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Bovine Tuberculosis in Swedish Farmed Deer

Detection and Control of the Disease

Helene Wahlström
Department of Clinical Sciences
Uppsala

Doctoral thesis
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Abstract

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Bovine tuberculosis (BTB) was introduced into Swedish farmed deer herds in 1987. Epidemiological investigations showed that 10 deer herds had become infected (July 1994) and a common source of infection, a consignment of 168 imported farmed fallow deer, was identified (I).

As trace-back of all imported and in-contact deer was not possible, a control program, based on tuberculin testing, was implemented in July 1994. As Sweden has been free from BTB since 1958, few practising veterinarians had experience in tuberculin testing. In this test, result relies on the skill, experience and conscientiousness of the testing veterinarian. Deficiencies in performing the test may adversely affect the test results and thereby compromise a control program.

Quality indicators may identify possible deficiencies in testing procedures. For that purpose, reference values for measured skin fold thickness (prior to injection of the tuberculin) were established (II) suggested to be used mainly by less experienced veterinarians to identify unexpected measurements. Furthermore, the within-veterinarian variation of the measured skin fold thickness was estimated by fitting general linear models to data (skin fold measurements) (III). The mean square error was used as an estimator of the within-veterinarian variation. Using this method, four (6%) veterinarians were considered to have unexpectedly large variation in measurements.

In certain large extensive deer farms, where mustering of all animals was difficult, meat inspection was suggested as an alternative to tuberculin testing. The efficiency of such a control was estimated in paper IV and V. A Reed Frost model was fitted to data from seven BTB-infected deer herds and the spread of infection was estimated (< 0.6 effective contacts per deer and year) (IV). These results were used to model the efficiency of meat inspection in an average extensive Swedish deer herd. Given a 20% annual slaughter and meat inspection, the model predicted that BTB would be either detected or eliminated in most herds (90%) 15 years after introduction of one infected deer. In 2003, an alternative control for BTB in extensive Swedish deer herds, based on the results of paper V, was implemented.

Keywords: cervidae transmission, modelling, intra-observer variability, tuberculin test, reference intervals, meat inspection, Sweden, epidemiological investigation, surveillance

Author's address: Helene Wahlström, Department of Disease Control, Swedish Zoonosis center, SVA, SE-751 89 UPPSALA

To Moa, Malin and Björn

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Appendix

Papers I-V

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

- I. Bölske, G., Englund, L., Wahlström, H., de Lisle, G. W., Collins, D. M., Croston, P. S., 1995. Bovine tuberculosis in Swedish deer farms: epidemiological investigations and tracing using restriction fragment analysis. *Vet. Rec.* 136, 414-417.
- II. Wahlström, H., von Rosen, D., Öhagen, P., Vågsholm, I., Englund, L., Engvall, A., 2004. Evaluation of measurements of skin fold thickness with manual calipers in the comparative cervical tuberculin test in Swedish farmed deer: 1. Reference intervals. *Submitted for publication*.
- III. Wahlström, H., von Rosen, D., Öhagen, P., Vågsholm, I., Englund, L., Engvall, A., 2004. Evaluation of measurements of skin fold thickness with manual calipers in the comparative cervical tuberculin test in Swedish farmed deer: 2. Within-veterinary variance. *Submitted for publication*.
- IV. Wahlström, H., Englund, L., Carpenter, T., Emanuelson, U., Engvall, A., Vågsholm, I., 1998. A Reed-Frost model of the spread of tuberculosis within seven Swedish extensive farmed fallow deer herds. *Prev. Vet. Med.* 35, 181-93.
- V. Wahlström, H., Carpenter, T., Giesecke, J., Andersson, M., Englund, L., Vågsholm, I., 2000. Herd-based monitoring for tuberculosis in extensive Swedish deer herds by culling and meat inspection rather than by intradermal tuberculin testing. *Prev. Vet. Med.* 43, 103-116.

Papers I, IV and V are reproduced by permission of the journals concerned.

Abbreviations

APHIS	Animal and Plant Health Inspection Service
BTB	Bovine tuberculosis (tuberculosis caused by <i>Mycobacterium bovis</i> irrespective of the host)
CCT	Comparative cervical test
cdf	Cumulative distribution function
CMI	Cell-mediated immune
CT	Cervical test
DTH	Delayed type hypersensitivity
IR	Incidence rates
ELISA	Enzyme-linked immunosorbent assay
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PFGE	Pulsed Field Gel electrophoresis
PPD	Purified protein derivate
PGRS	Polymorphic GC-rich sequence probe
REA	Restriction endonuclease analysis (Restriction enzyme analysis)
RFLP	Restriction fragment length polymorphism
SBA	Swedish Board of Agriculture
SIT	Single intradermal test
TB	Tuberculosis
USDA	US Department of Agriculture
VNTR	Variable-number tandem repeat typing

Introduction

The present description of bovine tuberculosis (BTB) covers mainly disease in cattle on account of knowledge about the disease in deer being scarcer. However, differences between cattle and deer that are considered relevant to the present thesis are included.

The term bovine tuberculosis is used to describe infection caused by *Mycobacterium (M.) bovis*, irrespective of the host. This is a commonly used approach although BTB really should be confined to tuberculosis in cattle (Collins, 2000).

Bovine tuberculosis in Sweden

BTB was probably imported to Sweden with infected breeding stock in the middle of the 19th century (Lagerlöf, 1962). At that time, the dairy industry developed rapidly and at the end of the 19th century it became common to return unpasteurized skim milk to farms for feeding of calves. This resulted in an increased spread of BTB among cattle (Lagerlöf, 1962). This route of infection was partially arrested in 1925, when pasteurisation of milk used for feeding livestock became compulsory (Myers & Steele, 1969). Interestingly, pasteurisation of milk for human consumption did not become compulsory until 1937 (www.slv.se; accessed 20-Aug-2004).

Control for BTB, based on tuberculin testing and slaughter, was initiated in the early 20th century. In 1930-1940 the control was intensified and in 1958 Sweden was, as one of the first countries in the world, declared free from BTB (Myers & Steele, 1969).

The effect of the control program was reflected in the prevalence of cattle with tuberculous lesions at slaughter. In 1937, 1947 and 1957, 30%, 10% and 0.01%, respectively, of cattle at slaughter had tuberculous lesions (Lagerlöf, 1962). After 1958, sporadic cases have occurred in cattle, the most recent in 1978 (September 2004). The compulsory tuberculin testing of all cattle was abolished in 1970 and the national BTB control is now based on meat inspection (Sjöland, 1995).

BTB, as well as tuberculosis caused by *M. tuberculosis*, has been notifiable under the Swedish legislation for epizootic diseases since 1958 (Anonymous, 1999a; Anonymous, 1999b). If *M. bovis* is confirmed in a herd the whole herd is depopulated. The Swedish Board of Agriculture (SBA) then performs an epidemiological investigation to identify the source of infection and any potential spread. Farmers get full compensation for any loss due to actions taken by the SBA. An investigation to identify humans that may have had contact with infected animals is also performed by the Regional medical officer for infectious disease control.

In Swedish wildlife, only two cases of BTB have been reported, both in free-living moose (*Alces alces*) (Pedersen Mörner & Mörner, 1990). No case has been diagnosed in dogs during the last 25 years (Anonymous, 1998) but in cats one case

was diagnosed in 1990. In the latter case, the source of infection was the owner who had a reactivated *M. bovis* infection (Hillerdal, Källenius & Pedersen Mörner 1991).

In 1991, BTB was diagnosed for the first time in farmed fallow deer in Sweden. Epidemiological investigations identified a consignment of deer imported in 1987 as the source of infection (Bölske *et al.*, 1995) (I). As all imported deer could not be traced, a voluntary control program was implemented in July 1994 and in June 2003 the control program became compulsory (Anonymous, 2003a; Anonymous, 2003c). The program is described in paper III. In brief, a herd obtains BTB-free status (A-status) after three consecutive whole herd tuberculin tests of all deer older than one year, with negative results. Only herds with A-status may sell live deer and to maintain the A-status all female deer have to be tested after two years and then every 3rd year without positive findings (Anonymous, 2003a). BTB-free status can also be obtained by slaughter of the whole herd and repopulation with deer from BTB-free herds. Herds that do not continue to test are downgraded to BTB-free herds with B-status.

Since the program's inception it has become evident that, on certain large extensive deer farms, it is difficult to muster all animals in the herd. Therefore, owners of farms larger than 100 hectares and where no deer have been added to the herd after 1985, may apply for an alternative control for BTB, based on slaughter and meat inspection. This alternative control is based on the results of papers IV and V. In these herds, at least 20% of the herd (equally distributed over sex and age classes) shall be slaughtered annually for at least 15 years and the carcasses submitted for meat inspection (Anonymous, 2003a). Furthermore, all other deer that are killed or die due to other reasons shall be meat inspected/autopsied.

In December 2003, there were 605 registered deer herds in Sweden, 585 of these were affiliated to the control program (pers. comm. Eriksson, 2004). In all, 488 herds had obtained BTB-free status and 26 of these continue to test to maintain their A-status (pers. comm. Eriksson, 2004). The remaining 97 herds are in the process of becoming BTB-free either by tuberculin testing, by slaughter of the whole herd and meat inspection, or the owners have applied for an alternative BTB-control. Such applications have been submitted to the SBA for 35 herds; 14 applications have been accepted, 17 rejected, two decisions are pending and the remaining two have been withdrawn (www.sjv.se; accessed 10-Aug-2004).

Meat inspection is compulsory for farmed deer since 1990, and in 1991 the requirements were extended to include all organs including the intestines and stomach. In 1994 meat inspection became compulsory also for free-living red- and fallow deer.

In humans, less than 10 cases of *M. bovis* are notified annually in Sweden. Most of these are elderly people, infected in their youth before BTB was eradicated in Sweden, or in immigrants from areas where BTB is still common (Anonymous, 1998; Anonymous, 1996-2002).

Deer farming in Sweden

Fallow deer is the deer species most commonly farmed in Sweden. Deer farming has traditions back to the 16th century when fallow deer were introduced and kept for their attractive appearance and for hunting in royal parks. Fallow deer is not an indigenous species in Sweden, but feral populations now occur in several parts of the country. To a lesser extent red deer, indigenous to Sweden, are also farmed.

The interest in deer keeping increased in the 1990s when government subsidies to promote alternative use of farmland made deer farming more profitable. At present (January 2004) there are approximately 20,000 farmed deer distributed between 388 herds. In addition, there are 217 empty, depopulated farms, bringing the total number of registered deer farms to 605 (pers. comm. Eriksson, 2004). Most deer are kept for venison production. A few herds sell live deer and in certain large herds (game parks) deer are kept for shooting purposes. Velvet production is not allowed due to reasons of animal welfare.

The calving period for fallow deer is between mid June and mid July and for red deer one month earlier. Female deer are sexually mature at about 16 months of age and may then become pregnant given that their body condition is good. Although male deer are sexually mature at the same age, it is usually male deer aged 7-10 years that sire the offspring. The reproductive life of female deer is usually about 15 years but it has been reported to be as long as 20 years (Johansson, 2001). For male deer, reproductive life is somewhat shorter, usually between 9-11 years. Live adult weights are 42-62 kg (female fallow deer), 80-130 kg (male fallow deer), 90-120 kg (female red deer) and 130-250 kg (male red deer) (Johansson, 2001).

In Sweden, deer farming is an extensive production. Farmed deer are not allowed to be kept on areas smaller than 5 ha. The size of the deer herd is required to be adjusted to the size and biotope of the deer enclosure. During the plant-growing period there must be sufficient natural vegetation in the deer enclosure to keep all deer well nourished without supplementary feeding.

Mycobacterium bovis

Mycobacteria belong to the order *Actinomycetales*, the family *Mycobacteriaceae* and the genus *Mycobacterium*. The genus *Mycobacterium* contains 95 species (Garrity, Bell & Lilburn, 2003). Based on their clinical importance the species can be divided into three groups. i) strict pathogens, including *M. tuberculosis* and *M. bovis* ii) potential pathogens, including the *M. avium* complex and iii) rare pathogens, including the saprophytes. Due to practical purposes, as the classification can be done in most laboratories, mycobacteria can also be divided into two groups: *M. tuberculosis* complex (including *M. tuberculosis*, *M. bovis*, *M. africanum* and *M. microti*) and mycobacteria other than the *M. tuberculosis* complex (Rastogi, Legrand & Sola 2001).

The mycobacterial taxonomy has been improved by the use of genotypic characteristics (mainly sequencing the 16SrRNA genes) (Rastogi, Legrand & Sola 2001). Recently a new subspecies, *M. tuberculosis* subsp. *caprae* has been proposed (Aranaz *et al.*, 1999). However, a more correct designation appears to be

M. bovis subsp. *caprae* (Niemann, Richter & Rusch-Gerdes, 2002; Garrity, Bell & Lilburn, 2003). Aranaz *et al.* (2003), however, later suggested that the strain should be elevated to species status and named *M. caprae*.

Mycobacteria are aerobic, acid-fast, rod-shaped, non-motile bacteria (Rastogi, Legrand & Sola 2001). Historically, they were considered as unencapsulated organisms but it is now known that pathogenic mycobacteria contain a “capsular structure” that protects the bacteria from microbiocidal activities of the macrophages and also contributes to the permeability barrier of the mycobacteria cell envelope (Rastogi, Legrand & Sola 2001).

Mycobacteria belonging to the *M. tuberculosis* complex are intracellular pathogens and can grow inside phagosomes and phagolysosomes (Rastogi, Legrand & Sola 2001). The cell envelope (bacterial cytoplasmic membrane, the cell wall and the mycobacterial capsule) is important to enable mycobacteria to survive and grow intracellularly. It is also important for the ability of mycobacteria to modulate the immune response in the host (Rastogi, Legrand & Sola 2001). Due to the high content of lipids in the cell wall, the bacterium is robust and has a long survival in the environment (Anonymous, 2003b). The bacteria resist drying but are killed by sunlight, ultraviolet radiation and pasteurisation (Hirsch & Zee, 1999).

In vitro, most mycobacteria belonging to the *M. tuberculosis* complex or the *M. avium* complex are slow growers, having a mean division time of 12-24 hours. A culture thereby requires about 15 to 28 days. The rare pathogens, including the saprophytes, are fast growers with a culture available within two to seven days (Rastogi, Legrand & Sola 2001).

M. bovis may have a long survival in the environment (Kelly & Collins, 1978; Morris, Pfeffer. & Jackson., 1994; O'Reilly & Daborn, 1995; Scanlon & Quinn, 2000). Several factors influence survival, such as the initial number of bacteria present, the organic matter, pH, temperature, sunlight, humidity and possible interactions with other microorganisms (Scanlon & Quinn, 2000). The survival of pathogenic bacteria is greater in soil or sub-soil than in the soil-surface or on herbage. *M. bovis* can be expected to survive up to two years in sub-soil or in slurry-treated soil (Kelly & Collins, 1978; Morris, Pfeffer. & Jackson., 1994), in faeces up to five months in winter (in England) and shorter during warmer periods, less than two months in summer (Stenhouse, Williams & Hoy, 1930). However, Menzies & Neill (2000) conclude that under natural conditions it appears that survival in the environment (in Northern Ireland) is only a few weeks.

Epidemiology of bovine tuberculosis

Occurrence

M. bovis has one of the broadest host ranges of all pathogens and BTB occurs worldwide (de Lisle, Mackintosh & Bengis, 2001; Cousins, 2001). The disease is of major public health importance but it also has a detrimental effect on animal

health (Rastogi, Legrand & Sola 2001). Due to the zoonotic nature of the disease, control and eradication programs have been implemented. However, in developed countries, at present the emphasis is laid on trading implications as the risk for human health usually is low (Collins *et al.*, 2001). In most developed countries, control and eradication program for BTB are in place and the disease is either absent or occurs at a very low prevalence (www.oie.int; accessed 17-July-2004). This was achieved at a time when cattle herds were small and when intensity of production, including the frequency of live animal movement, was lower and before significant wildlife reservoirs existed (Collins *et al.*, 2001). In areas where BTB is not eradicated, reservoirs in wildlife or feral animals often exist although infected cattle and deer also contribute to retain the infection (Neill *et al.*, 2001, www.aphis.usda.gov/vs/pdf_files/strat_plan.pdf; accessed 28 Sept-2004).

In most developing countries *M. bovis* is present in animals and, as control and surveillance activities often are inadequate, many epidemiological and public health aspects of *M. bovis* remain unknown (Cosivi *et al.*, 1998).

Maintenance and spill-over hosts

Animals may be considered either as maintenance hosts (the infection can be maintained in this population) or spill-over hosts (the infection will die out if the source of infection is removed). Cattle are the classical maintenance host. Several wild species may also act as maintenance host, e.g. badgers (*Meles meles*) in England and Ireland, brushtail possums (*Trichosurus vulpecula*) in New Zealand, buffaloes (*Syncerus caffer*) in South Africa, water buffaloes (*Bulbalis bulbalis*) in Australia, bison (*Bison bison*) in North America and several species of deer (both wild and farmed) (O'Reilly & Daborn, 1995; Anonymous, 1996; Radostitis *et al.*, 2000; de Lisle, Mackintosh & Bengis, 2001;). Swine and goats have also been suggested as maintenance host (Aranaz *et al.*, 1999; Parra *et al.*, 2003). The importance of the maintenance host as sources of infection for cattle may vary, but infected badgers and brushtail possums are known to be significant in the epidemiology of BTB (Krebs, 1997; Lugton *et al.*, 1997; Livingstone, 2000). In principle, all mammals, including man, can act as spill-over hosts, but they are not considered important in the epidemiology of BTB.

Transmission in cattle

The implementation of BTB-control programs, including regular tuberculin testing, has changed the relative frequency of modes of transmission for cattle. Modes that either require large infection doses or occur late in the course of the disease, such as ingestion of infected milk, occur less frequently, whereas airborne infections have become even more dominant (Morris, Pfeffer. & Jackson., 1994).

Modes of infection

Inhalation is considered the principal mode of transmission, especially in housed cattle but also for those at pasture (Radostitis *et al.*, 2000; Neill *et al.*, 2001). Inhalation of small numbers of mycobacteria can probably initiate lesions in cattle (Neill *et al.*, 2001). It has been suggested that only one organism may be sufficient

to cause infection in the lungs Francis, 1947; Neill; O'Brien & Hanna, 1991). However, the size and consistency of aerosol droplets is probably also of importance for establishment of an infection in the lungs (Neill *et al.*, 2001).

Infection by ingestion requires a large infective dose (several thousand to one million organisms) and is obviously more probable at pasture where faeces may contaminate feed, feed troughs and drinking water (Menzies & Neill, 2000). Although *M. bovis* can survive for prolonged periods at pasture, it is difficult to estimate the infectivity of pastures as experiments to clarify this have been performed under varying conditions (Radostitis *et al.*, 2000). Jackson, de Lisle & Morris (1995) concluded that, for cattle and deer (under New Zealand conditions), transmission at pasture was of minor importance compared with aerosol transmission. Menzies & Neill (2000) also suggested that the environment is not a significant source of infection. However, as environmental contamination often is cited to be important in badger-cattle transmission, Menzies & Neill (2000) conclude that further investigation of the importance of environmental contamination is warranted. Drinking water may also be a source of infection and has been shown to be infectious up to 18 days after use by tuberculous cattle (Radostitis *et al.*, 2000). Ingestion of infected milk by young animals was previously an important route of infection. However, in countries with advanced control programs this is not a common mode of spread nowadays, as mammary infection occurs late in the course of the disease (Radostitis *et al.*, 2000) and recycling of unpasteurized milk has been stopped.

Other less common routes of infection are cutaneous, congenital and genital transmission (Jubb & Kennedy, 1970).

M. bovis shedding

Infected cattle are the main source of infection and the bacteria are usually excreted in the exhaled air. It has been stated that cattle may excrete bacteria from the inception of a lung lesion (Neill *et al.*, 2001). In a significant proportion (9 - 19 %) of reactors *M. bovis* has been demonstrated in respiratory tract secretions (Menzies & Neill, 2000; Neill *et al.*, 2001). However, this is probably an underestimation, as shedding of *M. bovis* may be intermittent (Neill *et al.*, 2001). Infected cattle should therefore always be considered as a potential source of infection (Menzies & Neill, 2000). As the disease is progressive, excreting carriers may (if not removed) be infectious for months or even years (Cousins, 2001).

M. bovis may also occur in sputum, faeces, milk, urine, vaginal discharges and discharges from open peripheral lymph nodes (Radostitis *et al.*, 2000). In developed countries this is, however, of minor importance for spread of disease (Menzies & Neill, 2000).

Recently, it has been found that lesions or findings of *M. bovis* in the tonsillar region or upper respiratory tract of infected animals are not uncommon. It has been suggested that spread of organisms from these sites may be of importance for spread by the aerogenous route (Cassidy, Bryson & Neill., 1999; Menzies & Neill, 2000).

The time interval from infection to excretion may vary. Neill, O'Brien & Hanna (1991) found that there is an inverse relationship between the (experimental) infection dose and the time to first excretion. In natural infection, a regression analysis indicated that if infection was caused by one organism, excretion began about 87 days after infection (Neill; O'Brien & Hanna, 1991).

It is probable that only certain BTB-infected animals and under certain conditions act as effective disseminators of the bacteria (Morris, Pfeiffer. & Jackson., 1994).

Within-herd transmission

Transmission rates for *M. bovis* may vary, but it is generally considered that the spread of *M. bovis* is a relatively slow process. Menzies & Neill (2000) report that in the majority of herd breakdowns only one reactor animal is found, suggesting a low within-herd transmission. In intensive production, such as dairy cattle housed indoors, the prevalence of BTB may be high, reflecting a high transmission rate. In contrast, the prevalence is usually lower in extensive production, such as beef cattle, as they usually are kept in open range conditions. However, high herd prevalences may also occur in extensive farming, for example when large numbers of animals gather when drinking from stagnant water holes during the dry season (Radostitis *et al.*, 2000; Rastogi, Legrand & Sola., 2001).

Transmission of M. bovis in other species

Brushtail possum

Tuberculous possums were first found in New Zealand in 1968 (Ekdahl, Smith & Money., 1970). Today, the brushtail possum is the most important wild life host and a significant source of infection for cattle and deer in New Zealand, as it is abundant, very susceptible to BTB, and shares its habitat with domestic animals. The most common sites of infection are the superficial lymph nodes and the respiratory tract. Affected lymph nodes, which may rupture, often contain pus with large numbers of *M. bovis* (de Lisle, Mackintosh & Bengis, 2001). Cattle and deer probably become infected when investigating terminally ill possums (Morris & Pfeiffer, 1995). However, transmission from cattle/deer to possum is a rare event (Morris & Pfeiffer, 1995). It is more probable that deer are responsible for seeding *M. bovis* into possum populations than cattle. This conclusion is based on epidemiological evidence, knowledge that possums may den in sheds or barns in deer farms and that deer, especially feral, deer, are more infectious than cattle (Morris & Pfeiffer, 1995).

Badgers

In 1971, *M. bovis* was isolated from a badger carcass in South-West England and three years later badgers infected with *M. bovis* were found in Ireland (de Lisle, Mackintosh & Bengis, 2001). Subsequent investigations have shown relatively high prevalence of BTB in badgers in the UK (about 17 %) and a pathology consistent with excretion of large numbers of bacteria (Delahay, Cheeseman &

Clifton-Hadley, 2001). Cattle farming practices and the use of land in the UK brings badgers into close contact with domestic animals and increases the probability of disease transmission (Delahay, Cheeseman & Clifton-Hadley, 2001). An independent scientific review has identified badgers as a significant source of infection for cattle in the UK (Krebs, 1997).

Transmission between badgers probably is mostly aerogenous but the exact route of transmission from badger to cattle remains uncertain (Krebs, 1997). Environmental contamination with urine, sputum and faeces from infectious badgers is, however, thought to be the main transmission route to cattle (Delahay *et al.*, 2002).

Deer

The first case of BTB infection in farmed deer was identified in 1978 in New Zealand (Beatson, 1985) and by the early 1980s BTB was recognized as a significant problem in New Zealand (de Lisle, Mackintosh & Bengis, 2001). It has been suggested that deer may be more susceptible to *M. bovis* than cattle. Furthermore, deer appear to be more infectious for other species than cattle (Towar, 1965; Morris, Pfeffer. & Jackson., 1994; Munroe *et al.*, 2000).

In contrast to cattle, the alimentary route of infection is considered to be the most common route of infection, followed by the aerogenous route. This is shown by the distribution of lesions as well as results from inoculation studies (Lugton *et al.*, 1998; Palmer, Whipple & Olsen, 1999; Palmer, Waters & Whipple, 2003).

As in cattle, in areas where routine tuberculin testing is performed the within-herd prevalence is usually low and infected animals usually have single lymph node lesions (de Lisle, Mackintosh & Bengis, 2001). However, outbreaks with up to 50 % within-herd prevalence may occur (de Lisle, Mackintosh & Bengis, 2001). In many severe outbreaks of BTB, deer with discharging lesions have been present but have been absent when disease transmission has been low (Lugton *et al.*, 1998). It is probable that in BTB as well as in many other diseases, such as, for example bovine virus diarrhoea, certain animals are responsible for the main spread of disease (Roeder, Harkness & Wood, 1986; Lugton *et al.*, 1998).

Pathogenesis

Pathogenesis in cattle

Infection

Infection with *M. bovis* may be followed by a latency period where the bacterial load is not large enough to be cultured and no visible lesions are present. The length of this period of latency is unknown but it is assumed to be considerably shorter in cattle than in humans, where it may be lifelong. It is assumed that a large proportion of infected cattle will progress to clinical disease (Vordermeier *et al.*, 2004).

The manifestation of the disease is largely determined by the route of infection, the host immune response, the infection dose and virulence of the bacteria (Neill *et al.*, 1994b). Infection with *M. bovis* gives rise to a primary focus, from where the bacteria spreads to the regional lymph node causing a “primary complex”, consisting of a lesion at the point of entry and in the regional lymph node. From the primary focus, a post-primary dissemination may take place causing either miliary BTB or causing nodular lesions in various organs.

As most cases of *M. bovis* in cattle are acquired by inhalation, the most common sites of infection are the lungs and their regional lymph nodes.

Recently it has been suggested that the tonsillar region and the upper respiratory tract may also be an independent site of infection caused by infection either by the respiratory or the oral route (Cassidy, Bryson & Neill., 1999; Menzies & Neill, 2000) giving rise to a primary complex in these tissues and the retropharyngeal, submandibular or the parotid lymph nodes (Cassidy, Bryson & Neill., 1999). At times the primary complex is incomplete *i.e* there is no lesion at the point of entry. The fact that experimental intratonsillar inoculation in cattle causes lesions similar in distribution to natural BTB, and the knowledge that tonsils are considered an important site of infection in deer, supports this suggestion (Lugton *et al.*, 1997; Palmer, Whipple & Olsen, 1999; Palmer *et al.*, 1999).

Oral infection is less common than infection by inhalation and usually gives rise to an incomplete primary complex with lesions in the retropharyngeal or mesenteric lymph nodes (Neill *et al.*, 1994b ; Radostitis *et al.*, 2000). Although tonsillar and intestinal ulcers may occur, lesions at the site of entry are unusual (Jubb & Kennedy, 1970; Radostitis *et al.*, 2000).

Experiments have been performed to establish the sequence of infection. After intranasal inoculation, *M. bovis* was recovered from the lymph nodes of the upper respiratory tract, the tonsils and the caudal part of the lung three days post inoculation. Somewhat later, 7-11 days post inoculation, microscopic lesions were observed in the upper respiratory tract and/or lungs and regional lymph nodes (Cassidy *et al.*, 1998). Macroscopic lesions were found at the same sites 14-17 days post inoculation and in the tonsils after 21 days (Cassidy *et al.*, 1998). After intratonsillar inoculation *M. bovis* could be isolated from the medial retropharyngeal lymph node after four hours and typical microscopic lesions were found in these tissues after six weeks (Palmer *et al.*, 1999).

Lesions

The TB-lesion (tubercle) usually appears as a small whitish firm nodule. It consists of a central necrotic focus surrounded by epithelioid and giant cells (Langerhan’s type). Later, plasma cells, lymphocytes and monocytes surround the tubercle. Subsequently, the tubercle becomes surrounded by fibroblasts and a central necrosis develops and sometimes mineralisation occurs in the caseous necrotic area (Neill *et al.*, 2001).

Before the implementation of control programs, the majority of tuberculous cattle had lung lesions (Neill *et al.*, 2001). However, at present lesions are most commonly found in the thoracic lymph nodes (Corner *et al.*, 1990; Neill *et al.*,

2001). The number of cattle with lung lesions might, however, be underestimated as lung lesions may occur singly and be extremely small (< 1cm) and thereby easily being missed at meat inspection (McIlroy, Neill & McCracken, 1986; Neill *et al.*, 2001). Tuberculous lesions may also exist without concomitant lesions in the regional lymph nodes (Cassidy, Bryson & Neill., 1999). Medlar (1940) showed that approximately 10 % of reactor cattle ($n=200$) with small lung lesions had no macroscopic lesions in regional lymph nodes and in some cattle with chronic BTB-pneumonia, lesions in the regional lymph nodes may heal and therefore be absent (Neill *et al.*, 2001).

Lymph node lesions in the head region are the second most frequent site (Neill *et al.*, 2001), the medial retropharyngeal lymph node being the most common site of infection outside the thorax (Corner *et al.*, 1990; Crews, 1991; Lugton *et al.*, 1998). Lesions in this region reflect either a respiratory or an oral route of infection (Morris, Pfeffer. & Jackson., 1994).

TB-lesions occur less frequently in the mesenteric lymph nodes, reflecting an alimentary infection or swallowing of *M. bovis*-contaminated mucus from the respiratory tract (Neill *et al.*, 1988).

Recently, lesions have also been reported to occur at a relatively high frequency in the tonsils in naturally infected cattle (Cassidy, Bryson & Neill., 1999). The rarity of reports of lesions in the tonsils may correspond to the limited number of heads examined in naturally infected animals (Neill *et al.*, 2001). Lesions may also, less commonly, occur elsewhere in the body (Neill *et al.*, 2001).

Clinical findings

Infection with *M. bovis* causes a progressive disease with an underlying toxemia causing weakness and eventually death of the animal. Clinical signs may vary depending on the localisation of tubercles. Progressive emaciation, capricious appetite and fluctuating temperature are the most common symptoms. Pulmonary involvement is characterized by a chronic, low suppressed, moist cough. Mastitis is uncommon, affecting only 1% of tuberculous cattle, usually in advanced cases of BTB. In developing countries where advanced cases of BTB are more common (Menzies & Neill, 2000), mastitis is of major importance as the milk can spread BTB to humans and calves. However, in developed countries advanced cases of disseminated BTB in cattle are now rarely seen (Menzies & Neill, 2000; Radostitis *et al.*, 2000; Collins, 2000).

Pathogenesis in other species

Deer

In deer, lesions are most commonly found in the lymph nodes in the head region, the medial retropharyngeal lymph node being the one most commonly affected (Hathaway *et al.*, 1994; Mackintosh & Griffin, 1994; Morris, Pfeffer. & Jackson., 1994; de Lisle, Mackintosh & Bengis, 2001). In a study of tuberculous deer ($n=688$), BTB-lesions were most commonly found in of the retropharyngeal (52%), the ileo-jejunal (21 %), bronchial (11 %) and in the mediastinal and ileo-cecal lymph nodes (10%) (Hathaway *et al.*, 1994).

The tonsils are considered to be an important site of infection in deer, reflecting either an oral or aerogenous route of infection (de Lisle, Mackintosh & Bengis, 2001). Examination of 34 naturally infected wild red deer in New Zealand showed that tonsils were the most common site of infection (Lugton *et al.*, 1998), and experimental intratonsillar infection has been shown to closely mimic natural infection (Palmer, Whipple & Olsen, 1999). Although infected with mycobacteria, the tonsils often remain free from gross visible lesions, which might explain why the occurrence of *M. bovis* in the tonsils has previously been underestimated.

In general, the appearance of BTB-lesions in deer does not differ from that in cattle, however thin walled abscess-type lesions containing pus but with little calcification are more common in deer than in cattle (Beatson & Hutton, 1981; Clifton-Hadley & Wilesmith, 1991). Especially in the mesenteric lymph nodes, abscesses containing up to three litres of pus have been reported (Fleetwood *et al.*, 1988). Abscesses may also occur in superficial lymph nodes, which may rupture and cause significant spread of infectious material (Beatson, 1985).

In deer, there may be no clinical signs throughout the animal's life, but progressive emaciation may occur, which however may remain unnoticed until the condition is advanced or until the animal dies. Clinical signs are probably also more difficult for the owner to observe in an extensive production such as deer farming. When lung tissues are involved, coughing may occur but this symptom seems to be less prominent in deer than in cattle (Clifton-Hadley & Wilesmith, 1991). Involvement of superficial lymph nodes may be observed as swellings, mainly in the head region (Griffin & Buchan, 1994).

Zoonotic aspects of M. bovis

Only a very small proportion of tuberculosis (TB) in humans is caused by *M. bovis*. Instead, the major pathogen in human TB is *M. tuberculosis*. In developed countries, infection with *M. bovis* is now very rare (Grange *et al.*, 2001).

Cattle are considered the major source of *M. bovis* in humans (Grange *et al.*, 2001). However, at present, transmission from cattle to humans in developed countries is an uncommon event (Radostitis *et al.*, 2000). Historically, unpasteurized milk is regarded as the principal mode of transmission of *M. bovis* to humans (Grange & Yates, 1994). Less common infection routes are inhalation of aerosols from infected cattle or aerosols generated during the handling of tuberculous carcasses (Grange *et al.*, 2001). The risk of contracting BTB from meat in developed countries where hygiene at meat inspection is good is considered negligible (Francis, 1973).

In an evaluation of the public health risks of deer farming it was concluded that the risks of contracting BTB from cervidae were of occupational type; hunters, herd owners, livestock regulatory officials and abattoir workers being at risk (VanTiem, 1997). Observations on human health in an outbreak of *M. bovis* in farmed wapiti (*Cervus elaphus* var *canadensis*) supported this evaluation. It was concluded that aerosols may be created by infectious animals coughing, at examinations of lesions, or by clean-up activities (Nation *et al.*, 1999). It was also

concluded that in countries where control programs have significantly reduced the number of *M. bovis* cases, and where personal and food hygiene is adequate, the main route of exposure for humans to *M. bovis* may now be aerogenous (Nation *et al.*, 1999).

Other animals may also infect humans. For example, goats, farmed elk, seals and rhinoceros are reported to have infected humans (Fanning & Edwards, 1991; Dalovisio, Stetter & Mikota-Wells, 1992;). BTB-infected wildlife may be a risk for hunters and, furthermore, BTB-infected exotic animals kept in captivity may also be of public health significance (VanTiem, 1997; Radostitis *et al.*, 2000).

Although humans are not reservoir hosts, they may still transmit the disease. Most commonly reported is transmission by aerosol from humans with open BTB to cattle but transmission from humans with genitourinary BTB urinating in cowsheds has also been described (Magnusson, 1941; Lesslie, 1968; Grange & Yates, 1994). Investigation into the source of infection in Swedish cattle herds between 1955 and 1961 showed that between 1957 and 1961 on average two cases of BTB in cattle herds were detected annually where the source of infection was humans infected with *M. bovis*. The author noted that when the prevalence of BTB in cattle decreased, this type of transmission became more evident (Nilsson, 1962). In the most recent case of BTB in cattle in Sweden (1978), a cowman with renal tuberculosis (of unknown species of mycobacterium) had taken care of the cattle. Although it may be suspected that the cowman infected the cattle, the true source of infection was never clarified (pers. comm. Hugoson, 2004).

Human to human transmission of *M. bovis* appears to be an exceptional event (Grange *et al.*, 2001). However, in immunosuppressed people transmission might be facilitated. The potential impact of the HIV-epidemic on the epidemiology of *M. bovis* in developing countries has caused some concern (Grange *et al.*, 2001). Given a high incidence of HIV-infection in rural areas, it can not be excluded that a full cycle of transmission could be established with transmission from cattle to human beings and back to cattle again (Grange *et al.*, 2001). Such an event would lead to serious public health and economic consequences.

In humans, *M. bovis* causes a disease identical, both clinically, radiologically as well as pathologically, to that caused by *M. tuberculosis* (Grange *et al.*, 2001). However, due to differences in infection route, alimentary infection being the most common, *M. bovis* is less likely to cause pulmonary lesions. A survey performed in England and Wales between 1901 and 1932 showed that cervical lymphadenopathy due to *M. bovis* was particularly common in children, reflecting milk-borne infection (Grange *et al.*, 2001). Furthermore, it has been reported that pasteurisation of milk markedly reduced the incidence of alimentary BTB but had little effect on pulmonary TB due to *M. bovis* (Schmiedel, 1968). In rural areas, however, pulmonary disease was reported to be relatively more common than in urban areas, possibly reflecting aerogenous infection from infected cattle or from dust originating from contaminated cow sheds (Jensen, 1953 cited by Collins, 2000; Grange *et al.* 2001).

However, in late reactivation of TB due to *M. bovis*, the distribution of lesions has been reported to differ from those in primary disease. The relative incidence of

pulmonary BTB is much higher (23%) and genitourinary infection was also more common, accounting for 23% of 232 investigated cases (Grange *et al.*, 2001). This is an interesting feature when considering the possibility for humans to infect cattle.

It is often assumed, but not proven, that *M. bovis* is less virulent for humans than *M. tuberculosis*. Differences in virulence, for example, severity of disease, the ratio of infection to clinical disease, the incidence of human transmission, and the probability of endogenous reactivation, have been difficult to quantify (Grange *et al.*, 2001).

Diagnosis of *M. bovis* infection

Post mortem diagnosis

Detailed necropsy has been shown to be a sensitive method for detecting BTB-lesions in cattle. In 140 cattle detailed necropsy detected 85 % of all lesions identified by histological and bacteriological examination (Corner *et al.*, 1990). Similar figures were obtained by Norby *et al.* (2004). Routine meat inspection, used as the main surveillance method for BTB in many countries that are free or nearly free from BTB, has a lower sensitivity, estimated to be approximately 33-67% (de Kantor *et al.*, 1987; Corner *et al.*, 1990; Overton, 1994; Anonymous, 1995; Palmer *et al.*, 2000;). As could be expected, the sensitivity of meat inspection and necropsy is higher for cattle with more advanced disease. Norby *et al.* (2004) showed that necropsy detected 80 % of cattle ($n = 27$) with one lesion and all cattle ($n = 16$) with two or more lesions identified by bacteriological culture and/or PCR.

A presumptive diagnosis of mycobacterial infection can be made on microscopic examination. In direct smears from clinical samples stained by the Ziehl-Nielsen method, *M. bovis* can be seen as acid-fast rods and in histological examination of tissues, the bacteria can be seen as acid-fast rods (Ziehl Nielsen staining) surrounded by a characteristic granulomatous lesion (Anonymous, 2003b). However, a definite diagnosis of *M. bovis* requires culture and species identification. Standard bacteriological procedures, requiring about three to four weeks for the primary isolation of *M. bovis*, are used in Sweden. The Accuprobe (Gen Probe) is used to identify if the isolate belongs to the *M. tuberculosis*-complex. Complete identification of mycobacteria is done by standard procedures including Ziel-Nielsen staining, colony morphology and a panel of biochemical tests. The identification procedure requires an additional three to four weeks (Bölske *et al.*, 1995).

A more rapid confirmation of *M. bovis* can be obtained by using a PCR methodology on formalin-fixed, paraffin-embedded tissues including lesions with acid-fast organisms. The PCR-test has been reported to have a sensitivity of 93 %, using bacteriological culture as golden standard (Miller *et al.*, 1997; Norby *et al.*, 2004). However, in Sweden, PCR is not an approved method for confirmation of *M. bovis* in animals.

To identify different subtypes of *M. bovis*, genotyping can be used. It provides “added value” to conventional epidemiological methods and improves understanding of maintenance and transmission of *M. bovis* (Adams, 2001). Such methods have provided valuable information on the epidemiology of *M. bovis* in several countries, especially in New Zealand, where molecular strain typing is an integral part of the BTB-control program (Skuce *et al.*, 2001).

The progressive development of genotyping of *M. bovis* is given in Adams (2001). Initially, methods such as RFLP and REA, highly discriminating but cumbersome, were used. At present, spoligotyping is the most widely used fingerprinting method for *M. bovis* (Anonymous, 2003b). This method is simple and highly reproducible, although at present only moderately discriminating. As the method includes a PCR amplification it only requires a small amount of isolate for analysis. Further refinement of these methods is underway and elucidation of the *M. bovis* genome sequence (http://www.sanger.ac.uk/Projects/M_bovis) may offer new possibilities for strain differentiation (Adams, 2001).

For *M. tuberculosis*, an internationally accepted standard procedure has been devised and extensive strain type databases are available to facilitate global comparisons of strains. Although there is a need for a unified approach for strain typing also of *M. bovis*, no such standard procedures or databases are available (Skuce *et al.*, 2001; Adams, 2001).

Two different recommendations on a standardized protocol for fingerprinting of *M. bovis* have been proposed. The Tuberculosis in Animals Subsection of the International Union Against Tuberculosis and Lung Diseases (IUATLD) (Cousins *et al.*, 1998) recommended the use of IS6110-R restriction fragment length polymorphism (RFLP) on selected strains from geographical regions to define the *M. bovis* strains. If they contain more than three copies of IS6110, IS6110-R RFLP is sufficient for differentiation of DNA-types. For strains with three or fewer copies of IS6110 (the majority of strains), spoligotyping was recommended. If additional differentiation is needed on strains with common spoligotypes, polymorphic GC-rich sequence probe (PGRS) RFLP was recommended (Cousins *et al.*, 1998). Subtyping of *M. bovis* has also been considered in a multicenter evaluation (Skuce, 1998 cited by Skuce, 2001). The consortium recommended that spoligotyping should be used as an initial screening test. If further discrimination is needed, variable-number tandem repeat typing (VNTR), RFLP and Pulsed Field Gel electrophoresis (PFGE) methods were recommended.

In the present thesis, restriction enzyme analysis (REA), a highly discriminating method, was used (Collins & De Lisle, 1984; Collins & de Lisle, 1985). REA was first used as an epidemiological tool in New Zealand in the 1980s and is now used on a routine basis in the BTB control program in New Zealand. This method has also been used in other countries to increase understanding of disease transmission (Collins, 1999).

Ante mortem diagnosis

The tuberculin test

The tuberculin test has been used for more than 100 years and this test, applied in various formats, remains the mainstay of eradication programs for *M. bovis*. Control programs based on the tuberculin test have resulted in freedom from BTB in many countries. The test is based on the delayed type hypersensitivity (DTH) reaction, a component of the cell-mediated immune CMI response (Wood *et al.*, 2001) which is the main immunological reaction observed in *M. bovis* infection (Thorns & Morris, 1983).

The principle of the test is that a mixture of mycobacterial antigens containing purified protein derivate (PPD) is injected intradermally. After 72 hours the skin reaction of the animal is inspected visually, by palpation and (in some formats of the test) also by measurement of the skin fold thickness at the site of injection.

The major screening test is the single intradermal test (SIT). The site of injection can vary, common sites are the caudal fold (CF) (in cattle) and the cervical site (CT) (in cattle and deer). In cattle, the sensitivity to injected tuberculin has been reported to vary between different sites on the body, the cervical site being more sensitive than the caudal fold.

Table 1. *Estimated sensitivities and specificities of the tuberculin test in cattle and deer obtained from the literature.*

Species	Test	Sensitivity (%)	Specificity (%)	References
Cattle	CT	92	85	(Anonymous, 1994b)
	CF	65.5-81.9	96.3-98.8	(rev Monaghan <i>et al.</i> , 1994; Wood <i>et al.</i> , 1991; Francis <i>et al.</i> , 1978)
	CCT	77-95	96	(rev Monaghan <i>et al.</i> , 1994; Anonymous, 1994b)
Deer	CT	85	99.5	(Carter <i>et al.</i> , 1985)
	CCT	80-97	80-98.7	(Palmer <i>et al.</i> , 2001; Kollias <i>et al.</i> , 1982; Corrin <i>et al.</i> , 1993; Stuart <i>et al.</i> , 1988; Anonymous, 1990; Costello <i>et al.</i> , 1997)

The sensitivity of the tuberculin test in cattle and deer is rather low and therefore the test should mainly be used as a herd test. It is, however, due to the high specificity, suitable for screening purposes.

In herds where non-specific reactors are common, the specificity of the SIT may be considerably lower (Carter *et al.*, 1985). Such false positive reactions may, for example be caused by mycobacteria belonging to the *M. avium* complex. To avoid this, a comparative skin test (CCT) can be used. In this test both avian and bovine

PPD (PPD prepared from *M. avium* subsp. *avium* and *M. bovis* respectively) are injected (at different sites) and the host responses are compared.

The CCT is used as a complementary test to the SIT when non-specific reactors are suspected or as a primary screening test in herds or countries where non-specific reactors are common. For example, in the UK and Ireland six to 12% of cattle would be classified as reactors if a SIT were used as the primary test (Lesslie & Nancy Herbert, 1975; Monaghan *et al.*, 1994). In Sweden, the CCT is used as a primary test in cattle and other species, as avian reactors are not uncommon. The sensitivity of the CCT in cattle and deer is lower than that of the SIT but the specificity in herds with non-specific reactors is higher.

It should be noted that estimates of sensitivity and specificity of the tuberculin test obtained from different studies are difficult to compare. Many factors such as differences among the reference population (stages of the disease, exposure for environmental mycobacteria), technical variation of the test (type of tuberculin used), choice of golden standard and interpretation of the test (Monaghan *et al.*, 1994; Greiner & Gardner, 2000) may influence the test results. This is, for example, highlighted in a report from the US Department of Agriculture (USDA) where the sensitivity of the CCT in deer was reported to be higher than the CT (Anonymous, 1992).

There are several limitations of the tuberculin test. False positive reactions may be caused by non-specific reactions due to infection with, for example, *M. avium* subsp. *avium*, infection/vaccination against *M. avium* subsp. *paratuberculosis*, or occurrence of skin tuberculosis. In this context, *M. avium* subsp. *paratuberculosis* is, however, not a major problem in Sweden. Findings of *M. avium* subsp. *paratuberculosis* are compulsorily notifiable and no known infected herd exists (August 2004) (Sternberg & Viske, 2003). False negative reactions may occur in advanced cases of BTB, in early cases approximately 20-50 days after infection, 4-6 weeks after calving, in old animals, and in animals desensitized by tuberculin administration during the preceding 8-60 days, or after treatment with drugs such as dexamethazone. Furthermore, using tuberculin of low or reduced potency or injecting an insufficient amount of tuberculin may also cause false negative reactions (Francis, 1947; Monaghan *et al.*, 1994; Krebs, 1997).

Monaghan *et al.* (1994) concludes that although the specificity of the tuberculin test is high false positives may still cause problems in countries with low prevalence of BTB. Estimates of the sensitivity of the tuberculin test varies between 68 and > 95%, but these values may be reduced under field conditions. Consequently, although the intradermal test is useful for detection of infection on a herd basis, there is a need for other tests with higher sensitivity and specificity and where re-tests can be done with short intervals. Furthermore, especially in wildlife species such as farmed deer, there is a need for a test where the animals only have to be handled once (Wood & Rothel, 1994; Adams, 2001).

Antibody-based diagnostics

Antibody-based diagnostics such as the enzyme-linked immunosorbent assays (ELISA) are based on the humoral immune response, which is associated with

production of antibodies. However, in BTB there is a predominance for cellular rather than humoral immune response. Antibodies are characteristically only seen during the advanced stages of BTB and usually cannot be detected in the early stages of disease (Griffin, Nagai & Buchan, 1991; Neill *et al.*, 1994b; Wood & Rothel, 1994; Collins, 1995; Adams, 2001).

Antibody-based tests are appealing for use especially in deer but also in cattle as they require only a single handling event and, if required, can be repeated with short intervals (Livingstone, 2000; Waters, Palmer & Whipple, 2002). Furthermore, serum samples are simple to take and simple to handle on a large scale.

Studies on antibody-based tests for *M. bovis* have been performed for many years. However, mainly due to poor sensitivity they have not been able to replace cell-mediated tests (Vardaman & Larsen, 1964; Lepper, Pearson & Outteridge, 1973; Pollock *et al.*, 2001).

The sensitivity and specificity of virtually all antibody-based tests for BTB are relatively poor, possibly on account of a high degree of polymorphism in the antigen recognition and variable kinetics of the antibody response. Moreover, as antibodies are usually found only in later stages of disease, recently infected animals will not react to the test. In addition, cross reactivity with other mycobacteria may cause a low specificity (Adams, 2001). The sensitivity may be improved by prior tuberculin testing of cattle, which induces an anamnestic response about 6-7 to 16 days after the skin test (Mallman & Robinson, 1964; Wood & Rothel, 1994; Lightbody *et al.*, 1998). Lately, the use of more defined antigens such as rMPB70 have been shown to improve the specificity of the test (Fifis *et al.*, 1989).

As chronically-infected animals are occasionally anergic to the skin test, antibody-based tests could complement cell-mediated immunity tests (Collins, 1995; Adams, 2001). Such tests have been approved for use as a complementary test for BTB in New Zealand and in the USA (Livingstone, 2000; Waters, Palmer & Whipple, 2002).

Interferon-gamma assay

The interferon-gamma (IFN- γ) assay is an *in vitro* test based on the cell-mediated immune response. It detects IFN- γ in PPD stimulated whole blood cultures (Vordermeier *et al.*, 2004).

The test has a high sensitivity, can be used repeatedly and requires only a single handling of animals. Disadvantages are high costs and the need to perform the analysis within 30 hours of sampling (Livingstone, 2000).

The test was developed in 1985 and large field trials were first conducted in Australia in 1989 and 1990 (Wood *et al.*, 1991; Wood *et al.*, 2001). Eventually it was developed into a commercial kit (BOVIGAMTM) and since 1991 it is an official test for BTB in cattle in Australia (Wood *et al.*, 2001). The IFN- γ has also been approved in New Zealand as a confirmatory test for reactors, 7-30 days after caudal fold testing (Wood *et al.*, 2001).

The sensitivity of the IFN- γ assays (with PPD) has been reported to be equal to, or higher than, that of the intradermal test. In trials, the sensitivity has been estimated to be 77-96 % (Wood *et al.*, 1991; Gonzalez Llamazares *et al.*, 1999; Livingstone, 2000; Ryan, Buddle & De Lisle, 2000; Pollock *et al.*, 2000; Wood *et al.*, 2001). The use of combinations of defined, specific antigens from *M. bovis* instead of PPD may improve the sensitivity without decreasing the specificity (Wood *et al.*, 2001; Buddle *et al.*, 2003a). The IFN- γ has been reported to detect infected animals earlier than the intradermal test (Neill *et al.*, 1994a) with experimentally infected cattle becoming positive in the IFN- γ 14-28 days post inoculation (Buddle *et al.*, 1995). This might explain the increase in sensitivity compared with the intradermal test (Wood *et al.*, 2001).

The IFN- γ , using PPD, has been reported to lack sufficient specificity for widespread use as a screening test (Lauzi *et al.*, 2000). However, more specific antigens may offer an opportunity to increase the specificity. Furthermore, such antigens may discriminate between vaccinated animals and those infected with *M. bovis* (Pollock *et al.*, 2001; Wood *et al.*, 2001).

The IFN- γ test is not negatively influenced by previous skin testing. In fact, in animals sensitised to *M. bovis* the intradermal test induces an immune response resulting in an increase in the IFN- γ that is evident up to 70 days after the skin test (Wood *et al.*, 2001). IFN- γ has been reported to be a practical ancillary test for re-testing cattle 8-28 days following skin testing (Ryan, Buddle & De Lisle, 2000). Adverse nutritional conditioning of cattle will not affect the IFN- γ response but immunosuppression induced by dexamethazone administration and parturition may suppress the IFN- γ response to PPD for one week and four weeks, respectively (Wood *et al.*, 2001).

The bovine IFN- γ test has also been reported to successfully diagnose BTB in other animals, *i.e.*, Asian (*Bubalus bubalis*) and African buffalo (*Syncerus caffer*), goats (*Capra hircus*) and kudu (*Tragelaphus strepsiceros*) (Wood *et al.*, 2001). IFN- γ kits have been developed for several species including deer (CERVIGAMTM) (Slobbe *et al.*, 2000). The test was evaluated on white tailed deer and 67 of 91 (74 %) tests performed on 20 experimentally infected deer were positive (Palmer *et al.*, 2004). The author concludes that the assay may be useful in diagnosing *M. bovis* in white tailed deer. However, further studies are needed to compare the IFN- γ test with the skin test to characterize the effect of prior skin testing on the IFN- γ response and to clarify the IFN- γ response in deer co-infected with other mycobacteria (Palmer *et al.*, 2004).

Modelling infectious disease transmission

Mathematical models are tools for thinking about things in a precise way (Anderson & May, 1991). Modelling is a representation of events in quantitative mathematical terms, allowing predictions to be made about the events (Thrusfield, 1995). For example, the spread of BTB is described in the present thesis in quantitative mathematical terms and predictions are made about the spread of BTB in an “average” extensive deer herd.

A model is constructed for a specific purpose (Noordhuizen, 2001). In the present thesis, a model was developed to i) increase understanding of available data, ii) perform predictions, and iii) support decision-making. Models can also be developed to iv) more accurately define a problem, v) organize present knowledge, and vi) test knowledge (Noordhuizen, 2001).

For modelling purposes, infectious agents can be classified in two groups according to their generation dynamics, microparasites (for example bacteria and viruses) and macroparasites (for example helminths) (Thrushfield, 1995). Microparasitic infections are often modelled by a prevalence model which considers the absence or presence of infection in the hosts (Thrushfield, 1995). Macroparasites are usually modelled with density models that include information on the distribution of parasites among hosts (Anderson & May, 1991). Density models are not considered further in the present thesis.

Models can be classified as linear/non-linear, static/dynamic and deterministic/stochastic (Noordhuizen, 2001). In a static model the population is constant, whereas in a dynamic model the population size changes and the outcome is dependent on the population size and on individuals introduced or excluded from the population (Noordhuizen, 2001). In a deterministic model, the outcome relies only on the values of the parameters, therefore the output for a given set of inputs is always the same (Martin, Meek & Willeberg, 1987). If the parameter(s) vary by chance then the model is stochastic (Noordhuizen, 2001). Each run creates a different result due to chance and the average of these reflects the most probable outcome (Giesecke, 2002). A deterministic model may predict the eradication of a disease, the stochastic version may predict that eradication will occur only in 60% of simulations (Smith *et al.*, 2001). In a model, time can be treated as a discrete or continuous variable. A model where time is discrete can only have outputs at specific points in time. A model where time is continuous can have output at any given time and the model will consist of differential equations (Noordhuizen, 2001). In the present thesis, non-linear, dynamic models were used, deterministic in paper IV and stochastic in paper V. In both models, time was treated as a discrete variable.

In state transitional or compartmental models, the population of hosts might be divided into several classes where the distribution of hosts over classes changes over time (Anderson & May, 1991; Thrushfield, 1995). Hosts may be split into several classes, e.g. susceptible (S), infectious (I) recovered (e.g. immune) or removed (R) and latent infection (E) (Noordhuizen, 2001). In the present thesis, an SI-model was used in paper IV and an SIR-model in paper V.

Models may have a constant probability of infection or variable transition probabilities (Noordhuizen, 2001). For example, in an SI-model with constant probability of infection, the number of individuals that become infected (at time $t+1$) depends only on the number of susceptible individuals (at time t) multiplied by a fixed probability (Noordhuizen, 2001). This system can be modelled using a process called the Markov chain (Martin, Meek & Willeberg, 1987). In the more general model with variable transition probabilities, the number of infectious animals has an effect on the transition probabilities. Thereby, the transition

probabilities are not constant over time. A well-known example of this is the Reed Frost model.

In models with variable transition probabilities, disease transmission can be frequency dependent or density dependent. In frequency dependent disease transmission, the infection rate, *i.e.* the average number of individuals that are newly infected from an infectious individual per unit of time, can be assumed to be constant and independent of population size. Thereby the *number* of contacts of each animal with other animals is independent of population size and the *probability* of contact decreases as population size increases (de Jong, Dieckmann & Heesterbeek, 1995; Begon *et al.*, 1999; Noordhuizen, 2001). Frequency dependent transmission has been reported to give a better description of the within-species transmission than density dependent transmission (Bouma, de Jong & Kimman, 1995; Begon *et al.*, 1999). In the alternative, density dependent disease transmission, the number of contacts between infectious and susceptible individuals is related to the population size. This seems unreasonable as territorial animals may make a relatively fixed number of contacts regardless of population size (Smith *et al.*, 2001). In the present thesis the infection rate is assumed to be independent of population size.

Assumptions underlying any model must be clearly stated as no model will be better than the assumptions on which it is built. A sound decision, based on the outcome of a model, can only be made by a user knowing the limits of, and the assumptions underlying, the model (Noordhuizen, 2001; Giesecke, 2002).

Modelling BTB in cattle and in deer

Numerous papers modelling transmission of BTB in wildlife have been published. Fewer have modelled transmission of BTB in cattle and even fewer in (farmed) deer (Smith *et al.*, 2001).

The within-herd transmission of *M. bovis* has been modelled with a density dependent SEEI¹ model and the transmission rate was estimated based on historical data from New Zealand cattle. The model predicted that in a herd of around 200 cattle the contact rate (number of infectious contacts made per infectious cow per year) was about 2. It also predicted that external input was needed to maintain the infection (Barlow *et al.*, 1997). Furthermore, Barlow *et al.* (1998) developed a complementary deterministic SI model including between-herd transmission. The model predicted that the rate of detection of infected herds only slightly exceeded the rate of between-herd transmission. Reducing the testing interval to one year at the most would effectively reduce the percentage of herds under movement control due to BTB. The model contributed to the adoption of yearly testing in the investigated region in New Zealand.

A density dependent, deterministic, SEEI-model, including external infection and vaccination, predicted that external infection must be reduced in order to decrease the prevalence of BTB in cattle in New Zealand. A combined strategy

¹ Four classes, one susceptible (S), two latent (E) and one infectious (I)

including vaccination of cattle and vector control was suggested as the most efficient strategy (Kao, Roberts & Ryan., 1997).

Kean, Barlow & Hickling (1999) used a density dependent (cattle) and density independent (wildlife) model to describe transmission of BTB in cattle in New Zealand and concluded that within-herd transmission accounted for 20-32 % of all infections and supported the policy of wildlife control as the most effective method for reducing BTB in cattle in such areas.

Perez *et al.* (2002a) used a stochastic, density dependent, SEIR Reed Frost model, similar to the models used in papers IV and V, to estimate the transmission of BTB in Argentine dairy herds. The same model was used by Perez *et al.* (2002b) to evaluate different control strategies in dairy herds, all based on a test and cull approach with different tuberculosis tests. The model predicted that eradication was most efficiently achieved using the CF or IFN- γ test. However, when prevalence was $\geq 22\%$ depopulation of the whole herd was most efficient.

Models describing transmission in deer are scarce. McCarthy & Miller (1998) used a stochastic model, where disease transmission was density independent, to describe the spread of BTB in free-ranging white-tailed deer in Michigan, USA. Fawn survivorship was adjusted, sex-biased transmission was introduced and pseudo-vertical transmission allowed to obtain a better fit of the model to observed historical data. The model predicted that the prevalence would rise from approximately 3% to about 21% over 25 years and that lowered survival or transmission would not eradicate the disease.

Epidemiological aspects of detection of infection and documentation of freedom from infection

The interest in documentation of disease status has increased with the establishment of World Trade Organisation and the Sanitary-Phytosanitary agreement requiring that any animal health related trade restriction must be based on risk assessment (Hueston & Yoe, 2000). International agreements are available for several diseases, defining criteria for obtaining and maintaining freedom from infection on a herd or a country basis (www.oie.int/eng/normes/mcode/a_00058.htm; accessed 22-Aug-2004, http://europa.eu.int/eur-lex/sv/consleg/reg/sv_register_035030.html; accessed 9-Sept-2004). Often these requirements define fixed testing regimes, which is an inflexible and probably not always the most cost-efficient approach (Cannon, 2002).

Documentation of freedom from infection usually relies on passive surveillance (Toma *et al.*, 1999; Doherr & Audige, 2001) as active systems such as screenings with tests often require unrealistically large samples to obtain a reasonable level of confidence. Even if tests with a high specificity are available, large screenings may cause problems with false positive results (Baldock, 1998; Crauwels *et al.*, 1999; Paisley, Tharalden & Jarp, 2000). As the quality of passive surveillance may vary, there is a need for evaluation of such a self-reporting system (Baldock, 1998; Doherr & Audige, 2001).

There is also a need to be able to estimate the combined effect of passive and active surveillance. Although not available at present, analytical methods to combine surveillance data from several sources to estimate a probability of disease freedom, are under development (Doherr & Audige, 2001). Such systems would allow for more flexibility in demonstrating freedom from infection than existing international rules, as they would focus more on “what needs to be shown” rather than “how to do it” (Cannon, 2002).

Control strategies for *M. bovis* and documentation of freedom

For a disease like BTB, which can lie dormant for many years, it is difficult to define when a country is free, *i.e.* when *M. bovis* is eradicated from the surveilled population. The OIE definition of freedom does not require absolute freedom from infection but requires that 99.8% of herds shall have been officially free from BTB for at least the past three years. The World Trade Agreement requires countries to prove absence of disease but no agreement has been reached regarding the acceptable level or duration of surveillance (Cousins, 2001).

Test and slaughter strategy

Control and eradication programs based on tuberculin testing and removal of infected animals or herds have led to the eradication or major reduction of the prevalence of BTB in cattle in many countries (Cousins, 2001). Later, other tests such as IFN- γ , ELISA have been included in control programs as ancillary tests to the skin test (Vordermeier *et al.*, 2004). However, the existence of wildlife reservoirs has shown to be a serious barrier to eradication of BTB. Collins *et al.* (2001) suggested that a complementation of the classical test and slaughter strategy with a judicious use of new tests, new information technologies such as geographical information systems, better risk communication and a rational approach to removal of wildlife reservoirs would offer a better opportunity for success (under Irish conditions).

A test and slaughter strategy have successfully been applied to farmed deer to eradicate BTB in this species in Sweden and Denmark (www.sva.se; accessed 20-Aug-2004; <http://www.dfvf.dk>; accessed 9-Sept-2004) and to control *M. bovis* in deer in New Zealand, USA and Canada (de Lisle, Mackintosh & Bengis, 2001; <http://www.inspection.gc.ca/>; accessed 22-Aug-2004; <http://www.aphis.usda.gov/vs/nahps/tb>; accessed 28 Aug-2004). Ancillary tests such as IFN- γ , ELISA are also used in control programs in deer (Vordermeier *et al.*, 2004).

Abattoir inspection methods

Monitoring of lesions consistent with BTB at postmortem examination is an important part of any BTB control program in cattle and in farmed deer. Furthermore, after completion of an eradication program, documented freedom from infection usually relies on meat inspection. However, meat inspection can also be used for surveillance when routine testing of live animals is not possible, for example, in farmed and wild deer (V) (<http://www.aphis.usda.gov/vs/nahps/tb>;

accessed 28 Aug-2004; O'Brien *et al.*, 2004). If abattoir inspection is to be effective, inspectors must show diligence, be well trained, examine the correct tissues and submit identified granulomas for laboratory examination (Cousins, 2001). The efficiency of the surveillance also needs to be evaluated. For example, in the USA, APHIS has identified the need for enhanced surveillance at slaughter (increased submission rate of granulomas) to identify remaining pockets of infection (http://www.aphis.usda.gov/vs/nahps/tb/tb_surveillance.pdf; accessed 10 Sept-2004).

Vaccination and wildlife control

In areas where a wildlife reservoir exists, eradication of *M. bovis* may not be achieved by a test and slaughter strategy. In such areas, vaccination of cattle and farmed deer may contribute to the eradication of BTB (Buddle *et al.*, 2003b). In the UK, an independent scientific review panel concluded that the development of a vaccine for cattle would be the best option for long-term control of BTB in cattle (Krebs, 1997).

However, in the UK, the problem is only finally solved when the wildlife reservoir is controlled (Buddle *et al.*, 2003b). The UK report also recommended that vaccination of badgers should be considered (Krebs, 1997). In New Zealand, where possums are the major wildlife reservoir for *M. bovis* infection, wildlife control has been shown to be effective. Possum control from 1994 to 2001, using poisoning, has resulted in a large reduction in BTB in cattle and farmed deer (Livingstone, 2002). But there are several problems associated with poisoning, for example areas with endemic BTB can rapidly be re-colonized with possums when poisoning is discontinued. The most promising option for eliminating BTB in possums therefore probably includes vaccination of this species (Skinner *et al.*, 2001).

Vaccination of cattle with BCG seems to slow down the spread of disease but not prevent infection. It has been found to decrease the infectiousness of infected animals (by reducing the number of lesions and the burden of mycobacteria). However, in some studies, vaccination of cattle with BCG has shown little effect (Suazo, Escalera & Torres, 2003). In one trial, vaccination of deer with BCG was found to be highly effective against disease (Griffin *et al.*, 1999). Moreover, a drawback of the BCG vaccine is that vaccinated animals may react positively to the skin test (Buddle *et al.*, 2003b). However, new vaccines that do not interfere with the skin test, and new tests that can discriminate between vaccinated and infected animals, may become available in the future (Suazo, Escalera & Torres, 2003)

Aim of the present dissertation

The overall aim of this dissertation has been to address questions arising in connection with eradication of BTB in farmed deer herds in Sweden.

- i) To describe the introduction of BTB in Swedish deer farms and the epidemiological investigations performed to identify the source and the spread of infection (I),
- ii) To establish quality indicators (reference values for the measurements of the skin fold, prior to injection of the bovine tuberculin) for the comparative cervical intradermal tuberculin test in deer (II),
- iii) To establish quality indicators (a method to evaluate the within-veterinarian variation when measuring the skin fold, prior to injection of the bovine tuberculin) for the comparative cervical intradermal tuberculin test in deer (III),
- iv) To describe the within-herd spread of BTB in seven extensive Swedish deer herds by using a Reed-Frost model (IV), and
- v) To predict the spread of BTB in an average extensive Swedish deer herd given the introduction of one infected animal and to evaluate the efficiency of slaughter and meat inspection as a tool for surveillance for BTB in such herds (V).

Summary of materials and methods

For a more detailed account of the materials and methods used in these studies the reader is referred to relevant sections of papers I-V.

Paper I

An epidemiological investigation was performed to identify the source and the spread of infection with *M. bovis* introduced into Swedish farmed fallow deer herds in 1987.

All animals in the ten Swedish deer herds infected by July 1994 were destroyed and selected necropsies performed to determine the source of infection. To further characterize the *M. bovis* isolates, RFA as described by Collins & de Lisle (1985) and Collins & de Lisle (1984) were performed on eight isolates from five of the infected herds.

Moreover, after a consignment of 168 imported fallow deer had been identified as the source of infection, all identifiable imported deer from this consignment were killed, submitted for autopsy, histopathology and bacteriological culture.

A thorough investigation was performed to identify all in-contact herds of the tuberculous herds. In-contact herds were investigated by tuberculin testing, slaughter and meat inspection, or killing and necropsy of varying numbers of animals, including whole herds.

Paper II

Reference values for the measurements of the skin fold in the tuberculin test, prior to injection of the tuberculin, were established by fitting a general linear model to data on farmed deer, tuberculin tested within the BTB-control program between December 1991 and April 2003. The unit of concern was the measurement of the skin fold thickness prior to injection of the bovine tuberculin.

Variables considered for analysis were: *measured thickness of the skin fold* (ST), *the age of the tested deer* (AGE), *gender of the deer* (SEX), and *species of the deer* (SPC) and *testing veterinarian* (VET). Analyses were performed on data in dataset 2A ($n=6,374$) (Figure 2 and Table 1 in paper II) including only measurements of the first tuberculin test on each deer. As adult male red deer had a larger variance (in measurements of the skin fold thickness), the dataset was divided into two subsets (3A $n=6,211$ and 3B $n=163$) and separate analyses were performed on these datasets. The variables AGE, SEX, SPC and VET were included as fixed factors in a general linear model.

The model was formulated as:

$$ST = AGE + SEX + SPC + VET$$

The statistical analysis was performed with SAS version 8e (SAS, 2002).

Predicted values for each of six groups of deer were calculated. To obtain predicted values of the skin fold thickness for an “average” veterinarian the median parameter values for the variable VET (in dataset 3A and 3B) were used in the calculations.

The 95 % population intervals of the measured skin fold thickness were calculated using the mean square error (*MSe*)-values as estimators of the variance, obtained when fitting the model to datasets 3A and 3B, respectively.

The population intervals were calculated as:

$$\text{Predicted value} \pm 1.96\sqrt{\text{MSe}}$$

To assess the usefulness of the obtained population intervals, they were validated on dataset C, $n=7,613$.

Paper III

The within-veterinarian variation for each of 64 testing veterinarians was estimated by fitting a general linear model to data on skin fold measurements in farmed deer for each veterinarian.

The study population and the unit of concern were the same as in paper II. Variables considered for analysis were *measured thickness of the skin fold* (ST), *the age* (AGE), *gender* (SEX), and *species of the deer* (SPC) and *herd* (HERD). Analyses were performed on data by each of the 64 veterinarians in datasets 3A:1-3A:64 (Figure 1 in paper III), including only unique records of the first tuberculin test on each deer. Variables AGE, SEX, SPC, HERD were included as fixed factors in a general linear model. The model was formulated as:

$$\text{ST} = \text{AGE} + \text{SEX} + \text{SPC} + \text{HERD}$$

Estimates of the within-veterinarian variation (*MSe*) for each veterinarian were obtained by fitting the models to data obtained by each of the 64 veterinarians.

To evaluate if any of the 64 *MSe*-values were extreme, assessment of the *MSe*-values was performed in two ways. First the *MSe*-values were depicted as a histogram and subsequently a more comprehensive evaluation was performed in the following way.

Suppose we have a sample, *i.e.* a set of independent, identically distributed random observations from a distribution with a known cumulative distribution function (cdf). The cdf for the i^{th} largest observation in an ordered sample is given by

$$F_{[i]}(x) = P(X_{[i]} \leq x) = \sum_{j=i}^n \binom{n}{j} F^j(x) (1 - F(x))^{n-j}$$

$$i = 1, \dots, n$$

where $F(x)$ is the cdf of the individual observations.

The cdfs of the ordered sample can be used to decide if an observation, in a sample of the same size sampled from the same distribution, is indeed to be considered to be an outlier.

In the present study, to evaluate if the *MSe*-values were in fact extreme, they were standardized and compared with the 95th percentile of the distributions of the highest to the fifth highest observations from the standard normal distribution $N(0,1)$.

To evaluate if the difference in the magnitude of the *MSe*-values could be caused by the unequal distribution of the proportions of deer species tested by the veterinarians, the models were also fitted to data by 45 veterinarians in dataset B ($n=5,192$) (Figure 1 in paper III). Dataset B is a subset of dataset 3A including only measurements on fallow deer. Similar analyses of the *MSe* values were performed on these 45 *MSe* values.

Paper IV

The within-herd spread of BTB in seven Swedish extensive deer herds were described by fitting Reed-Frost models to data from these herds.

A total of 13 BTB-infected deer herds were identified in Sweden by December 1997 and all animals were killed. Epidemiological investigations including comprehensive autopsies of selected animals and tuberculin tests of in-contact herds were performed to identify the source and spread of infection. A deer was considered infected if *M. bovis* was isolated. Based on these results, the number of introduced and subsequently infected deer could be estimated in seven herds (herd nos. 1, 2, 5, 8 and 9 in paper I, and two of the three herds detected after publication of paper I).

Disease transmission was modelled using techniques described by Carpenter (1984).

A modified Reed-Frost model:

$$C_{t+1} = S_t * (1 - q^{CC_t})$$

was constructed, where C_{t+1} = number of newly infected deer in time period $t+1$, S_t = number of susceptible deer in time period t and $1 - q^{CC_t}$ = the probability that a susceptible animal will become infected in time period $t+1$ (Abbey, 1952).

The model consisted of two states: infectious (I) and susceptible (S). Input variables for the model were: number of introduced infected deer (C) and number of infected deer at depopulation ($CC_{(depopulation)}$), population size (N), number of calves born per time period (B), number of slaughtered animals per time period (L). A time interval (t) of one year, reflecting the average length of the incubation period, was used.

The k -value (the number of effective contacts made by an individual during time period t that would result in an infection if one individual was infected and one

susceptible) that optimised the fit between the predicted and observed incidence of BTB in each herd were evaluated.

The effect of imperfect tests on the k -values was evaluated by calculating the true number of newly infected deer, assuming a sensitivity of 0.7 for the tuberculin test and for necropsy. This calculation was performed on data from the three herds where spread of infection was observed.

Paper V

The expected within-herd spread of BTB in an “average” Swedish extensive deer herd after introduction of one infected deer was described by a Reed-Frost model.

The model consisted of three states: susceptible (S), infectious (CC) and slaughtered (L). Input variables for the model were: number of effective contacts (k), population size (N), number of calves born per time period (B), percentage of animals slaughtered per time period (L), the sensitivity for meat inspection (se_m). The time period (t) was set to one year.

By applying the binomial distribution to the Reed-Frost equation used in paper IV, we allowed the number of deer becoming infected to be random. The number of newly infected deer was calculated by using @RISK (Palisade Corporation, Newfield, NY), an add-in program to Excel (Microsoft, Redmond, WA):

$$C_{t+1} = \text{RiskBin}(S_t, 1 - q^{CC_t})$$

where C_{t+1} = number of newly infected deer in time period $t+1$, S_t = number of trials, where we think of a trial as determining whether a susceptible individual becomes infected or not, and $1 - q^{CC_t}$ = the probability that a susceptible animal will become infected in time period $t+1$.

The estimate for k used in the present study was described by a discrete probability distribution (Vose, 1996) using the eight estimates of k obtained from paper IV. In the model, a single k_t was randomly selected from the above-mentioned distribution for all time periods for each herd (*each simulation*). Estimates of the sensitivity of meat inspection for detecting BTB were based on information from the literature. The overall sensitivity of meat inspection in time t was assumed to follow a BetaPert distribution (Vose, 1996). When running the model, a new estimate was sampled from this distribution each time period (t) in each herd (*each simulation*) because the sensitivity of meat inspection was assumed to be randomly distributed between time periods as well as between herds.

The number of slaughtered infected deer was assumed to follow a hypergeometric distribution (Vose, 1996). The annual probability of detecting a BTB-infected herd through meat inspection was calculated according to the binomial distribution.

An estimate of the probability of a percentage of herds (*simulations*) either detecting or eliminating BTB was obtained by using @RISK. The model was run to simulate 15 years.

Finally, a sensitivity analysis was performed to evaluate how the outcome of the model was affected by changes in four variables: number of effective contacts (k), meat-inspection sensitivity, percent of deer slaughtered annually, and herd size.

The output of the model was the probability that either BTB was detected in a herd at meat inspection or that all infected deer in the herd were culled. The results were expressed as the minimum probability of the occurrence of this event in different proportions of herds (*simulations*).

Summary of results

For a more detailed account of the results of these studies the reader is referred to relevant sections of papers I-V.

Paper I

The epidemiological investigation identified 10 infected deer herds (by July 1994). All infected herds contained deer that had been part of, housed or farmed with, a group of 168 farmed fallow deer imported into Sweden in 1987.

All analysed *M. bovis* isolates from the infected herds showed identical patterns of DNA fragments. Identical isolates had previously been found in the country of origin of the imported animals although no direct connection could be identified with the farms of origin and the group of deer imported into Sweden. No other possible common source of infection could be identified. The consignment of 168 imported deer was therefore considered to be the source of infection.

Paper II

The 95% population intervals of the measured skin fold thickness for an “average veterinarian” were 2-4.5 mm (female red deer), 1-6 mm (young male red deer), 2.5-7 mm (adult male red deer), 1.5-4 mm (female fallow deer), 1.5-4 mm (young male fallow deer) and 2.5-5 mm (adult male fallow deer).

Validation of the population intervals on dataset C showed that 89 % (female red deer), 94 % (young male red deer), 92 % (adult male red deer), 98 % (female fallow deer), 87 % (young male fallow deer) and 88 % (adult male fallow deer) of the measurements in dataset C were included in the estimated 95 % population intervals.

Paper III

Descriptive statistics indicated that measurements by one veterinarian had unexpectedly large within-veterinarian variation, as reflected by the *MSe*-value, and an additional four veterinarians seemed to have unexpectedly large within-veterinarian variation (datasets 3A:1-3A:64). In datasets B:1-B:45, measurements by two veterinarians appeared to have unexpectedly large within-veterinarian variation.

Results of the further assessment of the *MSe*-values are given in Table 3 in paper III. Four standardized *MSe*-values from the subsets of dataset 3A were judged to be unusually large, *i.e.* lay outside the 95th percentile. In the subsets of dataset B, the highest *MSe*-value was large (outside the 88th percentile) and very close to the 2nd largest *MSe*-value, which was considered an outlier. It therefore seemed reasonable to consider both the two highest *MSe*-values as outliers.

Paper IV

The number of infected deer could be estimated in seven of the 13 BTB-infected herds. The number of effective contacts made by an individual, in a specified time interval, which would result in an infection if one deer were infectious and the other deer susceptible (k), was used as a measure of disease transmission.

The estimated k 's, obtained when fitting a modified Reed-Frost model to data from the seven herds, varied between ≤ 0.07 and 0.6. When accounting for an assumed test sensitivity of 0.7 in the three herds where spread of BTB was observed, the k 's increased and varied between ≤ 0.07 and 0.9. As the changes in obtained k 's were small, we decided to use a test sensitivity of 1.

In four of the seven herds where no spread of infection could be observed, the k that corresponded to transmission of disease to one deer at the time of depopulation was calculated. It was concluded that the estimated k 's in these herds were equal to or smaller than this value.

Paper V

Simulation results produced measures of the probability of a given percentage of herds achieving either detection or elimination of BTB in a given period of time. For example, assume that in 1000 of the 10,000 iterations (simulated herds), the probability of either detecting or eliminating BTB after five years was at least 88%. This would be reported as "in 10% of the herds there was an 88% or better probability that BTB would be detected or eradicated after five years".

Our model predicted a 100% probability of detecting or eradicating BTB seven years after its introduction in 50% of the herds. Furthermore, it predicted that 15 years after introduction of BTB, in the vast majority (90%) of herds the infection would be detected or eliminated, and in almost all herds (95%) it would be detected or eliminated with a high probability ($\leq 94\%$).

The sensitivity analysis showed that if k increased the probability of detecting BTB would also increase. It also showed that if the sensitivity of meat inspection decreased or if the herd size was under estimated (resulting in a decreased annual percent of animals slaughtered), the probability of detecting BTB would also decrease.

General discussion

Epidemiological investigation of the BTB outbreak (I)

After BTB was first detected in farmed deer in Sweden, an epidemiological investigation identified a common source of infection, a consignment of 168 fallow deer imported in 1987 (Bölske *et al.*, 1995) (I). At present, (August 2004) a total of 13 infected herds have been identified, the most recent in 1997. Additional information obtained after publication of paper I supports the hypothesis of a common source of infection (Figure 1).

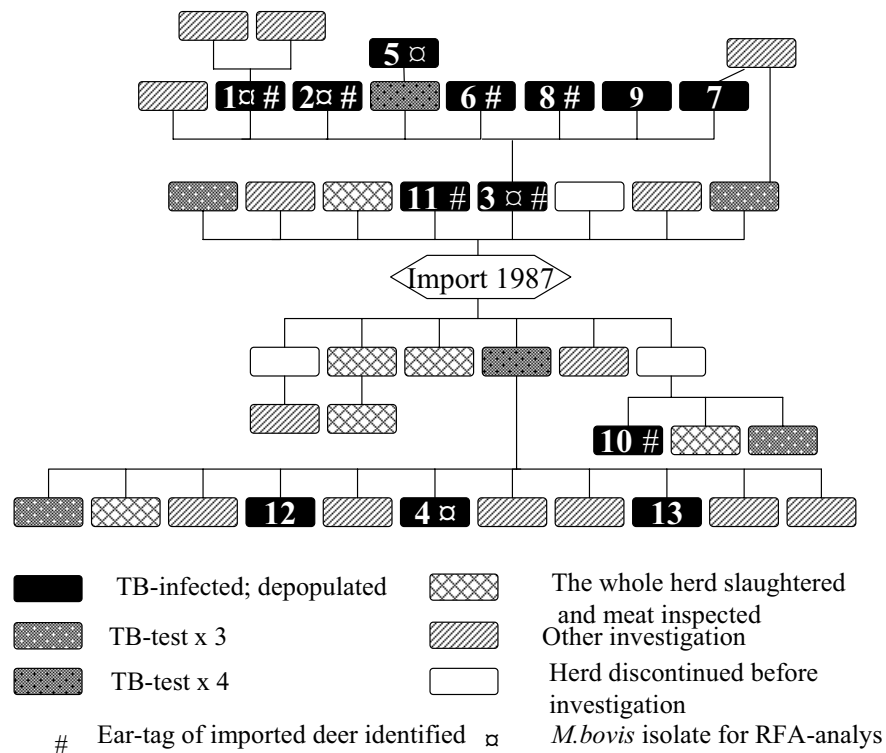


Figure 1. Deer farms with direct or indirect contact with a group of imported fallow deer identified as the common source of infection of 13 BTB-infected deer herds. Each line represents suspected or proved movement of deer from quarantine and outwards. The order of the number reflects the order of detection and herds (Englund, L., unpublished results).

Based on the results of paper I it seemed desirable to perform a complete trace-back on all imported deer. However, due to deficiencies in identification of deer and lack of herd records this was not possible. Mandatory individual identification of animals is a prerequisite for traceability and thereby also a prerequisite for disease detection and investigation capabilities of any country (Lees, 2004). Had mandatory identification of deer been in place, as was the case in an outbreak of BTB in farmed deer in Canada, all in-contact herds might have been identified

(Nation *et al.*, 1999). However, as such regulation did not exist, a control program involving all Swedish deer herds had to be implemented to be able to identify remaining infected herds and document BTB-freedom in Swedish farmed deer.

Results obtained in paper I were used for optimal allocation of resources, *i.e.* investigations were concentrated on imported deer and their possible contacts, and thereby some infected herds were detected earlier. Any potential spread between herds was stopped as movement of live animal from deer herds was prohibited. However, the more frightening scenario, spread of BTB from deer to badgers could not be neglected. It was therefore vital to identify and depopulate all infected herds as soon as possible. Increased surveillance on badgers has been implemented and fortunately there are no indications that such spread has occurred.

Reflections on the quality of surveillance systems for BTB (II, III and V)

Control and eradication as well as surveillance aiming at documenting freedom from BTB on herd or country basis rely mainly on three tests: the tuberculin test, meat inspection and, to a lesser extent, clinical surveillance. The latter is upheld mainly through the vigilance of veterinarians and owners and their ability to identify suspected cases of clinical BTB. In this thesis, the word “test”, as suggested by Somoza & Mossman (1992), means any device that reduces the uncertainty about the disease state.

The sensitivities of these three tests are often uncertain as they largely depend on human judgement and human performance of the tests. Especially in tests such as meat inspection and clinical surveillance, where the non-detection rate may be high, observed changes in prevalence may, in fact, be due to changes in the sensitivity of the tests (Toma *et al.*, 1999; Doherr & Audige, 2001; Enoe *et al.*, 2003).

The tuberculin test

At present, there is no single test with a high sensitivity as well as a high specificity allowing for efficient screening of BTB. This is in contrast to other diseases such as classical swine fever and Aujeszky's disease, where tests performed in laboratories with approved quality assurance systems ensuring high precision (reproducibility) and accuracy (validity) are available. The sensitivity and specificity of the tuberculin test have been evaluated several times (Monaghan *et al.*, 1994; Adams, 2001) and estimates obtained vary substantially depending on several factors, among which are human judgement and human performance. Examples of deficiencies in performing the test are: inadequate preparation of the site of injection, variation in the pressure applied to a manual calliper, variation in the amount of tuberculin injected, lack of experience and lack of carefulness when performing the test (Hunter, 1984; Monaghan *et al.*, 1994). Lack of carefulness may, for example, be due to lack of confidence in a BTB-control program or in the tuberculin test itself (for example if numerous false positive reactors occur)

(Monaghan *et al.*, 1994). Other factors that may influence the consistency of tests are poor facilities for restraint and perhaps unsuitable working environment, such as testing deer during winter months (Collins, 1985). As it is easier to gather deer during winter when pasture is scarce, most testing in Swedish deer herds is done during this period (Figure 3 in paper I).

Downey (1992) concluded that “defective and careless testing have undoubtedly contributed to the lack of progress” in the Irish BTB-program (cited by Anonymous, 1994a) and Menzies & Neill (2000) pointed out that it is very difficult to determine the diagnostic deficiencies of skin testing. Furthermore, Monaghan *et al.* (1994) stressed the necessity of continuous monitoring of the tuberculin test to minimise factors that could reduce its efficiency.

To reduce variation, it is essential that the tuberculin test is performed with great care (Griffiths, 1990; Hunter, 1984). However, in measurements involving human judgement, variation can be large and difficult to control (Fletcher, Fletcher & Wagner, 1996). Application of the tuberculin test in deer is technically more challenging than in cattle because deer have thinner skin requiring greater precision for the intradermal injection to be carried out properly (Carter *et al.*, 1984; Hunter, 1984).

Reports on evaluations of the act of performance of the tuberculin test are scarce. Hunter (1984) reported that examined the preparation of the test site shortly after testing identified deficiencies. No evaluation of the repeatability of the tuberculin test has, to the author’s knowledge, been published. Such an evaluation is also difficult to perform, compared with serological tests, as the tuberculin test cannot be repeated until after 40-60 days (Monaghan *et al.*, 1994). However, in New Zealand a quality assurance program for BTB in deer, states that testing veterinarians shall be practically assessed, being able to repeat a measurement of the skin fold to the satisfaction of an assessor and also within 0.5 mm over three measurements.

To obtain more reliable results from surveillance, a built-in validation is needed (Toma *et al.*, 1999; Doherr & Audige, 2001; Enoe *et al.*, 2003). In paper II we estimated reference values for pre-measurement of the skin fold thickness in red deer and fallow deer. These reference values can be used especially by veterinarians with less experience in tuberculin testing as self-evaluation to identify unexpected measurements and correct potential deficiencies in the testing procedure.

In paper III we propose a method for estimating the quality of performed tuberculin tests. Using such an approach, it is possible to identify veterinarians with unusually large variation in pre-measurements and thereby possible deficiencies in their testing procedure may be corrected. However, it is probable that implementation of such a control also might have an additional effect. When veterinarians are aware that their measurements are evaluated, testing might be performed with (even) greater care.

However, closer studies of the testing procedures of the veterinarians with high *MSe*-values might reveal other factors, not considered in our study, that may be associated with high *MSe*-values. In that case, such variables could be included in

the model and the analysis repeated. It is, however, important to point out that the four veterinarians with unusually high *MSe*-values in paper III were identified because their measurements were abnormal as defined by Fletcher, Fletcher & Wagner (1996). The relation between being abnormal and having deficits in the testing procedure remains to be documented. Therefore, this relation should be clarified before using *MSe*-values for quality control on a larger scale.

It is suggested that the results of papers II and III can be used to improve the over-all quality of the performance of the tuberculin-tests in the BTB control program in Swedish farmed deer.

Post mortem inspection

In the present thesis, the evaluation of the quality of tests focuses on the tuberculin test. However, meat inspection, considered an efficient tool for BTB-surveillance and therefore the main test used in the final stages of an eradication program and for documenting freedom from BTB, also relies on human skill and judgement (Lees, 2004). The sensitivity of a post mortem examination may vary and routine meat inspection has been shown to have a lower sensitivity for detecting BTB than autopsy (McIlroy, Neill & McCracken, 1986; Corner *et al.*, 1990).

As for the tuberculin test, a built-in validation is needed to obtain more reliable results (Toma *et al.*, 1999; Doherr & Audige, 2001; Enoe *et al.*, 2003). Such systems are in place in the USA, Canada and Australia (Anonymous, 1992; <http://www.aahc.com.au>; accessed 9-Sept-2004; <http://www.inspection.gc.ca/>; accessed 22-Aug-2004; <http://www.inspection.gc.ca/>; accessed 22-Aug-2004). Submission rates (number of submissions of BTB-suspect lesions (granulomas) divided by the number of slaughtered cattle) are continuously compiled and evaluated. A minimum submission rate (one lesion per 1000 or per 2000 adult cattle slaughtered) has been established (<http://www.aahc.com.au>; accessed 9-Sept-2004; <http://www.inspection.gc.ca/>; accessed 22-Aug-2004). In the USA, submission rates have been shown to vary substantially between slaughterhouses (Anonymous, 1992). Although parts of this variation may be due to factual differences in the occurrence of granulomas in slaughtered cattle, for example due to differences in exposure to bacteria causing granulomatous lesions, it is possible that the sensitivity of the meat inspection varies between slaughterhouses. Financial incentives have been implemented to increase submission rates (http://www.aphis.usda.gov/vs/naahps/tb/tb_surveillance.pdf; accessed 10 Sept-2004). Monitoring of granuloma submission rates has also been identified as a means of quality control of meat inspection in an opinion of the Scientific Panel on Biological Hazards of the European Food Safety (Anonymous, 2003b).

In paper V, it was shown that if the sensitivity of the meat inspection decreased then there was also a decrease in the probability to detect BTB. To ensure the validity of the prediction of paper V, submission rates for deer should be compiled and analysed herd-by-herd and veterinarian-by-veterinarian. If possible, a minimum submission rate should be specified. In addition, it would be desirable to evaluate the sensitivity of meat inspection under present conditions to ensure that it is not lower than that used in the assumptions of the model.

Such monitoring would probably detect changes in the awareness of inspecting veterinarians. If the disease prevalence decreases, and thereby the probability that granulomatous lesions are indeed caused by *M. bovis*, it may be expected that the awareness of the inspecting veterinarian also decreases. A compilation of the annual number of submission of lesions from deer to the National Veterinary Institute for *M. bovis* investigation (smears or culture) indicates that the awareness of veterinarians for BTB was probably low prior to 1991 (Figure 2). This is supported by the fact that when the first BTB-infected deer was autopsied in 1991, its generalized granulomatous lesions, including extensive lung lesions, were assumed to be caused by *M. avium* until culture proved otherwise. However, when the first BTB-case was detected in 1991 the number of submissions increased, but in the late 1990s submissions decreased, possibly reflecting decreased awareness.

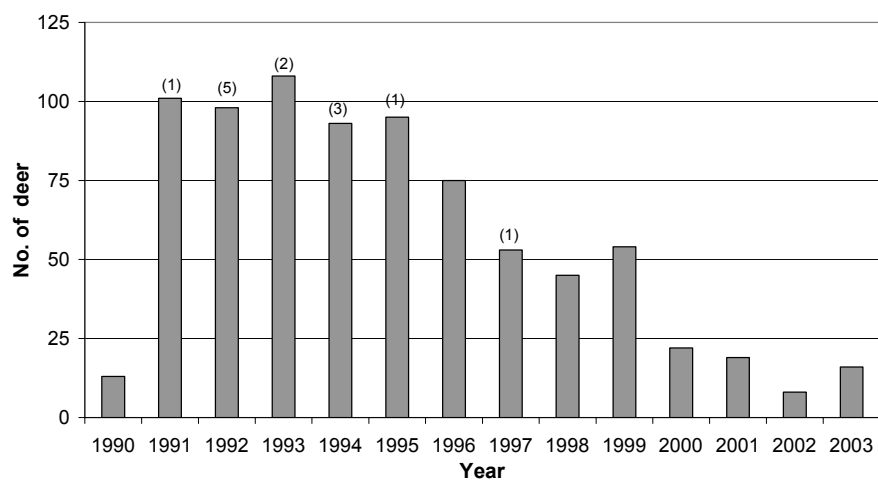


Figure 2. Numbers of deer where material was sent to the bacteriological laboratory at the National Veterinary Institute for BTB investigation (smears and/or culture) and number of annually detected BTB-infected deer herds (in brackets) 1990-2003.

One may ask if BTB could have been detected earlier than four years after its introduction if meat inspection had been compulsory and continuously evaluated by the authorities and a minimum submission rate specified?

We may conclude that a built-in validation of meat inspection in farmed deer is desirable to ensure the validity of the predictions of paper V.

Clinical surveillance

Finally, clinical surveillance as well as the skin test and meat inspection should also be continuously evaluated, although it is very difficult to evaluate and compare the sensitivity of passive surveillance of the same disease between different regions or countries (Doherr & Audige, 2001). As BTB seldom gives rise to clinical signs, passive clinical surveillance is not particularly efficient for this disease (Doherr & Audige, 2001; Lees, 2004). In Sweden, only one of the 13 BTB-infected deer herds was detected in this way, when the owner observed a

subcutaneous swelling on a deer (unpublished results). However, continuous evaluation of clinical surveillance may identify, and might prevent, lack of awareness of BTB. An example of lack of awareness was reported from Canada, where a veterinarian treated a deer with a tuberculous abscess several times without suspecting BTB (Fanning & Edwards, 1991).

Modelling spread of disease for the design and evaluation of control programs (IV, V)

Epidemiological tools such as risk analysis and modelling are increasingly used to understand the epidemiology of BTB, to ensure that control programs remain focused and relevant and to evaluate existing and future programs (Perez *et al.*, 2002a; Perez *et al.*, 2002b; Griffin & Collins, 2004). For example, in Australia, a cost-benefit analysis was applied to the BTB-eradication program to examine what the future extent of the program should be (Stoneham & Johnstone, 1987). In the USA, an evaluation of the risk factors for BTB was performed when the control program managed to decrease the prevalence to very low levels but not eradicate the infection. In the Netherlands a large outbreak of BTB in bovines indicated that the applied surveillance might have been insufficient and the sensitivities of different surveillance methods were modelled (Van Roermund *et al.*, 2004).

Also in Sweden, it was evident in the mid 1990s that it was impossible to muster all deer for testing in certain extensive deer herds. In order to be able to declare all Swedish deer herds free from BTB an alternative strategy for documenting freedom from BTB was needed. The authorities wanted to be able to estimate the probability of freedom from BTB that could be obtained through surveillance by meat inspection before implementing it as a part of the control program.

Modelling disease transmission

A model was used to estimate disease transmission (IV). Obtained estimates of the spread of disease (the number of infectious contacts per infectious individual per time unit) were low and similar to that found in cattle (Barlow *et al.*, 1997; Perez *et al.*, 2002a). However in our model, disease transmission was density independent, *i.e.* independent of population size, which means that an infected deer infects a certain *number* of animals per time unit. Models by Barlow *et al.* (1997) and Perez *et al.* (2002) were density dependent, *i.e.* dependent on population size, which means that an infected animal infects a certain *proportion* of animals per unit of time. Therefore, estimates of spread of disease are difficult to compare between these models when herd size changes.

Current knowledge indicated, however, that disease transmission was higher in farmed deer than in cattle (Towar, 1965; Morris, Pfeffer. & Jackson., 1994; pers. comm. Livingstone, 1997).

More recently, results from a density independent model describing the within-herd transmission of BTB in wild deer in the USA has been published, supporting this knowledge (McCarty & Miller, 1998). Three different transmission coefficients were obtained (number of infectious contacts per infectious individual

per year), one for the transmission between infectious individuals and female or offspring, one between infectious individuals and males and one between infectious females and their offspring. The estimated transmission coefficients (0.5, 8.1 and 0.25, respectively) were higher than the expected value of our estimates (0.26) (paper V).

However, comparisons of estimates of transmission from different studies may be difficult, as assumptions of the model affecting the estimates of transmission may be different. For example, Barlow *et al.* (1997) pointed out that the length of the incubation period (latent period) may affect the obtained estimates. In the model by McCarthy & Miller (1998), a latent period of two years and estimated average life spans of two years (male) and four years (female) were used. In our model (IV), a latent period of one year was used and it was assumed that no infected deer were removed from the herd prior to detection of BTB. If McCarthy & Miller (1998) had used a shorter latent period and assumed that no deer had been removed from the herd, a lower estimate of disease transmission would have been obtained.

Furthermore, Munroe *et al.* (2000) reported an incidence rate (IR) of BTB in farmed deer of 9.3 new cases per 100 animal-years and concluded that this was substantially higher than the IRs that could be calculated from our study (0-7.7 per 100 animal years). These estimates (0-7.7) were probably based on the population size at depopulation in paper IV. If this is the case, these calculated IRs are too low, as herd size, in paper IV, increased over time. However, comparing IRs between herds is probably not relevant as the infectious pressure varies between herds, for example depending on the number of introduced infected deer.

Following this reasoning, the findings of McCarthy & Miller (1998) and Munroe *et al.* (2000) are not considered to contradict our finding.

Our estimate of disease transmission might however, be underestimated as the whole herd was depopulated when BTB was identified. As stated in paper IV, in herds where deer with generalized BTB were found at depopulation, it is possible that an increase in disease transmission had occurred recently, which was not detected by our investigation. Although all infected animals should be regarded as potentially infectious (Menzies & Neill, 2000), certain animals are probably more infectious (Neill *et al.*, 1989; Morris, Pfeffer. & Jackson., 1994). It has been reported that in heavily infected herds usually at least one deer with discharging lesions has been present (Lugton *et al.*, 1998). If our estimate of disease transmission is underestimated it will not affect the predictions of paper V negatively, as the probability to detect BTB would simply increase.

Furthermore, our model, as well as the model by McCarthy & Miller (1998), is probably oversimplified. Data needed for a more realistic model were not available from the seven BTB-infected herds. Investigations on wild white-tailed deer populations suggest that the estimation of a transmission coefficient under a random mixing assumption may not be valid (McCarty & Miller; 1998 O'Brien *et al.*, 2002). It is known that farmed deer gather together with flocks of adult males separated from females and offsprings for the major part of the year. The sizes of the flocks depend on several factors such as availability of feed, weather, time of

the year, the biotope of the enclosure and the extent to which they are disturbed. Under different conditions, flock sizes may vary between less than 10 deer to more than 100 deer (Johansson, 2001). Therefore, the random mixing assumption may not be the best option when modelling spread of BTB in farmed deer herds and it may result in both over- and under-estimation of disease transmission.

Although the observed contact rates (k) of BTB in paper IV were low they are still judged to be relevant for extensive Swedish deer herds. Comparisons should not be made with farmed deer herds in general. The conditions for extensively held Swedish farmed deer are probably more similar to wild deer, in regions where winter-feeding of wild deer occur, than to many intensively managed farmed deer herds. In Swedish extensive deer herds the population density usually is low as the deer are mainly kept for shooting purposes. Furthermore, handling does not take place and supplementary feeding only occurs during winter months. Increased population density, for example by supplementary feeding in wild deer, has been shown to increase transmission of BTB (Neill *et al.*, 1989; Miller *et al.*, 2003). Transmission of BTB in extensive Swedish deer herds is, and should be, expected to be lower than in farmed deer in countries where herds with a high population density is common, where deer often are gathered and handled, and where supplementary feeding is more commonly applied, as is often the case in New Zealand.

Estimates of disease transmission vary between different studies. This emphasizes the importance of performing the modelling on the population on which the conclusions are to be used. This approach was used when evaluating different control strategies for BTB in cattle in Argentina (Perez *et al.*, 2002a; Perez *et al.*, 2002b). This is also highlighted by the fact that, if estimates of the spread of BTB in paper V would have been based on knowledge from other countries instead of evaluating the observed spread of disease in Swedish extensive deer herds (IV), the effect of the control program shown in paper V would have been overestimated.

Predictions of paper V

Results of several models describing the spread of BTB and the evaluation of different control strategies, mainly in wildlife but also including interaction between wildlife and cattle, have been published (Smith *et al.*, 2001). However, to the author's knowledge, only results from one model describing the efficacy of meat inspection as a tool to detect BTB has been published (Van Roermund *et al.*, 2004).

Our model predicted that in 50% of the herds BTB would be eliminated or detected (with 100% probability) within seven years from the introduction of one infected animal into the herd. This is a slightly longer time than the results of the model by Van Roermund *et al.* (2004), which predicted that meat inspection would detect BTB 5.7 years after its introduction in the cattle population in the Netherlands. However, Van Roermund *et al.* (2004) used a higher estimate of disease transmission (0.1 new infections per infectious animal per week). Although not known, it is probable that a higher estimate of the sensitivity of meat

inspection was used by Van Roermund *et al.* (2004), which would also reduce the time until detection. In paper V, we used a low estimate of the sensitivity of meat inspection of farmed deer (0.53) as meat inspection for deer was considered to be performed under less optimal conditions than meat inspection of cattle. Furthermore, it may also be assumed that Van Roermund *et al.* (2004) assumed that a larger proportion of the herd was submitted to meat inspection than in our model, where we assumed that only 20% of the deer were slaughtered annually. These (possible) differences between the models may explain the difference in prediction of the duration before BTB is detected.

It is concluded that, based on the available knowledge of the underlying assumptions of the model, the results of Van Roermund *et al.* (2004) do not contradict our findings.

Results from both models (Van Roermund *et al.*, 2004) (I) may raise the question if the present meat inspection is sensitive enough to detect new introductions of disease rapidly enough. Furthermore, as highlighted by the Scientific Panel on Biological Hazards of the European Food Safety, the adoption of palpation, instead of palpation and incision, at meat inspection would decrease the sensitivity of meat inspection (Anonymous, 2003b).

The model for cattle by Van Roermund *et al.* (2004) showed that meat inspection combined with annual bulk milk tank ELISA would reduce the period to detection down to 0.5 years. It was concluded that if bulk milk tank ELISA was feasible, the combination of meat inspection and annual bulk milk tank ELISA would be superior to meat inspection only (Van Asseldonk *et al.*, 1999). Some EU member states are currently evaluating the benefits of an ELISA on pooled sera collected randomly at slaughter (Anonymous, 2003b). Another approach to increase the efficiency of the BTB surveillance may be to increase and ensure the quality of meat inspection as proposed earlier (Enoe *et al.*, 2003; Anonymous, 2003b; <http://www.aahc.com.au>; 9-Sept-2004).

It may be concluded that both evaluation of the sensitivity of meat inspection and modelling the efficacy of meat inspection as a tool to detect BTB are important in clarifying the present effect of such surveillance in different animal populations.

Concluding remarks

i) The source of infection of the outbreak of BTB in farmed deer herds was identified as a consignment of 168 imported fallow deer. The trace-back investigation highlighted the need for mandatory animal identification as a prerequisite for traceability.

ii, iii) Two quality indicators of the skin test were established. First, reference values for the pre-measurement of the tuberculin test were established and suggested to be used mainly by less experienced veterinarians as “self-evaluation” to identify possible unexpected measurements. The measured thickness of a “standard” skin fold was also shown to be a subjective measurement personally set by each veterinarian. However, as long as the veterinarian is consistent in both pre- and post-measurements, such differences are not considered to affect test accuracy.

Secondly, the within-veterinarian variation *i.e.* the consistency of measurements of the skin fold thickness by each of 64 veterinarians, was estimated. A large variation in measurements might affect the accuracy of the test. Four veterinarians with unexpectedly large variation in measurements were identified. This highlights the need for evaluation of the quality of performed tuberculin tests.

iv) The within-herd spread of *M. bovis* in seven extensive Swedish farmed deer herds was estimated. Estimates of disease transmission were lower than those observed in farmed deer herds in other countries. This highlights that the spread of BTB in Swedish extensively farmed deer herds differs from that seen in more intensively farmed deer herds and that extrapolation of disease transmission cannot directly be performed between herds with different management systems.

v) The expected spread of BTB in an average extensive Swedish deer herd, given infection with one deer, was simulated. Furthermore, the efficiency of meat inspection as a tool to either eliminate or to detect BTB in such herds was evaluated. Results from our study were not considered to be contradictory to those reported by Van Roermund *et al.*, (2004). However, the importance of the underlying assumptions when modelling the spread of disease was highlighted, concluding that a cautious approach should be applied when using results from the literature to model diseases in other populations.

A sensitivity analysis of the results of the model (V) showed that if the sensitivity of meat inspection decreases, the probability to detect BTB also decreases. This highlights the need for an improved quality control of meat inspection, to ensure the validity of predictions in paper V.

In conclusion, these studies (I, II, III, IV and V) have contributed to increased knowledge of the introduction, spread and control of BTB in Swedish deer herds and facilitated the epidemiological investigations performed in these herds. Results of papers IV and V have been implemented in the TB-control program for farmed deer.

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