Bovine tuberculosis in deer: Update from the NADVet Conference, Austin, Texas, 19-20 Feb, 2002

C Mackintosh

Abstract

Two papers outlined the history and current situation of Tb in deer and wildlife in USA.

There has been a cattle Tb eradication programme in the USA since 1917. The all state prevalence of cattle Tb was reduced to <0.5% by the 1940s. However there was a serious outbreak of Tb in cattle in Michigan in the 1960s. A single case of Tb was diagnosed in a free-living white-tailed deer (WTD) in 1975 and then again in the early 1990s. Since then ~400 cases have been diagnosed in ~90,000 (0.5%) hunter-killed WTD and 4 of ~1000 elk in Michigan. Surveys of wildlife have found Tb in 7/213 black bear, 4/54 bobcat, 13/321 coyote, 2/291 opossum, 2/243 raccoon, and 2/25 red fox. It is believed that Tb spread from cattle to WTD in the 1960s and the sudden appearance of Tb is due to the great increase in numbers of WTD in the last 20 years. Increased feeding of WTD in the winter has improved their survival, but has probably led to increased transmission at these feeding stations and a higher incidence. Tb has not been diagnosed in free-living deer in other states.

Peter Wilson presented the paper "Deer tuberculosis in New Zealand: National and on-farm control", which described the current New Zealand deer Tb situation and experiences. It also discussed a few key points of epidemiological research that has contributed to our ability to control and manage Tb on individual deer farms.

Colin Mackintosh presented the paper "Tuberculosis research in deer in New Zealand", which discussed the research undertaken and advances made over the last 20 years in areas of diagnosis, epidemiology, immunology, vaccines and genetic resistance in deer.

"History of tuberculosis in cervids and wildlife in North America"

Joseph van Tiem (Senior Veterinarian, APHIS)
presented by Dan Baca, USDA, APHIS, Texas, USA

1917 - USA Tb eradication program introduced using skin testing – 5% reactor rate

1940s – All state prevalence <0.5%

1962 – Series of Tb breakdowns in cattle in Michigan.

1989 – Tb diagnosed in captive cervids in USA and Canada.

1991 – USDA meeting decided that the caudal fold test was not valid in cervids due to low sensitivity. The MCST was introduced and the BTB approved.

Since 1991 there have been 37 Tb infected herds identified, mostly elk and fallow, with a few WTD. Of these 37, 11 were quarantined and test-and-slaughtered clear, while 26 were depopulated. There were only two infected farms in Texas: a fallow herd in 1991 and an elk herd in 1993.

Today – Tb officially "eradicated" in all farmed livestock. The USDA policy is for whole herd depopulation and full compensation (95% appraised value with max. US$3000) for reactors and exposed animals. For a state to be declared accredited Tb-free it must not have had Tb for 5 years. However, Tb is present in free-ranging WTD and elk in Michigan.

"Tb in Michigan"

Colleen Bruning-Fann, *USDA, APHIS, VS, East Lancing, Michigan, USA*

History of Tb in captive deer in Michigan:

In 1997 a captive WTD herd with Tb (258 WTD and 4 elk) was diagnosed with Tb and the whole herd was depopulated and subsequently restocked.

History of Tb in wild deer in Michigan:

1975 – A hunter killed 9-year-old female WTD found to have bovine Tb

1994 – A hunter killed 4-year-old male WTD found to have Tb

1995 – Tb found in a number of WTD

Since then, extensive surveys of non-cervid wildlife have revealed Tb in 7/213 black bear, 4/54 bobcat, 13/321 coyote, 2/291 opossum, 2/243 raccoon, and 2/25 red fox. No infected American badgers, feral cats or skunks were found from >20 of each sampled.

In the 1990s all hunter-killed animals were examined throughout Michigan and 398 of 87,919 (0.5%) hunter-killed WTD were found to have found Tb. In the year 2000, 53 of 25,858 (0.2%) WTD were found to be Tb positive, although the apparent prevalence in the "core area" of northeast Michigan was 2.3%. This may be an underestimate of the prevalence because only suspect visible lesions were investigated the sensitivity of finding gross lesions at necropsy has not been determined.

The wild elk population in Michigan is estimated to be approximately 900 animals. Since 1996 a total of 1,051 hunter-killed and road-killed elk have been examined and two Tb infected animals have been found.

Cause: The apparent reason for the recent appearance and/or increase in prevalence of Tb is the dramatic increase in numbers of WTD in the last 20 years. It is believed that Tb probably passed into the WTD population in the 1960s when there was a serious rise in Tb in cattle, but the WTD population was relatively small and only the occasional case of Tb was detected. There has also been an increasing use of feeding stations, which have helped deer to over-winter and may have encouraged the deer-to-deer spread of Tb.

Control: The aim is to reduce the numbers of WTD in the state by increasing hunting pressure and reducing feeding and baiting. Feeding will be prohibited in the 12 high risk counties. If feeding is done outside these areas, then it should be at multiple small feeding stations.

Research: Colorado Station is investigating PCR test and a fluorescent polymerase test. Michigan State is investigating risk factors for Tb infected farms, the sensitivity and specificity of skin testing in deer, use of ultrasound to diagnose Tb lesions in head lymph nodes, evaluating rodents (especially prairie voles) as reservoirs of Tb, the effectiveness of deer repellents on cattle supplementary feed, the genetic relatedness of Tb infected WTD and elk, the genetic structure of the wild deer herd, deer ecology and movement, Tb in bears and factors affecting prevalence in the Tb core area. They are also investigating a gamma interferon test for deer and electronic tagging.

"White-tailed deer (WTD) pathogenesis study"

Mitchell Palmer, *National Animal Disease Centre, Ames, Iowa, USA*

In the last 5 years they have used the intra-tonsil Tb challenge model, developed at Invermay, to infect nearly 150 WTD and it produces lesions very similar to natural Tb.

In the most recent pathogenesis trial they used 300 cfu and this produced microscopic lesions at 28 days post infection (dpi), gross lesions at 42 dpi and there was a change from caseo-necrotic lesions to liquefied abscesses at 9-12 months pi. The majority of lesions were in the head, especially the medial retropharyngeal LN, and 40% also had pulmonary lesions. They detected Tb organisms in nasal secretions, saliva, faeces and uncommonly in urine. They have demonstrated deer to deer and deer to cattle transmission through sharing food.

The LT, ELISA and MCST are the main tests used to monitor infection. They are developing a gamma interferon test and various ELISA tests

"Tuberculosis research in deer in New Zealand"

***Dr Colin Mackintosh**, AgResearch Invermay, Mosgiel, NZ*
***Professor Frank Griffin**, Dept. Microbiology, University of Dunedin, NZ*

Introduction

Deer farming started in New Zealand in the late 1960s and it has grown exponentially so that today there are over 2 million deer on approximately 5000 farms. Over 90% are red deer, with the balance being wapiti, wapiti/red hybrids and fallow deer. In 1978 the first cases of bovine tuberculosis (Tb) caused by *Mycobacterium bovis* were diagnosed in farmed deer in Canterbury in the South Island of New Zealand (Beatson, 1985). Despite early efforts to isolate infected herds and control it, Tb spread widely throughout New Zealand as deer farming was expanding rapidly, deer were live-captured from the wild in large numbers and the significance of wildlife vectors was underestimated. Since the early 1980s a national Tb control programme for deer has been implemented and by October 1999 the point prevalence of Tb in infected farmed deer herds in areas containing infected wildlife and in areas with no infected wildlife was 5.23% and 0.14%, respectively (de Lisle et al., 2001). Over the last 20 years considerable research efforts have been undertaken in areas of diagnosis, epidemiology, immunology, vaccines and genetic resistance in deer and these are outlined below.

Diagnostic tests

The intradermal tuberculin skin test, developed for Tb diagnosis in cattle, has been used extensively for the control of Tb in farmed deer herds, together with Movement Control, whereby animals cannot be moved from infected herds except to slaughter, the thorough inspection of lymph nodes at slaughter plants and trace back of infected animals. Early trials showed that cervical site was the most sensitive for skin testing in deer (Beatson, 1985). The mid cervical skin test (MCST), using 1mg/ml bovine PPD, has a sensitivity of around 80%, if it is applied carefully and interpreted conservatively (Corrin ***et al.***, 1987). The MCST has limited specificity, especially when used in an environment with high levels of saprophytic mycobacteria present. This has required the introduction of the comparative cervical skin test (CCT) (Corrin ***et al.***, 1993) and ancillary blood tests have been developed to improve the precision of the MCST for the Tb diagnosis in deer. A composite immune cell and antibody test has been developed for Tb diagnosis in deer (Griffin ***et al.***, 1994***)***. The blood test for Tb (BTB), measures lymphocyte transformation (LT) of circulating T cells and antibody levels using an ELISA assay and has consistently high levels of sensitivity (>90%) and specificity (>98%). A modified ELISA test, which measures IgG1 antibodies, has been evaluated recently for Tb diagnosis in deer. The test shows has high sensitivity (>90%) for Tb diagnosis, and acceptable levels of specificity (97%) (Griffin, unpublished).

Histopathological examination of suspect lesions in a range of tissues is routinely used to confirm a diagnosis of Tb ***post mortem.*** The spectrum of pathology seen in deer may be more extensive than is seen traditionally in cattle. However, because of the similarity in lesions caused by a range of mycobacteria, microbiological culturing is still regarded as the "gold standard" for Tb diagnosis (de Lisle and Havill, 1985), and strain typing of ***M. bovis*** is carried out using DNA restriction enzyme analysis (Collins ***et al.***, 1993). The PCR test, specific for

M. bovis, is proving a useful and rapid alternative to culture. An array of new cytokine assays, including those for IL2, IL4, and gamma-interferon, and other immunological tests such as macrophage killing assays, are being developed for deer and will provide essential tools to probe the immune system and assist in understanding the differences between protective and non-protective responses (Hook *et al*., 1996).

Infected deer research farm

In 1990, approval was given by the Chief Veterinary Officer in New Zealand for the establishment of a quarantine deer farm where Tb research could be conducted safely. This "Infected Deer Farm" (IDF) had electrified "possum-proof" fencing around the perimeter and other safeguards to prevent the escape of Tb from the farm, which might put surrounding properties at risk. This 20 hectare property has allowed a range of epidemiology, pathogenesis, vaccine and genetic resistance studies to be carried out efficiently and safely over the lat 10 years. Groups of up to 90 red deer are usually used for trials and this has allowed treatment subgroups of 10 – 20 animals to be used. Access to a commercial Deer Slaughter Plant has facilitated the slaughter and thorough necropsy of trial animals at the completion of each Tb infection study.

Epidemiological studies

Observations of Tb lesion distribution in naturally infected farmed deer and experience with experimental infections has led to the hypothesis that the oro-pharyngeal tonsil is the most common site of primary infection in farmed deer in New Zealand (Mackintosh et al, 1995a). The preponderance of tuberculosis (Tb) lesions in the retropharyngeal lymph nodes of naturally infected deer and the close simulation of natural Tb by the experimental inoculation of the palatine tonsil, strongly supports this hypothesis.

Lugton *et al.* (1998) found the oropharyngeal tonsil to be the most commonly infected site (61%) in a series of 58 ***M. bovis*** infected wild red deer examined, although typical gross Tb lesions were present in only 3% of the infected tonsils. The medial retropharyngeal node is the commonest site of tuberculous lesions, outside the thorax, in cattle (Francis, 1958). Intratonsillar inoculation of cattle with ***M. bovis*** resulted in isolation of the organism from the medial retropharyngeal lymph node within 4 hours of inoculation and carriage of bacilli in the oropharyngeal tonsil for at least 8 weeks (Palmer et al., 1999). This evidence, and the recent isolation of ***M. bovis*** from one third of 31 cattle showing gross lesions of the medial retropharyngeal lymph node (Cassidy et al., 1999), supports the hypothesis that the tonsil is one of the most important primary sites of natural infection in both cattle and deer.

True aerosol spread appears to be much less common in farmed deer than in housed dairy cattle or in humans. Direct contact with infected deer or wildlife and indirect spread via infected dust, fomites, food or water appear the most likely forms of transmission. Spread of infection to other deer under field conditions occurs only slowly during the first 6-8 months of disease, although evidence of spread has be seen in in-contact animals 60 to 80 days following exposure to infected animals (Griffin and Mackintosh, 2000).

Stress also appears to play a significant role in making animals more susceptible to Tb, reducing the immune response and increasing the severity of disease (Thomson et al., 1995).

Intra-tonsil infection model

The first experimental Tb infection model for in deer in New Zealand used the intra-tracheal route of inoculation (de Lisle et al, 1983). However, this model produced severe lung lesions, which are relatively uncommon in farmed deer. In 1992/93, we conducted a trial at the IDF, which compared intra-tracheal, intra-nasal and intra-tonsil routes of inoculation with 2 doses of ***M. bovis*** (2×10^2 and 2×10^4 c.f.u.) in 6-month-old red hinds. When slaughtered 8 months later it was found that intra-tracheal inoculation produced moderate to severe lung lesions and thoracic lymph node (LN) involvement in 4/5 low dose and 5/5 high dose animals. Intra-nasal inoculation resulted in 1/5 with lesions in the low dose group and 5/5 with lesions in the

high dose group, with 3 animals having head LN and extensive lung and thoracic LN involvement. The low dose intra-tonsil group had one with No Visible Lesions (NVL) and 4/5 with retropharyngeal abscesses only. The high dose intra-tonsil group had 5/5 infected; one NVL but tonsil infected, 3 with retropharyngeal LN abscessation and one with retropharyngeal and ileocaecal LN abscessation (Mackintosh et al., 1993). Previous surveys of naturally infected deer have shown that over 50% had head LN involvement only and 10-20% with lung involvement. Therefore it was concluded that the low dose intra-tonsil inoculation produced the most natural lesions. Subsequent studies showed that doses as low as 8 cfu could infect 50% of a group, but doses of 100 – 500 cfu of virulent ***M. bovis*** in a 0.2 ml dose introduced onto the mucosal surface of the crypt of the tonsil, reliably resulted in infection levels in 70 - 100% of animals and disease in >50% (Mackintosh et al., 1995b). This challenge system has been used in all subsequent trials involving over 800 male and female deer of varying ages in 16 experiments, and it has been found to be highly repeatable and efficient. The intra tonsil model has also been used successfully in white-tailed deer and cattle in USA (Palmer et al., 1999; 2000) and in Cape buffalo in South Africa (L-M.. De Klerk, pers. comm..).

Vaccine studies

The Tb challenge model has also been used to test Tb vaccines in deer. It has been have shown that two doses six weeks apart of ***M. bovis*** BCG vaccine gave significant protection against Tb, whether given by subcutaneous injection, by instillation into the tonsil or by aerosol into the mouth (Griffin ***et al***., 1995; 1999). Injection of killed vaccine with an oil adjuvant gave no protection, but stimulated a high non-protective antibody (B cell) response. Unfortunately, vaccination with ***M. bovis*** BCG tends to make the animal reactive to the normal Tb skin test, and therefore vaccination is unsuitable at the present time for routine use on deer farms. However, vaccination of wild deer is likely to be a useful control option if a suitable oral bait delivery system can be developed (Griffin and Mackintosh, 2000).

Genetic resistance

A 3-year study was undertaken to demonstrate and measure the heritability of resistance to Tb in red deer. Six red deer stags were selected from 39 on the basis of their apparent susceptibility to Tb challenge, with two having no lesions, two with moderate lesions and two having severe lesions. Offspring were bred from these 6 stags by artificial insemination of randomly selected commercial red deer hinds. The 71 offspring were challenged with Tb at 9 months of age. In general terms, the offspring of the "resistant" sires had mild to moderate lesions, those of the "susceptible" sires had moderate to severe lesions and those of the intermediate sires ranged from mild to severe. The result shows that there is a strongly heritable basis to the natural resistance/susceptibility of red deer to Tb ($P< 0.01$) with an estimated heritability of 0.48 (95% CI 0.22 - 0.75). This is believed to be the first time the heritability of Tb resistance has been measured in a domesticated animal (Mackintosh et al., 2000).

We are now actively seeking immunological and genetic markers which would allow us to screen out highly susceptible animals and select for highly resistant herd sires in areas where infected wildlife pose a risk of Tb introduction (Mackintosh et al, 1997).

It is hoped that these genetic and immunological markers will lead to the discovery of the genes responsible for Tb resistance and the mechanisms of protective immune responses to mycobacterial infections.

References

Beatson N.S. (1985) Tuberculosis in red deer in New Zealand. In Biology of Deer Production, eds P. F. Fennessy and K. R. Drew. Bulletin, Royal Society of New Zealand, Dunedin. 22: 147 -150.

Cassidy J.P., Bryson D.G., Neill S.D. (1999) Tonsillar lesions in cattle naturally infected with Mycobacterium bovis. Vet. Rec.; 144:139-42.

Collins, D.M., Erasmuson, S.K, Stephens,D.M., Yates, G.F., de Lisle, G.W., (1993) DNA fingerprinting of Mycobacterium bovis strains by restriction fragment analysis and hybridization with insertion elements IS1081 and IS6110. Journal of Clinical Microbiology 31:1143-1147.

Corrin, K.C., Carter, C.E., Kissling, R.C. & de Lisle, G.W. (1987) Short interval skin testing in farmed red deer (Cervus elaphus) inoculated with Mycobacterium bovis. New Zealand Veterinary Journal 35: 204-207

Corrin, K.C., Carter, C. E., Kissling, R. C. & de Lisle, G. W. (1993) An evaluation of the comparative tuberculin skin test for detecting tuberculosis in farmed deer. New Zealand Veterinary Journal 41, 12-20

de Lisle, G.W., Welch, P.J., Havill, P.F., Julian, A.F., Polle, W.S.H., Corrin, K.C. & Gladden, N.R. (1983) Experimental tuberculosis in red deer (Cervus elaphus). New Zealand Veterinary Journal 41: 213-6

de Lisle, G.W. & Havill, P.F. (1985) Mycobacteria isolated from deer in New Zealand from 1970 - 1983. New Zealand Veterinary Journal 33: 138-140

de Lisle, G.W., Mackintosh, C.G. & Bengis, R.G. (2001) Mycobacterium bovis in free-living and captive wildlife, including farmed deer. Rev. sci. tech. Off. Int. Epiz. 20 (1): 86-111

Francis, J. Tuberculosis in animals and man. A study in comparative pathology (Cassell and Company Limited, London, 1958).

Griffin, J.F.T., Cross, J.P., Chinn, D.N., Rodgers, C.R. & Buchan, G.S. (1994) Diagnosis of tuberculosis due to Mycobacterium bovis in New Zealand red deer(Cervus elaphus) using a composite blood test and antibody assays. New Zealand Veterinary Journal 42: 173-179.

Griffin, J.F.T., Mackintosh, C.G. & Buchan, G.S. (1995) Animal models of protective immunity in tuberculosis to evaluate candidate vaccines. Trends in Microbiology 3: 418-424

Griffin, J.F.T., Mackintosh, C.G., Slobbe, L., Thomson, A.J. and Buchan, G.S. (1999) Vaccine Protocols to Optimise Protection against Tuberculosis Infection and Disease. Tubercle and Lung Disease, 79:135-143.

Griffin J.F.T. & Mackintosh C.G. (2000) Tuberculosis in deer: perceptions, problems and progress. The vet. J. 160: 202-219.

Hook, S., Griffin, F., Mackintosh, C. & Buchan, G. (1996) Activation of an interleukin-4 m RNA-production population of peripheral blood mononuclear cells after infection with Mycobacterium bovis or vaccination with killed, but not live BCG. Immunology 88: 269-74.

Lugton I.W., Wilson P.R., Morris R.S., Nugent G. (1998) Epidemiology and pathogenesis of Mycobacterium bovis infection of red deer (Cervus elaphus) in New Zealand. New Zealand Veterinary Journal 46:147-56.

Mackintosh, C.G., Waldrup, K., Labes, R.E., Griffin, J.F.T., Buchan, G.E., Cross, J.P. & de Lisle, G.W. (1993) Experimental Mycobacterium bovis infection in deer. Proceedings of the New Zealand Veterinary Association Deer Branch, Course No. 10: 297-304

Mackintosh, C.G. & Griffin, J.F.T. (1994) Epidemiological aspects of deer tuberculosis research. Proceedings of the New Zealand Veterinary Association Deer Branch, Course No. 11: 106-115.

Mackintosh, C.G., Waldrup, K. & Griffin, F. (1995a) A new perspective on the epidemiology of tuberculosis in farmed deer. In Griffin, F and de Lisle, G (eds) Tuberculosis in Wildlife and Domestic Animals. Otago Conference Series 3, University of Otago Press, Dunedin, pp. 270-272.

Mackintosh, C.G., Waldrup, K., Labes, R., Buchan, G. & Griffin, F. (1995b) Intra-tonsil inoculation : an experimental model for tuberculosis in deer. In Griffin, F and de Lisle, G (eds) Tuberculosis in Wildlife and Domestic Animals. Otago Conference Series 3, University of Otago Press, Dunedin, pp. 121-122.

Mackintosh, C.G., Qureshi, T., Waldrup, K., Labes, R.E., Dodds, K.G. & Griffin, J.F. (2000) Genetic resistance to experimental infection with Mycobacterium bovis in red deer (Cervus elaphus). Infection and Immunity 68: 1620-5

Palmer M.V., Whipple D.L., Rhyan J.C., Bolin C.A., Saari D.A. (1999) Granuloma development in cattle after intratonsillar inoculation with Mycobacterium bovis. Am. J. Vet. Res. 59: 310-315

Palmer, M.V., Whipple, D.L., Olsen, S.C. & Jacobson, R.H. (2000) Cell mediated and humoral immune responses of white-tailed deer experimentally infected with Mycobacterium bovis. Research in Veterinary Science 68: 95-8

Thomson, A., Mackintosh, C.G. & Griffin, F. (1995) The effect of dexamethasone on the protective efficacy of *Mycobacterium bovis* BCG in deer. In Griffin, F and de Lisle G (eds) *Tuberculosis in Wildlife and Domestic Animals. Otago Conference Series 3,* University of Otago Press, Dunedin, pp. 131-134.