"Epidemiological aspects of deer tuberculosis research"

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INTRODUCTION

In order to effectively and efficiently control a disease it is essential to understand the epidemiology of that disease in the target animal. Disease is the result of an interaction between the causative agent, the host and the environment. With tuberculosis (TB) in deer and cattle, much is known about the organism *Mycobacterium bovis*. Research at Invermay is centred on deer, the host, the factors that affect the transmission of TB to deer and the host responses to infection. A better understanding of the key factors will allow more accurate modelling of the disease in deer and assist in the development of control measures. This paper deals primarily with transmission of TB in deer.

TRANSMISSION

Transmission of infection is one of the key factors in understanding the epidemiology of any infectious disease and TB is no exception The most important factors that are likely to directly affect transmission from one animal to another are degree of infectivity of the host, environmental factors, degree of contact or exposure and susceptibility of the host. These factors will be discussed in turn.

1. DEGREE OF INFECTIVITY

Information on this topic is based on observations of naturally and experimentally infected deer.

Site of lesion: This gives a clue to both the site of entry of TB and the mode of excretion. In one study of TB infected deer from four properties, the majority of lesions were found in lymph nodes (LNs) of the head only (53%), followed by head and abdomen (11%), thorax only (9%), abdomen only (7%), head and thorax (6%) and head, thorax and abdomen (6%). The single most commonly affected LN was the medial retropharyngeal (45%). Altogether, 76% had head lesions alone or with other lesions (Beatson, 1985 citing Livingstone, 1980). In another report on 71 confirmed cases of TB in deer examined at a Deer Slaughter Plant (DSP) (Wilcockson, 1986) 27% had head only lesions (20% retropharyngeal, 6% submandibular and 1.4% parotid LNs), 31% were abdominal only and 4% thoracic only. The rest had two or more sites involving head, thorax and abdominal nodes. In two animals lesions were found in the precrural LN only Similarly, an analysis of data on tuberculous deer slaughtered in licensed DSPs throughout New Zealand from 1990 to 1993 again showed the retropharyngeal LN the most commonly affected (52.3%) followed by ileojejunal LN (20.8%), bronchial LN (11.1%), mediastinal LN (10%) and ileocecal LN (9.7%) (Hathaway et al, 1994).

In a serious outbreak of TB on a deer farm near Invermay, the entire herd was slaughtered. The most seriously affected were killed on farm while the rest were killed through a Deer Slaughter Plant and 123 out of 250 deer had lesions. Of these 46.3% had lesions in the head, thorax and abdomen, 31.7% had head only lesions, 13% thorax only lesions, 5 7% abdomen only lesions and 3.3% had carcass only lesions, mainly in the popliteal LN (Griffin, pers comm.). Of the six seriously diseased animals that were slaughtered on the farm, two had open discharging lesions on the neck and one had a large area of necrotic underrun skin over the shoulder that was discharging clear fluid from which *M. bovis* was isolated.

Experimental infections: In order to investigate TB transmission and to develop an experimental challenge system that closely mimics natural TB in deer, an experiment was conducted in 1992/93 comparing intra-tracheal, intra-nasal and intra-tonsil inoculation in groups of five weaners with either low dose (2×10^2) or high dose (2×10^4 c.f.u.) live virulent *M. bovis*. The deer were slaughtered eight months after inoculation and preliminary findings have been reported elsewhere (Mackintosh *et al*, 1993) but, in summary, intra-tracheal inoculation produced moderate to severe lung lesions and thoracic LN involvement in 4/5 and 5/5 in low dose and high dose groups respectively. One high dose animal died 13 weeks after inoculation with severe pulmonary TB. Intra-nasal low dose resulted in four animals with no visible lesions (NVLs) and one animal with anterior mediastinal abscesses but no gross lung lesions whereas intra-nasal high dose caused disease in all five, with three animals having submandibular and retropharyngeal LN abscesses and extensive lung and thoracic LN involvement. The other two had either retropharyngeal and anterior mediastinal LN abscessation or retropharyngeal and ileocecal LN abscessation. The intra-tonsil low dose group had one NVL and 4/5 with retropharyngeal LN abscesses only. The high dose intra-tonsil group had 5/5 infected, with one NVL but tonsil infected, three with retropharyngeal LN abscessation only and one with retropharyngeal and ileocecal LN abscessation. The conclusion reached was that neither intra-tracheal nor intra-nasal inoculation resulted in TB typical of that seen in the majority of naturally infected deer, whereas intra-tonsil inoculation reliably resulted in typical TB in the majority of animals, affecting especially the retropharyngeal LNs

During this trial, two animals from each group were sampled and *M. bovis* was isolated from lung lavage samples from 5/12 animals tested at 10 weeks after inoculation. Ten red deer weaners were run in contact with these inoculated animals for eight months and one became infected after about three months and had a typical retropharyngeal LN abscess at slaughter five months later. This control animal was infected shortly before one of the high dose intra-tracheally infected animals died of serious pulmonary Tb. This latter animal would have been highly infectious over this period, which was August - September, when the animals were grazed in one to two hectare paddocks and supplemented daily with barley and meadow hay. The animals were yarded and bloodsampled every two weeks over this period, providing additional close contact in covered yards

A further 10 weaners were added to the group after five months, but none of them appear to have become infected over the last three month period of the trial which was over the summer when the animals were grazed in 1-2 hectare paddocks without supplementation and were yarded every four weeks on average

This season (1993/94), two similar experimental infection studies were conducted. In one trial three groups of eight red deer weaners were given approximately 10^1, 10^1 plus dexamethasone (D) or 10^2 *M. bovis* organisms by intra-tonsil injection. Eight non-inoculated weaners were run in contact with the inoculated animals for the duration of the eight month trial (June-February). TB infections were produced in 5/8, 5/7 and 8/8 of the 10^1, 10^1 + D and 10^2

groups respectively. The latter two groups each had two animals severely infected with extensive or generalised TB lesions involving head and thoracic, or head, thoracic and abdominal lymph nodes. Two of these severely affected animals were euthanased in October, four months after inoculation. One of the eight control animals became infected in September/October at a time when these two seriously affected animals would have been highly infectious The control deer had typical retropharyngeal and mesenteric LN lesions.

Concurrently, three groups of two-year-old stags were inoculated in June with 10^1, 10^2 and 10^4 *M. bovis* organisms by the intra-tonsil route, and a non-inoculated group of eight stags ran with them. TB infections resulted in 4/7, 7/8 and 7/7 inoculated animals respectively with little difference in severity between 10^2 and 10^4 groups. None of the control stags became infected (all were NVL and LN culture negative). One of the inoculated stags developed severe lung involvement, in addition to head lesions, with several large abscesses and many miliary abscesses through-out the rest of the lungs, and was euthanased in December, six months after inoculation. One other stag had a small lung lesion plus retropharyngeal LN abscesses and one had a mesenteric LN abscess in addition to two retropharyngeal abscesses. The other 14 animals had lesions confined to the head with mild to moderate retropharyngeal abscesses and three also had an abscess in the left tonsil confirmed as tuberculosis. These latter three animals are likely to have been infectious in addition to the animals with lung and abdominal lesions.

In a recent vaccination trial, one experimentally infected animal developed a very enlarged left medial retropharyngeal lymph node (around 80 mm across) which burst, discharging its purulent contents It partially healed, leaving a sinus draining from the node onto the skin surface of the throat just proximal to the angle of the jaw Large numbers of acid fast organisms were demonstrated in the retropharyngeal lymph node lesion at slaughter No information is available on the number of infected organisms excreted from this lesion, but similar lesions in possums may contain up to 10^7 organisms per gram of material discharged.

<u>Discussion</u>: Classically, transmission of TB in humans and cattle is considered to be by aerosol via the pulmonary route (Francis, 1958). From the distribution of lesions found in naturally and experimentally infected deer it appears that they are exposed mostly through the nasal and oral cavities, by aerosols or infected discharges. Relatively few animals develop lung infections directly. In ruminants the lymphatic drainage from the lips, rostral nasal cavity, hard palate, gums and tip of the tongue is via the submandibular and parotid LNs and efferents go to the lateral retropharyngeal LN. Lymph from the tongue, floor of the mouth, hard and soft palate, gums, pharyngeal and palatine, tonsils, caudal nasal passages, sinuses, larynx and pharynx drains to the medial retropharyngeal LN which has efferents to the lateral retropharyngeal LN, which also receives lymphatic drainage directly from the tongue mucous membranes of the oral cavity, gums, lips and hard palate Its efferents form the Tracheal Trunk which empties with the Thoracic Duct into the jugular (Sisson and Grossman, 1975). TB abscesses in the tonsil appear to be uncommon even in animals directly inoculated with *M. bovis* into the tonsillar crypt (Mackintosh, unpub). In most cases the organisms transit to the medial retropharyngeal. However, at least some of those animals that do develop a tonsillar abscess excrete *M. bovis* into the oro-pharynx and may be infectious from saliva, aerosols or via faeces This may also result in secondary lesions in the lungs and gastrointestinal tract and mesenteric and ileocecal LNs.

It appears that a proportion of deer with primary infections in the retropharyngeal LNs develop progressive disease which is likely to spread via the lymphatic system, especially to the lungs and thoracic lymph nodes. Lymph from the medial and lateral retropharyngeal LN efferents drain into the jugular, and the blood is then pumped from the right side of the heart to the lungs where *M. bovis* appears to get trapped and form abscesses. These animals are likely to then become infectious via aerosols.

A few deer also develop abscesses in LNs which burst onto the skin surface and heal to form draining sinuses which discharge organisms onto the skin surface. Direct contamination of cuts and abrasions on the limbs of deer may explain some of the cases where only a single carcass node, such as a popliteal, precrural, prescapular or axillary LN are affected.

In terms of excretion, heavily infected lungs (aerosols), infected tonsils (saliva) and draining sinuses (discharges) are the most likely to be involved. Some organisms may be passed in faeces.

2. ENVIRONMENTAL FACTORS

Our group has not conducted any experiments in this area, however environmental survival of *M. bovis* organisms appear short-lived (days) under open pasture conditions and moderate (weeks) when protected from the elements (light, desiccation, etc) (Jackson pers. comm.). Under ideal conditions such as frozen in tissue they may persist for months. Because of faecal avoidance it is unlikely that TB organisms in pasture contaminated by infected faeces will remain viable long enough to be a significant source of infection.

3. DEGREE OF CONTACT/EXPOSURE

The amount of direct contact between farmed deer is dependent on inherent factors such as age, gender, season, natural behaviour and imposed factors which are largely the result of management such as stocking rate, grazing systems, food supplementation, mixing of groups, yarding etc.

It is very difficult to control all of these factors in order that their influence on transmission can be studied. Most of our information is based on observations and inference.

Observations: Young weaner deer are probably more likely to transmit TB by close contact than stags because weaners readily "mob up" when approached and there is much closer contact in yard situations compared with stags which maintain much more paddock separation and resent close contact with each other in yards especially when they are in hard antler. In two weaner studies (referred to in Section 1), 1/10 and 1/8 in-contact controls were infected within four months of the start of the trials whereas in one stag trial 0/8 control stags were infected over a similar period In all studies apparently infectious animals were present in the groups, the animals were yarded every 2-4 weeks and were grazed on pasture and supplemented with hay and grain over the winter (for the first three months). Obviously, the numbers are too small to demonstrate a higher relative risk for weaners than stags, but they are compatible with that hypothesis

Other obvious behavioural factors that affect contact are hind/calf association prior to weaning and stag/hind (and hind/hind) associations over the breeding season.

The most obvious management factors that increase contact are yarding, mob-stocking, feeding supplements, indoor wintering and the transporting of animals. The act of drenching or administering oral boluses for anthelmintic or trace element treatment may facilitate transfer of infected saliva.

4. SUSCEPTIBILITY

The number of *M. bovis* organisms required to cause infection is affected by a number of environmental and behavioural factors. If they are taken up at susceptible mucous membrane surfaces in the head or lung it appears that few are required to initiate infection i.e. <100 c.f.u. Experimental infections have resulted from as few as eight c.f.u.s instilled into the tonsillar sac (Mackintosh, unpub. data), and from around 100 c f.u.s instilled into the trachea (Mackintosh et al, 1993)

It is hypothesised that two of the most important genetically controlled factors that affect susceptibility/resistance are (a) innate and (b) acquired immunity (Griffin et al, 1993). The innate immunity is related to the ability of the animal's macrophages to kill *M. bovis* intracellularly. Acquired immunity relates to the Major Histo Compatibility (MHC) system and T lymphocyte responses. In mice, at least, the innate resistance seems to have a relatively simple genetic control and it may be a single gene effect, with susceptibility being recessive. Acquired immunity is likely to have a much more complicated genetic basis which will be reflected in a spectrum of degrees of susceptibility/resistance.

Overlaid on this genetic susceptibility will be the affects of stress. A small study showed that the administration of a chronic low level of dexamethasone mimicking chronic stress at the time of *M. bovis* challenge increased the number and degree of severity of TB in young red deer (Thomson, *et al*, 1994). The most common causes of stress on deer farms are nutritional, climatic, behavioural or management/handling. It has been shown in mice that stress, mediated by increased corticosterone, can increase the susceptibility of some strains to *M. avium* (Brown *et al*, 1993).

Deer are very seasonal animals and go through reproductive and physiological cycles annually. The liveweight of stags varies dramatically, with them increasing in weight over the spring and summer to a pre-rut peak and then suddenly declining over the mating season, with maintenance at a minimal level over the winter This cycle, which has a basic endogenous rhythm entrained by day length, results in elevated testosterone levels in late summer, autumn and winter when the animal is in "hard antler" There is some evidence that androgens can be immunosuppressive and they may result in increased susceptibility to a number of diseases, including TB (Ansar-Ahmed, 1985). Likewise hinds mate seasonally and late pregnancy in spring/early summer is also associated with a degree of immuno suppression.

Concurrent disease is also likely to make animals more susceptible.

CONCLUSIONS/HYPOTHESES

1. Transmission of TB in deer is largely by exposure of susceptible animals to infected aerosols, fomites or discharges. Infected faeces are probably not a common source of infection.

2. The majority of animals first develop gross lesions in the retropharyngeal lymph nodes after the TB organisms have been carried there in the lymphatic drainage subsequent to crossing the mucous membrane especially in areas of lymphoid aggregations such as tonsils of the nasal or oral cavity. A small proportion develop primary lesions in the parotid, submandibular, mesenteric, ileocecal LNs or lung/mediastinal LNs (see Fig. 1).

3. Initially, the animal is probably not very infectious. A proportion of animals develop progressive disease whereby infection spreads from the retropharyngeal lymph node to the lungs, the abdominal cavity or body LNs.

4. Deer with serious lung, tonsil or draining sinus lesions are likely to be the most infectious.

5. Other major factors affecting transmission relate to the degree of contact/exposure and the susceptibility of exposed animals.

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Fig. 1 **Working hypothesis of Tb transmission in deer**