S FAMILIAR NAME IF GIVEN = BARNAME

4.AGE: IN MONTHS AT THE ENTRY DATE = AGE
The convention which has been chosen for animals that are not given an exact age is as follows:

calf = 3 mo.
yearling = 12 mo.
heifer = 18 mo.
adult = 99 (age to be assigned later)

5. SEX = SEX

1 = FEMALE
2 = MALE
3 = NEUTERED ANIMAL

6. BREED: BREED OF ANIMAL = BREED SEE # 2 ABOVE

7. ENTRY CODE = ENTRYCD

1 = PRESENT AT THE STUDY START DATE*
2 = BORN INTO THE HERD, AFTER STUDY START DATE
4 = BOUGHT FROM A NEGATIVE HERD
5 = BOUGHT FROM A NEGATIVE HERD WITH REACTORS
6 = BOUGHT FROM A POSITIVE HERD
7 = OTHER - IF ANIMAL WAS NOT PRESENT AT THE BEGINNING OF THE STUDY THEN WE DO NOT KNOW THE EXACT ENTRY DATE EITHER. HOWEVER WE CAN USE THE DATE OF THE FIRST TEST ON THE ANIMAL AS AN ENTRY DATE. FOR EXAMPLE WE COULD HAVE AN ENTRY CODE OF '7' AND AN ENTRY DATE LATER THAN THE DATE OF THE FIRST TEST IN THIS HERD.

Numbers 4, 5, and 6 imply that #1 is also true but these other codes give us more information

*The study start date is the date of the first recorded test on the herd as a result of the outbreak investigation.

8. ENTRY DATE: dd/mm/yy = ENTRYDT
For all animals present at the beginning of the study (entry code = 1) the entry date is the date of the first test. We can get earliest exposure date from the herd information. For all the other entry

codes, the entry date is when the animal comes into the herd through sale, birth, etc. Doing the dates this way allows us to get data on length of time from exposure to test.

9. DEPARTURE CODE = DEPCD
    1 = NEGATIVE
    2 = SOLD
    3 = DIED
    4 = SLAUGHTERED (NON REACTOR ON TEST (MAY BE CF POSITIVE) BUT SLAUGHTERED ANY WAY)
    5 = SLAUGHTERED (A REACTOR ON SKIN TEST- THIS IMPLIES A REACTOR TO A MID-CERVICAL OR SINGLE CERVICAL TEST)
    6 = DEPOPULATED
    7 = SLAUGHTERED WITH DAM
    8 = OTHER/UNKNOWN
    9 = INDEX CASE

10. DEPARTURE DATE: dd/mm/yy = DEPDT

11. TEST REACTION;
    1 = NEGATIVE
    2 = SUSPICIOUS
    3 = POSITIVE

C. MISCELLANEOUS ACRONYMS

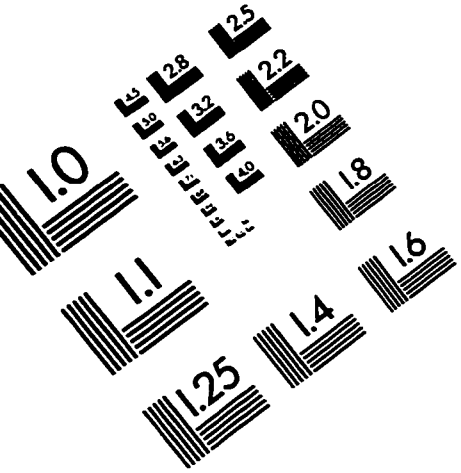
1. ALL MISSING VALUES = -1 (DATA EXIST BUT ARE NOT THERE)
2. DATA DO NOT EXIST, USE BLANK
3. TEST CODES:

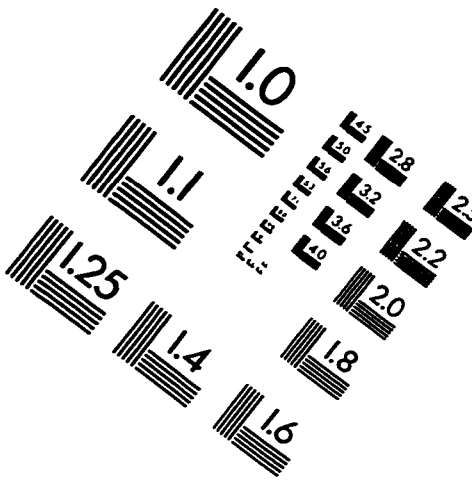
CF = CAUDAL FOLD
CFDATE* = DATE OF CF TEST
CFR* = REACTION TO CF TEST

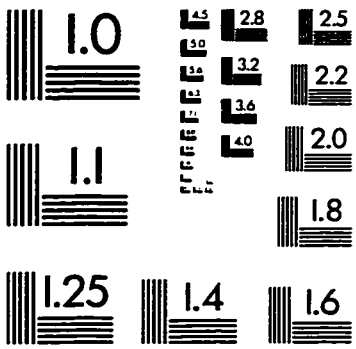
CC = COMPARATIVE CERVICAL
MC = MID-CERVICAL
GP = GROSS PATHOLOGY
HT = HISTOLOGY
CT = CULTURE
RA = ROUTINE ABATTOIR INSPECTION
EL = ELISA
GA = GAMMA INTERFERON
LS = LYMPHOCYTE STIMULATION
BTB = BLOOD TB TEST
OT = OTHER

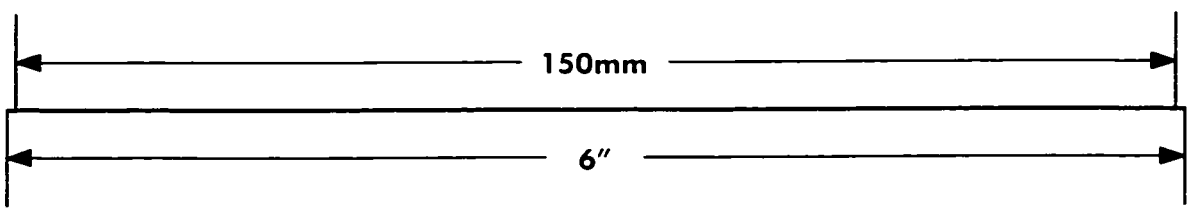
*NOTE: ALL TESTS WILL HAVE A DATE AND REACTION FIELD. THE RESULTS FOLLOW THE CONVENTION FOR TEST RESULTS OUTLINED ABOVE

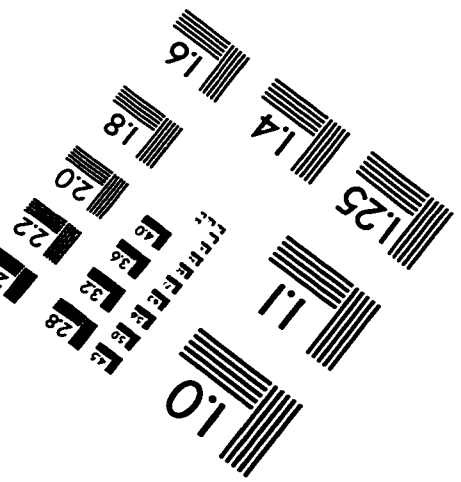
IMAGE EVALUATION
TEST TARGET (QA-3)

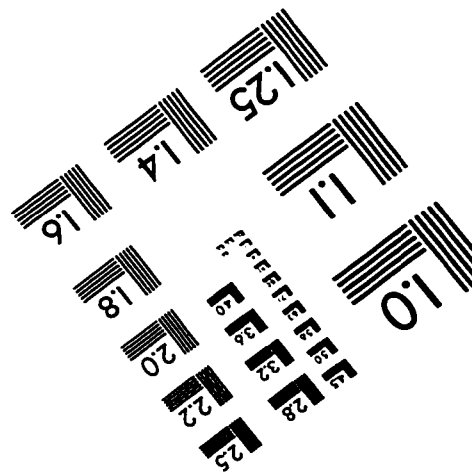












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