Johne's Disease
in New Zealand Farmed Deer

Johne's Research Group

Bulletin Three November 2004

Update of Current Research on Johne's Disease in Deer in New Zealand

The purpose of this Bulletin is to explain the research that's being carried out in New Zealand on Johne's disease (JD) in deer, and to explain how this research is likely to help diagnose and control the disease on New Zealand's deer farms.

In a nutshell, the purpose of all the research is

- To help make diagnosis quicker and more accurate
- To develop vaccines to prevent JD
- To promote a better understanding of the way JD spreads and causes disease within herds (ie its epidemiology).

continued overleaf

Other publications produced by the JRG include:

JRG Bulletin One:
"Johne's disease in New Zealand farmed deer. What does this mean for you and your farm in 2004?"

JRG Bulletin Two:
"Detained carcasses: Johne's disease lymph node lesions in slaughtered deer and their implications."

JRG Information Leaflet:
"Johne's disease in farmed deer. Dr C G Mackintosh, Invermay, AgResearch (August 2002)."

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Comment on *vaccine research*

- Research aims to produce a cost-effective vaccine that is acceptable to our markets.
- No licensed vaccines are currently available for use in deer.
- An ideal JD vaccine would not cause vaccinated deer to react positively to Animal Health Board (AHB)-approved Tb tests (MCT, CCT, BTB, ELISA and ETB).
- 'Gudair' (and its predecessor 'Neoparasec') are JD vaccines for sheep and cattle, but they can cause false positive reactions to the skin tests for tuberculosis.
- Gudair vaccination is likely to interfere with Tb testing in deer. The AHB is unlikely to permit it to be used in deer retained as herd replacements, but may allow it to be used in deer destined for slaughter. These would be "Works Monitored" for Tb (ie examined for Tb at the DSP) rather than Tb-tested.
- The long term objective is to develop a vaccine that stimulates protective immunity, that doesn't cause infection or disease, and doesn't interfere with Tb tests. Two types of vaccine are showing promise:
  - New vaccines containing attenuated (ie weakened) organisms. This work is being carried out by Geoff de Lisle's group at Wallaceville.
  - Subunit vaccine based on key proteins or molecules in the JD organism. A group at Massey headed by Dr Alan Murray is following this line of research.

Research programmes are being carried out at

1. AgResearch Invermay
2. Massey University (the veterinary faculty, now known as IVABS)
3. AgResearch Wallaceville
4. The Disease Research Laboratory, Otago University, Dunedin

These programmes are integrated. The researchers communicate freely with one another, so effectively they work as a team with the mutual aim of understanding, detecting and preventing JD in deer.

1 AgResearch Invermay (Colin Mackintosh et al.)

Invermay research aims to help understand how JD spreads and causes disease within herds (its epidemiology) and to develop a useful and effective vaccine.

Epidemiology

Dose effects

- Signs of clinical JD (loss of weight, scouring etc) developed in a group of yearlings that had been dosed at 4 months of age with a large dose of organisms by mouth, but not in those that were given a medium or low dose, although most of these were infected

Advice to farmers

- Cull deer that show signs of the disease (scouring and weight loss) as soon as possible, especially hinds that will pass infection onto fawns
- If possible, cull hinds that are infected with JD (ie PARALISA blood test positive or faecal culture positive)

Strain differences

- There are two main types of JD bacteria – the bovine (cattle) and the ovine (sheep) strains.
- The bovine strain is the cause of most outbreaks of JD in deer, and it appears to cause more severe disease than the ovine strain.
- The bovine strain is well adapted to deer, and probably spreads from farm to farm in deer.
- It took twice as many organisms of the ovine strain as of the cattle strain to cause the same amount of infection and damage in the intestine of experimentally infected deer.

Advice to farmers

- JD is probably brought onto farms mainly in infected deer. Ideally bought in deer (particularly breeding stock) should be negative to the PARALISA blood test and/or faecal culture.

Vaccine testing

- Currently Invermay research is looking at the effects of vaccination with Gudair and another oil-based vaccine, 316F
- 316F is a vaccine based on an attenuated (weakened) strain of the JD organism.
- 316F is being tested to make sure it cannot cause disease and does not cause false positive reactions to the Tb skin test.
- A water-based vaccine has been tested – it gave some protection but not enough to be useful

2 Massey University (Peter Wilson, Cord Heuer, Jaimie Glossop)

Massey University in partnership with the Johne's Research Group is undertaking a major epidemiology project.

Epidemiology

- The project will lead to a better understanding of the way JD spreads and causes disease within herds
- It will look at risk factors and evaluate control measures.
- The project has just begun, and it will be completed in 4 years' time.
- The work will be carried out on over 200 'volunteer' deer farms all over New Zealand, and so far it has involved affected and unaffected farms in Canterbury and Otago.
- It is hoped the project will provide management and control recommendations for deer farmers.

3 AgResearch Wallaceville (Geoff de Lisle)

JD research at Wallaceville is aimed at developing better on-farm diagnostic tests, studying JD epidemiology and developing more effective vaccines.

Pooled faecal culture test

- This is a promising new technique for testing herds rather than individual animals.
- It has a moderate level of sensitivity (ie it can detect very small numbers of bacteria) and does not give false positive results for JD.
- It involves mixing 10 to 25 individual faecal samples and taking one subsample for testing.
- Pooled faecal culture is significantly cheaper than individual faecal culture.
- A small farm trial is underway in Canterbury
- Pooled faecal culture could potentially be used in Market Assurance Programmes or QA Programmes.

Advice to farmers:

- Farmers who want to show that they don't have JD can do repeated pooled faecal cultures on their deer to show that none is shedding JD organisms.
- Farmers who want to buy JD-free deer could purchase from these herds.

PCR test and epidemiology study

A PCR test (based on DNA analysis) has been developed to distinguish between the sheep (ovine) and cattle (bovine) strains of the JD bacterium *(Mycobacterium paratuberculosis)*.

- A trial is underway using the PCR for this purpose in JD-infected deer herds throughout New Zealand.
- Preliminary results show that while the bovine strain is far more common, the ovine strain is present in a small number of infected herds.

Vaccine research

- New live vaccines are being developed by carefully treating JD bacteria and selecting for attenuated (ie weakened) organisms that cause protective immunity to develop but don't cause disease.
- Specific genes can be deleted from bacteria to make them less likely to cause disease and less likely to cause false positive reactions to the skin tests while preserving their ability to give protection against JD.
- Similar vaccines are being developed for Tb.

4 Disease Research Laboratory, Otago University

(Frank Griffin, Chris Rodgers)

The purpose of much of the DRL research on JD in deer is to improve diagnostic tests and to investigate the immune response.

Currently, projects are underway:

- To develop laboratory assays to look at cellular immunity (protective immunity) and antibody reactivity (a measure of disease).
- To develop techniques to look at the differences in the tissues (eg lymph nodes) of infected and non-infected animals
- To compare the immune responses of naturally infected and experimentally infected animals
- To compare the immune responses in sheep and deer that have been experimentally infected with 'ovine' and 'bovine' strains of JD.

PARALISA

The lab has developed a new blood test for Johne's disease.

- It detects antibodies (a type of marker) in blood samples from Johne's-affected deer.
- Results are reported as positive, suspicious or negative
- It is more sensitive and specific for Johne's than other antibody tests
- It detects earlier cases of Johne's infection

DEFINITION OF TECHNICAL TERMS

The AHB-approved tests for Tb in deer are the MCT, CCT, BTB, ELISA and ETB.

Tests used for JD diagnosis are PARALISA, Indirect ELISA, PCR, BACTEC culture and pooled faecal culture (PFC).

BACTEC culture – A sensitive culture system for growing JD organisms from samples of faeces and body tissues such as intestine and lymph node. JD organisms tend to be very slow growing in culture but this system is significantly quicker than the older system and a result is usually obtained in 3 to 8 weeks.

BTB – The blood test for TB. It is the most sensitive and specific test available for Tb. It can be used as the first (primary) test for Tb in deer, or it can be used as a secondary test on deer that have tested positive to the MCT (single-shot skin test).

The BTB can be carried out 2 to 4 weeks after the skin test whereas the CCT can't be carried out within 90 days of the MCT.

Unfortunately, when deer with JD are tested using the BTB they can sometimes give a false positive result for Tb.

CCT – Comparative cervical test. This is a two-shot skin test. Two sites are prepared by clipping two patches on the neck, and avian tuberculin is injected into the skin at the upper site and bovine tuberculin at the lower site. Any increase in skin thickness (reaction) is measured after 72 hours and the difference between the two sites determines whether the original CCT positive was a reaction to avian or bovine types of Mycobacteria.

Deer with bovine Tb usually react more at the bovine site. Deer with avian Tb and deer with JD usually react more at the avian site

ELISA – A blood test for Tb, approved by the AHB, for use with skin testing in herds that are experiencing an outbreak of Tb.

ETB – A follow-up (secondary) blood test for Tb for use on reactors. The ETB is almost as sensitive and specific as the BTB but quicker and cheaper.

The ETB includes a specific test for Tb and tests for bovine Tb, avian Tb and JD. In deer with JD, the response to the JD test is usually greater than those for bovine Tb and avian Tb, and a diagnosis of probable JD can be made. However AHB policy is that if the bovine response is greater than the avian response the animal must be culled, even if the JD response indicates that the cause is probably JD, not Tb.

Indirect ELISA – This test has been developed by Gribbles' Laboratory Palmerston North for the diagnosis of JD in sheep cattle and deer. Useful for confirming JD in deer with signs of the disease but may not be as sensitive as the PARALISA for detecting subclinical cases (deer that are not yet showing signs of the disease).

MCT – Single-shot mid-cervical test, sometimes called the skin test (ST). This is the standard test for Tb in deer. A patch is clipped on the neck, bovine tuberculin is injected into the skin and the site is examined 72 hours later for any visible or palpable increase in skin thickness.

Deer with bovine Tb, deer with avian Tb and deer with JD can all give false positive results to the MCT. When deer react positive to the MCT the farmer can kill them on the farm, send them to a DSP or re-test to confirm using the CCT, MCT, CCT or BTB.

PARALISA – A blood test for JD. Available from the DRL Dunedin. It is more sensitive (detects deer at an earlier stage of disease) and more specific (very few false positives) than other blood tests. It detects antibodies (a type of marker) in blood samples from Johne's-affected deer. It can be used to test for JD in individual deer suspected of having JD, to test for JD in deer being presented for sale and to screen breeding hinds so that affected hinds can be culled.

PCR – Polymerase chain reaction. This is a relatively quick test for a specific sequence of DNA (genetic material) in particular bacteria or viruses in blood or body tissue samples. It is not as sensitive as culture for Mycobacteria, but it is usually very specific if carried out carefully.

PFC – Pooled faecal culture involves pooling faecal samples from up to 25 animals, mixing, and taking a sample for BACTEC culture. Useful for screening groups of animals cost-effectively.

ST – Skin test. The name sometimes given to the MCT (see above).

Note: A more comprehensive and detailed glossary of the technical terms used in these Bulletins will be included with Bulletin Four.