# Prospects and issues for Johne's vaccination of farmed deer

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## Abstract

This paper addresses the key questions of "why vaccinate deer", "what vaccines are available in NZ", "how effective are they" and "what are the main issues relating to use of Johne's vaccines in deer". Johne's disease is an emerging problem in farmed deer and other domestic livestock in New Zealand and overseas, and vaccination offers a degree of control in most situations. Currently available vaccines contain either killed or live attenuated whole cell *M. paratuberculosis* organisms with an oil adjuvant. None of the vaccines developed so far produce sterile immunity, but they significantly reduce the severity of disease and reduce faecal shedding. However, they all provoke a vigorous injection site reaction and cause cattle and deer to give a positive response to the bovine tuberculin (Tb) test. Problems of sensitising deer to the Tb test may be overcome by vaccinating only animals destined for slaughter as yearlings, thus avoiding the need to Tb test, or only vaccinating deer in low-risk areas where a comparative Tb test is acceptable.

**KEY WORDS:** Deer ,Johne's disease, *M. paratuberculosis*, vaccines, farm, tuberculosis testing interference, injection site reactions, efficacy

**Why vaccinate deer?**

Johne's disease has emerged as a serious problem on deer farms in New Zealand (Mackintosh et al 2004a; Mackintosh et al 2004b), there are no proven cost-effective means of eradication and control is difficult. There are a number of direct management steps that can be taken to attempt to control Johne's disease on deer farms and reduce the severity of the problem, including culling affected stock and changing from breeding to a venison finishing operation. Some herds have also used extensive testing of breeding hinds using an IgG1-based ELISA (Paralisa™) to subsequently reduce the incidence of clinical disease in yearlings (Bell 2005; Rodgers et al 2005), although the cost-effectiveness of these practices requires further clinical and research evaluation. However, for many herds the options for cost-effective control are limited. If a Johne's vaccine was available that was inexpensive and efficacious, was used in a way that did not interfere with the national bovine tuberculosis control programme, and did not cause unacceptable carcass blemishes or downgrading, then targeted or whole-herd vaccination could provide practical means of Johne's disease control on deer farms in New Zealand. That the majority of disease appears to be in animals less than one-year-of-age suggests that there may be a role for a controlled programme of vaccination of deer that are destined for slaughter at a young age, and don't reach Tb testing age eligibility.

**What Johne's vaccines have been used in livestock worldwide and how effective are they?**

Vaccines fall into three main types:

- Whole-cell live vaccines containing live attenuated strain(s) of *Mycobacterium avium* subsp. *paratuberculosis* plus an adjuvant,
- Whole-cell inactivated vaccines containing varying quantities and strains of heat killed *M. paratuberculosis* plus an adjuvant, and
- subunit vaccines, which contain purified antigens derived from *M. paratuberculosis* plus an adjuvant, although none has ever been produced for routine commercial use.

The subject of Johne's vaccines was reviewed by Wilesmith (1982) and more recently by Emery and Whittington (2004). It was also the subject of a workshop at the 8th International Colloquium on Paratuberculosis (2005, Proceedings *in press*).

***Whole cell live vaccines***

Vaccination with live *M. paratuberculosis* was first used in 1926 (Vallée and Rinjard 1926) cited by (Emery and Whittington 2004). One of the earliest commercial Johne's vaccines was produced by the Central Veterinary Laboratory, Weybridge, United Kingdom and used in cattle in that country UK (Doyle 1964; Stuart 1965). It comprised two live attenuated strains of *M. paratuberculosis* with an adjuvant containing olive oil, liquid paraffin and finely ground pumice and was given by subcutaneous injection to very young calves. Although it caused an unsightly reaction at the injection site, it was effective in experimental studies and on dairy farms (Wilesmith 1982). Vaccination reduced clinical disease but it did not completely eliminate infection with *M. paratuberculosis*, although it did reduce the rate of infection by 33% (Stuart 1965). This vaccine was also used in cattle herds in New Zealand in the 1950s because it offered a promising means of controlling Johne's disease, but a high proportion of vaccinated cattle gave false-positive reactions to the caudal fold test (Chandler 1957a).

Argente (1991) reported on the use of "Neoparasec"™ vaccine in cattle in France. It was another live attenuated *M. paratuberculosis* Strain 316 vaccine made by the Pasteur Institute, but it had a water-in-mineral oil adjuvant. "Neoparasec" vaccination of 2073 cattle, plus disease management strategies, reduced faecal shedding rates by 85% compared with 23% by management strategies alone with 1281 animals. In 1987, "Neoparasec" was licensed for use in cattle and sheep in New Zealand. Its use in cattle was restricted to areas where bovine tuberculosis (Tb) was not present in wildlife, because of the potential problems of distinguishing cattle vaccinated against Johne's disease from those infected with *M. bovis* and this curtailed its use. Sales of Johne's vaccine were modest for use in sheep, mainly because it was recommended for use in lambs 2-4 weeks old and this resulted in the vaccination of some lambs destined for slaughter at 3-5 months of age. Many of these lambs had severe injection site lesions, which necessitated extensive trimming at slaughter and there was a risk that this could have affected the export value of the carcasses. Even though "Neoparasec" had major limitations, it was a useful tool for controlling clinical Johne's disease (de Lisle 2002). Although live vaccines appear to be efficacious, public health concerns related to accidental self-inoculation, the short period (24 hours) in which Neoparasec had to be used once it was reconstituted and the possibility of spread of the vaccine strain *M. paratuberculosis* from vaccinated cattle have precluded their continued use (Emery and Whittington 2004). "Neoparasec" was withdrawn from sale in New Zealand in 2002.

In Scotland, vaccination of deer with the Weybridge live attenuated vaccine has been used successfully to prevent clinical disease and it is believed that, with continued vaccination over an extended period of time, the infection levels within the herd will decline and may approach zero (Goddard et al 1994; Fawcett et al 1995). A small trial was conducted in red deer in New Zealand to assess the local and systemic responses of deer to vaccination with "Neoparasec" (see later).

***Whole cell inactivated vaccines***

Johne's disease was introduced into Iceland in the 1930s and caused a high incidence of clinical disease in sheep. A number of small field trials with an oil adjuvanted killed vaccine in the 1940s led to a large field trial in the early 1950s. The vaccine, containing 5 mg of two bovine strains *M. paratuberculosis* (Teps strain and Strain 18), was given to half the lambs on 141 farms in 1950 and 1951 and the clinical outcomes were monitored for 6 years. Over 6400 lambs were involved and the vaccination reduced Johne's disease mortality (confirmed by histopathology) to 0.3-0.8% in the vaccinates compared with 8-12% in the controls (Sigurdsson 1960). Subsequently wide scale

vaccination of lambs has almost eliminated clinical Johne's disease in Iceland. However, once vaccination was stopped, the disease reappeared (Fridriksdottir et al 2002).

Experimental challenge studies in the late 1950s showed that subcutaneous injection of sheep with killed *M. johnei* (*M. paratuberculosis)* vaccines gave significant protection against clinical disease, but oral vaccination was ineffective (Brotherston et al 1961).

There have been a number of reports on the use of a whole cell inactivated vaccines as a water-in-oil emulsion, given to calves <30 days old. They have shown that although vaccination reduced the number and severity of clinical cases and reduced faecal shedding, it did not eliminate infection and it was recommended that hygienic practices remain essential in herd management (Kalis et al 2001).

"Gudair"™ vaccine is a whole cell inactivated vaccine containing heat killed *M. paratuberculosis* Strain 316F plus a mineral oil adjuvant, which is manufactured by CZV in Spain and it was originally marketed in New Zealand and Australia by CSL and is currently marketed by Pfizer Ltd A recent review of Australian trials with "Gudair" vaccine showed that vaccination delayed the onset and reduced the incidence of mortalities and faecal shedding of *M. paratuberculosis* by approximately 90% (Windsor 2006). A rapid decrease in losses occurred in a very high prevalence flock following whole flock vaccination and numerous management changes. Persistent injection lesions were common in vaccinated sheep and although human exposure to the vaccine was uncommon, accidental self-injection of vaccine may produce lesions requiring surgical intervention (Windsor et al 2005). Recent research in sheep flocks in Australia has shown that vaccination delayed onset of clinical disease, reduced clinical and sub-clinical disease incidence rates, bacterial faecal shedding rates and lesion rates at slaughter, and over a longer period resulted in improved fleece weights (Eppleston et al 2005 and Eppleston unpublished). Disease modelling suggests that annual vaccination of lambs will lead to reduced transmission and a marked reduction in within-flock prevalence of infection over a 10-year period (Sergeant 2003).

A small trial was conducted in farmed deer at Invermay to measure the efficacy of "Gudair" vaccine and assess potential interference with Tb testing (see later).

"Gudair" has also recently been used on four Johne's infected deer farms in South Australia, in conjunction with other control measures, in order to reduce clinical losses (van Wijk, pers comm.). The additional control measures include serological testing and removal of suspect clinical cases, herd profiling, age segregation and feeding off the ground. Two of these herds have subsequently undergone voluntary destocking programmes and the other two herds are likely to phase in voluntary destocking at some point. The efficacy of the vaccination is currently being assessed.

***Subunit vaccines***

There are a number of groups internationally that are working on the development of subunit vaccines, and although some vaccines have shown promise, none have been licensed for use in domestic animals. They target individual DNA, proteins or antigens that are believed to be associated with virulence or promote a protective host response, and are chosen because they are unique to *M. paratuberculosis* and/or are unlikely to provoke a positive bovine skin test reaction. One such experimental vaccine contains a recombinant mycobacterial 70 kD heat-shock protein (HSP70) and it has shown give significant reduction in faecal shedding in cattle (Koets et al 2006)

**What are the negative (or potentially negative) aspects of Johne's vaccines?**

***Interference with Tb testing***

It has been shown repeatedly since Johne's vaccines were first used over 60 years ago that they sensitised cattle to the bovine tuberculin (Tb) skin test (Chandler 1957b; Munday 1959; Doyle 1964; Kohler et al 2001) and vaccination has usually been recommended only in Tb-free herds. In most cases vaccinated cattle react more strongly to avian (or Johnin) site than the bovine site in the comparative skin test (CCT), although some results may be equivocal (Chandler 1957b). A trial was carried out in the USA in which cattle that had been vaccinated with a killed Johne's vaccine six months earlier were experimentally infected with *M. bovis*. Prior to the *M. bovis* challenge the CCT response was clearly biased towards the Johnin site, whereas after challenge the CCT response became clearly bovine (Larsen et al 1969).

In Scotland, red deer calves vaccinated with the live Weybridge vaccine were skin tested and blood sampled for serological testing twice, as yearlings, and a high proportion (86-92%) reacted to the avian PPD skin test, compared with 32% the previous year in unvaccinated yearlings. Vaccination also increased the proportion of animals that were seropositive for antibodies to a *M. paratuberculosis* antigen (Goddard et al 1994). Unfortunately no bovine skin tests or serological tests were carried out.

Two vaccination trials have been carried out in young red deer at AgResearch Invermay, the first with "Neoparasec" and the second with "Gudair" vaccine. In the "Neoparasec" study, 14/15 deer reacted to the mid cervical bovine Tb test (MCT) 12 weeks after vaccination. When a CCT was conducted 24 weeks after vaccination, 2/15 animals were positive (in relation to Tb), but by Week 36, all animals were CCT-negative (Mackintosh et al 2005). In the "Gudair" trial, 30 deer were vaccinated and 30 were unvaccinated controls, and all animals were challenged with live *M. paratuberculosis* 10 weeks later to measure the efficacy of the vaccination. All the animals were skin tested at Week 23 (10 weeks after challenge) and over 90% of deer in both groups were MCT-positive, and there were no significant differences between groups. In the CCT at Week 37, two "Gudair" and one Control deer were bovine Tb positive, but in the CCT at Week 57 the level of reactivity at the bovine site had declined overall and there were no Tb reactors in either group (Mackintosh et al, in prep.). These results suggest that deer are likely to respond to skin testing in a similar way to cattle and that over time their reactivity to the MCT may recede, but a proportion may remain positive for a prolonged time. However, the CCT is likely to be effective for differentiating vaccinated deer that are not infected with bovine Tb but are MCT-positive. It remains to be seen how deer that are Johne's vaccinated and Tb infected respond to the CCT. A complicating factor with deer infected with Johne's disease and vaccinated with Johne's vaccines, is that they produce significant amounts of *M. bovis* antibody as well as avian/johnin antibody. This has the potential to interfere with ancillary Tb serological test such as the ETB.

***Injection site reactions***

One of the earliest Johne's vaccines contained olive oil, liquid paraffin and finely ground pumice, which produced an extremely vigorous host reaction at the injection site (Doyle 1964). Subsequently, the adjuvants have been refined somewhat, although all current commercial whole cell Johne's vaccines contain various mineral oil formulations and the result is very similar to Freund's complete adjuvant, which is well known to cause severe reactions at the injection site in most animals. Attempts have been made to find alternative adjuvants, but none have become commercial realties.

"Neoparasec" vaccination in 2-4 week-old lambs in the early 1990s in New Zealand resulted in many severe injection site lesions in lambs at slaughter 3-4 months later and in some cases the draining lymph node had a caseous Tb-like lesion that was AFO-positive on histopathological examination. This caused problems with meat inspection and required extensive trimming of the carcasses, with loss of value and increase in processing costs. Some of these problems were caused by faulty vaccination technique resulting in intramuscular rather than subcutaneous injection.

However, vaccination of very young lambs requires great care. Also the vaccination of lambs at 2-4 weeks resulted in the unnecessary vaccination of lambs destined for slaughter within 3-4 months. Subsequently, "Neoparasec" has been withdrawn from the market and "Gudair" vaccine introduced. It is recommended that "Gudair" be used at weaning (3 months of age) and only in replacement animals. This has resulted in minimal problems of vaccinated animals entering the food chain soon after vaccination.

Limited experience with the vaccination of fifteen 2-3 month-old red deer with "Neoparasec" has shown that this vaccine was moderately well tolerated (Mackintosh et al 2005). It produced hard, indurated lumps >5 mm thick and 8–23 mm in diameter (mean 16.8 mm) at the vaccination site 3 weeks after injection, and these remained as hard lumps, averaging 14.6 mm in diameter at 9 weeks after injection. At slaughter 10 months after vaccination there were a few nodules on the carcasses that were easily trimmed and no involvement of the prescapular lymph node.

A challenge trial involving the vaccination of 30 deer with "Gudair" vaccine and then subsequent experimental infection with *M. paratuberculosis* has recently been completed at Invermay. The vaccination site lesions were similar to those of "Neoparasec", and the full results of the trial will be reported elsewhere (Mackintosh unpublished).

***Interference with diagnosis of Johne's disease***

One of the consequences of using a whole cell oil adjuvanted Johne's vaccine is that none of the existing immune-based diagnostic tests can subsequently be used in a vaccinated deer that develops clinical signs of Johne's disease to confirm if that animal has the disease.

***Acceptability of venison from vaccinated animals in some overseas markets***

Venison exporting companies and their customers will need to be assured that venison from vaccinated animals will be acceptable in our overseas markets. This will rely on any Johne's vaccine for deer being fully licensed and approved by the New Zealand Food Safety Authority (NZFSA). Deer in New Zealand already receive a range of vaccines against yersiniosis, leptospirosis and clostridial diseases without concern from overseas markets, and it is technically logical to assume that a licensed Johne's vaccine will be treated in the same way. In a marketing context, involving exporters and consumers, a balance must be maintained between possible advantages of vaccination in terms of animal health and welfare, compared with the perceived disadvantages of using animal remedies.

***Public health concerns***

There are public health concerns regarding serious injuries caused to people who have been accidentally injected with mineral oil adjuvanted vaccines (Windsor et al 2005). Because of their similarity to Freund's complete adjuvant, they can be expected to cause severe reactions at the injection site and, although human exposure to "Gudair" vaccine in Australia has been uncommon, accidental self-injection with this vaccine has caused serious lesions requiring surgical intervention (Windsor et al 2005). However, other vaccines also sometimes cause severe lesions in some people after accidental self-injection.

***Cost versus benefits***

If a Johne's vaccine is licensed for use in deer in New Zealand it will be sensible for each farmer to do a cost-benefit analysis to assess the justification for its use. This is a complex question and needs to be considered in relation to the entire production system and its goals and objectives, and cannot be evaluated simply on whether there will be an "economic response".

***Live vaccine reversion***
There has been some concern about the possibility of spread or transmission of *M. paratuberculosis* from animals vaccinated with a live vaccine, especially if there is some reversion of attenuated vaccines strains of *M. paratuberculosis* to a more virulent form (Emery and Whittington 2004). However, the risk of reversion appears to be small because live Johne's vaccines have been used extensively in a number of countries over many years without any evidence of reversion to a virulent form. Nevertheless some countries only accept killed Johne's vaccines.

**What vaccines are available in NZ currently?**

At the time of writing (May 2006) the only Johne's vaccine currently licensed in New Zealand is "Gudair" and it is only licensed for sheep and goats. However, Pfizer Ltd is currently in the process of licensing another similar vaccine, called "Silirum"™, for use in cattle in Australia and New Zealand. They hope that this vaccine will be available in mid to late 2006, and investigations are underway to extend the license for use in deer. The "Silirum" formulation differs from that of "Gudair" with respect to the amount of whole cells and the type of oil adjuvant.

**What are the main issues relating to use of Johne's vaccines in deer?**

***Age at vaccination***
Deer are likely to be exposed to *M. paratuberculosis* infection from an early age and it seems logical to try to vaccinate them prior to exposure. However, it is impractical to aim to vaccinate deer before February on the majority of deer farms in New Zealand. Evidence from sheep, with the current whole cell vaccines, suggest that vaccination at 2-3 months of age should be effective at reducing clinical disease, even if the animals have already become infected (Eppleston et al 2005).

***Strategies to avoid interference with Tb testing***
Because vaccination of deer with any of the current whole cell oil adjuvanted Johne's vaccines is likely to sensitise them to the MCT, it may be necessary in the first instance to limit vaccination to deer that are destined for slaughter before 15 months of age, and rely on "works monitoring" for Tb rather than annual Tb testing. This is fraught with potential problems because farmers may subsequently change their plans and sell vaccinated deer or keep them as replacements. If these animals are kept and skin tested it is likely that a proportion of them will react to the MCT. There is limited data on the longevity of the sensitisation of vaccinate deer to the MCT, but research in cattle suggest that the reactivity to bovine tuberculin declines somewhat, but may never disappear in some animals. In these animals it would be necessary to use an ancillary test. A CCT should give an avian response, but the ETB may give a bovine positive response, unless the interpretation of the result can be modified by use of additional Johne's antigens. This is the subject of discussions with the Animal health Board currently. Some herds that have a low risk of Tb may be permitted to use the CCT as the primary screening test for Tb in vaccinated animals, but this will increase the cost of Tb testing significantly.

If vaccination were to be permitted in animals destined for slaughter only, it would be imperative to have a tagging (eg. electronic implant and/or ear tag) and recording system that permitted their life-time identification. This should be linked to the AHB identification system and database, allowing identification and differentiation if they were Tb test positive, and allowing immediate trace-back at slaughterhouses.

***Injection site reactions***
It is anticipated that there will be injection site reactions to any of the current whole cell oil adjuvanted Johne's vaccines. These should peak within the first 3-4 weeks after vaccination and then decline to small lumps or nodules by the time of slaughter. Correct vaccination technique

should ensure that the nodules or blemishes on the carcass are superficial and easily trimmed. It would be sensible for the meat inspector at the DSP to incise (or excise) the draining prescapular lymph node in vaccinated deer to ensure that there are no lesions present.

***Market issues***

If a vaccine is licensed by NZFSA it is up to the venison marketing companies to assure the markets that the vaccination of deer with Johne's vaccines is one of a range of routine vaccinations and animal health procedures, which will actually protect their clients and alleviate their animal health and welfare concerns, rather than having any negative connotations. However, one potential downside of widespread use of Johne's vaccines is that it may signal to the market that Johne's is a problem in New Zealand farmed deer.

**What would be the ideal Johne's vaccine?**

The ideal Johne's vaccine would be one with the following properties:

- minimal injection site reaction
- no issues with meat inspection or export markets
- no interference with Tb testing
- only a single dose needed
- effective when given to young deer at around 3 months of age
- complete efficacy,
    - preventing clinical disease
    - preventing establishment of infection if given prior to challenge
    - achieving "cure" if given after challenge.

It remains to be seen if research and development can ever achieve this lofty goal. It will not be easy, because a number of these ideals are in conflict. There are a number of groups internationally trying to develop such a vaccine since JD is of global concern and international markets are substantial.

**Johne's vaccine research in deer undertaken, currently underway or planned?**

A "Neoparasec" immunisation study was completed in 2001 (Mackintosh et al 2005). A challenge study involving a non-adjuvanted live attenuated 316F vaccine is to be published shortly. A "Gudair" challenge study was completed in 2005 and a manuscript is in preparation. There is a current AgResearch Invermay trial of "Silirum", the new Johne's vaccine, being undertaken in 2006/7. A Massey University study on cross-reactivity with Tb testing 1 and 2 years after vaccination will commence shortly after publication of this paper, and a collaborative Massey University/Agresearch Invermay field trial of "Silirum" in deer is planned for 2007/8.

**Conclusion**

This paper has reviewed the history and current status of JD vaccination and key issues surrounding its use, with particular reference to deer. Experience in other species suggests vaccination may have a role in some circumstances in the deer industry in limiting the financial and other impacts of JD. Researchers and the deer industry are working together to establish whether Johne's vaccines are appropriate in deer herds in New Zealand, and if so, how best to use them. There are many issues to be addressed, and a significant amount of research must be conducted before a vaccine is available for either restricted or general use.

Thus, even if the currently available vaccines are shown not to be appropriate or efficacious in deer, future developments may yield suitable alternatives. Research in New Zealand and overseas, using advanced biotechnology, is searching for vaccines that can overcome many or all the problems associated with the presently available vaccines.

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