JD in deer: Practical steps towards taking control

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Abstract

As our knowledge of Johne's Disease (JD) in deer increases through a variety of sources including the Massey University 2005 nationwide case-control study currently under analysis by the author, it is becoming apparent that clear guidelines for a herd classification system are necessary. Pertinent details concerning the biology of *M. paratuberculosis,* including transmission routes likely to be applicable to farmed deer, are outlined. A nationwide case-control study of 174 properties throughout New Zealand in 2005 found *M. paratuberculosis* is geographically widespread on deer properties throughout the North and South Islands of New Zealand. *M. paratuberculosis* was identified, via pooled faecal culture, from deer on some properties reporting no typical clinical signs of JD and could not be cultured from some properties with typical clinical signs. A proposed herd classification system based on the diagnosis of *M. paratuberculosis* is outlined to allow discourse between purchasers and vendors of live deer in New Zealand. Although there is no current accreditation program for JD in the New Zealand deer industry, this classification system may form the basis discussion for a future program.

Key Words: Johne's Disease, deer*, Mycobacterium paratuberculosis*, herd status, faecal culture

Introduction

Johne's Disease (JD) is a chronic, debilitating enteritis predominately of ruminants, caused by the facultative intracellular, acid-fast bacterium *Mycobacterium avium* subspecies *paratuberculosis* (*M. paratuberculosis*) (Huda and Jensen, 2003). In the last 5 years, the prevalence of clinical cases of JD in the New Zealand deer industry appears to have increased substantially and the disease is now a serious economic burden to many farmers, particularly in the South Island (Wilson, 2002; de Lisle et al 2003).

This paper presents discussion of strain types found in deer, transmission pathways and a number of factors that require consideration in a management programme. The geographic distribution of *M. paratuberculosis* positive properties as determined by pooled faecal culture in a nationwide case-control study is also presented, along with preliminary data of herd status. The presence or otherwise of clinical signs typical of JD within a deer herd is not sufficient to accurately diagnose that herd's JD status. A preliminary herd JD status classification system is proposed for discussion to provide a framework for the purchase of live animals in the deer industry and as a basis for a possible future market assurance program for JD in deer.

M. paratuberculosis strains

Through the use of pulsed-field electrophoresis (PFGE) and IS900-restriction fragment length polymorphisms, *M. paratuberculosis* isolates have been divided into distinct types: Type I and Type II (Stevenson et al., 2002). Type I (S or ovine strain) *M. paratuberculosis* comprises very slow-growing and predominately pigmented isolates which form smooth and uniform colonies and have been largely isolated from sheep and other small ruminants (Dohmann et al., 2003). This strain has been found to be extremely difficult to isolate in culture, resulting in problems with transmission and pathogenesis studies, antemortem test validation, prevalence studies and effectiveness monitoring of control programs (Stehman, 1996). Type II (C or bovine strain) *M. paratuberculosis* comprises faster-growing, non-pigmented isolates which form rough and non-uniform colonies. This strain has been commonly isolated from cattle but exhibits a very broad host range including wildlife (Stevenson et al., 2002; Dohmann et al., 2003). It has been found that deer can become infected with both strains of *M. paratuberculosis*, although the Type II or bovine strain appears to be more prevalent and virulent, causing the "outbreak" forms of the disease in weaner and yearling mobs.

Transmission pathways of *M. paratuberculosis*

Extensive research in cattle and sheep has found that there are a number of possible transmission routes for *M. paratuberculosis*. Based on current knowledge, the 3 main pathways by which *M. paratuberculosis* can be transmitted to a fawn are:

In utero

van Kooten et al. (2006) demonstrated that eight of nine (89%) hinds affected with clinical signs of JD passed *M. paratuberculosis* to their foetuses. Further research has shown that 14 of 18 (78%) dams which were not showing clinical signs of JD but were positive to an IgG1 ELISA (Paralisa™) also infected their fetus, indicating this is a significant method of transmission (Mackintosh et al., these proceedings).

M. paratuberculosis has also been recovered from the uterus and placenta of infected cattle. In slaughterhouse studies, the percentage of foetuses from infected cows that were also infected ranged from 26.4% to 63.9% (Gay and Sherman, 1992; Clarke, 1997). However, although intrauterine infection of the foetus occurs, classical lesions of paratuberculosis have not been recognised in infected bovine foetuses (Clarke, 1997).

Transmammary

M. paratuberculosis is shed in the milk of infected cattle and sheep and it is very likely that infected hinds can also cause transmit infection to their fawns through the transmammary route (Collins, 2003). Mackintosh et al. (these proceedings) have cultured *M. paratuberculosis* from the mammary gland and associated lymph nodes from 12 of 18 (67%) sub-clinically infected hinds.

Faecal-oral

A challenge trial completed by Mackintosh et al. (2004) definitively demonstrated

that the oral intake of a sufficient dose of *M. paratuberculosis* resulted in clinical signs of JD in deer including carcass lesions and typical clinical signs. *M. paratuberculosis* is shed in the faeces of infected animals, particularly in scouring animals, and the predominant sources of bacterial shedding onto pasture are likely to be other deer, species other than deer (e.g.: sheep and cattle) and possibly wildlife. Although deer can become infected with both the bovine and ovine strains of *M. paratuberculosis*, it appears that the bovine strain is more prevalent and possibly more virulent, being the predominant cause of "outbreaks" in weaner and yearling mobs.

JD in deer: Five essential factors

When considering the development of a management program for the control of JD, there are five essential factors to consider. Further information is needed to provide a full understanding some of these factors in deer.

Age barrier

Research in cattle has found that infection with *M. paratuberculosis* is affected by an "age barrier" at approximately 6-10 months, beyond which an animal is less susceptible to becoming infected with the bacteria and subsequently developing clinical disease (Clarke, 1997). Similar research in sheep indicates this species may be susceptible in infection with *M. paratuberculosis* throughout life, although lambs and hoggets may be relatively more susceptible. Anecdotal evidence in deer suggested that naïve yearlings that were apparently exposed to *M. paratuberculosis* for the first time at 20-22 months of age demonstrated an "outbreak" of clinical signs of JD. Thus, whether deer follow the cattle or sheep pattern is yet to be determined.

Clinical signs

Clinical signs and mortality due to JD do not occur in sheep and cattle until they are 2-5 and 2-10 years of age, respectively, although yearling animals may show signs of disease if reared in heavily infected herds/flocks or heavily contaminated environments (AAHC, 2004). Deaths tend to occur sporadically rather than as an outbreak, and cases tend to occur more commonly at times of stress, such as during drought and just after calving or lambing. However, deer can develop clinical signs of JD from 6 months onwards and signs can vary significantly. A proportion of infected deer remain apparently unaffected (i.e.: no apparent weight loss/scouring), but may show carcass lesions at slaughter, while others may develop the full range of clinical symptoms. The clinical signs of JD may include:

Early-stage clinical signs:

- Separation from the mob
- Rough, light coat with a "moth-eaten" appearance/retention of the winter coat
- Good appetite, bright attitude, sometimes diarrhoea

Mid-stage clinical signs:

- Persistent diarrhoea
- Weight loss/ wasting/ ill-thrift
- Little to no response to treatment (eg: drench, antibiotics)

Late-stage clinical signs:

- A soft swelling under the skin (oedema) of the brisket or under the jaw (submandibular)
- Persistent diarrhoea
- Emaciation
- Death within weeks or months despite treatment

Sub-clinical or "carrier" animals

Animals infected with *M. paratuberculosis* will not immediately or may never develop clinical signs of the disease. Once ingested, *M. paratuberculosis* targets the mucosa-associated lymphoid tissues of, preferentially, the upper gastrointestinal tract, where it is endocytosed by the M cells of the ileal Peyer's patches and subsequently phagocytosed by subepithelial and intraepithelial macrophages (Harris and Barletta, 2001). *M. paratuberculosis* bacilli then remain in the phagosome for weeks to years, where they multiply intracellularly. The animal may therefore be infected but will not demonstrate clinical signs of the disease (i.e.: be sub-clinical) until periods of stress or other factors cause the bacteria to emerge from the cells. The initiation of a cellular immune response by nearby lymph nodes then creates intestinal granuloma and leads to the typical clinical signs associated with JD (Harris and Barletta, 2001).

In dairy operations it has been found that economic losses due to sub-clinical effects of JD can occur (Wells et al, 2002). These sub-clinical effects may include reduced milk production, premature culling and reduced bodyweight in culled cows. Further research is needed to determine whether a similar economic loss is being experienced in the deer industry due to sub-clinical JD.

Faecal contamination

Clinically affected animals appear to shed significantly more *M. paratuberculosis* than sub-clinically affected animals (Clarke, 1997; Collins, 2003). Clinically affected cattle may shed in excess of 10^8 bacilli/g of faeces and sheep have been reported to excrete 1.09×10^8 viable bacteria/g of faeces (Daniels, 2003). Early diagnosis and culling of clinically affected animals is, therefore, an essential step in the long-term management of this disease.

Longevity in the environment

Although *M. paratuberculosis* is thought not to be free-living (i.e.: able to grow and multiply) in the environment, a thick capsule confers significant resistance to environmental effects, enabling the bacteria to survive for up to 11 months in soil (Gay, 1992; Collins, 2003). It has also been shown that *Mycobacterium* species have a higher survival rate in acidic environments (Ward et al., 2004). Environments rich in organic matter that have a low pH, such as sandy loam soils, may provide conditions that enhance survival of *M. paratuberculosis* and therefore environmental persistence (Johnson-Ifearulundu and Kaneene, 1997).

Whittington et al. (2003) found the analytical sensitivity of culture from environmental samples is less than that from faeces. Approximately 20% of 163 soil-pasture, water and sediment samples taken on Australian sheep properties infected with *M. paratuberculosis* recovered the bacteria. Positive samples were located from sites where faecal contamination was concentrated by gradient and runoff. Re-culture of the same soil sites approximately 5 months later found only 1 was positive and none were culture positive over 12 months later. However, three sediment samples

were positive after destocking of sheep and goats for 9-24 months and recontamination from cattle or water could not be excluded (Whittington, 2003).

Massey University epidemiological research

In 2003, the New Zealand deer industry, in conjunction with Massey University and the Johne's Research Group (JRG), commissioned a comprehensive research project into the epidemiology of JD in farmed deer commencing in September 2004. A major component of this research has been a nationwide case-control study, for which sampling began in July 2005. The primary aims of the study were:

- To determine risk factors at the herd level for:
    a. Infection with *M. paratuberculosis* and
    b. Clinical or other signs of JD (e.g.: scouring/wasting; carcass lesions; Tb skin test non-specific reactors)
- To investigate management practices that may be employed to control these risk factors
- To determine regional distribution of the presence of *M. ptb* and clinical signs of JD
- To increase general knowledge of JD in deer

Study design

One hundred and seventy four properties grazing deer were enrolled in the nationwide case-control study, based on their willingness to participate. Sixty-one (61) had a positive tissue or faecal culture for *M. paratuberculosis* prior to study commencement. The remainder (113) were faecal and blood sampled between August and November 2005, to establish the presence of *M. paratuberculosis* at the herd level. Using an equation described by Christensen et al. (2000), the pooling and BACTEC culture of faecal samples from 60 targeted adult breeding hinds in 6 pools of 10 was estimated to be the most sensitive and cost-effective method for the diagnosis of *M. paratuberculosis* at the herd level. This methodology resulted in an estimated herd level sensitivity of 68% and a specificity of 100%.

Potentially infected animals (e.g. animals which are scouring, wasting and/or demonstrate a poor quality hair coat) and non-specific reactors to a previous TB skin test were targeted for sampling to increase the likelihood of detecting infection in the herd (Smith and Slenning, 2000). Preliminary results are highlighted in Table 1.

Table 1: The number of study herds without prior culture evidence of infection (n=113) that were culture positive or negative for *M. paratuberculosis* in the North Island (NI) (n = 51), South Island (SI) (n = 64) and overall New Zealand (NZ) as determined by pooled faecal culture, in relation to the presence or absence of reported clinical signs typical of JD.

	***M. paratuberculosis* (pooled faecal culture) Herd Status**							
	Positive			**Negative**			**TOTAL**	
Clinical status	**NZ**	NI	SI	**NZ**	NI	SI	Number	%

Positive	**NZ**	**36**			**8**			**44**	**38**
	NI		12			2		14	27
	SI			24			6	30	47
Negative	**NZ**	**14**			**57**			**71**	**62**
	NI		3			34		37	73
	SI			11			23	34	53
TOTAL	Number	**50**	15	35	**65**	36	29	**115**	
	%	**43**	29	55	**57**	71	45		**100**

Note: *The data presented cannot be used to accurately determine the regional or national prevalence of M. paratuberculosis as property selection was not random and the sample size is insufficient to obtain an accurate estimate.*

The geographic distribution of properties sampled was approximately equal between the two islands with 55% (64) located in the South Island. Although 43% (50/113) of properties sampled were culture positive for *M.paratuberculosis*, there was an apparent difference between the North and South Islands with 55% (35/64) of the South Island properties culture positive in comparison to 29% (15/51) in the North Island. Approximately half (30/64) of the South Island properties sampled showed clinical signs typical of JD at the time of sampling in comparison with only 27% (14/51) of the North Island properties. On South Island properties 31% (11/35) culture positive did not report clinical signs typical of JD, compared with 20% (3/15) in the North Island. Of the 14 properties in the North Island which demonstrated typical clinical signs of JD, 14% (2/14) did not have a positive *M. paratuberculosis* culture result, compared to 20% (6/30) of properties in the South Island.

Data in Table 1 indicates that the presence or absence of clinical signs typical of JD in a herd is not sufficient to have confidence in predicting the presence or absence of *M. paratuberculosis* within that herd

Definition of herd status

The deer industry does not have an accreditation program for JD requiring determination of herd status. The herd categories below are a suggested classification to allow discourse between herd owners, particularly during the purchase of replacement or finishing stock.

- Confirmed infected
- High risk
- Low risk
- Unconfirmed
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*Confirmed infected****:*** A herd is classified as confirmed infected following one or more individual tissue or faecal sample or pooled faecal sample culture test positive.

Note that the IgG1 ELISA (Paralisa™) may be used to identify animals that are most likely to be infected, and therefore targeted for the definitive culture test, either on faeces or intestinal tract and lymph node tissue.

Thus, the confirmed infected classification refers to the presence of *M. paratuberculosis* in the deer herd. It does not indicate whether there is economic loss due clinical signs typical of JD within that herd.

High risk.

Herds that are not confirmed infected but which have experienced a Paralisa test positive result without confirmation by culture. Other factors to consider are clinical disease resembling JD without veterinary investigation to exclude other potential causes, those that are known to have JD in sheep and/or cattle grazing on the same property, and those that have purchased deer from known infected herds.

Note: The concept of differentiation of risk status is presented here to prompt discussion. Establishment of measurable risk criteria is complex and would require detailed consideration.

Low risk

Herds that have tested the recommended number of animals (see below) using on-farm diagnostic tests (e.g. Paralisa™ and/or individual or pooled faecal culture) with a negative result, and from which the following criteria ar fulfilled:

- No clinical signs typical of JD (i.e. scouring and/or wasting) in any ruminant (deer, sheep or cattle) on the property in the previous 24 months
- No Tb-like carcass lesions that subsequently tested culture-negative for Tb
- Tb skin tests were negative, or positive but subsequently ETB-negative for JD

As there is no diagnostic test for *M. paratuberculosis* which is 100% sensitive, it is not possible to prove conclusively that a herd is "free" of the bacteria. Thus confidence in this category will depend on the rigour of the testing regime: ie: the number and type of deer tested or observed, the frequency of testing and the tests used.

Unconfirmed

All deer farms that do not confirm to the aboe criteria would be classified as unconfirmed.

Establishing herd status

Figure 2 outlines the testing options available to the owner/manager of a herd with an "unconfirmed" status determine the JD status of a deer herd.

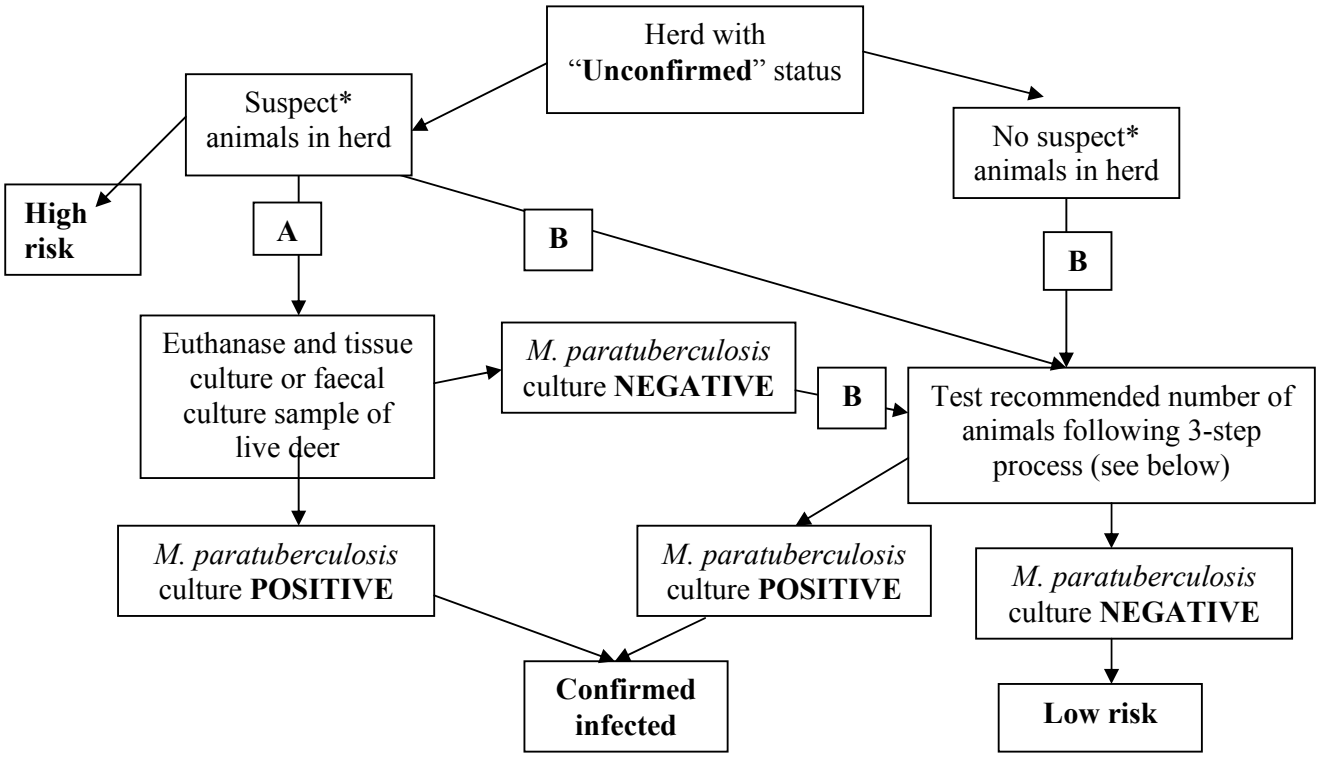

Figure 2: Decision tree outlining the testing options to determine the herd status for *M. paratuberculosis*

* e.g. Clinical signs or lesions of disease, Paralisa positive, non-specific Tb test reactions

Testing Option A

Euthanasia and tissue culture, or faecal culture of a live deer suspected of suffering from JD, particularly with clinical signs of the disease, may be a low-cost option for determining herd status since only one culture positive for *M. paratuberculosis* is necessary to classify a herd as confirmed infected. However, if the selected animals tested are found negative for *M.paratuberculosis*, the herdowner/manager would need to undertake testing option B before a low risk classification can be determined.

Testing Option B

Unfortunately, it is not possible to be 100% confident of a negative JD status of a herd due to the nature of the disease and the limitations of current tests. However, following the three steps outlined below will give a high level of confidence of herd status:

1. Choosing sample size based on estimated herd prevalence of JD

To determine the appropriate sample size to establish the JD status of a herd, the prevalence of the disease (ie: low, medium or high) must be estimated, based on the

parameters outlined in Table 2. These guidelines relate to recent observations and indings, preferably within the previous 24 months

Table 2: The estimated herd prevalence of JD prior to recommended testing based on the parameters outlined.

Estimated herd JD prevalence	Clinical signs	Carcass lesions (Tb culture negative)	Non-specific reactors (MCT)	ETB (JD suspicious)
Low (<2%)	Nil	Nil	<2%	0
Medium (2-10%)	<10%	<2%	2-10%	≥ 1
High (>10%)	>10%	>2%	>10%	Not applicable

2. Choose a diagnostic test

A diagnostic test is then chosen based on test:

- Sensitivity (ability to be test positive in infected animals) and specificity (ability to be test negative in non-infected animals
- Cost
- Logistics (e.g.: time, labour and facilities)

The only diagnostic tests currently available to confirm diagnosis of JD in deer herds, and hence their status as above, are individual faecal culture, pooled faecal culture and tissue culture.

3. Appropriate sample size

The appropriate number of deer to test to determine a herd's JD status will also depend on the current herd size, including adults, rising 2-year-olds and weaner hinds and stags. Tables outlining the appropriate sample size for estimated low, medium and high prevalence herds using the available diagnostic tests are available in the Johne's Research Group's JD manual (2006).

For a more comprehensive description of the steps involved in testing option B, refer to "Johne's Disease: The Way Forward", a JD manual for deer, published by the Johne's Research Group (2006).

Conclusions

Johne's Disease has become a serious source of economic loss to the New Zealand deer farmer in the last 5 years as the apparent prevalence of clinical signs of the disease have escalated. A nationwide case-control study throughout New Zealand in 2005 found *M. paratuberculosis* is geographically widespread on deer properties throughout both Islands. A herd classification system based on the diagnosis of *M. paratuberculosis* was outlined to allow discourse between purchasers and vendors of live deer in New Zealand. Although there is no accreditation program for JD in the New Zealand deer industry, this classification system may form the basis of a future program.

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