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# **Assessment of surveillance and control of Johne's disease in farm animals in GB**

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The purpose of this report was to assess the surveillance and control of Johne's disease in farm animals with a view to recommending appropriate systems of surveillance and control for Great Britain. This was to be carried out by considering not only what had been achieved in this country, but also to take into consideration the international situation. Surveillance and control was reviewed for various countries, notably Australia, the Netherlands and the USA. An assessment of the losses attributable to paratuberculosis and the costs of the proposed surveillance and control was to be made. Deficiencies in the current knowledge base would be identified and recommendations made.

The first step in this exercise was to review the literature on paratuberculosis to establish the current limitations in understanding the disease and how these impact on diagnosis, and control. This review demonstrated that the long time course of the disease from infection to shedding the infective organism in the faeces, and eventually the development of clinical disease made research into paratuberculosis extremely difficult and expensive. Large gaps in the understanding of both the host parasite interaction and the epidemiology of the disease exist. The causal organism, *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) is able to survive for lengthy periods in the environment, but the importance this has for the epidemiology of the infection is unknown (Chapter 8, page 54). The prolonged time interval between infection and development of the disease also means that diagnostic tests are limiting in sensitivity. These deficiencies impact adversely on surveillance, but have a greater effect on control programmes, rendering eradication of the disease extremely difficult.

Fundamental to the control of any disease is the need to identify the reservoirs of infection and to determine their significance for maintaining infection on farms or spreading the infection to previously uninfected farms. At present the importance of interactions between the different species of domestic ruminants and camelids is unknown and opinion on the possible significance appears to differ between countries. In GB where sheep and cattle populations mix on many farms this question is critical to control. A range of species of wildlife has also been found to be infected and to develop lesions of paratuberculosis. Once again the significance of this for control of paratuberculosis remains to be established (Chapter 5, page 31).

Australia and the USA have carried out work to develop and validate diagnostic tests (Chapter 4, page 22). The ability of the tests to detect the presence of infection (sensitivity) in these situations has been around 50%. In contrast, the number of uninfected animals falsely identified as positive is low (specificity of close to 100%). The poor sensitivity limits the value of the diagnostic tests for a test and cull programme for the eradication of paratuberculosis, although they may be effective in demonstrating absence of infection at the herd level. The differing climatic conditions and therefore possible difference in the rate of exposure of domestic ruminants to subspecies of *Mycobacterium avium* other than the subspecies that causes paratuberculosis (*Map*) may mean that the test has inferior specificity in GB conditions. False positives may

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then become a problem and so adversely affect both surveillance and control programmes. Therefore it is necessary to validate the two commercially available absorbed enzyme linked immunosorbant assays (ELISA) for the detection of antibody to this disease for GB conditions. Alternatively there is the option to develop and validate a new antibody ELISA. The same drawbacks exist for bacteriological tests and therefore automated culture systems should be validated for individual samples and for pooled faeces or bulk milk. The over thirty-month slaughter scheme, part of the bovine spongiform encephalopathy control measures, currently offers a readily available population in the target age range for the validation of these tests against the gold standard of necropsy (Chapter 8, page 55).

The current passive surveillance systems are capable of demonstrating changes in the annual trend of diagnoses, but cannot provide information on which to base an estimate of national prevalence. Such an estimate is necessary in order to determine which form of control is appropriate and to calculate the resource to be allocated. We therefore recommend that once tests have been adequately validated a national survey be carried out. We envisage a national survey that is repeated at five-year intervals (Chapter 9, page 57). Our view at this time is the absorbed ELISA for the detection of serum antibody should be used for cattle and all animals over two years of age in a selection of both beef and dairy herds should be tested. A similar study and interval is required for sheep, goats and camelids using the agar gel diffusion test (AGIDT) for the detection of serum antibody. (However it is possible to modify the ELISA for use with sera from species other than cattle and as ELISA systems can be readily automated and the AGIDT cannot be, then development of the former for sheep may be indicated). In deer younger animals are affected and consequently it would be appropriate to survey the disease by carrying out abattoir surveys, using the polymerase chain reaction (PCR) test for the presence of the organism in the mesenteric lymph nodes (Chapter 9, page 59).

Vaccination has been shown to reduce the number of clinical cases in an infected dairy cow herd and to deliver a positive cost benefit. Vaccination has also been useful in the control of the disease in sheep, but no study has demonstrated that vaccination is of value in beef cow herds. However vaccination does not significantly reduce the number of cattle that are infected in all herds and vaccinated animals that are sold to other herds will remain an effective route for the spread of the disease (Chapter 6, page 39) For this reason vaccination should only be considered as a control method in herds that do not sell stock for breeding purposes. This is unless vaccinated animals can be readily identified as such and the risk they pose made clear to prospective purchasers. In addition there are issues of safety for the operator when administering live vaccines. Vaccinated animals will also react to the avian component of the comparative intra-dermal tuberculosis test and therefore have potential to cause difficulty in the interpretation of tuberculosis herd tests.

For the control of the disease we recommend using similar methodology to that used in USA and Australia, assurance programmes based on annual testing of adult stock to demonstrate absence of disease. These are supported by biosecurity systems in the form of management rules on hygiene, the prevention of feeding calves milk or colostrum from cows other than their dam and the control of added animals. (Such programmes already exist in GB.) Herds that are free of infection can then sell accredited breeding stock as

free of paratuberculosis. This would have the effect of allowing the purchase of animals, with a low risk of introducing infection to the herd and so reduce the spread of the disease. In our opinion it is necessary to have wide support for this approach from within the industry, from the breed societies and the selling agents. Secondly the purchasers of the stock must demand assurances that they are buying stock that are free of infection. To achieve the latter, information on the disease must be provided in a clear way to the industry.

Such an approach will stigmatise infected herds and it is therefore necessary to offer test and cull programmes for infected herds. Currently there is no information available that demonstrates that test and cull is successful. This is largely because such schemes have not yet had sufficient time to produce results. Eradication of the disease from a herd is essentially a process that will take many years. There is also clear opinion that if these programmes are to achieve success improved hygiene and management of calves must accompany them. In dairy herds relatively straightforward new recommendations about colostrum feeding can be made, although much of the advice on colostrum contradicts that given in the past. This is not the case in the beef herd where calves remain with the cows in an infected environment during the period when they are most susceptible to infection. Relatively little improvement can be made other than to change the time of calving to summer, calve outside, fence off water courses and provide mains water for drinking. Embryo transfer and artificial insemination are techniques that may be used to salvage genetic material from healthy animals within an infected herd, however neither can be guaranteed to produce material that is free from infection with *Map* (Chapter 6, page 44).

In sheep, because the cost of screening is high in relation to the value of the animal, neither assurance programmes nor test and cull programmes are recommended. This position may change if it is found that either the disease is widespread in the national sheep flock or a particular problem within pedigree breeds where the value of the animals is higher. However at present paratuberculosis is thought to be less prevalent in sheep than in cattle. In infected flocks vaccination is advocated. However the absence of a commercially produced vaccine licensed for sheep in this country is an impediment to this. The vaccination currently used is a cattle vaccine that is considerably more expensive than equivalent vaccines available on mainland Europe. Vaccination is also considered to be the most suitable method for the control of the disease in goats and deer. Were these species shown to be reservoirs of infection for cattle this advice would not apply as vaccination could not be expected to remove the risk infected animals would pose to cattle.

By using modeling techniques we have calculated average current losses for an infected 100 cow dairy herd to be £2600 per annum. The depressive effect the disease has on milk yield is the critical factor in the dairy herd. For our standard dairy herd we estimated a milk yield depression of 10%, varying this by plus or minus 5% provided a range of £1700 to £3600 per herd. These calculations are dependent upon limited within herd prevalence data and the many assumptions discussed in Chapter 12. In beef herds the reduction in lifetime calf output is the major factor determining the losses incurred and £1617 is the estimated loss for the average 100 cow beef herd. This estimate is also limited by the lack of within herd prevalence data as well as the assumptions discussed in Chapter 11. These estimates are obviously

affected by the current value of the output and level of subsidy support in the case of the beef herd. The cost of the disease is therefore higher in pedigree and high genetic merit animals. The national loss can only be guessed at as no national estimate of herd prevalence is available, but using prevalence figures from other countries and the limited regional data that is available within GB a figure of 17.5% is offered as a reasonable estimate. If we vary this herd prevalence estimate by plus or minus 10% we can provide a range of losses. This gives annual losses due to paratuberculosis of £ 9.8 million (range £4.2 to 15.4 million) for the dairy herd at a milk yield depression of 10% and £3.1 million (range £1.33 to 4.88 million) for the beef herd. Clearer estimates of these costs will be possible once more accurate national prevalence figures are available.

A cost benefit analysis of the control options is not possible as there is no figure for time scale of eradication through test and cull or control through vaccination. Herds that are free of infection and enrol in an assurance programme have no immediate cost benefit unless the animals that are sold gain a premium over stock sold without a declaration of status. There is also the benefit of reduced risk of introducing infection. This cannot be estimated until national prevalence figures are known. From a national perspective we cannot at this time offer estimates of how successful assurance programmes will be in reducing the spread of the disease to previously uninfected herds. However we have constructed a framework that can be used for sensitivity analyses on the cost benefit of control programmes and we have demonstrated the results that could be expected using conservative estimates of efficacy for a test and cull programme and vaccination (Chapter 13). We would like to emphasise that our limited experience to date is that a test and cull programme will perform better than the estimates used in the examples given. It would therefore be prudent to extend the limited sensitivity analyses that have been carried out.

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# Chapter 1

# Introduction

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The objectives of this study are:

- To review Johne's disease control programmes in existence in other countries.
- To assess what features of the programmes might be applicable to GB.
- To assess what surveillance method would be necessary to monitor the prevalence of Johne's disease and the impact of control programmes.
- To carry out cost benefit analysis of surveillance and control programmes

Johne's disease is a chronic, debilitating, infectious disease of ruminants characterised by chronic weight loss and particularly in cattle by profuse diarrhoea. The disease has a worldwide distribution and is categorised by the OIE as a list B disease that is of serious economic or public health importance (OIE 2000). Several countries have national control policies for the disease in order to safeguard the productivity of their national herds and flocks and to safeguard their international market. In other countries control programmes have been initiated by the livestock industry in response to concern over the losses suffered by individual producers.

The cause of the disease is infection with *Mycobacterium avium subspecies paratuberculosis* (*Map*). The disease is also known as paratuberculosis and that terminology will be used throughout this report.

The first section of this report reviews the current literature on the disease encompassing the biology and diagnosis of paratuberculosis, without which an understanding of the control programmes would not be possible, before reviewing the control of paratuberculosis in farm animals (chapters 1 to 7). The proceedings from the first workshop make up appendix 1 where the subjects considered in the literature review are further examined through a series of presentations from invited experts with group discussion. Conclusions from this are presented. Chapters 8, 9 and 10 of the report present a series of conclusions covering the principal points, the gaps in the current understanding and an outline of the surveillance and control procedures that are considered applicable to GB. In the report for the second part of the study, which draws on the findings of the review, mathematical modelling is used to explore the cost benefit of the recommended control and surveillance strategies. Results are presented in chapters 11 to 16.

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# Chapter 2

## The organism

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### Classification

The Mycobacteriaceae are a large family of related microorganisms. The genus *Mycobacterium* includes obligate parasites, saprophytes and intermediate forms and the type species is *M. tuberculosis*. These aerobic, slow growing, bacteria are characteristically acid-fast (Sneath and others 1986, Barrow and Feltham 1993).

*M. avium* is the cause of tuberculosis in fowls and less frequently the cause of lymph node lesions of pigs and cattle. This bacterial species shows considerable overlap in taxonomic studies with *M. intracellulare* and *M. scrofulaceum* that may be isolated as commensal and opportunist pathogens from humans. Hawkins (1977) referred to *M. avium*, *M. intracellulare* and indeterminate strains as the *M. avium/intracellulare/scrofulaceum* (MAIS) complex. However, this is not an internationally accepted classification and there is good evidence to retain the distinct bacterial species.

- The species *M. avium* can be divided into three pathogenic subspecies using a wide range of biochemical tests (Thorel 1990), while an alternative classification is based on differences in the genome of the bacteria (Kunze and others 1992).
- *Mycobacterium avium subsp. avium* (*Maa*) is, for the most part, an opportunist pathogen, which may cause infection in debilitated patients. The DNA insertion elements IS1245, IS1110, IS1311 and IS1626 have been identified from this subspecies.
- *Mycobacterium avium subsp. silvaticum* (*Mas*) – formerly known as the wood pigeon strain - is capable of causing chronic enteritis not unlike paratuberculosis in calves, goats and deer. DNA insertion elements IS901 and IS902 have been identified in some isolates of this subspecies.
- *Mycobacterium avium subsp. paratuberculosis* (*Map*) is the causal agent of a chronic enteritis in a wide range of animals known as paratuberculosis or Johne's disease. DNA insertion element IS900 is unique to this sub-species and allows it to be differentiated from the other sub-species.

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## Characteristics

*Mycobacterium avium subsp. paratuberculosis* (*Map*) is an aerobic, non-spore forming, non-motile, acid-fast bacillus. However, some non-acid fast, lightly acid-fast and cell wall-deficient types are encountered. The bacilli generally occur in clumps linked together by a network of intercellular filaments. The type strain of *Map* is strain ATCC 19698 (Thorel and others 1990)

*Map* has a complex cell wall, which is relatively impermeable and rich in lipids, this confers acid-fast properties and may enhance its survival in the environment. A prominent lipopolysaccharide of the cell wall, lipoarabinomannan (LAM), is thought to have important consequences for hypersensitivity-type reactions and the formation of the granulomatous lesions of the disease. Different types of LAM in bovine and ovine isolates have been used diagnostically in an enzyme linked immunoassay (ELISA), as they are strongly immunogenic (Multharia and others 1997).

## Culture

In in-vitro culture, the organism is slow growing and fastidious, as are all the mycobacteria, when grown on artificial media, such as Herrolds egg yolk, Watson-Reid or Lowenstein-Jensen Middlebrook. Pigmented *Map* isolates and those from small ruminants are especially so. The colonies are usually non-pigmented, either smooth or rough and, with repeated sub-culturing, may change from one to the other. Primary visible growth usually takes between 5 to 14 weeks of culture.

The pathogenic mycobacteria require a source of organic iron for in-vitro growth. Exochelins chelate free iron extracellularly and then exchange it with membrane-associated substances termed mycobactins. Most strains of *Map* are unable to produce mycobactin, which must be provided exogenously for growth in culture. Reductase, an extracellular mycobacterial enzyme has been found in *Map* and is able to mobilise iron complexed in various forms, such as ferritin and lactoferrin (Sneath and others 1986; OIE 2000).

## Survival

The microorganism is resistant to drying, acid conditions and to many disinfectants, but is killed within 10 minutes in aqueous solutions of formalin (5%), calcium hypochloride (1:50) and phenol (1:40). The effectiveness of disinfectants is diminished in the presence of organic matter and decontamination of faeces requires the addition of high concentrations of detergents to allow penetration by disinfectants. The organism can survive freezing at temperatures of -14 deg C for a year or longer, but is killed by ultra-violet light after 100 hours of exposure or by boiling for two minutes.

*Map* can survive in the environment for long periods. For example, it can survive in river water for five months, in pond water for nine months and in soil for 47 months. Acid soils are said to enhance its survival.

Urine is bactericidal to the organism, *Map* can nevertheless survive in bovine faeces, including slurry and deep litter, for at least eleven months. Whilst it can survive outside the animal for a considerable time, *Map* is an obligate parasite. It is suggested that only relatively small numbers of organisms are necessary to initiate infection.

### Strain Differentiation

Initial observations on the growth characteristics of different isolates led to the suggestion that three distinct strains or groups of strains existed: bovine, pigmented (yellow-orange) and small ruminant. In vitro, the small ruminant strain or strains grow even more slowly than the bovine strains. The recent use of molecular techniques has provided further differentiation (Stevenson and Sharp 1997). Using restriction endonuclease analysis and DNA hybridisation (Collins and others 2000) and examination of DNA restriction fragment length polymorphisms (Whipple and others 2000) has shown two distinct groups of strains. The first is represented by strains from cattle and other species and the second contains strains from sheep. In these studies isolates from North America, Australia, New Zealand and Norway were investigated. These tools appear to be essential for the further investigation of the epidemiology of *Map* infection and the application of molecular techniques to strains isolated from cattle, small ruminants and wildlife in the UK is indicated.

# Chapter 3 Paratuberculosis in Cattle: Pathology and Clinical Disease

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## Pathogenesis

The current hypothesis on pathogenesis of *Map* infections is largely based on models developed for *M tuberculosis* and *Maa* (Valentin-Weigand and Goethe 1999). Recent work on the cytokine production of peripheral blood mononuclear cells of subclinically infected compared to clinically infected animals (Stabel 2000) and cytokine production in the ileum of subclinical cases compared to that in clinical cases (Sweeney and others 1998) has supported this.

The underlying feature of the pathogenesis is the prolonged survival of *Map* within the lysosomes of macrophages that results in the slow development of lesions in infected animals and the eventual appearance of clinical disease. *Map* and its products are not known to cause any toxic effects on host cells (Benedixen and others 1978) and the lesions of paratuberculosis are assumed to be largely due to excessive production of pro-inflammatory cytokines by infected host cells.

Following ingestion by the host, *Map* organisms are carried through the gut in the ingesta. Those that are not swept away in the gut contents must then establish close contact with the mucosa. In doing so they must survive the non-specific defences of the host, that include bile (microbicidal), acidity, digestive enzymes, lactoferrin, peristalsis, competition from and dilution by other gut flora and the protective properties of mucus.

In calves, the oesophageal groove reaction operates when they are drinking milk and the ingesta bypasses the immature rumen. This may be one of the factors that make young calves more susceptible to this infection than adult animals (Clarke 1997).

Once in contact with the mucosa, the organisms undergo a process of endocytosis by M-cells of the dome epithelium overlying the gut-associated lymphoid tissue of Peyer's patches, particularly of the ileum, but also of the jejunum (Momotani and others 1988 and Garcia Marin and others 1992). M-cells are specialised absorbent mucosal epithelial cells that, unlike enterocytes, lack a brush border, digestive enzymes or surface mucus (Featherstone 1997).

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Living *Map* microorganisms traverse the M-cells by a process of transcytosis and are expelled on the basolateral side of the cell, to be scavenged by macrophages or dendritic cells in the subepithelial and intraepithelial areas of Peyer's patches and the adjacent lamina propria (Stabel 1999). It takes only 20 hours from M-cell ingestion to the mycobacteria being phagocytosed by macrophages.

Peyer's patches are at their most developed stage at birth. In calves, almost nine percent of the small intestine comprises lymphoid tissue, of which two thirds is found in the ileum and the remaining third in the upper small intestine. As the animal ages, the lymphoid tissue involutes, except in the region of the ileo-caecal valve where it may still be found in adults. This may offer a further explanation why young calves are more susceptible than adults to mycobacterial infection (Nisbet and others 1962).

Once inside the macrophages, the mycobacteria are not exposed to the humoral antibody response and replicate relatively unhindered by the cell's attempts at destruction. There are various mechanisms described for intracellular pathogens to escape macrophage killing: 1. escape of the phagosome and multiplication in cytoplasm, 2. prevention of fusion of phagosome and lysosome, 3. resistance to the effects of lysosome and phagosome enzymes (Riott 1993). The exact mechanism for *Map* is unclear but mechanism number 3 is considered most likely (Valentin-Weigand and Goethe 1999). The success of *Map* 'in evading the macrophages' killing and degradation activity is a prerequisite for persistence and eventually for the development of paratuberculosis (Clarke 1997). Indeed, the majority of animals naturally exposed to infection do not develop disease, probably due to the effective intracellular killing mechanisms. Animals may then become resistant after the development of only minor lesions in the gut (Perez and others 1996).

The earliest lesions develop in the intestinal Peyer's patches where they may persist for extended periods before spreading to other parts of the intestine, especially the terminal ileum, to produce the granulomatous enteritis characteristic of the disease. Infected macrophages can be found in the local lymphatics, regional lymph nodes and even in the early stages of infection in peripheral blood.

The presentation of mycobacterial antigen to T-lymphocytes may initiate a complex cascade of cell and cytokine activity that influences the fate of the intracellular microorganisms and is pivotal in the production of the immunopathological effects seen in paratuberculosis (Kunkel and others 1989). The host's initial response to invasion of tissues by *Map* is a pronounced cell mediated immune (CMI) response, in which CD4<sup>+</sup>T-helper type 1 cells (Th1) appear to be important in controlling the progression of infection (Lepper and others 1989). These cells produce the cytokine gamma-interferon, which activates macrophages, which in turn produce other cytokines. This co-ordinated action, if effective, will clear the macrophages of bacteria and produce a resistant state in the animal. This is assumed to occur in a significant, but unknown proportion of the exposed population.

Intracellular bacteria, such as the mycobacteria, do not elicit a humoral (antibody) response in the early stages of infection. Only when the cell mediated response wanes and infected macrophages lyse and release bacteria, is a strong antibody response initiated (Merkal and others 1970 and Milner and others

1990). This usually occurs in advanced clinical cases of paratuberculosis. The progression of the disease is thought to involve a shift from a Th-1 like immune response to a Th-2 like reaction.

In a population of infected animals a spectrum of immunological responses to *Map* will be seen that are presumed to be the result of the individual host's response to infection. This spectrum of response has in turn been related to the spectrum of pathology observed in affected animals. In the lymphocytic or tuberculoid form associated with a strong CMI response only small numbers of focal lesions are found on examination. As the CMI response wanes the disease progresses, and the humoral response, initiated by the presence of bacilli released by lysed macrophages, becomes predominant. This is the opposite end of the spectrum of observed pathology and is referred to as the lepromatous form. However the severity of the clinical signs of the disease do not invariably correlate with the changes in the immunological response nor do they necessarily correlate with the severity of the pathology found at necropsy (Chiodini and others 1984).

In between the tuberculoid and lepromatous forms are so-called borderline cases. In the borderline tuberculoid types (with a strong CMI response) there will be few, if any, *Map* microorganisms present (paucibacillary) whereas borderline lepromatous animals (with a strong humoral response) will have numerous bacilli present in the tissues (multibacillary). The latter is the most common form of the disease found in cattle.

The response of an animal to any particular diagnostic test will depend upon where it lies in this immunological spectrum. Early in the disease, cell mediated immunity may be detectable via delayed-type hypersensitivity skin reactions in cattle. These reactions become progressively weaker as the pathological changes advance. Serum antibody concentrations tend to rise as CMI responses fade and can be detected by complement fixation test (CFT), agar gel immunodiffusion (AGID) or enzyme linked immunosorbent assay (ELISA). It is thought that as CMI wanes, there is a proliferation of bacilli within macrophages, which eventually lyse and release *Map* antigens. Antibodies produced in response are however unable to neutralise any remaining intracellular organisms (within macrophages) which, in a waning CMI environment, multiply virtually unhindered.

The systemic nature of the disease is underlined by the evidence of foetal infection. For some time it has been recognised that foetal infection occurs in a significant proportion of clinical cases and isolates from foetal tissues have been used successfully to infect calves via the intravenous route (Doyle 1958). (These animals went on to develop intestinal lesions, illustrating the tropism of the organism for the intestinal tract.) Even in animals showing no clinical signs of paratuberculosis foetal infection has been confirmed (Sweeney and others 1992). *Map* has also been isolated from both the male and female reproductive tracts and from the semen of infected bulls (Larsen and Kopecky 1970). The epidemiological significance of these features of the disease has not been determined and may well be minor relative to the higher frequency with which the organism is shed in faeces, colostrum and milk (Streeter and others 1995).

The macroscopic lesions in cases of naturally occurring paratuberculosis are usually confined to the terminal ileum and only rarely extend beyond the ileocaecal valve to the colon, caecum or rectum. Occasional lesions are found in jejunum or even duodenum. There is a chronic granulomatous enteritis, chronic intestinal lymphangitis and mesenteric lymphadenopathy. The intestinal mucosa is thickened and corrugated and has a soft, velvety appearance and touch. The lymphatic vessels are dilated and tortuous and the mesenteric lymph nodes are enlarged, oedematous and pale, with little corticomedullary distinction evident. These changes are especially evident in the region of the ileocaecal valve. Focal granulomas, sometimes noted in the liver, may be difficult to identify on gross examination. In some severe clinical cases macroscopic changes found at necropsy may appear relatively mild and even be difficult to detect (Barker and others 1993). Secondary pathology includes atrophy of skeletal muscle and fat, serous effusion into body cavities and vascular changes such as endocardial and aortic calcification.

At the histological level the observed changes may range from the subtle to the obvious. In the former the changes are restricted to infiltration of the lamina propria with lymphocytes, plasma cells, large numbers of eosinophils and few macrophages. In the latter the lamina propria of the mucosa is packed with macrophages and the villi take on a club-shaped appearance due to the distortion. Macrophages also infiltrate the submucosa but not the muscle layers. The earliest lesions are diffuse and multifocal, but as the disease advances these coalesce and obliterate the intestinal crypts. Macrophages and giant cells (principally of the Langham's type) are generally filled with acid-fast bacilli, the more so as the lesions increase in severity. Lymphatics and lymph nodes show signs of inflammation and the dilated lacteals may fistulate into the lumen of the gut. The loss of functional villi in the ileum that results from these changes leads to an overload of the absorptive capacity of the colon and diarrhoea. Malabsorption and protein leakage into the intestinal lumen is the other significant consequences of the disease process.

Granulomata may be seen microscopically in the liver and hepatic lymph nodes. In advanced cases of the disease, granulomatous lesions may also be found in the lungs, kidneys and carcass lymph nodes. Widespread dissemination via the lymphatic and venous drainage may account for the detection of acid-fast bacilli in the mononuclear cell-rich fraction of blood and tissue fluids (Van der Giessen and others 1994).

## The Clinical Disease in Cattle

Paratuberculosis has a protracted incubation period, which generally lasts for many months or years, during which the disease progresses in the absence of clinical signs. The majority of affected animals become clinically ill from between two and six years of age although the range is from 4 months to 15 years. The long and variable incubation period dictates that even in a herd with a high prevalence of infection, clinical cases occur only sporadically and a number of animals may be culled for other reasons and never recognised as paratuberculosis cases. It has been suggested that for every clinical case of paratuberculosis

in a herd there are a further 25 infected animals (Whitlock and Buergelt, 1996).

As a consequence of both the prolonged incubation period and the infrequent occurrence of clinical disease in immature animals, the disease does not appear to have an important adverse effect on growth rate. In the lactation prior to the onset of clinical disease milk yield has been found to be lower than performance records would have predicted (Malmo 1995). There has been some suggestion that infected animals may be more prone to other diseases such as mastitis and possibly less fertile than their contemporaries (Johnson-Ifearewun and Kaneene 1997) but this evidence is not convincing.

It has been observed that several factors may precipitate the onset of the clinical phase of the disease. These include inadequate dietary supply of macro or micro nutrients, concurrent infection, parasitism, parturition or peak lactation, or following transportation or introduction to new premises (Downham 1950, Macindoe 1950 and Smyth 1935). Immunosuppression as a result of infection with bovine viral diarrhoea virus (BVD) may also contribute to triggering the clinical condition (Allen and others 1986). Remission of clinical disease and progressive weight gain may occur during pregnancy, followed by relapse after parturition. It is suggested that the mechanisms, which protect the foetus, may also protect the dam from clinical paratuberculosis (Chiodini and others 1984a).

Gradual loss of condition and a change in the consistency of the faeces are the earliest signs of disease. In dairy cows a drop in milk production is often noted before the onset of scouring. The diarrhoea may be continuous or intermittent. Animals become unthrifty and develop a rough coat and dry skin but generally retain a reasonable appetite until the advanced stage of the disease. Thirst is increased as a result of the fluid loss with the diarrhoea. Heart rate and respiratory rate remain normal, but there may be an intermittent fever. The most significant blood biochemical finding is a progressive hypoalbuminaemia as the clinical condition of the animal deteriorates. At this stage of the disease most animals will be positive on faecal culture and show raised levels of serum antibody. Animals shed billions of organisms per day at this stage (Whitlock and others 1996).

In the final stages of the disease, which may follow weeks or possibly a few months after the onset of clinical signs, animals are very lethargic and weak and appetite is lost. Profuse, watery diarrhoea (sometimes containing frank blood) causes a rapid deterioration in body condition to the point of extreme emaciation. Ventral oedema and submandibular oedema are present as a result of the hypoproteinaemia. Death normally follows after only a few days in this severely debilitated state.

# Chapter 4

## Diagnosis of Paratuberculosis

G Caldow<sup>1</sup> and C J Low<sup>1</sup>

### Introduction

Diagnosis of *Map* infection should include detection of the preclinical and the clinical phases of the disease (OIE, 1996). The latter is straightforward and the available diagnostic tests have levels of sensitivity and specificity that compare reasonably well with those available for many other diseases of economic importance. In comparison the diagnosis of the preclinical stage of *Map* infection in cattle is difficult, largely because of the prolonged incubation period and slow progression of the disease in the animal.

In the early stages of infection there are few, if any organisms shed in the faeces and little or no detectable humoral immune response. The consequence is low test-sensitivity for detection of preclinical infection. Thus the available diagnostic tests cannot identify infection in young, recently infected animals nor can they identify every infected adult animal in a herd. Confusion also exists in the literature over the test status of animals that have been infected but recover or those exposed but uninfected. The high specificity of the serological tests suggests that if recovered animals exist they do not normally test positive and therefore this should not be a practical concern. There is also a possibility that animals that are infected in-utero may develop a tolerance, be unable to recognise *Map* and so be unable to mount a measurable immune response. This may explain the observation that around one in ten clinical cases do not show any detectable antibody response.

The sensitivity of a test is its ability to correctly identify the truly infected animals as test positive. The specificity of a test is its ability to correctly identify animals that are uninfected as negative. These values, usually expressed as percentages, can only be calculated if it is known which animals are infected and which are not. A definitive test or gold standard is required to define true positives for comparison with the test results. For paratuberculosis this gold standard has been obtained from a combination of pathological and microbiological findings at necropsy. The histological characteristics of the granulomatous enteritis and lymphangitis for a definitive diagnosis have been well-described (Barker and others 1993) and culture of tissues is far more successful than culture of faeces from the live animal (see below).

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Specificity has most frequently been estimated by testing large numbers of animals in herds of known paratuberculosis-free status, without reference to the gold standard. While for sensitivity animals from populations where *Map* infection is endemic have been tested and then their infection status immediately confirmed by slaughter and necropsy. In general the available tests have been adequately validated in North American and Australian cattle populations, however the sensitivity and specificity estimates may not be relevant to the cattle populations of Britain and independent validation under local conditions is required (Greiner and Gardner, 2000). This is further underlined by the recent criticism of the validation procedures used for the antibody ELISA (Whitlock and others 1999). As the gold standard involves the slaughter, necropsy and histological and microbiological examination of a large number of animals this will be an expensive exercise. However, without accurate sensitivity and specificity estimates any test has limited value for surveillance or disease control.

## TESTS FOR DIRECT IDENTIFICATION OF THE PATHOGEN

### **Faecal Smears**

The microscopic identification of the infectious agent in the faeces of infected animals is rapid, cheap and simple. Ziehl Nielsen staining is used and a positive result is when clumps (three or more) of small, strongly acid-fast organisms are seen. The test is too insensitive in the preclinical phase of infection, but can be of value in the diagnosis of clinical disease. However as few as one third of true clinical cases can be expected to be positive on the examination of a single smear (OIE, 1996). In the hands of the inexperienced the presence of other acid-fast bodies in the faeces may lead to false positives. The test therefore has poor sensitivity and specificity that is operator dependent.

### **Bacterial Culture of Faeces**

The culture of the organism from faeces is considered by most authorities to be the most sensitive and highly specific test available for the diagnosis of the preclinical phase of *Map* infection in the live animal. A specificity of 100 % is generally accepted where the identity of isolates is confirmed by polymerase chain reaction (PCR) (see below). However estimation of sensitivity is problematic, as comparisons are difficult due to variation in techniques used. The loss of cultures through overgrowth with fungal contaminants is a particular problem, while the excretion rate in infected animals varies to such an extent that the number of organisms excreted will fall below detectable levels on some occasions. A sensitivity of around 50% is generally accepted, but levels above 60% in animals in the subclinical phase of infection have been reported (Sockett and others, 1992). A surprisingly low level of 70% was achieved in diagnosis of clinical cases (Egan and others, 1999). The reported variations in the success of culture almost certainly reflect the lack of standardisation in decontamination methods, selective concentration and other procedures.

The technique is also slow with traditional culture methods on solid media taking up to 6 months (Whipple and others 1991). Growth is inhibited to some extent by the decontamination techniques that are essential to prevent overgrowth of the media with fast growing organisms (Collins and others 1990). Hexadecylpyridinium chloride (HPC) incorporated into Herrold's Egg Yolk Medium (HEYM) is the most efficient decontaminant for killing non-mycobacteria. An alternative is to use oxalic acid and NaOH in Lowenstein-Jensen medium. Culture systems have been fully described elsewhere (OIE, 1996). Limited confirmation of the identity of the colonies is achieved by plating them onto a mycobactin-containing medium, a process that takes a further month (Vary and others 1990). However this can now be achieved with more precision and in a single day by using a polymerase chain reaction (PCR) amplification of the IS900 insertion element that is unique to *Map* (see below).

Centrifugation (this concentrates the organisms and so increases the number of infected animals detected) and incubation of paired cultures (this decreases the risk of contamination) both improve the performance of the test. Occasionally, cultures may be lost through overgrowth with contaminants following these methods.

An alternative technique for the isolation of *Map* is the BACTEC system, which uses a liquid culture medium incorporating a radio isotope-labelled nutrient source (CO<sub>2</sub> labelled palmitate) (Collins and others 1990). A special instrument (BACTEC 460) reads the results by measuring carbon-14 labelled CO<sub>2</sub> released from the metabolised substrate. The BACTEC system, together with a technique for filter concentration of faeces samples, is able to detect positive samples in half the time and is more sensitive than conventional culture techniques (Collins 1996). However, because the test is radiometrically based it is used in only a few laboratories.

A number of rapid fluorescence-based tests have recently been introduced, but they suffer from overgrowth problems and have not been fully evaluated to date. (BACTEC 9000, MGIT – Becton Dickinson and MBBact – Organon.)

If animals ingest large numbers of organisms from a heavily contaminated environment, it is possible for them to pass through the gut without replication within the host. The current tests are insufficiently sensitive to detect this. In future there may be a danger that improved culture techniques tests will detect this passive shedding and as a result, increase the number of positives (Seitz and others 1989, Streeter and others 1995, Sweeney and others 1992 and Taylor and others 1981).

Isolating the organism from tissues is generally more sensitive than from faeces as it is subject to less contamination. However its use, without surgical intervention, is limited to the examination of slaughterhouse material or necropsy specimens.

The use of bacteriological isolation of *Map* as a diagnostic technique has a number of advantages. Positive faecal culture correlates well with increased likelihood of the excretion of organisms in colostrum and milk and also to the transmission of infection to the foetus *in-utero* (Whipple and others 1992). Infected animals can be immediately removed from the herd. Isolation of the organism allows further molecular techniques to

be applied, which may assist in the study of the epidemiology of the disease.

The use of culture as a routine diagnostic technique is also disadvantaged because the collection of faeces samples itself is more time consuming than collecting blood samples and the laboratory test is more costly than antibody detection by a factor of 5. For these reasons it is often considered unsuitable for use as the primary test in control and eradication programmes although it is used as a second or confirmatory test.

### **Polymerase chain reaction (PCR)**

The insertion element IS900 is considered unique to *Map* and can be used in a PCR gene amplification technique for diagnosis. The test can be completed within a day as opposed to several months for culture, a high specificity can be achieved and no samples should be lost through fungal overgrowth. However, PCR technology has proved to be difficult to use in routine diagnostic tests, particularly where a significant proportion of the samples under test may be positive, as the test is vulnerable to cross contamination at sampling and within the laboratory. In these situations extreme care is required to prevent the generation of false positives.

Problems are also encountered in the examination of faeces samples as the detection limit is around  $10^4$  organisms/gm of clinical sample and because inhibitors of the PCR naturally occur in faeces further limit test sensitivity (Whipple and others 1992). The technique has been used to examine mesenteric lymph nodes collected at slaughter and it seems particularly suited to this type of investigation (Cetinkaya and others 1996).

PCR tests require skilled technical input and specialised equipment and are therefore expensive to perform. The organisms are not isolated so cannot be further tested and the technique is unable to distinguish viable from non-viable organisms. Contamination in the laboratory may contribute to the number of false positives and there is a considerable variation in accuracy between laboratories using the method. For these reasons the technique is not useful as a first test on faecal specimens, but it has an important role in the confirmation of isolates from faecal culture and will undoubtedly prove a useful research tool (Gwozdz and others 1997).

## **TESTS FOR THE INDIRECT DETECTION OF THE PATHOGEN**

### **A. Detection of serum antibody**

Serological tests are attractive for the mass screening of cattle. However, there are problems confronting serological tests for paratuberculosis. Antibody production is a relatively late occurrence in the progression of the disease and some animals that show clinical disease fail to produce antibody at all. Additionally, the specificity of serological tests is affected by cross-reactions with antigenically similar organisms and by the existence of cross-reactions to rheumatoid factors. The widespread presence of *Maa* in the environment in temperate regions is a particular concern.

There is little that can be done to overcome the inherent insensitivity of these antibody tests, but refining the test antigen and pre-absorbing sera with sonicate prepared from *Mycobacterium phlei* have proved very useful in improving the specificity of the tests (Milner and others, 1987).

### **Complement fixation Test (CFT)**

The CFT was the standard test for large scale screening of herds for paratuberculosis for many years despite its lack of sensitivity, considered lower than the absorbed ELISA. Numerous antigens and protocols are used in the test in different countries and laboratories and there is, therefore a lack of standardisation, which leads to confusion in the interpretation of results. The test is not therefore considered to give a reliable guarantee of the paratuberculosis status of an animal except when in the clinical stages of the disease. Nevertheless, the test is still called for as proof of disease status by a number of importing countries.

The USDA-licensed CF test has been improved through technical advances and a better understanding of the pathogen and now has a specificity of greater than 99% (Sockett and others 1992).

### **Agar gel immunodiffusion (AGID)**

As the detection system relies on the demonstration of lines of precipitin between the antigen and the sera sample it can be used without modification to test different species' sera. It remains the test most routinely used in small ruminants. The low costs are a further benefit.

### **ELISA**

The absorbed ELISA is now widely accepted as the standard serological test for paratuberculosis in cattle. Estimates on the sensitivity of the test vary but it is generally accepted that the sensitivity increases from a low level where infection is in its early stages to a maximum level in the clinical phase. A sensitivity of 45% was achieved using a commercially available ELISA at the point of slaughter where a pathological and microbiological examination was used to confirm infection. The sensitivity of the test was 87% for animals identified as clinically affected (Sweeney and others 1995). In animals identified as light shedders of *Map* with no clinical signs, a sensitivity of 15% was observed (Sweeney and others 1995). The difficulties in determining the sensitivity of diagnostic tests for paratuberculosis in cattle are demonstrated by studies that have involved sequential sampling of animals. In one such study 80% of animals were detected by an absorbed ELISA before the onset of clinical disease while 65% of faecal shedders were serologically positive on or before the first positive faecal culture (Cox and others 1991). In contrast, of six experimentally infected animals slaughtered between 21 and 29 months of age only one was positive by antibody ELISA (McDonald and others 1999). A sensitivity of 47% was found when serology results were compared for 106 known faecal culture positive animals (Reichel and others, 1999) and some have suggested that the true sensitivity of the ELISA may be in the region of 25%; as the sensitivity of faecal culture is believed to be around 50%. The difficulties with this line of thought are highlighted by the poor repeatability of culture and

therefore variable sensitivity found between laboratories. In a recent study where 84 animals from one herd showed clinical signs consistent with paratuberculosis 56 were confirmed by culture and pathology at slaughter while faecal samples collected immediately prior to slaughter identified 39 (70%) and serological examination 43 (77%) respectively (Egan and others 1999).

What is clear from these studies is that the sensitivity of the absorbed ELISA improves with the progression of the disease, but due to the variable antibody response the sensitivity will not exceed 90% even for clinical cases. In general it is accepted that the sensitivity of the antibody ELISA is 50% in all infected adults and this rises to 90% for clinical cases (Sweeney and others 1995). In a recent review it was suggested that the culture methods used in the 'gold standard' necropsy were insufficiently sensitive and as a result the estimates of sensitivity for the antibody ELISA may be considerably lower (Whitlock and others 1999). We understand that an extensive study to revalidate the antibody ELISA is due to be reported later this year (Whitlock and others Preventive Veterinary Medicine in press)

Calculating the specificity of the test is less complicated as populations known to be free from the disease do exist. A series of reports from North America and Australia have indicated a specificity exceeding 97% and frequently reported as 99% and above (Cox and others 1991 and Reichel and others 1999).

To overcome low test sensitivity, the ELISA and faecal culture can be used in parallel. When the tests are used in this way animals are classified as infected if positive to any one of the tests. The sensitivity of the parallel absorbed ELISA and faecal culture in cattle may be as high as 75%.

ELISA tests to identify antibodies to *Map* in milk do not correlate well with the ELISA results on serum from the same animals. Similarly, the bulk-milk ELISA test does not accurately predict the percentage of cattle in herds that test positive by ELISA on serum samples (Hardin 1995). The test has been used recently in a national survey, but was considered insufficiently robust for use as a tool for surveillance (Nielsen and others, 2000).

## **B. Detection of cell-mediated immunity**

### **Delayed-type hypersensitivity (DTH)**

The earliest response to mycobacterial infection is a cell-mediated immune response (CMI) generated by T-lymphocytes. Skin tests using mycobacterial antigens to measure CMI by measuring delayed type hypersensitivity (DTH) are similar to the skin tests for tuberculosis in humans and cattle. The *in-vivo* test is by injection of either avian purified protein derivative (PPD) or Johnin intradermally. (Both products are of comparable sensitivity and specificity.) An increase in skin thickness of 2mm over a 72-hour period is considered positive. However, *Map* has more antigens in common with environmental mycobacteria than do *M. tuberculosis* or *M. bovis*,. sensitisation to *M. avium avium* is widespread and neither PPD nor Johnin are highly specific (Gilot and others 1993). Consequently, the DTH test is of limited value and should not be relied upon for the testing of animals for control programmes except as a preliminary test to give a rough

indication of the number of sensitised animals in the herd. Because of the high number of false positives and false negatives, the DTH test should not be used on cattle for export (Collins 1996).

### **Gamma-interferon**

*In vitro* assays for cytokines (substances that modulate immune responses) can be used to measure CMI responses and so can be used to determine whether an animal has been infected or exposed to infection. For paratuberculosis, *M. avium avium*, which is antigenically similar to *Map*, has been used as the test antigen. Leukocytes harvested as buffy coat from freshly collected heparinised blood are exposed to the test antigen and an ELISA is used to detect gamma-interferon released as a measure of CMI response (Billman-Jacobe and others, 1992). *Map*-infected animals show positive on the assay before they do on serum antibody tests and before they become consistently positive by faecal culture. The test therefore has a higher sensitivity than other tests for detecting the preclinical phase of the infection and sensitivity as high as 93% has been reported (Billman-Jacobe and others, 1992). Despite high levels of specificity in initial reports the test has been shown to lack specificity, particularly in the younger animals that are the target population for the test. In a recent study all non-infected controls tested positive on at least one occasion when sequentially sampled (McDonald and others, 1999). Furthermore it is difficult to ensure that the delay between sample collection and performing the assay can be kept within the time limits required in order to ensure that the leukocytes are viable.

## **Conclusions**

When considering the value of diagnostic tests it is important to recognise the differences in performance of the tests in detecting the preclinical phase of infection or the clinical disease. Test sensitivity is limiting in the former.

The gold standard for the identification of true positives has been defined as necropsy with histological and microbiological examination. This has been used to validate the serological tests in Australia and North America. Validation under UK conditions has not been carried out.

Culture of the organism from faecal specimens is sensitive and specific, but there is a lack of standardisation of methodology. The procedures are technically demanding and the performance varies significantly between laboratories. Isolation of *Map* from faecal samples is time consuming and relatively costly, and so is of limited value as a screening test for paratuberculosis.

Polymerase chain reaction for the IS900 insertion element of *Map* is of little value for screening faeces, but is very useful for the rapid confirmation of the identity of suspect *Map* colonies after isolation. It is a useful technique when applied to samples collected at necropsy.

Measures of cell-mediated immunity are currently of no value for paratuberculosis screening.

Despite the difficulties of detecting the preclinical stage of infection with *Map* the available diagnostic tests are considered to have been adequately validated to allow their use in national control programmes (USAHA, 1998).

# Chapter 5

## Epidemiology

G Caldow<sup>1</sup>, D C Henderson<sup>5</sup> and C J Low<sup>1</sup>

### Prevalence

Paratuberculosis has been reported from many countries throughout the world. Surveys have been carried out on a national and regional basis and the presence of infection has been determined by several different methods.

Most of the surveys have been concerned exclusively with dairy cows and data on prevalence in beef herds is limited. Countries can be split into those where the disease is of low prevalence or almost absent such as Austria (Gasteiner and others 1999), Norway (Paisley and others 2000) and Sweden (Bolske and others 1999) and those where the herd prevalence exceeds 15% such as USA (Wells and Wagner 2000), Denmark (Nielsen and others 2000), Belgium (Boelaert and others 1999) and Costa Rica (Dolz and others 1999).

The percentage of animals detected as positive in the higher prevalence countries ranges from 1.2% in Belgium to 11.9% in Costa Rica. Within herd prevalence not unexpectedly shows a greater range and in one study carried out in New York State a within herd prevalence of 28% was reported on the basis of faecal culture (Obasanjo and others 1997).

National prevalence has been estimated in the USA by different methods. Where culture of ileocaecal lymph nodes was carried out in cull cows from 32 states in 1983 to 1984 the prevalence of infection was 1.6% and this split into 0.8% for beef cattle and 2.9% for dairy (Merkal and others 1987). In general abattoir surveys are limited to estimating individual animal prevalence and are affected by important biases, but the precision of the screening test is higher than achieved in surveys based on either faecal culture or serology. In a more recent survey using serology on a herd basis 21.6% of the herds were positive and 3.4% of all cows (Wells and Wagner 2000). This study represented 79% of the national dairy herd. Regional surveys carried out in USA have included both beef and dairy cattle and found a herd prevalence of 30% in Louisiana beef herds (Turnquist and others 1991) and 40% in Missouri beef herds (Thorne and Harden 1997). In the second study 14 of 19 dairy herds were considered to be positive. A study of culled cows in abattoirs in Canada arrived at an individual prevalence of 5.5% on the basis of culture and it was concluded that the paratuberculosis status of the Canadian dairy herd was similar to that of the USA (McNab and others 1991). These surveys confirm that the disease is of high prevalence in both the dairy and the beef herds of North America.

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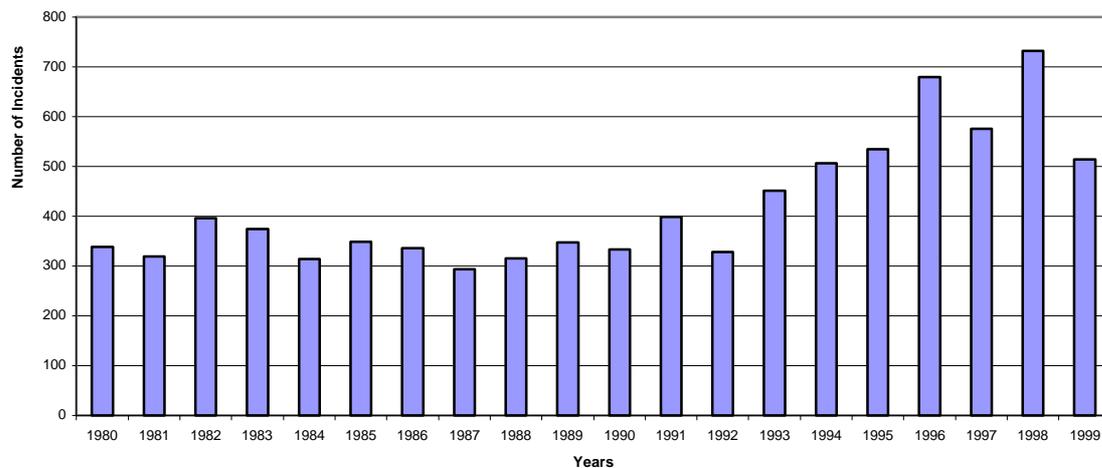
<sup>5</sup> Independent Consultant

Data from Australia confirms that the disease is present in 11% of herds, but information from beef herds is lacking (see Kennedy in Workshop 1).

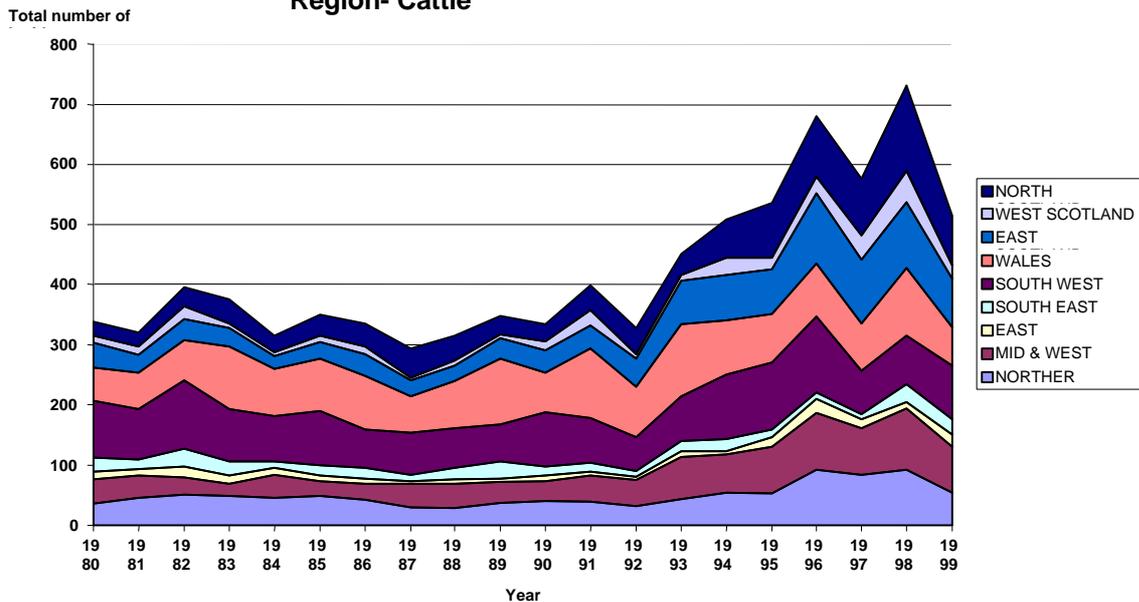
Several European Community (EC) countries with large cattle populations, notably UK, France and Germany have not carried out national surveys. However, there are recent reports from countries with a significant dairy industry. In Belgium 17% of herds were found to be infected in a study based on serology (Boelaert and others 1999). In Denmark the less precise test for bulk milk antibody was used to show that 70% of herds had evidence of infection (Nielsen and others 2000). In the Netherlands 55% of dairy herds had serological evidence of infection (Muskens and others 1999a).

In GB there are two sources of data. The first is the diagnostic data collected by the veterinary investigation centres (VIDA). These figures are limited by several important sources of bias and lack a denominator (Caldow and others 1993). It is possible to relate the individual diagnoses to farms and so to the number of holdings with the target species (Leonard and others 1993), but this has not been done for paratuberculosis and ignores the problem that VIDA information is derived from passive submissions. The VIDA figures do show an upward trend in the number of laboratory diagnoses made in the past 10 years (figures 1 and 2).

**Figure 1:Johnes Disease Incidents  
Total of all Great Britain -  
Cattle**



**Figure 2: Johnes Disease Incidents by Region- Cattle**



The second source of data is regional surveys carried out in the south west of Britain. Telephone and Postal questionnaires were used to survey veterinary practices and dairy farms respectively (Cetinkaya and others 1994, Cetinkaya and others 1998) to arrive at estimates of herd prevalence of 1% and 17.5%. The differing estimates underline the limitations of these approaches in accurately measuring prevalence.

In an abattoir study in the south west of Britain the polymerase chain reaction was used to identify the presence of *Map* in mesenteric lymph nodes of cull cows. This resulted in an individual animal prevalence of 3.5% (Cetinkaya and others 1996). The similarity of this figure to that achieved in the national survey carried out in the USA (Merkal and others 1987) suggests that at least in the south west of Britain the prevalence of paratuberculosis in cattle may be of a similar order to that in the USA.

## Transmission

### Within species transmission of infection

The most important source of paratuberculosis infection is the faeces of infected animals and cattle are most vulnerable during the first month of life and especially whilst feeding from their dam (Collins and others 1994). In an infected herd preclinical and, in particular, clinical cases of the disease shed billions of bacteria daily in their faeces, which contaminate food and water supplies and the general environment. Movements of stock, vehicles and personnel can therefore spread infection throughout the farm and between farms.

Of particular risk to the newborn calf is the soiling of the udder and teats from the dam's own infected faeces

of from faeces from another animal. It is likely that most calves born into a heavily contaminated environment will ingest *Map* whilst sucking or nosing around to find the teats. It is not known why the majority does not develop clinical disease. Though many beef herds in the UK and elsewhere are kept in extensive conditions, the cows and calves may be crowded together at calving time and feeding areas may be congested additionally favouring the transmission of infection.

From the early stages of the disease *Map* can be spread from the intestine to the reproductive tract or the udder (see Chapter 3). As a result up to one third of clinical cases and a significant proportion of asymptomatic subclinical cases of paratuberculosis shed organisms in their colostrum and milk (Meylan and others 1996). This is an additional risk for the offspring, but also as the common practice of pooling and feeding of colostrum may increase the rate of transmission of paratuberculosis in an infected herd (Streeter and others 1995). Cross suckling also occurs naturally in the suckler herd. Infection may disseminate to the uterus and transplacentally in a pregnant animal to the foetus itself. The organism has been isolated from around half the foetuses of clinical cases and from a smaller proportion of the foetuses of asymptomatic animals (Ridge 1994).

In advanced cases of paratuberculosis in the male, organisms may be found in the accessory sex glands and in semen in small numbers (Larsen and Kopecky 1970) and in theory infection may be transmitted to the uterus of the cow during mating or artificial insemination. However, the importance of this route of infection is unknown (Chiodini and others 1984).

*Map* may adhere to the embryos of infected cows even after ten washings *in-vitro*. Though embryo transfer may pose a risk of infection to recipients or foetuses this has not been recorded (Rohde and others 1990) but infected recipients of embryos are a significant risk to the foetus both *in-utero* and subsequently.

Calves infected at birth may show clinical signs as early as one year of age. Studies have shown that of calves on an infected farm naturally exposed to infection during the neonatal period, approximately half will become infected and around one third will eventually die of the disease (Hagan 1978). Naturally infected calves may therefore act as a potential source of infection for other susceptible calves.

The number of microorganisms ingested; the age of the animal and the efficiency of the host's defence mechanisms presumably determine whether infection progresses to clinical disease. Adult animals are apparently more resistant to infection, though in conditions of heavy challenge where there is poor hygiene some are believed to become infected and may develop clinical disease. This age related resistance is not

understood and is not the case in other naturally infected species such as sheep and goats, which may become infected at any age (Benedixin and others 1978, Doyle 1953, Doyle 1956, Hagan 1978, Larsen and others 1975, Rankin 1958, Rankin 1961<sup>a</sup>, Rankin 1961<sup>b</sup>, Rankin 1962 and Taylor 1953). Certainly, adult cattle dosed with high numbers of organisms are capable of becoming infected and progressing to the clinical state (Rankin 1962). Experience in high prevalence beef herds in Scotland appears to support the view that clinical disease may occur following infection of adults though the lack of known uninfected replacement breeding stock prevents this from being established in the field.

### **Between herd transmission of infection**

The purchase of breeding stock is an integral part of most cattle production systems and the most common way in which infection is considered to enter a herd is through the addition of infected animals. Screening replacements to exclude infected animals is ineffective, as the available diagnostic tests are insufficiently sensitive. Hiring bulls from other herds also carries with it a significant risk of introducing paratuberculosis.

The risk of infection from other species and from the wild population has not been determined, but may be of importance (see below). Most young calves in dairy herds are housed for the first few months of their lives and so are kept apart from most other species until they are of an age when the risk of infection is reduced. This is not the case for beef suckler herds, where calves may be reared outside and so contact the faeces of other species at an age when they are at highest risk of infection.

### **Interspecies transmission of infection**

Paratuberculosis is principally a disease of ruminants, both domestic and wild species. However, it is also known to occur naturally in a number of non-ruminant species including pigs, horses, macaque monkeys, and, most recently, it has been recorded in rabbits, foxes and stoats (Greig and others 1997; Beard and others 1999).

Interspecies infection with *Map* has recently been reviewed (Morgan 1999) and only limited and uncertain evidence that interspecies transmission may occur was found. There is no indication of how significant such transmission might be in the epidemiology of paratuberculosis. Experimental studies demonstrate that it is possible to induce infection in a particular species with *Map* strains from another species, but they do not provide any evidence that the cross infection may occur under natural conditions (Klausen and others 1997 and Mokresh and others 1990).

In Iceland ovine paratuberculosis was traced to the importation of 20 Karakul sheep (Sigurdsson 1956). Depopulation was employed on some farms but was often unsuccessful and this led to the suggestion that infection had spread to cattle and this was later confirmed.

There are several reports of sheep and goats becoming infected after grazing pasture that had been grazed

by infected cattle. In general clinical disease and gross intestinal lesions are not a feature of these reports and infection was often only identified on culture. In The Netherlands co-grazing of sheep and cattle is not uncommon but clinical paratuberculosis is rarely seen in sheep. However, recent work investigated sheep from farms where paratuberculosis has been confirmed in the cattle. Sheep in poor body condition and those found positive by serology were examined at post mortem and eight of 50 had macroscopic changes consistent with paratuberculosis. Altogether culture confirmed infection in individuals in ten flocks (Muskens 1999b).

Molecular epidemiological studies using such techniques as PCR and restriction fragment length polymorphism (RFLP) tend to indicate that specific *Map* strains may be adapted to a particular host species without being host specific (Stevenson and others 1997). These techniques can also demonstrate that genetically similar *Map* organisms may be isolated from different species, but this does not prove that interspecies transmission occurs in the field.

A recent study of wild rabbits on farms with cattle and sheep with a history of paratuberculosis, using pulsed-field gel electrophoresis (PFGE), IS900 restriction fragment length polymorphism (RFLP), and chemotype profiles, provided the strongest evidence yet of the possibility of interspecies transmission in the field (Greig and others 1999). Further studies under controlled conditions will be necessary to demonstrate that interspecies transmission occurs.

There is very little evidence in the literature regarding the spread of infection within a secondary species following infection from another species. Neither is there information as to whether the infection can be maintained in the secondary species to become a reservoir of infection for the primary species through contamination of the environment. The amount of contact and in particular the contact between susceptible younger animals and adults of other species or exposure to faecal material that takes place naturally between different species of grazing animals in farming systems is unknown.

An important gap in knowledge is whether wildlife may provide a reservoir of infection for domestic livestock and this is essential for the further development of plans for control or eradication of paratuberculosis.

## Risk Factors

The most widely used technique to determine potential risk factors for paratuberculosis has been through questionnaires in conjunction with prevalence studies. The majority of such work has been carried out in dairy herds. Two major sources of bias affect these studies: firstly husbandry practices within regions tend towards the uniform and secondly awareness of both the presence of the disease and the control measures that should be adopted will exist amongst the herd managers of positive herds. A further problem is that intuitively, the addition of infected animals must be the most common way in which a herd becomes infected

and therefore management factors will impact more on herd prevalence and the persistence or clearance of the infection from the herd. Earlier studies failed to address this effectively. To overcome these limitations the knowledge of whether the disease has been recognised in the herd and for how long it has been present along with within herd prevalence have been included in a recent study (Wells and Wagner 2000). Nevertheless until prospective studies are carried out both to investigate causal factors and to assess the success of intervention measures a clear picture on the magnitude of the risk factors that affect paratuberculosis in cattle will not emerge.

## Herd Factors

Large herd size has been recognised as a positive risk factor in several studies (Collins and others 1994, Goodger and others 1996, Jacobsen and others 2000, Wells and Wagner 2000). This may reflect the increased likelihood that an infected animal will be added to the herd or that conditions within large herds favour the maintenance and transmission of infection within the herd. In support of the former, the number of cows purchased from other herds has also been positively associated with the presence of the disease (Wells and Wagner 2000). Practices that increase the exposure of immature cattle to the faeces of both older cattle and that of their contemporaries are associated with a positive risk (Collins and others 1994, Goodger and others 1996, Obasanjo and others 1997, Wells and Wagner 2000). These factors include: group housing of calves before weaning, group housing of calves after weaning and practices that expose young calves to manure from older animals. Newborn calf care and the management of periparturient cows have been examined in several studies and group calving (Wells and Wagner 2000) and failure to remove calves immediately from cows (Goodger and others 1997) have been identified as risk factors.

## Individual Animal Factors

A high within breed prevalence has been recognised historically in Jersey cattle in Britain (Withers 1959) and demonstrated recently in Denmark (Jacobsen and others 2000). In Britain a number of breeds have the reputation of having a particular problem with paratuberculosis. This is of potential importance as several of the breeds in question are terminal sires used in beef production and therefore traded widely throughout the commercial beef and dairy herd. No data exists to support these concerns and no information on a genetic susceptibility to paratuberculosis can be found. Increased genetic susceptibility need not be the mechanism for any differences in breed prevalence as it may merely reflect endemic infection in a few popular and subsequently widely dispersed herds.

In the recent study from Denmark the probability of an animal testing positive for antibody to *Map* increased with parity and was strongly associated with the first month after parturition (Jacobsen and others 2000).

Higher producing dairy cows infected with *Map* are said to be more likely to develop clinical signs of paratuberculosis (Doyle 1956) Cows with paratuberculosis have been shown to have a greater potential milk production (as mature animals) than their herd average by some 8 - 9 % (Benedictus and others 1987) suggesting that there may be a relationship between the productive capacity of an animal and its likelihood of being culled as a result of paratuberculosis.

## Environmental Factors

A clear regional variation of within herd prevalence has been found in several studies (Collins and others 1994, Jacobsen and others 2000, Wells and Wagner 2000). No clear explanation for this emerges from the studies themselves, but associated local factors may offer an explanation. Soil acidity has long been considered a potential factor and has been reviewed (Johnson-Ifearegulu and Kaneene 1997). With increasing soil acidity iron becomes more available and as *Map* requires iron for growth the mechanism exists to explain this association. Both decreasing soil pH and increasing soil iron availability have been associated with an increased within herd prevalence of paratuberculosis (Johnson-Ifearegulu and Kaneene 1999). What remains to be explained is how soil acidity can impact on the host and as *Map* is an obligate intracellular pathogen this is clearly important.

Direct faecal contamination has been shown to be of importance in the transmission of infection and although information exists on survival times for *Map* in slurry, urine and on pasture (see Chapter 2) there is no clear information on how important environmental contamination is in the maintenance and transmission of *Map* infection.

*Map* is able to survive for prolonged periods in water (see Chapter 2) this aspect has seldom been examined as a risk and it would appear that there is no documented case where infection entered a herd via water courses (Clins N Am-to be confirmed). Anecdotal reports indicate that in GB where suckler herds have access to stagnant or slowly moving water or water that has flowed through another farm there is a higher prevalence of paratuberculosis. The provision of mains water to herds has resulted in a reduction of prevalence within herds (Wilesmith 1982 ).

## Economic Impact

While studies on economic losses have been carried out in several of the countries where there has been concern over paratuberculosis, several factors limit these studies. Firstly there is a prolonged subclinical phase of the disease during which detection of infection is limited and secondly there is an increased susceptibility to other diseases that leads to premature culling during the subclinical phase of infection

(Johnson-Ifearegulu and Kaneene 1997). Together these mean that animals in the subclinical phase of *Map* infection may be wrongly classified and losses either under estimated or not attributed. In addition any reduction in output is related to the initial level of production, which in turn is determined by management and is likely to vary between regions and countries. This is also true for the cost of production and the value of the produce.

In dairy cattle, losses include those from lowered milk production and shorter life expectancy (Sorensen and others 1967), reduced fertility, longer calving intervals and extensive culling (Buergelt and others 1980). Reduced production has been demonstrated to coincide with periods when cattle are shedding *Map* in the faeces (Larsen 1973).

Studies in the USA have recorded milk production reductions of 16% in their last lactation from cows with clinical paratuberculosis and 6% from sub-clinical animals as compared with their previous lactation (Buergelt and others 1980). Similar figures were achieved in the Netherlands where the reduction in yield was 19% in the lactation in which the animal showed clinical signs and 6% in the preceding lactation when compared to that of two years before culling (Benedictus and others 1987). In New Zealand a difference of 17% in milk production from faecal culture positive cows as compared to culture negative herd mates was recorded (deLisle and others 1989).

In the USA a National Animal Health Monitoring System study in 1996 estimated the economic losses in paratuberculosis-affected herds to be around US\$100 per cow as compared to non-infected herds as a result of reduction in milk yield and an increase in replacement costs due to premature culling (Ott and others 1999). For herds with a high prevalence of the disease (10% or greater culls of clinical cases) the losses were in excess of US \$200 per cow as a result of cows producing 700 kg less milk, higher culling rates and a reduced return for cull animals. Reduced production in high prevalence herds has been estimated to be US\$184 per cow greater than in low prevalence herds (Ott and others 1999). Across the USA, paratuberculosis is estimated to cost the dairy industry in the region of US\$250 million.

Animals with paratuberculosis have been shown to be more susceptible to other infections than non-infected animals. In one study of herds infected with *Map* only 30% of infected animals were culled as a result of paratuberculosis whilst 70% of infected animals were culled as a result of secondary complications, namely mastitis (16%), infertility (37%) and others (17%) (Kopecky and others 1967, Merkal and others 1975). In the Netherlands the economic losses attributable to paratuberculosis have been identified as reduced productivity (32%), treatment and veterinary costs (2%), idle production facilities (3%) and unrealised future income (43%).

Data on economic loss in beef herds is lacking. Reduced milk production will reduce calf growth rates, but increased cow wastage is of more importance as beef cows have a relatively long productive life when compared to dairy cows. As the peak incidence of clinical disease occurs at 3 to 5 years of age, affected animals may be culled from the herd at a time when they have achieved no more than a fifth of their potential output. Clinical disease in high value breeding bulls is a further loss of importance.

The indirect costs associated with paratuberculosis include testing of animals for movement or export purposes, constraints on the trading of breeding animals from infected herds and the loss of sale of semen or embryos. These have largely been omitted from the above studies.

# Chapter 6

## Control

G Caldow<sup>1</sup> and D Henderson<sup>5</sup>

### Introduction

The ability to control the spread of *Map* infection or to eradicate the disease at the herd or flock level is limited by several factors. The preclinical phase of infection is prolonged during which time the infected animal may produce large numbers of *Map* in the faeces. This is compounded by the lack of sensitivity of the available diagnostic tests during this phase. In dairy herds it is difficult to limit the exposure of susceptible animals to infected faecal material and in beef herds it may be impractical to do so. The impact reservoirs of infection in other domestic or wild species may have on control programmes is unknown, but may be of significance. Similarly *Map* can survive for lengthy periods in the environment, but it is not known what role this has on the epidemiology of paratuberculosis. Therefore the measures proposed for controlling *Map* infection are based on an incomplete understanding of the biology of the disease. Experimental studies to measure the efficacy of the most frequently proposed farm level control measures are generally lacking and where work has been done there are conflicting results. The high costs associated with the long-term prospective epidemiological studies required to answer these questions may explain why little work has been carried out. Several of the studies referred to below are either retrospective or based solely on questionnaires and the use of their findings is perhaps best used to construct hypotheses rather than to support widely recommended disease control programmes (Collins and others 1994; Centinkaya and others 1997; Wraight and others 1999).

The general consensus is that a two-pronged approach to the control of paratuberculosis on dairy farms is required. Firstly testing is necessary to identify infected herds and infected individuals and to identify herds free from the disease. The infected herds should be isolated and infected individuals culled. Secondly measures to reduce the risk of the spread of infection in infected herds and to reduce the risk of introducing infection into paratuberculosis-free herds must be implemented.

The unanimous message from the many authorities advising on the control of paratuberculosis is that the complexities of the disease, the differing individual circumstances of the herd and the objectives of the herd

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owner must all be considered and the suggested measures must be practical and straightforward to implement. There is agreement that a clear responsibility to point out the long-term nature of the process of eradication of paratuberculosis to herd owners exists. Eradication will take many years depending on the prevalence in the herd and the aggressiveness of the measures adopted.

## Treatment

Treatment is not an option on the grounds of cost and efficacy although isoniazid may be used in individual cases of high genetic merit to allow the collection of semen or ova (St Jean 1996). The demonstration the monensin sodium can be used to limit the development of intestinal lesions raises the possibility of inexpensive ionophores being used as a prophylactic measure in young cattle in infected herds (Brumbaugh and others 2000).

## Vaccination against paratuberculosis in cattle

In 1926 Vallee and Rinjard reported the use of a vaccine that contained live *Map* suspended in oil. The earliest vaccines were composed of non-virulent strains of living *Map* organisms in olive oil and liquid paraffin, with pumice powder as an irritant that produced a nodule at the vaccination site. Vaccines may also be prepared from lyophilised, live attenuated or heat-killed bacterins and may contain from one to three strains of *Map* (OIE 1996). The Weybridge vaccine used in the UK is a three-strain live vaccine in powdered pumice and oil and is essentially similar to the original. Vaccines made from killed bacilli have been used in an effort to increase and vary the number of antigens exposed to the immune system (Larsen and others 1978). However there are also concerns that the use of live vaccines may carry the danger of introducing infection to individual animals.

There is only sparse evidence to support the number of bacilli that should be included in a vaccine and very little data regarding the most appropriate route of administration. Vaccines should be administered within the first month of life by subcutaneous injection (OIE 1996). Originally it was planned to repeat vaccination throughout the life of the animal, but this is not practiced. In one study repeat vaccination at intervals longer than 1 year failed to confer any benefit over a single vaccination in the first month of life (Stuart 1965). Exposure of calves to *Map* in infected herds is likely to occur before vaccine has been administered, but there is experimental evidence to suggest that where vaccination precedes exposure the immune response from the gastrointestinal tract is superior to that in calves where infection precedes vaccination (Chilton and others 1999).

A number of reports on vaccine efficacy have been made and despite differences in type of vaccine used and management conditions all report a reduction in the number of clinical cases when compared to either before vaccination or in the experimental situation when compared to unvaccinated infected controls. In one 13 year study where the Weybridge vaccine was used, in the last seven years of the study the annual incidence fell from 2.2% to 0.3%. In the first period of the study no reduction was found (Spears 1959). Despite the overall apparent success of vaccination in reducing clinical disease there were individual herds where no improvement in this aspect was recognised (Spears 1959). The age at which cattle began to show clinical disease in this study did not appear to be affected as of 157 clinical incidents where the age was recorded 87% had developed the disease before 6 years of age. An altogether more optimistic picture was portrayed by a study based on questionnaires directed to the veterinary surgeons attending herds where the Weybridge vaccine had been used. A total of 83% of 175 herds were free of clinical disease six years after commencing vaccination of all calves (Wilesmith 1982). This extraordinary result is put into context by the finding that 37.7% achieved freedom in the year that followed vaccination, suggesting that either the data were unreliable or the herds were not endemically infected to begin with. No effort was made to contrast these findings with those of the earlier survey despite both authors being employed by the same organisation that manufactured and sold the vaccine.

In the Netherlands where a killed vaccine in oil and water was used over a seven-year period the annual culling rate attributed to clinical paratuberculosis fell from 7.8% to 1.8% (Wentink and others 1994). In another Dutch study, once more using a killed vaccine, clinical disease was reduced by 90% (van Schaik and others 1996). In Wisconsin, USA a comparison in infected herds with a) whole cell killed in oil, b) fractionated cell in oil, or c) no vaccine found the percentage of animals developing clinical disease was a) 1.1%, b) 4.9% and c) 12.6% (Larsen and others 1978).

Where other measures of efficacy were made studies indicate less effective control. In the Dutch study above when both histological and culture findings were considered together 21.8% of the non-vaccinates showed evidence of the disease compared to 25.9% of the vaccinated animals (Wentink and others 1994). In another Dutch study the faecal positive rate was 4.9% in herds that had been vaccinated for 12 years compared to 5.9% in unvaccinated infected herds (Kalis and others 1999). The Wisconsin study found 5.0% of the group given the whole cell killed in oil vaccine to be faecal culture positive and 8.2% for the fractionated bacilli in oil vaccine compared with 11.4% for the cattle that were not vaccinated (Larsen and others 1978). In Brittany after the use of a live vaccine for 16 years 68.6% of the herds still had faecal culture positive animals. In 4.7% of the total herds infection was so severe that whole herd slaughter and depopulation had been employed before freedom from infection was achieved (Hillion and Argente 1987).

These studies indicate that vaccination may have a role to play in reducing clinical disease in heavily infected herds, but vaccinated animals constitute a risk of infection when sold to other herds for breeding purposes. A significant cost benefit for vaccination in heavily infected dairy herds in the Netherlands has been demonstrated (van Schaik and others 1996). In the UK the live vaccine is expensive, currently costing £10.20 per dose, but the dead vaccine used in most other countries is considerably less expensive, in some

cases by a factor of three.

Beef cattle feature relatively rarely in the above studies and no conclusions on the efficacy of the vaccine in infected beef herds can be made.

Reactions to the vaccine occur in the form of localised nodules (OIE 1996) and for this reason the brisket is advocated as the site of administration (Spears 1959). In reports of vaccine efficacy these reactions are seldom attributed with significance. However the irritant nature of the vaccine is of particular concern where self-injection may lead to serious complications (Chiodini and others 1984).

In general the use of any vaccine is considered to have the potential to interfere with serological diagnostic tests for the particular disease. The Weybridge vaccine was found to produce a variable antibody response when measured by the unabsorbed CFT and it was concluded that it was impossible to assess the significance of a positive CFT result in a vaccinated animal (Spears 1959). In a more recent investigation where the absorbed ELISA was used, 13 of 15 calves became seropositive within two to six months of vaccination in the first month of life while unvaccinated controls remained seronegative. The authors concluded that the use of the vaccine might interfere with control programmes that are based on serological tests (Spangler and others 1991). The value of this assertion is limited by the failure to examine animals older than 15 months of age, as in most control programmes animals are two years of age before they are screened for the first time. Observations to date with control programmes in Britain indicate that animals that have been vaccinated with the Weybridge vaccine are most commonly seronegative when tested at above two years of age. In contrast, in Spain, a killed bacilli in oil vaccine that had been used successfully to control paratuberculosis in sheep and goats was used in cattle. The mean optical density reading of the group in an absorbed ELISA was positive at 300 days after vaccination (Lopez Cruz and others 1999).

Of more importance is the increased sensitivity to mammalian and avian tuberculin shown by vaccinated animals. The Weybridge vaccine was found to increase the sensitivity to both mammalian and avian tuberculin by a factor of eight, increasing the difficulty in interpreting the intra dermal comparative test (Herbert and others 1959). Killed vaccines in oil have also been found to create a problem in this respect (Larsen and others 1978, Lopez Cruz and others 1999) and their use in herds where screening for tuberculosis is required is not recommended (Larsen and others 1978). Avian responses appear to be the most exaggerated and therefore the test can still be interpreted, but the general recommendation remains that vaccination is not used to control bovine paratuberculosis in areas where bovine tuberculosis is present.

#### Husbandry measures in the control of paratuberculosis

Within infected herds the main objective of advice on husbandry and hygiene is to limit the exposure of calves to *Map* by reducing environmental contamination and breaking the link of infection between dam and offspring. This can be achieved by using the available diagnostic tests to identify and remove the animals

that test positive. Secondly by implementing sound husbandry and hygiene measures to reduce faecal contamination of the calves' environment and by ceasing the practice of feeding pooled colostrum or discarded milk. However compliance with these recommendations on farms where the disease is present is often poor (Wraight and others 1999).

The details of the hygiene and husbandry measures range from the complex and extensive (Rossiter and Burhans 1996) to the simple and concise (Socket 1996). The latter is used as a basis for further discussion, as the general advice is that measures should be practical and straightforward. The use of clean individual calving areas is recommended to prevent carry over of infection. Attention is also required to ensure that slurry does not contaminate such areas. Calving can take place outside, but the previous grazing history of the paddock must be considered. However an association between paratuberculosis being diagnosed on a farm and cows calving in individual pens as opposed to out at grass with the herd has been found (Centinkaya and others 1997). No assessment of the cleanliness of the calving areas was possible in this retrospective study.

It is also recommended that the calf should be removed from the cow within twelve hours of birth, but the ideal is immediate removal, and the calf should be fed uncontaminated colostrum. Several studies have failed to find a significant association between the diagnosis of paratuberculosis in a herd and the length of time calves are kept with their dams (Collins and others 1994; Centinkaya and others 1997). However in one report removing the calf within an hour of birth was associated with a significantly reduced likelihood of a herd being infected with paratuberculosis in a population where most herd owners removed calves within 12 hours of birth (Goodger and others 1996).

The recommendation to feed uncontaminated colostrum is fraught with difficulty. Even young cows with no test history of paratuberculosis may shed organisms in colostrum and in a herd where the prevalence of infection is high one fifth of faecal excretors also shed *Map* in colostrum (Streeter and others 1995). There is no pasteurisation procedure that will kill *Map* that does not also destroy the immunoglobulins present in colostrum. It is also important to ensure that colostrum is collected hygienically to avoid faecal contamination (Meylan and others 1996). This problem extends to the practice of feeding to calves any milk, that has been excluded from the bulk tank and it is therefore recommended that a high quality milk replacer should be used instead. This advice has cost implications for the farmer, but also contradicts the advisory messages of the past few decades designed to improve the quality of colostrum for calves in terms of volume and immunoglobulin concentration and to control neonatal enteritis, particularly rotavirus infection. This is underlined by an Australian study that found only 7% of calves were fed milk replacer, 90% were mostly fed on pooled colostrum and more than 70% of calves were fed milk that was excluded from the bulk tank for some reason (Wraight, 1999).

In a further effort to reduce the cross infection from one cow to another's calf it is recommended that calves are isolated from each other until 30 days of age. An association of herd infection with paratuberculosis and the practice of housing calves communally rather than in individual pens has been found (Collins and others 1994). However keeping calves in individual pens is not keeping them in isolation and may explain why in another study no such association was found (Centinkaya and others 1997). The current guidelines on

animal welfare in the UK preclude the individual isolation of calves.

For best practice the manure from adult animals should not be used on pasture where young stock will graze. Buildings for young stock should be free of faecal material from older animals. These recommendations are empirical and there is little literature to support or contradict them.

Drinking water may provide a medium for the spread of *Map* infection. An improvement in the disease status was reported in herds where contaminated water sources were fenced-off and piped water was supplied (Wilesmith 1982)

The literature on the control of paratuberculosis in cattle is almost exclusively concerned with the disease as it affects dairy cattle. In general the principles remain the same for control in beef herds, but much of the advice concerned with breaking the link of infection between cow and calf cannot be implemented as calves spend the first 6 to 9 months of their life with the cows. Anecdotal information from the beef herds in Scotland indicates that in such conditions even introduced adults may become infected with the subsequent development of clinical disease. This may be the consequence of longer productive life of beef cows as they live to produce 7 to 10 calves compared to the average of 2 to 4 calves in the dairy herd.

A further important difference for beef herds in the UK is that there is greater movement of cattle into the herd than occurs in dairy herds. Beef herds rely on natural service and so purchase or share bulls. The majority of beef herds also purchase replacement cows. Therefore perhaps even more importantly than is the case for the dairy sector, the identification of herds that are clear of infection should be an essential part of control. This will reduce the risk of introducing infection to an uninfected herd by providing a pool of disease free breeding replacements.

### Breeding Technology

Embryo transfer and artificial insemination may be used to introduce new genetic material to a herd or to allow genetic material to be salvaged from an animal that is either diseased or is from a herd that is infected. Both semen and embryos may be infected with *Map*, although the risk of transmission by this means is considered to be small. There is no report of the successful use of these techniques to effect salvage of genetic material from herds infected with *Map*. Artificial insemination centres do not screen semen for the presence of *Map*, nor do they require bulls to come from herds known to be free from paratuberculosis.

### Control Programmes

Formalised control programmes incorporate biosecurity rules, auditing procedures and accreditation of laboratories and testing procedures. Their primary objective is to demonstrate a herd is free from infection with *Map* to an acceptable level of confidence in order to facilitate trade in animals with a negligible risk of

introducing *Map* infection to a herd. This therefore is a method of reducing the spread of *Map* infection within a region or country. The secondary objective is to provide a framework for control in infected herds that will a) produce a positive cost benefit and b) lead to eradication of infection. Copies of specific control programmes from Australia, USA and Britain are to be found in appendix 3.

## Biosecurity Rules

Prevention of direct contact with either cattle of an unknown status for *Map* infection or animals from known infected herds is the most important rule. In the early stages of control programmes this can be a limiting factor, but in countries where there is a large pool of uninfected animals the rule can be complied with. The latter applies to Australia. Boundaries of farm units must be sound and so prevent the incursion of other stock. The American programme proscribes the co-mingling with other species of stock that are susceptible to paratuberculosis. This is in contrast to the Australian programme where ovine and bovine paratuberculosis are considered to be distinct disease entities, but alpaca deer and goats are considered to be susceptible to bovine strains of *Map* and may be a source of infection to cattle. Prevention of pooling of colostrum or feeding colostrum from a cow other than the calf's dam is the next most important and consistent rule. Manure that originates from other herds must not be used to treat pasture that will be grazed by the herd.

## Screening

The sensitivity of the antibody ELISA is such that random sampling of a limited number of the herd cannot be used to show freedom from infection (Jordan 1996). Despite this both the Australian and American programmes allow aggregate testing, but only when the herd size exceeds 210 in the case of the Australian programme and for the American where the total number of animals in their second or higher lactation exceeds 500. As the antibody ELISA has very low sensitivity in the latent phase of the infection the general recommendation is to limit testing to animals of two years of age and older. The American programme takes this back further to include animals in their second lactation or older. In herds where infection was thought to be absent second tests are used to confirm that animals that are antibody positive are infected. The second test is faecal culture on one or more occasions. Necropsy is used to confirm disputed cases in the American, Australian and British programmes. Alternatively in the American, biopsy of the ileum (full thickness) and a mesenteric lymph node for culture and histopathology along with faecal culture at the same time or faecal culture at monthly intervals on six occasions can be resorted to.

Once herds have had a clear test the testing interval is two years, but in addition the British programme requires any animal that is scheduled to be culled or that may show signs of disease that are suggestive of paratuberculosis must also be sampled. Where this cannot be complied with a herd test is carried out every year.

In addition to certifying that the testing procedure has not detected evidence of disease certification also states the length of time the herd has been certified for. This is considered of importance given the long incubation period of the disease.

#### Alternative levels of status

In the Australian and American programmes options exist for a lower level of testing with or without certification. This is designed to facilitate trade where herds consist of lower value stock where participation in the full programme is considered too costly. Clear differentiation between the different statuses is made in the market place.

#### Vendor declaration of paratuberculosis status of herd

Vendor declaration of herd status of paratuberculosis is voluntary in USA. In the areas of Australia designated as Residual or Control zones where herd prevalence is relatively high this is also the case. In the areas of Australia where paratuberculosis is absent or the prevalence of herd infection is low, known as Free and Protected zones, vendor declaration of the herd status of paratuberculosis is voluntary within areas of similar status, but compulsory for herds from Residual and Control Zones.

Where the declaration of status is voluntary (non-statutory) the responsibility effectively falls on the individual producer or on industry groups such as breed societies or on the authority acting as agent for the sale of cattle. In the case of the individual producer information on the effects of the disease and the risks added animals pose must be made available in an easily understood form; if not demand for declaration of status will not originate from this source. Where breed societies recognise the disease to be prevalent within the herds of their members there exists a powerful disincentive to call for vendor declaration. Where the disease is not considered to be prevalent there may be a tendency to see such declarations as an irrelevance and the requisite assurance programme as an unnecessary cost for their members. As government of the breed society is the sole responsibility of the members, who in turn are breeders of cattle for sale, an objective evaluation of the potential benefits of such an approach may be difficult to achieve. Preliminary consultation with the Institute of Auctioneers in Scotland suggests that vendor declaration of the disease status of the animals may in fact be in the interest of those acting as agents in the sale of stock, as it will effectively reduce the time spent in pursuit of the resolution of disputes that arise from the sale of animals that are subsequently found to be diseased.

## Infected Herds

As cattle in infected herds are not usually traded with a status there are no set rules and procedures for infected herds although recommended testing and culling procedures are usually given. It is accepted that the interval between herd tests should not exceed one year and for maximum progress this can be reduced to an interval of six months. Likewise it has been suggested that whole herd faecal screening could be used in alternate years (Collins 1996). It is assumed that the offspring of test positive females are at higher risk of being infected and it is recommended that these animals be removed from the breeding herd (Rossiter and Burhans 1996).

## Disposal of reactor animals

Removal of reactor animals and any other that are assumed to be at high risk of developing the disease creates a further problem in disease control. These animals may be sold to other herds where paratuberculosis is either not recognised or the addition of infected animals is not considered to be a risk. This may therefore act to spread the disease between herds at a higher rate than would occur without the control programmes. In the Australian programme such animals can only go direct to slaughter, but no such provision exists in the USA programme or in the existing GB programmes.

## Assessment of Progress

There is little evidence in the literature to indicate that the identification of herds free from *Map* infection has led to an improvement in regional or national disease status. This may be because the programmes have had insufficient time to achieve results. The Australian programme commenced in 1996 (Kennedy and Allworth 1999) and by June 2000 there were 1139 herds assured free from infection and 2134 known infected herds (Anon 2000). The USA programme began in 1993, but attracted relatively few participants and was redesigned and re-launched in 1998 (Appendix 2). No data is available on recent progress. British programmes have been running for over two years (Appendix 2). Details of national membership are not available although membership for the Premium Cattle Health Scheme Johne's disease Programme stood at 94 in October 2000.

The clearest question on the value of these programmes rests with the short period of time required to achieve disease free status. As the peak age related incidence occurs at three to five years and because most infections are considered to have occurred in calf hood, the minimum period before freedom from disease can be assumed should exceed five years. This is not the case for any of the programmes. The second area for concern is the low test sensitivity of the antibody ELISA used for screening, although

particularly in large herds this is more a problem for eradication of infection than demonstration of freedom. An interesting Dutch study where faecal culture rather than antibody ELISA was used found that of 100 herds assumed to be free from infection after four rounds of faecal testing at six month intervals only 58 showed no evidence of infection (Kalis and others 1999). For purposes of comparison it was unfortunate that the antibody ELISA had not also been used. Nevertheless this study caused real concern that creation of a sufficiently large pool of herds that were free from *Map* infection may not be achievable in the Netherlands (see also Workshop 1). Such a pool is necessary for practical purposes of trade, but also for the motivation of the industry.

Additionally for these programmes to be attractive to producers, in the event of a herd failing there must be access to eradication programmes that have a reasonable chance of success and are affordable. The probable long time course of such programmes alone is discouraging, although in those herds where the disease is causing significant financial loss there may be no economic alternative. Stigmatisation and loss of income for breeders of pedigree or high genetic merit dairy stock is a profound disincentive that must be recognised and allowances made for this where any national scheme is considered.

In the current literature there is no report of successful eradication of *Map* infection from a herd using a test and cull programme in conjunction with a management programme.

Of most concern is the finding that in the early stages of a control programme the age distribution of ELISA positive animals was the same as that for clinical disease (Jubb and Galvin 1999). This raises the question of whether the time gained in removing reactors before they develop clinical disease is significant in terms of control where testing is carried out annually. More frequent testing may be indicated. This study also demonstrated that until the programme had ran for four years there was no decline in the mean reactor prevalence, a finding that was related to the probability that most animals were infected prior to the onset of the programme (Jubb and Galvin 1999).

## National Control Strategies

The approach of countries to paratuberculosis is dictated by the known or perceived prevalence of the disease. In countries of high prevalence control is absent or voluntary and where control programmes are present the cattle producers themselves have often asked for them. In countries with a low national or regional prevalence the disease may be notifiable and infection stamped out by herd slaughter. In both high and low prevalence situations voluntary programmes may be in place to provide a source of breeding animals free from infection as described above. A strategy of vaccination is also promoted by at least one country. Many countries may also request pre-import testing of animals, but for trade within the EU paratuberculosis status cannot be used as a barrier.

### **Low Prevalence Countries**

Norway and Sweden offer the best examples of this. The disease is notifiable and in Sweden a stamping out policy exists (Bolske and others 1999). The farm buildings are disinfected and pasture is quarantined for three years. As Sweden is also in the EU there can be no national barrier to the importation of cattle from other EU countries on the basis of paratuberculosis status. *Map* infected animals have been imported from Denmark and current policy is to trace imported animals and test them and any in contacts. The possibility of whole herd slaughter is a powerful disincentive for the individual producer importing cattle that may carry the risk of introducing *Map* infection. In both Sweden and Norway various surveillance systems are in place to ensure that the low prevalence status is maintained. Sweden also has an assurance programme designed for beef herds selling terminal sires where testing is based on whole herd faecal culture.

In Australia paratuberculosis is endemic in cattle, sheep, goats and deer, but as large parts of the country were found to contain little or no infection, a policy of zoning on the basis of disease prevalence was adopted. Within zones where the infection was largely absent or of low prevalence (free and protected zones respectively) the disease is notifiable and movement restrictions are placed on infected herds such that animals can only move off them direct to slaughter or to feed lots, but not to other herds for breeding purposes (Appendix 1 and Appendix 2). Vaccination is prohibited within these zones. In zones where the disease is of moderate prevalence or the status is unknown (control and residual zones respectively) the disease is not notifiable and vaccination is permitted. With the exception of herds in the free zone, where there is no need, all can participate in the so-called CattleMAP assurance programme that provides a testing and certification programme as described above.

### **High Prevalence Countries**

Until 1999 the Netherlands was planning to implement a compulsory eradication programme based on whole herd faecal screening (Benedictus and others 1999). A number of factors have caused this position to be revised: the national prevalence survey found the percentage of dairy herds with serological evidence of infection to be 55%; results of the preliminary programme based on faecal screening was disappointing and the simultaneous problem with contamination of the marker vaccine used in the Bovine Herpes 1 Virus eradication programme had an adverse effect on how farmers perceived national disease control initiatives. They have now launched a voluntary programme where the emphasis is on management procedures with test and cull programmes limited to high prevalence herds.

In France a national initiative began in 1997 with the objective of reducing the prevalence of *Map* infection and there are prevention programmes in place in 23 of 96 departments (Guilbert and others 1999). A national vaccination programme supported by faecal screening and culling is planned.

The USA have recently reviewed their voluntary programme as a consequence of lack of progress and have drawn up a simplified model to be used by all state veterinarians as the basis for control for their state based herd certification programmes. This has been discussed above. In 1999 over 30 states had some

form of assurance programme in place (Rossiter and others 1999).

Japan has a test and cull programme in Hokaido, the main centre for their dairy industry. All adult cattle are tested twice within three months using the antibody ELISA and this is repeated annually. Herds must achieve four clear annual tests consecutively before they achieve free status. Positive cattle are culled and compensated at 80% of the market value.

# Chapter 7

## Small Ruminants

M V Cranwell<sup>3</sup>

### Introduction

This paper provides general information on paratuberculosis primarily in sheep but also goats and deer. The papers by Stockman (1911), McEwen (1939), Dunkin (1934) and Sigurdsson (1956) provide further detail. The main topics covered in this summary are:

- Clinical signs and experimental infection.
- Immunology and vaccination.
- Diagnosis - serology.
- Diagnosis - bacterial culture and molecular techniques.
- Paratuberculosis in goats.
- Paratuberculosis in deer.
- Interspecies transmission.

### Clinical signs

#### Natural infections

There are many descriptions of the clinical signs, gross pathology, and histological lesions (McEwen 1939, Stamp and Watt 1954, Carrigan and Seaman 1990, Clarke and Little 1996, Perez and others 1996, Clarke 1997). Early cases may only show a loss of condition but this usually progresses to more severe emaciation and an inconstant scour. In some animals the wool becomes brittle and is easily pulled out. There may be a marked reduction in serum albumin and calcium. The likelihood of detecting acid-fast bacilli is higher if the faeces are softer or more fluid than normal. Collections of clear fluid in the body cavities,

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emaciation, and atrophy are all found at necropsy. The walls of the lower small mesenteric attachments oedematous and the local lymph nodes swollen. Thickening may extend to involve the jejunum, caecum and colon. Caseation is more commonly seen in goat lymph nodes. The mucosa is thickened and often granular. A yellowish-orange pigmentation is present in some cases. Some animals may show minimal intestinal changes but exhibit pronounced clinical signs. Huge numbers of organisms are seen in Ziehl-Nielsen stained sections of intestines. Lesions, range across a spectrum between two extremes: those where epithelioid macrophage cells are numerous and these contain many acid-fast organisms (multibacillary or lepromatous), to those cases where there was a profuse lymphocytic contribution with small collections of macrophages and very few organisms (paucibacillary or granulomatous). Acid-fast organisms are more frequently found in faeces from the multibacillary cases and it is also these animals that are detected more reliably by serological tests. Paucibacillary cases have ranged from 60% to 12% of affected animals examined.

### **Experimental infection**

Historically, culture of the organism from ovine material was generally unsuccessful. To follow the course of an experimental infection, McEwen (1939) was successful in causing disease in lambs by dosing with minced infected intestines. Twenty years later workers at the Moredun Research Institute (Brotherston 1961) could reliably produce the disease by oral dosing of culture-derived bovine and ovine strains. Some animals apparently recovered. They developed a method to quantitatively determine the weight of infection in intestines and associated lymph nodes and showed that infection did not progress using a killed organism as a vaccine. It was also demonstrated that animals that developing a strong cell-mediated immunity (determined by using intradermal avian tuberculin) showed a low level of intestinal infection whilst those with a weak response had numerous organisms within intestinal macrophages. Juste and others (1994) in Spain also studied experimental oral infection of vaccinated and unvaccinated lambs and commented that early lesions were found in the interfollicular areas of Peyer's patches and that this tissue was greatly reduced in the adult animal. This may provide an explanation for the lower susceptibility that occurs with age.

### **Immunology**

In general cell-mediated immunity is strongest during the earlier stages of infection and humoral antibody responses increase over time. Burrells and others (1998) showed peripheral lymphocyte responses of clinically affected animals correlated with the type of histological response in the intestine those with a "paucibacillary" type of reaction showing a strong CMI whilst those with a "multibacillary" reaction have a weak CMI. This confirms the two extremes of response to this organism – tuberculoid and strong resistance to the pathogen with few or no organisms found and marked local inflammatory responses - and lepromatous, associated with weak CMI, numerous organisms and a diffuse macrophage response

### **Vaccination**

Vaccination has generally been successful in the control of clinical disease (Crowther and others 1976,

Cranwell 1993, Juste and others 1994). Vaccines may be prepared from heat-killed organisms or live avirulent strains. In the UK the latter form is used and the CVL Weybridge vaccine contains three strains combined with pumice, olive oil and liquid paraffin. In young lambs half a bovine dose is given subcutaneously in the brisket. In Iceland, the strain of organism that caused severe losses could not be cultured on artificial media and instead two heat-killed bovine strains were used in the vaccine. A significant reduction in death due to paratuberculosis when compared to unvaccinated flocks resulted. This was despite the fact that lambs were not vaccinated until four or five months old. Vaccine has also been used effectively in both sheep and goats in Cyprus and Spain. It should be noted that vaccination does not cause the organism to disappear and vaccinated animals may carry infection and pass the organism in faeces though possibly at a lower level.

## Diagnosis

### Serology

It is generally accepted that as the extent of the pathological processes occurring within the animal advances the cell-mediated immune response wanes and humoral antibody increases. This serological response can be detected by the complement fixation test (CFT); agar gel immunodiffusion test (AGIDT) or an enzyme-linked immunosorbent assay (ELISA). Serological testing performed on clinical cases of paratuberculosis, where infection status was confirmed by histological examination, showed that both the AGID and the ELISA performed well in detecting the multibacillary form. Control sera were all negative by both tests confirming a 100% specificity. However, detection of the paucibacillary animals less effective; 10% gave a positive ELISA result and 30% a positive AGID result.

### Bacterial culture

Sheep strains have proved more difficult to isolate than the bovine strain. However Map can be isolated from sheep using Herrold's egg yolk medium, Lowenstein-Jensen medium and Middlebrook 7H11. Lowenstein-Jensen medium without sodium pyruvate has also been used successfully for strains from sheep and goats. Recent work from Australia (Whittington and others 1998) has compared the use of a Bactec 12B system with conventional solid media for the detection of MAP in faeces and tissues from infected sheep. The results showed that colonies of MAP could be reliably isolated using solid media based on Middlebrook 7H10 and 7H11 agars with mycobactin and egg yolk. Culture was sensitive in detecting infection in tissues from both multibacillary and paucibacillary cases. However, only 48% of samples of faeces from 31 paucibacillary cases were culture positive. These isolations took longer than using Bactec 12B, which is a more expensive technique. Histological examination of tissues has always been the "gold standard" for determining infection status but this series of experiments suggested that culture could now become the "gold standard".

## Molecular techniques

The PCR technique using an IS900 probe is of most use in confirming that colonies isolated on artificial media are *MAP* (Cousins and others 1995). Whittington and others (1999) describes an IS900 PCR-based technique whereby archived histological material can be examined for the presence of *MAP* DNA. The test gave no false positives and the sensitivity of the technique for examining material prepared from cattle, sheep and goats was 88% using a 229 base pair assay.

## Paratuberculosis in Goats

The disease occurs worldwide in this species. Clinical signs and pathological findings being similar to those seen in sheep but diarrhoea may be more of a feature in the terminal stages (Thomas, 1985, Morin, 1982, Fodstad and Gunnarsson, 1979). The only difference from sheep is that necrosis or even calcification is more commonly seen in mesenteric lymph nodes. Diagnosis is commonly made by examination of Ziehl-Neelsen stained smears prepared from the faeces. The AGID test has been traditionally used to confirm clinical cases and identify infected animals. More recently an ELISA test system has been developed from the bovine test with a quoted specificity of 100%. Vaccination, using the CVL Weybridge product, has been shown to be very effective in reducing the incidence of clinical disease. On occasions adults have been vaccinated as well as kids, to good effect (Crowther and others 1976, Ullrich and others 1982) and Wilesmith, 1982 reported elimination of disease from a herd in 4-10 years.

## Paratuberculosis in Deer

Natural infections have been documented in farmed and wild deer species (Fawcett and others 1995, Power and others 1993, Gumbrell 1986) and wild species (Matthews and others 1981, Duker and others 1992). Gilmour and Nyange (1989) produced a review of clinical signs, pathology and diagnostic methods.

In farmed deer the clinical course of weight loss and diarrhoea may be more rapid than in cattle, sheep and goats and affects yearlings rather than the older animals. More importantly the same signs can also be shown by infection with *M avium* subspecies *avium* and *Mycobacterium bovis*. There may be a sudden onset of diarrhoea and death in 2-3 weeks or a more prolonged course over a few months with development of extreme emaciation. Some cases do not develop diarrhoea. The specific lesions are confined to intestine, mesenteric lymphatics and associated lymph nodes. Histological examination of the gut shows heavy infiltration of the mucosa and submucosa by large macrophages suffused with acid-fast bacilli. Diagnosis of infection is straightforward if acid-fast bacilli are present in the faeces in an animal with typical clinical signs, otherwise diagnosis is best made at post mortem examination when histology and cultures can be carried out. As tuberculosis in deer is a notifiable disease every effort should be made to determine the species of mycobacterium involved in these cases. This can be done by use of a specific

DNA probe and PCR technique. Neither skin tests nor serology are sufficiently sensitive or specific to detect subclinical infection as part of a control scheme. Vaccination appears to reduce the number of clinical cases, but evidence of disease will still be found when carcasses are examined.

### Ovine Partuberculosis Control: The Australian Situation.

The disease was first confirmed in cattle in 1925 and in goats in 1977. The first case in sheep (McCausland 1980) was seen in one of six yearlings that grazed pasture where clinical cases of bovine paratuberculosis had been held. The following year infection was confirmed in a flock in the central table lands of NSW, with no suggestion of a bovine link (Seaman and Gardner 1981). Further investigations in the region found another 6/111 infected flocks (Seaman and Thompson 1984). One observation was that flocks breeding their own replacements tended to have disease in animals as young as two years old. Because the number of infected properties appeared to be increasing, there were a number of meetings between members of breed societies and other farmers' groups. In 1989 one of these committees requested up-to-date information on OJD and an options paper was produced. The options suggested were: quarantine and sale for slaughter only, quarantine and vaccination, and slaughter and compensation. The committee opted for "surveillance and advice".

At a meeting in 1994 another committee, convened by NSW Agriculture, decided to take further action and the result was a strategic plan in 1996. By the end of 1997 there were 181 NSW flocks classified as infected and as of that same date 66 Victorian flocks were infected. In 1997 a meeting of the Australian Animal Health Council Limited and other bodies developed a proposal for a nationally funded programme to help owners of infected flocks to eradicate OJD if they wished. This was specifically by eradication and later repopulation. Because of issues concerning funding and compensation and concerns about the true extent of infection, it was decided that a national fund would not be supported.

Because of disagreement among producers about how OJD should be tackled, the Hussy-Morris report was produced. It identified knowledge deficits, which had to be remedied so as to facilitate disease management. With funding of 2.45 million dollars the State implemented an interim surveillance and research programme in 1998. Subsequently the National Ovine Johne's Disease Control and Eradication Programme (NOJDP) has been developed for immediate implementation throughout Australia. In March 1999 the Government endorsed it.

#### **Its objectives are to:**

- Provide by 2003 sufficient information to allow an informed decision on the national management of OJD, and especially the feasibility and cost-effectiveness of eradication. To control OJD during the research and evaluation programme the specific scientific programmes are:

- To evaluate existing and potential methods of detecting, controlling and eliminating Maptb in infected sheep flocks and populations.
- To evaluate and define the extent of infection of OJD nationally.
- To minimise further spread of OJD during the evaluation period.
- To implement an effective and efficient management structure for the programme.
- To communicate effectively and efficiently the objectives, process and outcomes of the programme and to ensure the programme achieves its objectives

By June 2000 there were 700 flocks with some level of assurance within the so-called SheepMap programme, while 515 flocks were recognised as infected. The screening test is faecal culture performed on pooled samples.

#### **Other Small Ruminant Control Programmes in Australia**

As discussed under the section of control in cattle in Australia, for the purposes of the control of paratuberculosis goats, deer and camelids are considered epidemiologically similarly to cattle and distinct from sheep. Assurance programmes also exist for goats and alpaca and as of June 2000 there are 35 goat herds and 10 alpaca herds with some level of assurance within the GoatMAP and AlpacaMap programmes. As for sheep the screening test is faecal culture and the programmes are not designed to support test and cull strategies for infected flocks.

# Chapter 8

## Recommendations: Research

C J Low<sup>1</sup> and G Caldow<sup>1</sup>

### Introduction

In reviewing the literature on paratuberculosis it became clear that there are significant deficiencies in knowledge of the biology of the bacterium, the pathogenesis and epidemiology of the disease. These deficiencies limit the development of control strategies. As a consequence of the prolonged pre-clinical phase of the disease and the gradual increase in faecal shedding of *Map* and the late increase in antibody production validation of diagnostic tests has proved difficult. In particular accurate sensitivity estimates have not been established. This too has an important impact on control programmes. We have approached this summary of areas for further research from the objective of improving control strategies.

### The Organism

- Work is required to catalogue and differentiate the strains of *Map* found in cattle, sheep, goats, deer and wildlife in GB.
- There is little recent literature on the survival of the organism in the environment. The fate of the organism in slurry, dung, on pasture and in watercourses needs to be explored under GB conditions both qualitatively and quantitatively. Crude survival times in these media are known but this alone is of little value.

### Pathology / Immunology

- The mechanisms that allow *Map* to survive within macrophages require to be elucidated.
- The factors that determine the progression from a Th1 dominated immune response to a Th2 dominated immune response are unknown and appear to be critical in dictating the progress towards clinical disease.
- Many young and therefore in theory susceptible animals are exposed to *Map* in sufficient numbers, but do not progress towards the infected state. The reasons for this are unknown.
- There remains debate over the importance of age-related resistance. It is important to know what proportion of naive adult animals can become infected when placed in an infected herd.

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<sup>1</sup> SAC Veterinary Science Division

## Epidemiology

- The rôle of other species including ruminants and wildlife in the epidemiology of infection requires study.

## Diagnostic tests

- The absorbed antibody ELISA has not been examined under GB conditions against the gold-standard of histopathology and culture.
- A validation study to examine sensitivity and specificity under GB conditions should be conducted. It may be necessary to develop tests for the detection of antibody specifically against *Map* and *Mas* should the validation reveal an inadequate specificity.
- Faecal screening by culture followed by identification using PCR appears to be the most sensitive method of detecting the infected animals in the pre-clinical phase of the disease. Work needs to be done to refine and standardise decontamination methods; to validate automated systems and to validate techniques using pooled faecal samples.
- The immunomagnetic separation/PCR technique requires to be examined for use with bulk milk samples and possibly faeces.
- A recent report has suggested that pooled faecal culture is as accurate as serological detection for the identification of infected sheep flocks (Whittington, and others 2000). This study requires confirmation.

## Control

- There has been no long-term critical evaluation of the use of vaccines to control paratuberculosis in beef herds. This should be considered.
- Research into vaccines is on going in the Netherlands. Development work on other vaccines directed against mycobacterial infection current in GB should be reviewed for relevance to this area.
- There has been no evaluation of a test and cull programme to eradicate *Map* infection from infected herds.

A summary of the research on ovine paratuberculosis (OJD) that is in progress or planned in Australia is detailed below. In the Netherlands research on the interface between host and *Map* and vaccine development continues.

## Summary of Current (and Proposed) R&D in Australia for ovine paratuberculosis

### Evaluation of Eradication Strategies

Objective

Can destocking and decontamination over a 15 month period eradicate OJD from infected properties which are undergoing an approved depopulation and eradication programme and, is it an economic option.

### **Genetic Preservation**

Objective

To identify means of 'safely' retaining valued genetics from sheep flocks or goat herds found to be infected with JD.

### **Control Strategy Evaluation**

Objective

To evaluate strategies to control and limit the impact of OJD in infected flocks  
by management  
by vaccination

# Recommendations

## Chapter 9

## Surveillance

G Caldow<sup>1</sup> and G J Gunn<sup>1</sup>

### Introduction

Current surveillance systems within GB are inadequate for the purposes of estimating herd or animal prevalence of paratuberculosis in cattle, sheep or other ruminants. Without accurate prevalence data the most suitable control strategies cannot be defined and the costs of the disease cannot be estimated. The diagnostic tools available are limited by sensitivity and therefore screening all adult animals at the farm level is required for cattle herds. The antibody ELISA is the test of choice for cattle, and although not validated in GB against a gold-standard, its use in a surveillance study would allow comparison of prevalence levels with other countries and would provide a bench mark for the future.

There are a number of technological developments on the horizon that offer opportunities to improve or decrease the costs of surveillance systems. Immunomagnetic separation with polymerase chain reaction (IMS-PCR), described by Dr Irene Grant (appendix 3), should be examined for use as a screening test for bulk milk samples. Despite the labour intensive nature of the test this may in future offer a highly specific and rapid test for paratuberculosis surveillance though only in the dairy herd.

Culture of pooled faeces has been reported as more accurate and cheaper than serological surveillance in sheep flocks. Although at the present time the available scientific literature supports the absorbed ELISA, the possibility of using the culture of pooled faeces for cattle should be examined further. Automated culture systems for faecal screening may require validation where large numbers of samples are collected for bacterial culture. As described above for milk, IMS-PCR may in future prove to be a useful technique for screening faecal samples.

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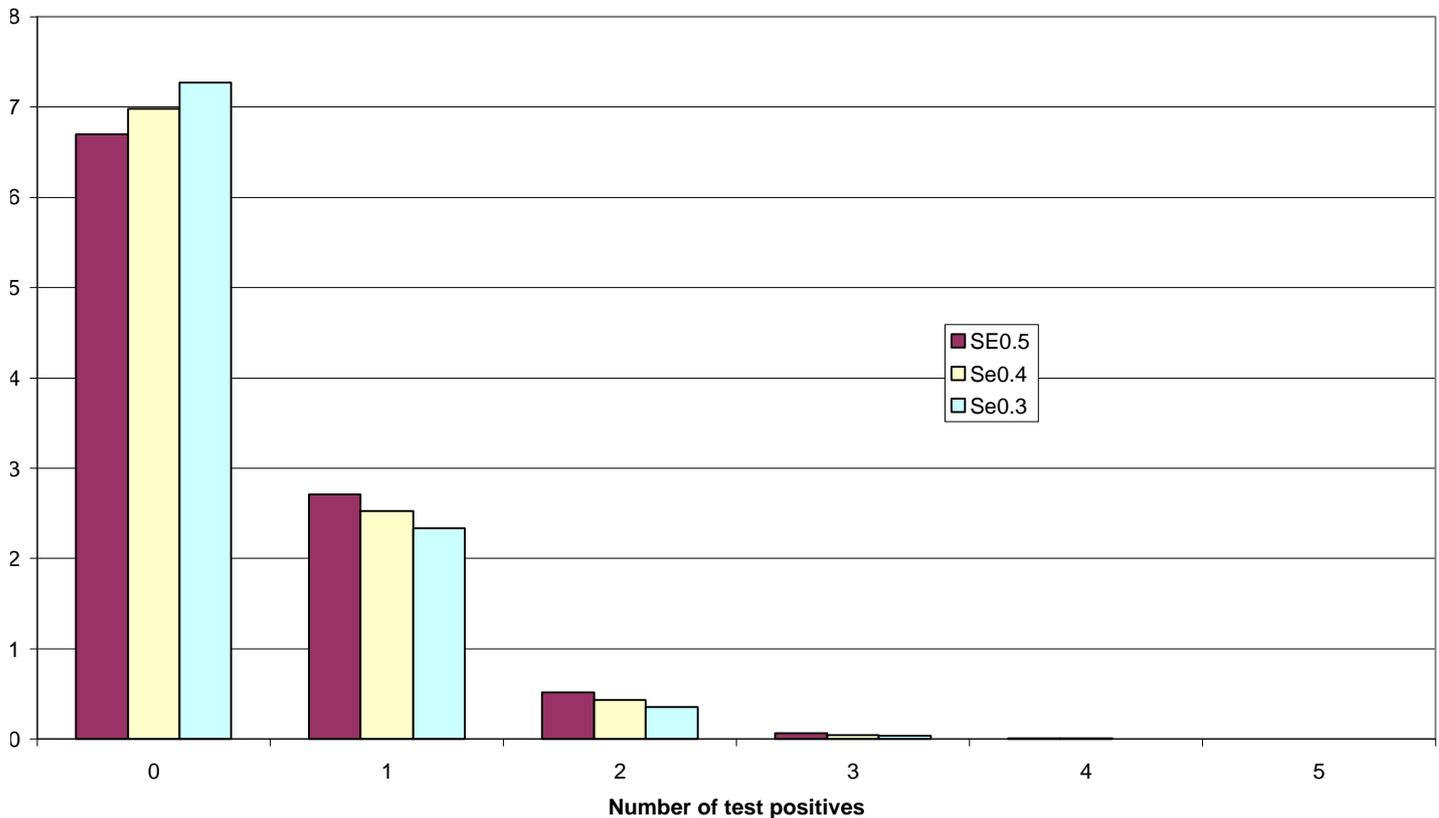
## Recommendations

The conclusions from workshop 1 are repeated here.

- Test Beef Cattle Using Brucella Blood Samples.
- Select Herds At Random.
- Test Animals Over 2 Years.
- Absorbed ELISA Satisfactory.
- Samples Tested Singly in ELISA.

Dairy herds to be tested using a similar survey design to that used in beef cattle, but samples would have to be collected specifically for the purpose.

It should be noted that the workshop 1 conclusion that samples collected for *Mycoplasma agalactiae* surveillance could be used for screening by AGID is not recommended further. The small sample size (20) is not adequate for a test of such limited sensitivity. We have calculated that where the sensitivity of the test is 50% in a flock with a prevalence of 2% only 33% of flocks would yield one or more positive result (fig 1).Figure 1. Probability distribution of the number of test positives from a sample of 20 given a background prevalence of 2%, sensitivities of 50%, 40% and 30% and a specificity of 99%.



For surveillance of sheep flocks it will be necessary to adopt the following recommendations but the sampling framework needs further consideration. As part of the modelling and costing exercise carried out in part 2 sampling tables for different levels of confidence and test sensitivity and specificity have been constructed.

- Flocks Selected Randomly.
- Apply AGIDT.

As *Map* infection has a long pre-clinical phase and infection is considered to occur in most cases within the first six months of life control programmes are unlikely to show a detectable improvement in the annual rate of reactors before 4 years have elapsed. It is therefore recommended that surveys to determine national prevalence in cattle be carried out every five years. A similar period would be applicable for sheep.

For other small ruminants a similar approach to sheep surveillance should be adopted with the exception of deer. In deer as clinical disease occurs at an earlier stage it can be difficult to differentiate the disease caused by *Map* from that caused by *Maa* or *M bovis*. For these reasons abattoir surveys of the slaughter generation using PCR may be more relevant.

**Accounting for sources of bias**

Surveillance operates upon two levels within the context of this study:

- herd and flock level prevalence (national)

- ii. within herd and flock level prevalence
- iii. it is assumed that there is no significant regional variation in paratuberculosis prevalence

The sensitivity and specificity of the surveillance tools, whether they are serological tests, skin tests or faecal culture is of fundamental importance to surveillance and the issue must be addressed before embarking upon any surveillance project. Methodology exists to accommodate the lack of test sensitivity and specificity as sources of bias if there are estimates available for these parameters (Rogan and Gladen 1978).

The recommendations for surveillance have been made above. It has also been emphasised that it is advisable to validate tests for GB conditions. It is assumed that all bovines over 2 years of age and all ovines over 12 months would be sampled in a randomly selected sample of herds and flocks.

### **Within herd prevalence**

The estimates on within herd prevalence could be adjusted to account for poor test sensitivity (as above). To design this study it would be necessary to establish the budget; the test to be used and how precise an estimate was required. The smaller the tolerance specified the larger the number of herds to be tested. The confidence in the final estimates would depend primarily upon the scale of the study and the variation in within herd prevalence found.

### **Prevalence of infected herds (herd prevalence)**

As herd owners would have to provide consent prior to samples being tested for paratuberculosis there would be a probable bias in compliance rate between herd owners who knew or suspected they had a paratuberculosis problem and those who did not. The importance of this source of bias cannot be estimated. The most logical way to proceed would be to draw a random list of holdings, request permission to collect samples and test them. Where owners refuse permission they will be asked to specify why they were not willing to comply. The response to the questionnaire would instruct the researcher as to the source and scale of any bias. Compliance would obviously be enhanced were the target population assured of confidentiality of the information on the disease status of their herd and answers to the questionnaire.

An alternative approach would be to design a small study to provide sufficient information to allow us to estimate true prevalence from survey figures. To do this it would be necessary to derive the following information:

- the probability that a herd owner agrees to have samples tested, if the herd is positive
- the probability that a herd owner agrees to have samples tested, if the herd is negative
- the probability that the herd is positive if the herd owner agrees to have his samples tested

The sampling biases, in theory at least, could then be estimated by a trial in which whole herd samples from

brucellosis herd tests were selected randomly and tested for antibody to *Map*. The herd owners would be retrospectively approached and their permission to test requested. By necessity the herd owner would need to be blind to his results: i.e. it would be necessary to test samples without permission to estimate the bias. this would provide the best estimator, but legal opinion should be sought on this approach.

# Chapter 10

## Recommendations: Control

G Caldow<sup>1</sup>

### Introduction

From the limited surveillance data available and the history of trade in live animals with the Netherlands, USA, Belgium and Canada herd prevalence in the dairy herd may be similar to these countries. Passive surveillance indicates that the disease is of increasing importance in the national beef herd. Against this background the policy followed by countries where the herd prevalence is low and the disease is notifiable is not considered to be appropriate for GB.

### Vaccination

Vaccination using the currently available vaccines is likely to produce a positive cost benefit response in infected dairy herds, but there is no information of its value in beef herds. Vaccination does not appear to significantly reduce the number of infected animals nor the number of animals that excrete *Map* and will not prevent the spread of infection between herds. Vaccination on its own is not recommended as a control programme applicable to GB. Where vaccination with faecal screening, as pursued by French authorities, is supported by movement restrictions from vaccinated herds, progress may be made.

### Assurance programmes

Assurance programmes through herd screening to demonstrate freedom from infection are used by Australia, Sweden and to a limited extent GB. These programmes offer animals that are at low risk of developing paratuberculosis or introducing *Map* infection to a herd. The degree of confidence that can be placed in such cattle being free from infection will always be limited by the prolonged incubation period of the disease and the low test sensitivity during this period. If the sensitivity of the antibody ELISA is as low as 30% rather than 45% this confidence will decrease further. However, the corollary of this is that animals from herds where infection has been demonstrated represent a high risk of introducing infection. Assurance programmes may therefore offer a pragmatic control method for countries where there is a high prevalence of paratuberculosis. Validating automated culture systems for faecal screening would allow a test of greater sensitivity to be used and so improve the robustness of the schemes.

Assurance programmes are usually voluntary and the Government may finance research to support the programmes. The difficulties encountered by infected pedigree or high genetic merit herds must be

recognised. It is likely that once they are identified as infected they will no longer be able to gain income from selling breeding stock.

## Management Programmes

Management Programmes are essential to limit the chance of introducing infection and to restrict the transmission of infection. They now form the most important part of the Netherlands approach to the disease. Much of the advice (on colostrum) contradicts previous general veterinary advice and the recommendations on ceasing to feed discarded milk to calves will be seen as a further cost. However, the management approach to control of paratuberculosis should be recommended to dairy herds in GB.

There is less opportunity to effect change in beef herds and the impact of management programmes may be limited. Fencing-off water sources in extensive grazing areas may be of most benefit, but prohibitively expensive for some farms.

## A strategy for GB within existing legislation

We recommend that assurance programmes based on the methodology used in both the Australian and American programmes are adopted. The Cattle Health Certification Standards (CHeCS) advocate a programme that was developed by SAC only after review of the relevant control programmes in other countries.

At the present time the available information supports the testing strategy based on the absorbed ELISA for blood antibody and the screening of all adults in the herd on an annual basis on two occasions before status is conferred. Thereafter the screening can revert to a biennial basis providing animals that are culled in the intervening period are also screened. Faecal culture should be used as the second test to confirm the presence of infection in herds that were thought to be free of infection prior to testing positive by the absorbed ELISA. As any alternative test (and the antibody ELISA) are validated for GB conditions as described in the preceding sections it will be necessary to review this strategy.

It should be a condition of membership of such assurance programmes that any animal that is removed from the herd, because it is suspected of being infected with *Map*, must be slaughtered directly and not sold on as a breeding animal.

A lower level of screening may be appropriate in some situations such as in herds that sell replacement breeding heifers to commercial herds. However this approach should not be advocated for high value animals.

In order to make significant progress it will be necessary to support such programmes with information campaigns to the vendors and purchasers of cattle. This has been recognised by the other countries that have adopted assurance programmes. Such information should take the form of instruction on best practice

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management to prevent the introduction of the disease to a herd and to reduce the chance of spread of infection by adopting the management programmes that have been detailed elsewhere for this purpose.

Breed societies and the professional organisations representing the auctioneers should be consulted with a view to developing a system that encourages vendor declaration of the paratuberculosis status of their herd and promotes the rights of the purchaser of cattle within the current legislation on the sale of goods. The appropriate experts in civil law should specifically explore this final point.

# PART 2: Economic Assessment of Paratuberculosis in GB

## Chapter 11 An economic evaluation of Johne's disease (paratuberculosis) in the beef herd using the Markov Chain model

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Cost-Benefit analysis for possible surveillance and control programmes

### Introduction

One of the four aims of this project was to carry out cost-benefit analysis, relating to animal health and welfare, on possible surveillance and control programmes for paratuberculosis in farmed species. It was specifically stated that “the analysis” was to exclude the “possible danger to human health”. To provide such a cost-benefit analysis it was essential for the research team to provide a best estimate, using currently available knowledge, for the cost of paratuberculosis at farm, national and international level from a GB perspective. To provide this estimate the team had to estimate the cost of paratuberculosis for each affected species and in addition agreed to give independent estimates for the beef and bovine dairy industries. Modeling paratuberculosis provides the best way of pooling together existing information; classifying the strength of the published information and highlighting the deficiencies in the existing knowledge base. At the same time modelling avoids the need to actually carry out expensive long term experimental infections in herds. Part of the project team was already evaluating the losses associated with paratuberculosis for Scottish Executive Rural Affairs Department (SERAD) using epidemiological/mathematical/economic modelling techniques. The preliminary results from this model have been included in the report to provide a summary of the detailed breakdown of losses with some sensitivity analysis.

Losses Associated with Paratuberculosis in the Beef Herd

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## Overview

Ideally the economic impact of any farm animal disease should be measured as the avoidable loss (McInerney 1996). This is the difference between total costs (output loss plus control expenditure) at current levels of prevalence and control and minimum total costs that can be achieved. In the case of paratuberculosis in the beef suckler herd there are currently three main control options. Animals with clinical disease may be identified, treated, diagnosed and culled until the herd settles down, in the long term, to a steady state of endemic infection. Vaccination can be used to reduce the number of clinically affected animals, but the herd will remain endemically infected. A test and cull strategy can be employed to remove both the infected animals that can be detected by this method and the offspring of infected females. The time scale and effectiveness of this last approach is unknown. For this study however we had insufficient epidemiological information to model the consequences of vaccination or test and cull strategy. Instead total losses due to paratuberculosis are calculated as the difference between the first control option above and the disease free state: that is believed to exist for many beef herds in GB. This is then the maximum investment that is justified in order to achieve the disease free state.

Earlier chapters in this report demonstrate the lack of reliable knowledge about the epidemiology of paratuberculosis in the field. For this reason the team used a combination of published data and expert based knowledge to build simulation models for paratuberculosis outbreak in a previously naïve herd. The output of these models detailed the losses as they occurred which in turn provided the basis for costing.

## Epidemiological Model for Paratuberculosis in the GB Beef Herd

### Methodology

A simple state-transition Markov Chain model (Agrawal and Heady 1972) incorporating a stochastic (Monte-Carlo) process has been developed for SERAD to represent the course of a paratuberculosis outbreak in a beef herd. The time interval between consecutive state vectors is 6 months. In this case the transition matrix is dynamic so the transition probabilities change with time and depend on the state vector at that time. In this model the state vector represents the state of the herd –I.E. what proportion of the herd is in each age class and disease state

The herd structure is simple and represents the 'typical' practice for beef herds in GB. All the animals are considered to run as a single unit so animals of all ages are exposed to the infectious agent. One calf is born per cow p.a. and calving occurs either in the autumn just as the animals are being housed or else occurs in the spring when they are being turned out. All calves are kept in the herd until 6 months of age at which point 70% of them are removed. The remaining 30% replacement heifers are kept until 24 months of age at which point half are removed and with only 15% selected to enter the cow herd. Cows are removed

voluntarily to make space for the incoming heifers. The model keeps a track of season and whether the animals are on winter grazing/housing or summer grazing. It is acknowledged that many GB beef herds buy in breeding replacements. However, given that we know almost nothing about the prevalence of paratuberculosis in GB beef herds we could not attempt to model the transfer of infected cattle between herds. The first priority is to understand the dynamics of the disease in a closed herd. Nevertheless the effect of purchasing infected breeding replacements is an area that is to be examined further as part of the SERAD funded project.

## Epidemiology

The disease model is based on four possible disease states plus death: Susceptible; Infected not clinical; Clinical and Resistant. The model assumes that the infectious agent is *Mycobacterium avium* subspecies *paratuberculosis* and that the level of exposure to this agent determines the probability of infection. The probability of infection (P(S-I)) is modelled using the Reed-Frost equation(Abbey 1952).

$$P(S - I) = 1 - q^{Exp} \quad (\text{Where } Exp \text{ is the level of exposure and } q \text{ a constant})$$

The probability of an infected-not-clinical animal is a fixed parameter and does not change with time. We have included a fixed probability of an infected dam infecting her calf *in utero* (Seitz and others 1989). We have defined the level of exposure for calves aged 0-6 months as the number of bacteria received in the colostrum plus the concentration of bacteria in the environment. The concentration of bacteria in the environment equates with the number of bacteria in the environment divided by the infected area. The level of exposure for animals over the age of 6 months is defined as the concentration of bacteria in the environment. The parameter  $q$  is set in the model to give appropriate probabilities of infection as the disease progresses. The value of  $q$  is higher for older animals than young animals reflecting the fact that younger animals are more susceptible to infection (Doyle 1956) but older animals can become infected (Doyle 1953). It is also possible, in this model, for young animals to succumb to clinical infection (Smythe 1950). We have included the possibility of bacteria surviving in the environment (Wray 1975). The number of bacteria in the environment is calculated from the number of bacteria produced in the previous six months in addition to those bacteria that survived from previous periods.

The model depends on various parameters (see Table 1) whose values determine the course of the disease. The following hierarchy set the "standard" values for parameters.

1. If there were reliable published data, these estimates were used.
2. Where expert opinion gave estimates for parameters, we used these to give us approximate values for the parameters.
3. unresolved parameter values were adjusted iteratively so that the output of the model satisfied three criteria: a) that the incidence of clinical cases in the 100 cow herd approximated 4 animals p.a.; b) The clinical cases consisted of approximately 3 cows and 1 heifer p.a. (see Figure 1); c) Equilibrium is

reached within 7 years.

One *a priori* hypothesis was that the dynamics of the disease would be particularly sensitive to the number of 0-6 month calves born to infected or clinical dams getting infected themselves. An element of stochasticity was introduced, using a binomial distribution, at that particular point to represent the variability of the disease. However, the model was highly robust to this introduction of randomness and in hindsight we believe that there may be more appropriate points in the model for the introduction of stochasticity. It is likely, therefore, that this model underestimates the true variation in the course of the disease.

**Table 1. Standard Set of parameter values for Markov Chain Model**

No animals initially infected	1
Herd size	100
number of calves up to 6 months old kept per 12 month period per cow	1
number of calves from 6 months of age kept per 12 month period	0.3
number of heifers kept to enter cow herd (per cow)	0.15
Spring Calving/Autumn Calving (0 or 1 respectively)	1
Number of female calves kept as replacements (per cow per year)	0.3
probability of an infected, but not clinical cow giving birth to an infected calf (Groenendaal and Galligan,1999, Seitz, and others,1989)	0.2
Probability of a clinical cow giving birth to an infected calf (Doyle,1958)	0.4
Initial environmental loading of bacteria	0
Initial proportion of herd infected	0.01
No. bacteria produced per animal per day (*10 <sup>12</sup> ) (Cocito, and others,1994, Whittington, and others, 2000)	1.5
The amount of loading in environment produced by an infected animal (*10 <sup>13</sup> )-summer	3.905
The amount of loading in environment produced by an infected animal (*10 <sup>13</sup> )-winter	5.804
The proportion of loading produced by sub-clinical animal	0.5
Survival rate of bacterial loading in the summer environment (the probability of a bacterium surviving 6 months)	0.001
Survival rate of bacterial loading in the winter environment (the probability of a bacterium surviving 6 months)	0.01
amount of loading (equivalent to *10 <sup>13</sup> bact per ha in environment) received through suckling 1 infected cow over 6 months	50
The q value in the Reed Frost equation for calves 0-6months	0.988
The q value in the Reed Frost equation for calves 6-12months	0.995
The q value in the Reed Frost equation for heifers 12-24months	0.999
The q value in the Reed Frost equation for cows over 24months	1
The probability of a calf going S->R directly	0.1
The probability of an older calf (6-12 months) going S->R directly	0.1
The probability of a heifer (12-24mnth) going S->R directly	0.1
The probability of a cow (>24month) going s->R directly	0.5
Probability of subclinical becoming clinical - 0-6month	0.01
Probability of subclinical becoming clinical - 6-12month	0.02

No animals initially infected	1
Probability of subclinical becoming clinical - 12-24month	0.03
Probability of subclinical becoming clinical - 24+month	0.1
Size of grazing/bacterial exposure area	1
Size of winter housing/bacterial exposure area	0.5
Involuntary removal rate over 6 months for susceptibles + resistants	0.01
Involuntary removal rate over 6 months for sub-clinicals	0.02
Involuntary removal rate over 6 months for clinical animals	1
Probability that sub-clinical animals become resistant	0.1

**Financial costing included:**

1. Costs of increased cow culling due to: a) infertility b) poor calf performance (as a result of lower milk yields) c) deaths due to clinical cases.
2. Increased replacement costs due to decreased cull cow carcass weights (as part of Over Thirty Month Scheme (OTMS)), increased cost of disposal (veterinary costs, labour costs), cost of a replacement heifer.
3. Lower calf growth rate due to reduced milk yields.

All the following price assumptions and the physical performance values on which they are based are from the SAC Farm Management Handbook 2000/2001 (Chadwick, 2000) unless otherwise stated.

**Suckler Gross Margin**

Price assumptions used to calculate the gross output for the typical suckler herd are given in Table 2. Subsidies are therefore the maximum rates currently available. Values of cull cows are based on the OTMS. The reduction in weight and hence value of cows culled due to clinical paratuberculosis was assumed to be about one condition score unit (100kg) (K.Sinclair, personal communication). All prices were multiplied by the corresponding number of disease states for calves and cows predicted by the epidemiological herd model. The total enterprise gross output each year throughout the time course of the model (10 years) was calculated.

Variable costs are given in Table 3. All are standard figures with the exception of an extra fixed veterinary charge against each clinical case. This veterinary charge reflects the cost of veterinary time and treatments plus farm labour etc. associated with diagnosis and treatment. It is an estimate based on informal communication with veterinary practitioners.

**Follower Gross Margin**

The gross output for the followers is based on the sale of newly calved heifers offset against the transfer-in charge of female calves from the suckler herd (Table 2). It was assumed that any clinically infected heifer was sold under OTMS at £225. Variable costs were as shown in Table 3. Note that no 'other livestock expenses' are charged against followers as the majority were transferred to the suckler herd thus saving any marketing costs.

## Herd Gross Margin

The herd gross margin was calculated as the sum of the two enterprise gross margins. The ten-year stream of such gross margins was discounted at a real interest rate of 5%. This allows future costs and income to be expressed in "today's terms" by taking into account investment opportunity costs (Boehlje and Eidman 1984). Test gross margins were then summed to give a net present value and annuity equivalent for the entire time horizon. This single annuity could then be used to compare with the disease-free gross margins and with other disease assumptions.

## Results and Discussion

The "standard" set of parameters is given in Table 1. Figure 1 provides output from the model illustrating the number of clinical cases from heifers and cows. The results from the standard model are presented in Tables 4 to 11. Tables 4-7 present the results on a per cow basis. Tables 8-11 present the results on a herd basis. Tables 4-5 and Tables 8-9 present the results as a NPV whereas Tables 6-7 and Tables 10-11 present the results as an annuity. Having selected our standard we then ran the model with different parameter values as a form of sensitivity analysis. Tables 4, 6, 8 and 10 demonstrate the difference due to time of calving. Tables 5, 7, 9 and 11 illustrate the change in losses for different herd sizes. The effects of changes in bacterial loading, infective area and the survival rate of the bacteria were also tested and are discussed.

Figure 1 demonstrates that in the standard model equilibrium is reached after approximately seven years, with 3 cows and one heifer clinically affected each year and that there is seasonality in the number of clinical cases. The reasons for the model having a seasonal effect are 3-fold:

- a) Calving is either all spring or all autumn calving and therefore there is a cohort of animals coming through each age step every six-month period.
- b) We have treated the farm as having two separate environments - a winter (housed) environment and a summer (grazing) environment. The bacterial loading of each environment is distinct. Furthermore we have allowed a difference in survival rate of bacteria for the two environments.
- c) Both the number of organisms and the infectious area over which they are spread determine the infectivity of bacteria. We have allowed the infectious area to differ between the two seasons and hence environments.

The estimated cost of the disease in our standard (autumn calving, 100 cow herd) model over the first 20 years following the introduction of the disease is £202 NPV per cow (Table 4) and £ 20152 per herd (Table 8). The equivalent annuity cost is £16 per cow (Table 5) and £1617 per herd (Table 10). There are a few papers estimating the cost of Johne's in the dairy sector ((Benedictus and others 1987, Hutchinson 1996,

Johnson-Ifeorulundu and others 1999, Ott and others 1999, vanSchaik and others 1996)) but there is little published for beef herds. Our estimated annual cost of £16 per cow over the first 20 years following infection is low when compared to the estimates for the dairy sector in the United States (e.g. approximately US\$100: (Ott and others 1999)). Our estimate of £16 can be presented as a proportion of the current £300 gross margin per beef cow at about 5%. Currently for a dairy cow £550 is a reasonable gross margin per cow and our own dairy cow estimate of £26 also represents 5% of GM i.e. identical to the beef result. In contrast the \$100 US\$ estimate represents 13% of GM (converting 100 US\$ to pounds at approx. 100/1.4=£71)

### **Time of Calving**

The cost of the disease over the 20 years was slightly less for a spring-calving herd than an autumn-calving herd (Tables 4,6,8 and 10). This was despite the fact that the disease progressed slightly faster for the former but reached a similar equilibrium. The reason for this was that the Gross Margin per cow is less for the spring-calving herd than the autumn-calving herd because the "Beef Special Premium" (BSP) is only claimed in the autumn-calving management system (page 175 of Farm Management Handbook 2000/2001). ). Although BSP is now available on all male calves over 9 months of age for the purposes of this study it was estimated that in a spring calving herd, such calves would be sold before reaching qualifying age.

### **Herd size**

The general effect of increasing herd size (Tables 5, 7, 9 and 11) is to increase the cost of the disease (Table 7; annuity per cow in a herd of 30 is £2 but in a herd of 200 is £18). This is unsurprising because of the importance both in the literature (Cocito and others 1994), and consequently in the model, attached to the number of bacteria shed into the environment. The more animals in an area the more potential shedders there are and hence the greater impact of the disease. When the model was adjusted for herd size, all other parameters were held constant including the "infectious area". In practice the infectious area would probably increase as herd size increases and hence "dilute" the bacterial exposure over a larger area. This observation therefore reflects the greater intensity of production rather than herd size per se.

### **Bacterial loading**

The bacterial loading of an infected animal is the number of colony forming units. (c.f.u) of *Map* excreted per animal per day. The value may vary greatly with weather, age and food. We were unable to find any precise data for this parameter (Cocito and others 1994), and have had to rely on data relating to sheep ((Whittington and others 2000)) and estimates of faecal output by cattle. Due to the lack of knowledge it was considered reasonable to alter this value by one order of magnitude either way. This showed that the bacterial loading had a great effect on the course of the disease. A ten-fold increase in bacteria excreted by infected animals caused the disease to develop faster although once at equilibrium the clinical effects of the disease were similar to the "standard" disease model. This resulted in an annuity of £2042 compared with £1617 in the standard herd. A ten-fold decrease in shedding rates made the disease become unsustainable

and die out rapidly. This would suggest that removing shedding animals from the herd would help control this disease.

### **Infective area**

The “infective area” is best defined as “the area over which the infective agent is shed and in which animals are exposed to that agent”. The Reed-Frost relationship in this model in part depends upon the infective area. Therefore for a fixed number of bacteria the smaller the area, the higher the concentration and hence the greater the likelihood of infection. It is unsurprising therefore that our model indicated that the larger the “infective area” the lower the costs of disease. When the “infective area” was increased five fold, the disease was unable to sustain itself. Therefore if this model represents the true mechanistic nature of the disease then extensification will help control the disease.

### **Survival rate of bacteria**

The survival of the *Map* is thought to be high relative to many other species of bacteria (Wray 1975). Published references about survival rate are not necessarily quantitative since they refer to the maximum length of time the bacteria has been shown to survive. In this model we have assumed an exponential decay of bacteria in the environment. Expert opinion suggested a decay rate of 90% over 2 months (equivalent to a survival rate of 1 per thousand over 6 months). Decreasing the survival rate of the bacteria slowed the rate at which the disease reached equilibrium in the herd. *In extremis*, a very low survival rate ( $10^{-9}$  per 6 months) caused the disease to die out. Therefore one possible control measure would be to decrease the survival rate of bacteria in the environment by physical or chemical means.

This modelling exercise was sponsored by SERAD as part of a ROAME “Development of farm animal health decision support systems”. They have agreed to the presentation of preliminary results in this report due to the perceived importance of paratuberculosis. This work however is not the property of MAFF. Future work should involve testing the model against field data. Many of the parameters used are estimates from experts or from members of the research team. We recommend that many estimates need to be improved through experimental work and surveillance projects.

## **Estimate of Losses Associated with Paratuberculosis in the Beef Herd at National Level**

The infected herd results reported above were aggregated to national level using the MAFF June Census figures (beef breeding herds) for 1999 ([http://www.maff.gov.uk/esg/m\\_index.htm](http://www.maff.gov.uk/esg/m_index.htm)) and an assumed prevalence level for paratuberculosis of 17.5% (Cetinkaya and others 1998) across and within all herd size categories. The results are presented in Table 12. Infected herds of size 40 cows or less were assumed to have costs per cow of £2 pa. The figure used for herd sizes above 100 was £18 pa, 50-100 £16 pa and size category 40-50 £11.3 (a weighted average of the two adjacent categories). These results are directly proportional to the assumed level of prevalence that is not well established for GB (see Chapter 5, page 29).

If we vary this herd level prevalence estimate by plus or minus 10% we can provide a range of losses from £1.3 million at 7.5% of herds affected to £4.88 million at 27.5% of herds affected. Alternatively if the prevalence is assumed to be just 1% (Cetinkaya and others 1994) then the GB total figure falls from £3.1m pa to £0.18m pa. Both prevalence figures are based on published surveys of dairy farms in the south of Britain. There seems to be no indication of the prevalence in beef herds or any information about possible variation of prevalence by herd size or location. Until such basic information is available there seems little point in developing more sophisticated modelling techniques to establish the nature and extent of the economic problem or best means of control.

It should be noted as pointed out in the overview above, that these national level figures are based on control option 1, i.e. toleration of chronic infection. These figures should be compared with the only alternative option that is eradication.

#### Estimate of Losses Associated with Paratuberculosis in the Beef Herd at International Level

With the BSE ban in place and now restrictions due to FMD there are currently no international implications for trade in livestock due to paratuberculosis in any of our farmed species. Assuming restrictions in trade due to both diseases were to be removed then it is unlikely that paratuberculosis will result in any significant change in the near future. The only identifiable penalty might operate at individual farm level in, for example, a Scandinavian country and we cannot predict such an effect.

**Table 2** Price assumptions used to calculate Suckler herd gross output

<b>Category</b>	<b>Weight at sale</b>	<b>Price (£/kg)</b>	<b>Total Value (£)</b>
<b>Sales:</b>			
Male calf	239	1.28	306
Female calf	221	0.90	199
Clinical calf	n/a	n/a	0
Cull cow	600	0.50	300
Clinical cull cow	500	0.50	250
<b>Replacements:</b>			
Heifer with calf			550
Share of bull & calf (per cow)			13
<b>Subsidies:</b>			
Beef Special Premium			55
Suckler Cow Premium			144

**Table 3** Variable cost assumptions (£/head)

<b>Cost item</b>	<b>Sucklers</b>	<b>Followers</b>
Veterinary cost	15	12
Extra vet costs for a clinical case	100	100
Feed cost	14	28
Forage cost	65	50
Other Livestock expenses	15.5	

### Number of clinical cases per 6 month period in Standard Herd over 20 years

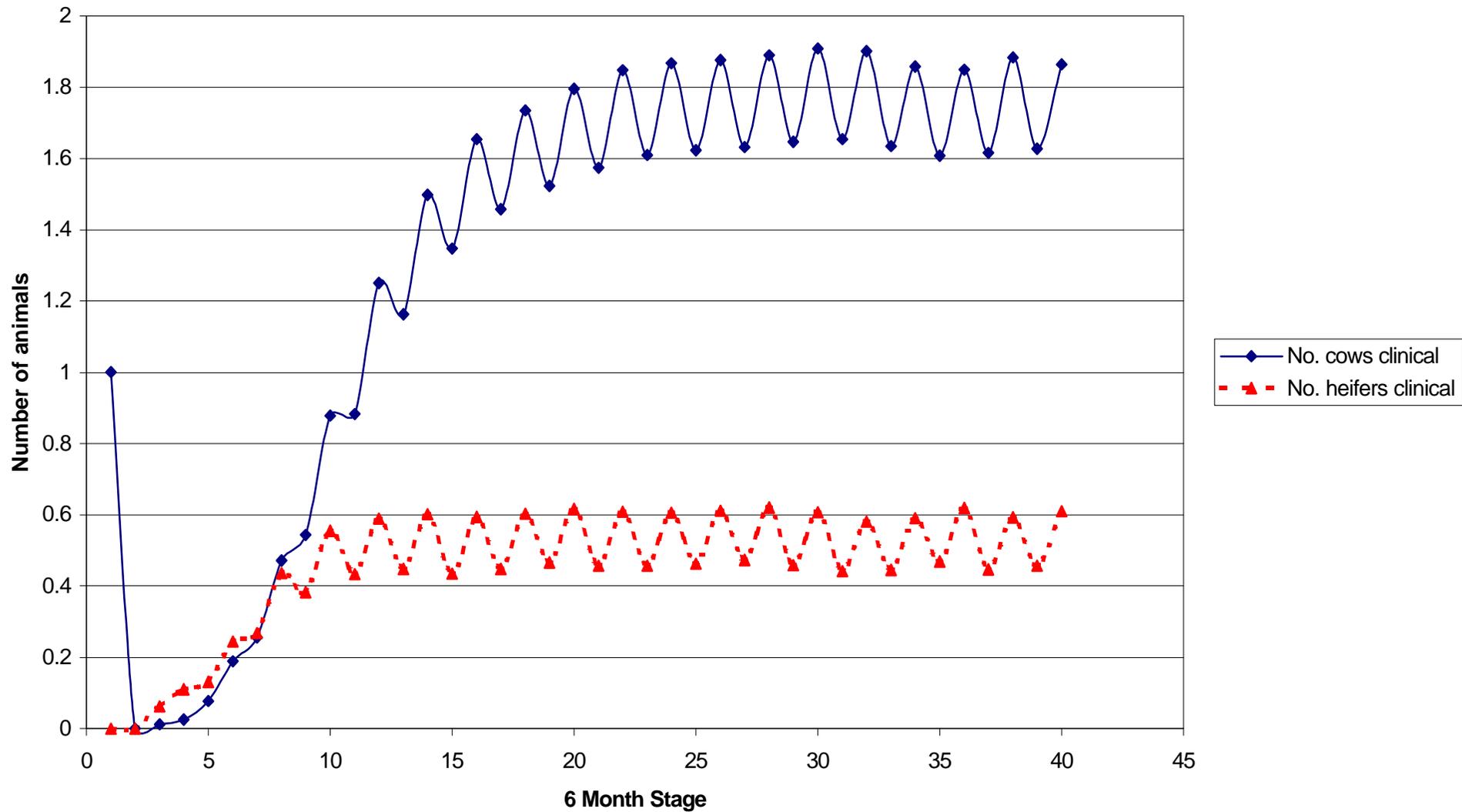


Figure 1. The number of clinical cases amongst the heifer herd and cow herd in the standard herd per six month period: demonstrating the attainment of equilibrium and the effect of season. This demonstrates approximately three cows and one heifer clinical cases per annum.

**Table 4.** Net Present Value (£) per cow with and without disease over 20 years following the introduction of Johne's disease for the standard autumn calving herd (herd size 100) compared with a spring calving herd (herd size 100).

	<b>Standard autumn calving Herd</b>	Spring Calving
No disease	<b>3499</b>	2818
With Disease	<b>3297</b>	2629
<b>Cost of disease</b>	<b>202</b>	<b>188</b>

**Table 5** Net Present Value (£) per cow with and without disease over 20 years following the introduction of Johne's disease for the standard autumn calving herd (herd size 100) compared with autumn calving herds of different sizes.

	HERD SIZE					
	30	50	70	<b>100 (standard)</b>	150	200
No disease	3499	3499	3499	<b>3499</b>	3499	3499
With Disease	3470	3373	3329	<b>3297</b>	3278	3272
<b>Cost of disease</b>	<b>29</b>	<b>125</b>	<b>170</b>	<b>202</b>	<b>221</b>	<b>226</b>

**Table 6.** Annuity (£) per cow with and without disease over 20 years following the introduction of Johne's disease for the standard autumn calving herd (herd size 100) compared with a spring calving herd (herd size 100).

	<b>Standard autumn calving Herd</b>	Spring Calving
No disease	<b>281</b>	226
With Disease	<b>265</b>	211
<b>Cost of disease</b>	<b>16</b>	<b>15</b>

**Table 7.** Annuity (£) per cow with and without disease over 20 years following the introduction of Johne's disease for the standard autumn calving herd (herd size 100) compared with autumn calving herds of different sizes.

	HERD SIZE					
	30	50	70	<b>100 (standard)</b>	150	200
No disease	281	281	281	<b>281</b>	281	281
With Disease	278	271	267	<b>265</b>	263	263
<b>Cost of disease</b>	<b>2</b>	<b>10</b>	<b>14</b>	<b>16</b>	<b>18</b>	<b>18</b>

**Table 8.** Net Present Value per herd with and without disease over 20 years following the introduction of Johne's disease for the standard autumn calving herd (herd size 100) compared with a spring calving herd (herd size 100).

	<b>Standard autumn calving Herd</b>	Spring Calving
No disease	<b>349871</b>	281768
With Disease	<b>329719</b>	262919
<b>Cost of disease</b>	<b>20152</b>	<b>18848</b>

**Table 9** Net Present Value (£) per herd with and without disease over 20 years following the introduction of Johne's disease for the standard autumn calving herd (herd size 100) compared with autumn calving herds of different sizes.

	HERD SIZE					
	30	50	70	<b>100 (standard)</b>	150	200
No disease	104961	174936	244910	<b>349871</b>	524807	699743
With Disease	104106	168661	233019	<b>329719</b>	491666	654469
<b>Cost of disease</b>	<b>855</b>	<b>6274</b>	<b>11891</b>	<b>20152</b>	<b>33141</b>	<b>45274</b>

**Table 10.** Annuity (£) per herd with and without disease over 20 years following the introduction of Johne's disease for the standard autumn calving herd (herd size 100) compared with a spring calving herd (herd size 100).

	<b>Standard autumn calving Herd</b>	Spring Calving
No disease	<b>28075</b>	22610
With Disease	<b>26458</b>	21097
<b>Cost of disease</b>	<b>1617</b>	<b>1512</b>

**Table 11.** Annuity (£) per herd with and without disease over 20 years following the introduction of Johne's disease for the standard autumn calving herd (herd size 100) compared with autumn calving herds of different sizes.

	HERD SIZE					
	30	50	70	<b>100 (standard)</b>	150	200
No disease	8422	14037	19652	<b>28075</b>	42112	56149
With Disease	8354	13534	18698	<b>26458</b>	39453	52516
<b>Cost of disease</b>	<b>69</b>	<b>503</b>	<b>954</b>	<b>1617</b>	<b>2659</b>	<b>3633</b>

**Table 12** Annual national costs of paratuberculosis in beef breeding cattle at farm level based on costings reported here aggregated using MAFF June 1999 census and a prevalence of 17.5%

<b>Herd size:</b>	1 - <10	10 - <30	30 - <40	40 - <50	50 - <100	100 and over	Total cost  (£/million)
<b>England</b>							
Number of holdings	11 533	11 262	2 667	1 797	3 419	1 122	
Number of cows	51 701	198 748	90 681	78 894	230 866	169 821	
Cows/herd	4.5	17.6	34.0	43.9	67.5	151.4	
Cost/cow	2.0	2.0	2.0	11.3	16.0	18.0	
Cost/ infected herd	9.0	35.3	68.0	496.6	1080.4	2724.4	
Cost all herds	18095	69562	31738	156170	646425	534936	1.457
<b>Wales</b>							
Number of holdings	3 422	3 777	960	606	934	188	
Number of cows	15 272	68 068	32 503	26 540	61 122	26 187	
Cows/herd	4.5	18.0	33.9	43.8	65.4	139.3	
Cost/cow	2.0	2.0	2.0	11.2	16.0	18.0	
Cost/ infected herd	8.9	36.0	67.7	491.7	1047.1	2507.3	
Cost all herds	5345	23824	11376	52141	171142	82489	0.346
<b>Scotland</b>							
Number of holdings	2 302	2 438	913	817	2 313	1 500	
Number of cows	10 109	44 548	30 976	36 075	163 693	235 038	
Cows/herd	4.4	18.3	33.9	44.2	70.8	156.7	
Cost/cow	2.0	2.0	2.0	11.5	16.0	18.0	
Cost/ infected herd	8.8	36.5	67.9	506.2	1132.3	2820.5	
Cost all herds	3538	15592	10842	72369	458340	740370	1.301
<b>Great Britain</b>							
Number of holdings	17 257	17 477	4 540	3 220	6 666	2 810	
Number of cows	77 082	311 364	154 160	141 509	455 681	431 046	
Cows/herd	4.5	17.8	34.0	43.9	68.4	153.4	
Cost/cow	2.0	2.0	2.0	11.4	16.0	18.0	
Cost/ infected herd	8.9	35.6	67.9	499.0	1093.7	2761.1	
Cost all herds	26979	108977	53956	281165	1275907	1357795	3.105

# Chapter 12 An economic evaluation of Johne's disease (paratuberculosis) in the dairy herd using dynamic programming

A W Stott<sup>2</sup>, G M Jones<sup>2</sup>, R Humphry<sup>2</sup> and G J Gunn<sup>1</sup>

## Introduction

An economic evaluation of Johne's disease (paratuberculosis) in the dairy herd using dynamic programming

We discovered through modelling paratuberculosis for the beef herd that there are two main constraints to an economic analysis of paratuberculosis. Firstly the necessary epidemiological data is lacking. Secondly the long time course of the disease makes a static economic analysis such as that described by McInerney (1987) unsuitable. For the dairy herd we used an alternative approach and applied a dynamic programming (DP) methodology. The technique was first described by Bellman (1957). Kennedy (1986) reviews DP applications to agriculture and includes a listing of the algorithms used in this study. More recently, DP has been used in the development of a national selection index for the dairy cow in the UK (Veerkamp and others 1995).

DP can be used to establish the returns to milk production in the long term under given physical and financial assumptions. As paratuberculosis primarily affects adult cows over a protracted period, the DP provides an appropriate framework for economic analysis. By altering the assumptions to represent an infected herd, the economic effects of the disease can be obtained by comparison with a control herd. The DP uses an optimum culling regime throughout (Stott 1994). Re-optimisation of the culling regime ensures that any effects of the disease on longevity are properly accounted for. The DP allows voluntary culling to be treated as the control expenditure undertaken in response to the output losses due to paratuberculosis. As voluntary culling is optimised, the extra control expenditure (culling) due to paratuberculosis is exactly balanced by the output losses saved (e.g. less clinical cases because more cows are culled before symptoms are observed) thus establishing the optimum point on the loss-expenditure frontier envisaged by McInerney (1987).

Applying DP to paratuberculosis in this way incorporates two important simplifications. First culling is treated as the only control option. This is not unreasonable because to date there is no effective way of treating an infected animal (European Commission 2000) and all other control alternatives need to be applied at the herd level (Rossiter and Burhans 1996). Any herd level controls can be treated as separate runs of the DP model, allowing a conventional loss-expenditure frontier to be constructed. Second all assumptions in the DP are fixed throughout the time horizon i.e. the model is stationary (Kennedy 1986). This means that neither time nor the culling of infected animals affects the disease risks to which remaining animals are exposed. Although this is

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obviously not the true position it reflects the difficulty of predicting the future not just for the epidemiology of paratuberculosis but also for other physical and financial assumptions made and the interactions between them. This DP approach may therefore be seen as a means to make an economic assessment of a fixed set of bio-economic assumptions related to paratuberculosis. It is not an epidemiological model of the disease but a means to systematically explore the relative importance of different aspects of our understanding of the disease. In this way it complements rather than competes with other models reported under the beef section of this report.

The DP objective here was to maximise the expected net present value (ENPV) from a current heifer and all its successors over a series of 20 annual stages. (As the annual returns are discounted, extending the time horizon beyond 20 years would have had little effect on the outcome). For convenience, the ENPV was expressed as an annuity equivalent (Boehlje and Eidman 1984).

The DP objective will depend on the expected net margins flowing from the current heifer and its optimum sequence of successors at each stage over the time horizon. These net margins are the margins of milk and calf sales over feed costs (adjusted for paratuberculosis except in the control herd), involuntary culling (enforced culling due to death, disease or infertility which will be affected by paratuberculosis) and other 'fixed' cost items. As future milk yield and involuntary culling outcomes are unknown, the range of possibilities was reflected (approximately) by 180 'states'. These were 15 milk yield states at each of 12 lactation states. The milk yield states represented 'bins' spread across the normal distribution of yield for each lactation. Given the mean (Table 1) and variance (Stott 1994) of this distribution it was possible to assign absolute values to each 'bin' and associated probabilities. For each milk yield state, margin over feed was calculated based on a least-cost diet formulated using SAC's 'Feedbyte' program as described in Logue and others 2000. The cost and probability of involuntary culling varied with lactation state (Table 2). All other costs and returns were considered fixed (Table 3). The expected net margin i.e. the probability weighted average margin from all states could then be calculated as the margin of milk and calf sales over feed costs and involuntary culling costs and all other 'fixed' costs.

If the decision is to 'keep' and the cow is not subject to involuntary culling then the probability of transition to any state in the next stage will be influenced by the state in the current and any previous stages. This fact will affect the expected net revenue and hence the DP objective. The extent of this effect will depend on the repeatability of milk yield (Kennedy & Stott 1993) which was set in this application to 0.48 (Stott 1994).

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**Table 1:** Average milk yield by lactation number. Figures are from Logue and others (2000) and based on a recent analysis of National Milk Records data

Lactation no.	Average Milk Yield (kg)
1	5913
2	6637
3	6976
4	7056
5	7033
6	6923
7	6861
8	6734
9	6562
10	6456
11	6266
12	5842

**Table 2:** Probability of enforced (involuntary) culling based on Forbes and others (1999) and cow cull value based on the Over Thirty Months Scheme (0.46 £/kg live weight), with adjustment for mortality, weight and milk yield losses in the case of involuntary replacement (see Logue and others, 2000)

Lactation no.	Prob. Involuntary culling	Cull cow value (£):	
		Voluntary replacement	Involuntary replacement
1	0.096	303	156
2	0.112	323	182
3	0.141	331	157
4	0.164	333	165
5	0.182	334	150
6	0.206	335	156
7	0.204	335	134
8	0.249	335	132
9	0.262	335	127
10	0.283	335	121
11	0.304	335	114
12	0.325	335	108

**Table 3:** Fixed financial assumptions used in the DP model

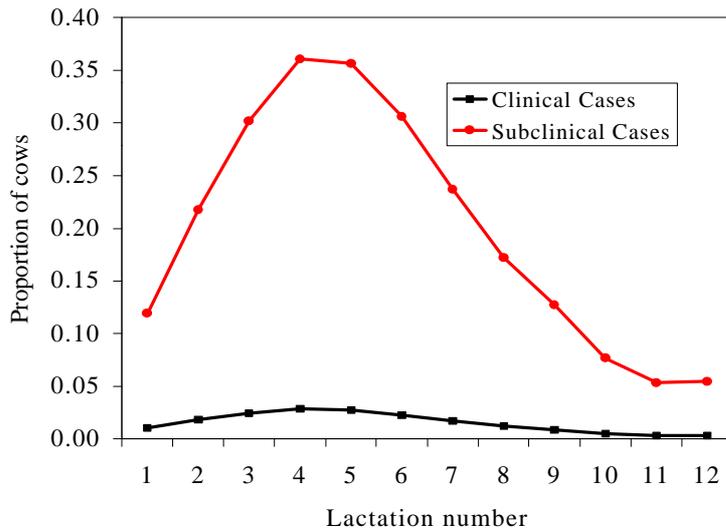
Assumption	Value	Units
Discount rate	5	%
Milk price	0.185	£/kg
Calf sale value	20	£
Replacement heifer price	700	£
Fixed and non-variable costs	500	£/cow

#### Adjustments for paratuberculosis

This disease was assumed to affect milk yield (Table 1) and hence margin over feed, probability of involuntary culling (Table 2) and the value of an involuntary cull (Table 2). For an infected herd therefore, all the above figures were adjusted and the DP re-run to see the effect on ENPV. In each case, a weighted average value was used based on the proportion of affected and unaffected cows thought to be present in each lactation of an infected herd.

Benedictus and others (1987) report the age distribution of 61 Holstein-Friesian animals culled from 11 dairy farms taking part in an organised paratuberculosis eradication scheme for showing clinical signs of the disease. They also report the age of 52 animals from 7 farms considered subclinical for paratuberculosis on the basis of annual allergic and serological examination. By using the age distribution implied by the DP, it was therefore possible to deduce the proportion of clinical and subclinical animals in each lactation of these affected herds from the total number of clinical and subclinical cases in the herd. This total number was assumed in the first instance to be 2 clinical cases and 25 subclinical cases (Cetinkaya and others 1998). The distribution of clinical and subclinical cases in each lactation was smoothed by fitting a Poisson distribution to the data (Figure 1). This distribution provided the proportions of healthy, clinical and subclinical animals in each lactation from which weighted average milk yields and involuntary culling probabilities were calculated for use in the DP.

For the standard infected herd it was assumed that subclinically affected cows suffer a 10% reduction in milk yield (Benedictus and others 1987, Collins and Morgan 1991, Groenendaal and Galligan 1999), and an 11% increase in the probability of involuntary culling (Wilson and others 1995). Clinical cases were certain of involuntary culling and their milk yield was reduced by 20% compared to the healthy equivalent (Table 1). In addition, each clinical case incurred a £100 fixed charge to cover veterinary attention, blood testing etc. To test sensitivity the model was run with changes to the above standard parameters. Furthermore the model was run with alternative numbers of clinical (1/100 or 5/100cows) and subclinical cases (20 or 30 animals). The sensitivity of milk price and cost of replacement was tested in both the control and infected herd.



**Figure 1:** Distribution of Paratuberculosis cases by lactation number for an infected 100-cow herd, assuming 2 clinical cases and 25 subclinical cases.

## Results

For the control herd, ENPV expressed as an annuity was £259/cow. The corresponding figure for an infected herd was £232/cow suggesting that paratuberculosis reduced net margins from milk production by about £26/cow (Table 4). This effect of the disease was very much dependent on milk price, but was not dependent on replacement costs (Table 5). There was a linear relationship between the annual cost of the disease per cow and the number of clinical animals in the herd with about a £2 increase per clinical animal within a range of 0-5 clinical animals/100 cows (Figure 2). An increase/decrease of 5 animals from 25 subclinical animals resulted in a £4 difference in annuity (Table 6). A change of 5% in milk yield depression had a much greater effect on the annuity, when it was for subclinical rather than for clinical animals (£9 versus £0.8). However, an increase in the % of involuntary culling due to subclinical animals had very little effect on the annuity.

### Estimate of Losses Associated with Paratuberculosis in the Dairy Herd at National Level

The above estimated cost of paratuberculosis in infected herds of £26/cow pa was aggregated to national level using the MAFF June Census figures (dairy breeding herds) for 1999 ([http://www.maff.gov.uk/esg/m\\_index.htm](http://www.maff.gov.uk/esg/m_index.htm)) and an assumed prevalence level for paratuberculosis of 17.5% (Cetinkaya and others 1998). The results are presented in Table 7. These results are directly proportional to the assumed level of prevalence that is not well established in the UK (see Chapter 5, page 29). If we vary this herd level prevalence estimate by plus or minus 10% we can provide a range of losses from £4.2 million at 7.5% of herds affected to £15.4 million at 27.5% of herds affected. Alternatively if the prevalence is assumed be just 1% (Cetinkaya and others 1994) then the GB total figure falls from £9.8m pa to £0.6m pa. Both prevalence figures are based on published surveys of dairy farms in the south of Britain. There seems to be no indication of the prevalence over the whole of the country or in different herd types or regions etc. where

prevalence may be quite different. Until such basic information is available there seems little point in developing more sophisticated modelling techniques to establish the nature and extent of the economic problem or best means of control.

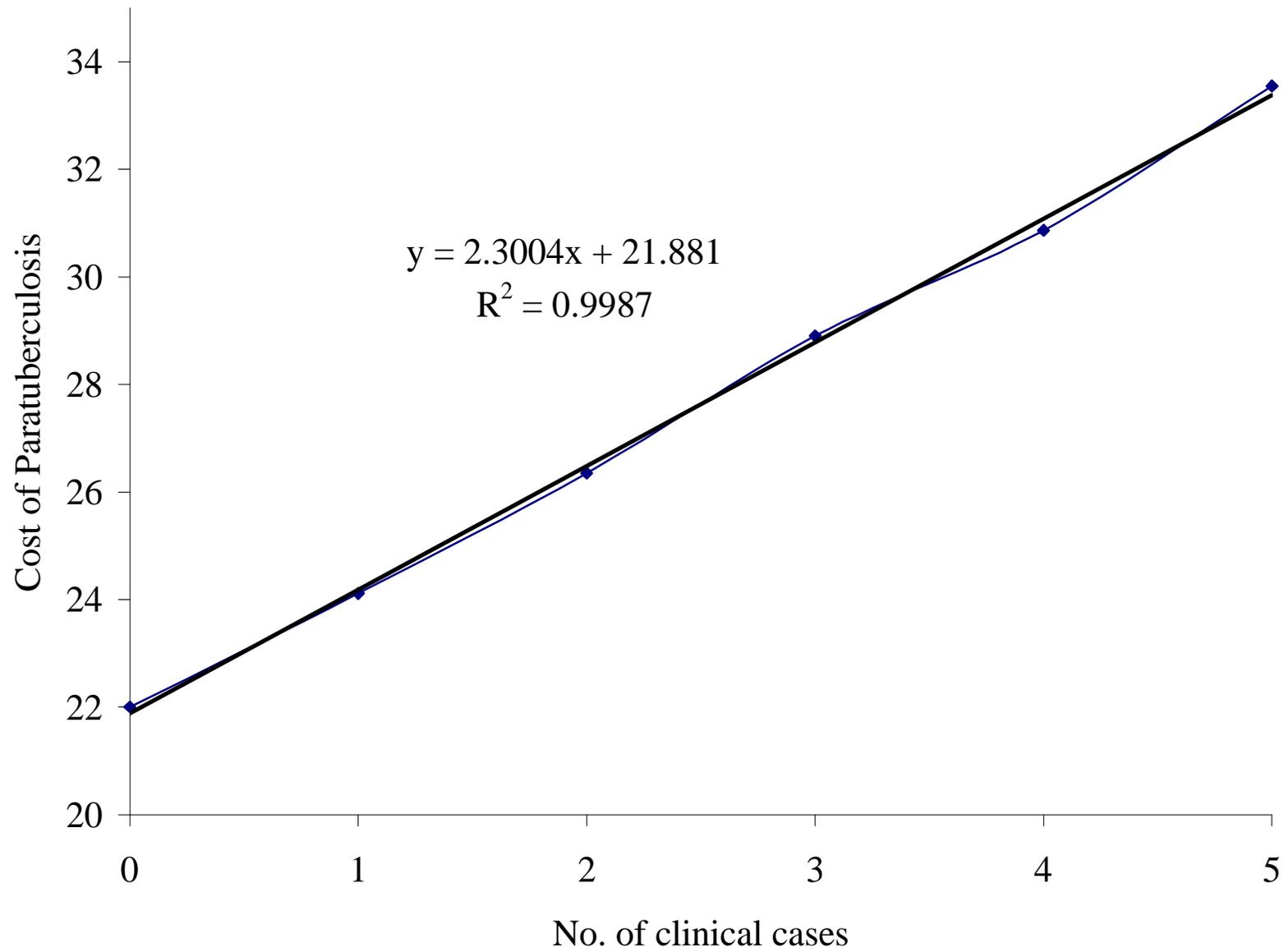
It should be noted as pointed out in the beef herd costings, that these national level figures are based on control option 1, i.e. toleration of chronic infection. These figures should be compared with the only alternative option, that is eradication.

### **Estimate of Losses Associated with Paratuberculosis in the Dairy Herd at International Level**

With the BSE ban in place and now restrictions due to FMD there are currently no international implications for trade in livestock due to paratuberculosis in any of our farmed species. Assuming restrictions in trade due to both these diseases were to be removed then it is unlikely that paratuberculosis will result in any significant change in the near future. The only identifiable penalty might operate at individual farm level in, for example, a Scandinavian country and we cannot predict such an effect.

**Table 4: The effect of paratuberculosis in a standard infected herd (2 clinical and 25 subclinical animals)**

	% involuntary culling	% Voluntary Culling	% Total Culling	Annuity (£)	Expected Milk Yield (kg)	Average age of herd (lactations)
Control Herd	15.5	4.1	19.6	258.6	6741	3.9
Standard Infected Herd	16.2	3.9	20.1	232.2	6546	3.8
Difference	-0.7	0.2	-0.4	26.4	195	0.1



**Figure 2:** Cost of disease in response to the number of clinical cases/100cows.

**Table 5:** Sensitivity of Paratuberculosis cost estimates to milk price and replacement cost assumptions

	Control herd	Infected herd	Difference	Sensitivity
	Annuity (£)	Annuity (£)	(Control-Infected)	(Difference from standard)
<b>Milk price</b>				
18.5ppl <sup>1</sup>	258.56	232.21	26.35	
20.5ppl	400.52	371.14	29.38	-3.03
16.5ppl	116.23	112.99	3.24	23.11
<b>Replacement cost</b>				
Heifer price £700 <sup>1</sup>	258.56	232.21	26.35	
Heifer price £ 600	279.98	254.12	25.86	0.49
Heifer price £800	238	211.35	26.65	-0.3

<sup>1</sup>Standard assumptions

**Table 6** Sensitivity of cost of paratuberculosis to changes in the assumptions for the number. of subclinical animals, milk yield depression (MYD) in sub-clinical and clinical animals and % increase in involuntary culling in subclinical animals in the standard infected herd (2 clinical animals, 25 subclinical animals, 10% milk yield depression in subclinical and 20% in clinical animals, 11% increase in involuntary culling in subclinical animals.)

Assumption	Infected herd	Difference	Sensitivity
	Annuity (£)	(Control <sup>†</sup> -Infected)	(Difference from standard)
Standard	232.21	26.35	
20 subclinical animals	236.34	22.23	4.13
30 subclinical animals	227.89	30.67	-4.31
15% MYD in clinical animals	232.99	25.58	0.78
25% MYD in clinical animals	231.43	27.14	-0.078
5% MYD in subclinicals	241.82	16.75	9.61
15% in subclinicals	222.33	36.23	-9.88
+ 1.5% increase in invol. culling of subclinical cases	231.97	26.59	-0.24

<sup>†</sup> Annuity for control herd = £258.56

Table 7: Annual national costs of paratuberculosis in dairy herds at farm level based on a cost of £26/cow in infected herds and a prevalence of 17.5%. Numbers of cows and holdings are from the MAFF June Census 1999.

### England

Number of holdings	21 326
Number of cows	1 659 210
Cows/holding	78
Cost/infected herd (£pa)	2023
<b>Cost all herds (£m pa)</b>	<b>7.5</b>

### Wales

Number of holdings	4 596
Number of cows	278 533
Cows/holding	61
Cost/infected herd (£pa)	1576
<b>Cost all herds (£m pa)</b>	<b>1.3</b>

### Scotland

Number of holdings	2 467
Number of cows	213 855
Cows/holding	87
Cost/infected herd (£pa)	2254
<b>Cost all herds (£m pa)</b>	<b>1.0</b>

### Great Britain

Number of holdings	28 389
Number of cows	2 151 598
Cows/holding	76
Cost/infected herd (£pa)	1971
<b>Cost all herds (£m pa)</b>	<b>9.8</b>

## Conclusions

Paratuberculosis is one of 13 cattle diseases costed by Reading University and reported on the web at <http://www.rdg.ac.uk/AcaDepts/ae/AEM/livestockdisea/cattle.htm>. This study estimates the direct costs of each disease for Great Britain. By using the assumptions given it is possible to convert these costs into the costs per cow in an infected herd so as to make a comparison with the figures reported here. Using the mid-value costs given by Reading University the costs per cow in

an infected herd are either £65 or £125 depending on which clinical disease incidence level (0.00023 or 0.00012) is used to convert national costs into costs per infected herd. Repeating the conversion for the alternative assumptions used by Reading University (low and high) gave a range of costs from £18/cow to £240/cow in an infected herd.

The above exercise suggests that the figure reported here (£26/cow) is within the scope of the Reading University study but it also illustrates just how difficult it is to obtain a precise estimate of the costs of paratuberculosis. This problem is a reflection of the lack of information available relevant to costing the disease but it probably also reflects the considerable variation in the financial impact of paratuberculosis experienced in practice. Other diseases exhibit a wide and skewed distribution of costs with the majority of farms suffering below average costs while a significant minority experience extreme losses (Stott and Gunn 1995). This may lead to complacency and a willingness to tolerate endemic disease inconsistent with the business risks involved. Without further research it is not possible to quantify the importance of this risk effect in the case of paratuberculosis. However, given the stability of the DP results above to wide variation in most of the assumptions used, it seems unlikely to be very significant.

Average net farm income per cow on English dairy farms taking part in the Farm Business Survey was approximately £140 in 1998/9 and £230 in 1997/98 (SAC, 2000). This is in line with the control herd annuities obtained from the DP model using milk price assumptions of 16.5 ppl and 18.5 ppl respectively (see Table 5). Farm gate milk prices reported by MAFF ([http://www.maff.gov.uk/esg/Work\\_htm/Notices/milk.pdf](http://www.maff.gov.uk/esg/Work_htm/Notices/milk.pdf)) confirm this downward trend. These observations suggest that the DP model results are consistent with current dairy farming experience. Furthermore, the extreme sensitivity of paratuberculosis costs to falling milk price (Table 5) suggests that the current trend in milk prices has important implications for the disease. At 18.5 ppl paratuberculosis costs represent about 10% of the annuity from dairying whereas at 16.5 ppl this has fallen to less than 3%. This suggests that there is now less incentive to control the disease at farm level than there was a few years ago. This, coupled with a lack of resources to invest in control will discourage farmers from taking action against paratuberculosis so long as the down turn in milk prices persists.

At £26/cow, paratuberculosis will cause dairy farmers less concern than several other major diseases and associated inefficiencies. Subclinical mastitis for example costs about £100/cow in total, £40 of which is avoidable (Yalcin and others 1999). In Bennett and others (1999) national costs of 5 major diseases of dairy cattle are reported (BVD, lameness, leptospirosis, mastitis and summer mastitis). All are attributed with costs considerably in excess of those reported by the University of Reading (see website given above) for paratuberculosis. The poor and declining level of fertility in the UK is also a cause of concern (Royal and others 2000). The inter-quartile range for dairy cow fertility in the UK reported by Kossabati and Esslemont (1995) is worth about £120/cow (Stott and others 1999). All these reports suggest that paratuberculosis is of relatively

minor concern compared with other sources of loss on dairy farms. The priority for scarce resources to remedy each situation however will depend not only on these estimated costs but also on the cost and scope for improvement. This point has been addressed for subclinical mastitis (Yalcin and others 1999) and infertility (Stott and others 1999) but not for the other diseases. The prospects for paratuberculosis in this area however are not good. The difficulty of identifying carriers, the small proportion of clinical cases, the effort required to reduce transmission and the lack of a wholly effective treatment or vaccine will all encourage farmers to concentrate on other sources of loss where responses are higher, easier to obtain and more assured.

The figure of £26/cow can be used to compare with the likely costs of controlling the disease. In a 100-cow herd this represents £2,600 which could be invested in capital projects to reduce transmission rates, for example, better housing or more hygienic water supplies. Some of the cost could be offset against the opportunity cost of using farm labour to reduce the spread of the disease, e.g. by separating calves from cows at birth. Given that none of these control measures are likely to eradicate the disease from an infected herd, it's unlikely that they will be justified on purely financial grounds. Even control by culling that is built into the DP analysis seems not to be justified in this analysis (Table 4). This is because the disease increases involuntary culling which reduces the scope for further voluntary culling to control the disease. It should be noted however that information on the impact of control measures on the epidemiology of paratuberculosis is extremely limited. Until further research is done it is difficult to give more specific recommendations on the best strategies for on-farm control. With information from such research, the DP framework reported here could be used to provide optimum culling strategies that ensure the best balance between the costs of premature culling and the benefits of removing a source of infection.

# Chapter 13 Cost-Benefits of Control for Paratuberculosis in Cattle Herds in GB

G J Gunn<sup>1</sup> and A W Stott<sup>2</sup>

## Introduction

The study team has provided estimates of the losses associated with paratuberculosis in cattle herds at farm, national and international levels. We believe that these estimates when taken together with the ranges also provided are fair, based upon the limited epidemiological knowledge we can obtain about this disease in ruminants in GB. The estimates must however be viewed in the light of our limited knowledge base. With regards to the benefits we are not able to cost the welfare benefit of reducing clinical disease or sub-clinical disease. We have not estimated a loss for a herd classified as paratuberculosis positive when it embarks on a control option, nor the benefit that herd might accrue once it can declare itself monitored free for however many years. We cannot cost the premium a farmer might gain for selling accredited stock when there is currently no identifiable market. We cannot provide an estimate for the long-term benefit of safeguarding future sales of beef and milk. Perhaps the most important deficiency in our costs is we cannot estimate the national benefit of reducing any future spread of this disease. The figures that we do provide however can be taken as a rough guide as to the cost-benefits associated with either vaccination or test and cull.

### Losses associated with paratuberculosis in Dairy Cattle:

Herd Level (100 Cow Herd):	£2600
National Level (assuming 17.5% herd prevalence):	£9.1million
International Level:	No cost

### Losses associated with paratuberculosis Beef Cattle:

Herd Level (100 Cow Herd):	£1600
National Level (assuming 17.5% herd prevalence):	£3.1 million
International Level:	No cost

### Control Options

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<sup>2</sup> SAC Agricultural and Food Economics Division

The only control options are vaccination; test and cull or do nothing. As discussed elsewhere both the proactive options are imperfect. There is no clear evidence of just how effective either option is in reducing paratuberculosis in cattle herds in GB. We have also included the likely costs for a paratuberculosis free herd demonstrating freedom from the disease using an existing assurance programme.

## Vaccination

Vaccination for paratuberculosis is given as a single injection to each bovine as a neonate. The current cost of vaccination includes the vaccine cost of £10.20 per dose plus the time involved in the process of vaccination. These costs assume two options (i) that all calves born are vaccinated and (ii) that only female replacement heifers are vaccinated. We believe that vaccination probably only reduces the number of clinical cases on a farm but have included an option where sub-clinical cases are also reduced. It is important to stress that benefits will only accrue after a lag phase when vaccinated replacement cattle mature and enter the adult herd. This lag phase would be between 24 and 30 months for most GB cattle systems.

## Dairy

In some circumstances all cattle are often vaccinated to help limit environmental contamination. If only female replacements are vaccinated then about 30 calves will be vaccinated each year, i.e. the vaccination charge per cow is approximately £3.

### First scenario - assume 50% reduction in clinical disease

- For the 100-cow dairy herd, the standard assumption was 2 clinical cases per 100 cows per year
- Each clinical case added approximately £2/cow/year to the cost of disease in an infected herd
- A 50% reduction in clinical disease would therefore reduce the output losses to £24/cow/year

**Table 1:** Total cost of paratuberculosis assuming control is by vaccination which results in a 50% reduction in clinical disease.

Vaccinate:	All calves	Replacements only
Output losses	£24/cow/yr	£24/cow/yr
Cost of vaccine	£10/cow/yr	£3/cow/yr
Total cost (infected herd)	£34/cow/yr	£27/cow/yr
Total cost (100-cow herd)	£3400 pa	£2700 pa
National (GB) total cost: (assuming 17.5% herd prevalence)	£12.8m pa	£10.2m pa

### Second scenario - assume 100% reduction is clinical disease

- For the 100-cow dairy herd, the standard assumption was 2 clinical cases per 100 cows per year
- Each clinical case reduces the loss by approximately £2/cow/year in an infected herd
- A 100% reduction in clinical disease would therefore reduce the output losses to £22/cow/year

**Table 2:** Total cost of paratuberculosis assuming control is by vaccination which results in a 100% reduction in clinical disease.

Vaccinate:	All calves	Replacements only
Output losses	£22/cow/yr	£22/cow/yr
Cost of vaccine	£10/cow/yr	£3/cow/yr
Total cost (infected herd)	£32/cow/yr	£25/cow/yr
Total cost (100-cow herd)	£3200 pa	£2500 pa
National (GB) total cost: (assuming 17.5% herd prevalence)	£12.0m pa	£9.4m pa

### Third scenario - assume 100% reduction is clinical disease plus 50% reduction in subclinical disease

- For the 100-cow dairy herd, the standard assumption was 2 clinical cases per 100 cows per year
- Each clinical case reduces the loss by approximately £2/cow/year in an infected herd
- A 100% reduction in clinical disease would therefore reduce the output losses to £22/cow/year
- Sensitivity analysis showed that a reduction from 25 to 20 cases per 100 cows reduced output losses by £3.77. Assuming that this trend continues down to a 50% reduction in subclinical cases then the equivalent reduction in losses would be £9.00.

**Table 3:** Total cost of paratuberculosis for a dairy herd assuming control is by vaccination which results in a 100% reduction in clinical disease and a 50% reduction in subclinical disease.

Vaccinate:	All calves	Replacements only
Output losses	£13/cow/yr	£13/cow/yr
Cost of vaccine	£10/cow/yr	£3/cow/yr
Total cost (infected herd)	£23/cow/yr	£16/cow/yr
Total cost (100-cow herd)	£2300 pa	£1600 pa
National (GB) total cost: (assuming 17.5% herd prevalence)	£8.7m pa	£6.0m pa

This then is the only vaccination scenario that produces a saving in total cost over the original unvaccinated scenario. However, as discussed in the introduction any beneficial effects that a national vaccination programme might have on disease transmission both within and between herds and hence on herd prevalence are not considered. On the other hand, these figures are for the 'steady state'. In reality costs would be higher than this in the early years until the effects of a calf vaccination programme worked through into the dairy herd.

## Beef

In some circumstances all cattle are often vaccinated in the belief that it will help limit environmental contamination. If only females are vaccinated then about 50% calves will be vaccinated each year i.e. the vaccination charge per cow is approximately £5. We can only consider the scenario where a herd breeds it's own replacements ie pedigree herds or larger beef herds.

Assume 50% reduction in clinical disease after 5 years if breed own replacements

- once paratuberculosis has reached a steady-state in the infected herd the beef herd model predicts approximately 4 clinical cases per 100 cows p.a.
- if the vaccine were to reduce clinical cases by 50% then the annual rate would be reduced by approximately 2 clinical cases per 100 cows p.a.
- each clinical case costs about £150 (£100 in extra vet charges plus £50 loss in cull value)
- the cost per clinically affected cow is therefore about £3 ( $150 \times 1.8 / 100$ )
- this means that the output loss due to paratuberculosis which was estimated at £16/cow/year would fall to about £13/cow/year if a vaccination programme was adopted.

**Table 4:** Total cost of paratuberculosis for a beef herd assuming control is by vaccination which results in a 50% reduction in clinical disease.

Vaccinate:	All calves	Replacements only
Output losses	£13/cow/yr	£13/cow/yr
Cost of vaccine	£10/cow/yr	£5/cow/yr
Total cost (infected herd)	£23/cow/yr	£18/cow/yr
Total cost (100-cow herd)	£2300 pa	£1800 pa
National (GB) total cost: (assuming 17.5% herd prevalence)	£6.3m pa	£4.9m pa

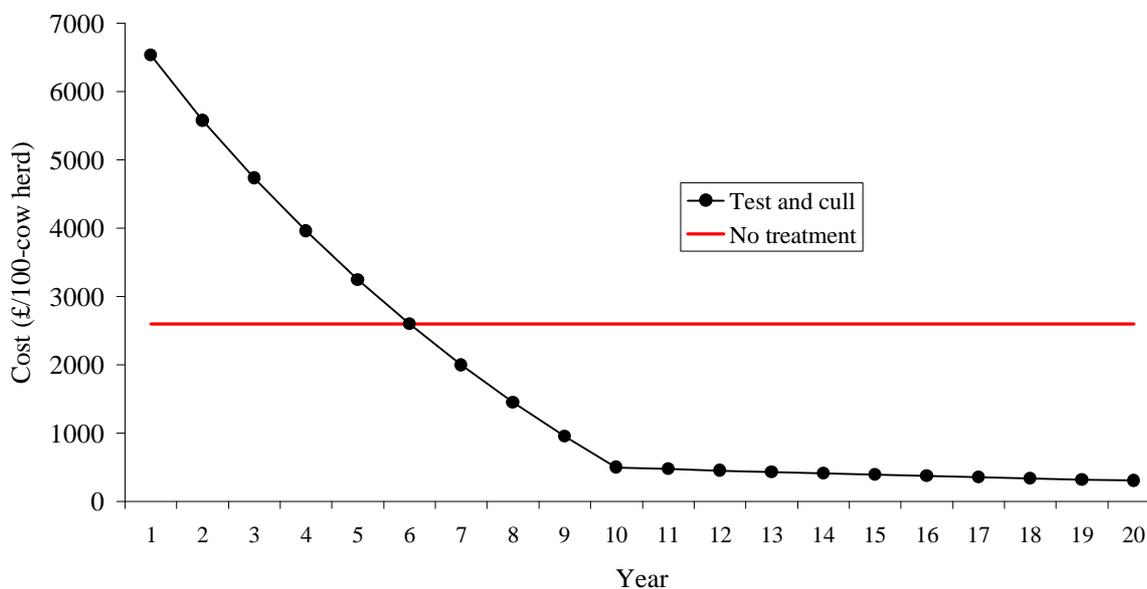
It would appear working with the assumptions we have made and given the caveats stated within the introduction that there would be no immediate gain to the beef sector through the partial control

of paratuberculosis using vaccines. In this case we have not been able to account for all the epidemiological effects of reducing the number of clinical cases but it is possible that we are overestimating the losses due to the disease in this case.

## Test and Cull Programmes

The current CHeCS approved paratuberculosis programme involves testing all the animals in the affected herd aged 2 years and above each year and removing any positive animals plus any of the progeny of any positive females from the herd. For this analysis we assumed a 50% reduction in clinical cases by 5 years with complete eradication by year 10.

Figure 1: The discounted total cost of test and cull in a 100-cow dairy herd



**Figure 1** shows the total discounted cost of paratuberculosis in a 100-cow dairy herd committed to eradicating the disease using a test and cull policy. This policy is assumed to reduce the cost of this disease progressively to zero over a 10-year period. Output losses without control are set at £26/cow as previously established. Costs of test and cull include an initial £100 subscription to the health scheme with £50 pa thereafter. Test costs are £6.60 per cow pa plus £100 per herd pa for faecal screening. The number of sub-clinical cows is assumed to decline by 10% each year from a peak prior to eradication of 25 cows per 100. New sub-clinical cases are also assumed to decline in the same way from a peak of 6 per year. Of these, 50% are detected, using the low sensitivity tests currently available, each year and culled. As normal replacement rates in a dairy herd are about 25%, 75% of these culls are considered to be extra replacements at a net cost of £350 each. In practice the extra replacement costs may be higher than assumed here because female progeny as well as the positive reactors themselves will be culled. However, given the current value of dairy-

bred calves this element was considered small enough to ignore. The test and cull scheme has a payback period under these assumptions of 13 years.

Calculations for the beef herd are the same as for the dairy with the exception that output losses are only £16/cow in the first instance but 85% of culled sub-clinical cases will be considered 'extra' due to the increased culling rates compared with normal beef herds. Under these assumptions, the programme does not break even within the 20-year time horizon.

## Conclusions

The problem with these calculations is that the true state of nature is unknown. It is possible for instance that some of the sub-clinical cases self-cure and no longer shed *Map* or suffer any long-term consequences of infection. If they do not shed or sero-convert then they would not be detected as cases and disappear from the equation. If the number of detectable cases falls then the payback period would be reduced for either the dairy or the beef herd, however whatever assumptions are made then the payback period is likely to be many years. Our experience in paratuberculosis eradication to date suggests that test and cull programmes result in an immediate reduction in clinical cases and reactors that is maintained beyond the first year. Our experience is currently limited to three annual tests in the herds that are attempting eradication. If this improvement were to be maintained then a more optimistic prediction could be made and therefore a more positive cost benefit would be expected.

## Assurance Programme

The current CHeCS approved herd assurance programme for paratuberculosis involves testing all the animals in the affected herd aged equal or greater than 2 years, each year. After 2 consecutive clear annual tests the herd moves on to screening all animals aged equal or greater than 2 years every second year. In addition all cull animals are serum-screened on the interim years.

The costs involved are assumed to be:

- membership fee -£100 for year one
  - £50 per year thereafter
- obtaining blood sample £3 per animal
- cost of test £3.60 per animal
- increased replacement rate
- add £100pa faecal screening etc

- assumed 25% culls for the dairy herd
- assumed 15% culls for the beef herd

The NPV of assurance for a disease free 100-cow dairy herd is £7454 over 20 years. This has an annuity equivalent of £598. This equates with a cost of £6.00 per cow per annum.

For the beef herd we are assuming the herd breeds all its own replacements ie it is a large commercial herd or a pedigree herd. The NPV of assurance for a disease free 100-cow beef herd is £7095 over 20 years. This has an annuity equivalent of £569. This equates with a cost of £5.50 per cow per annum which is less than for the dairy herd due to the lower cull rate for the beef herd.

# Chapter 14 Losses Associated with Paratuberculosis in Sheep

S Ashworth<sup>2</sup> and G J Gunn<sup>1</sup>

## Introduction

Paratuberculosis is known to occur in sheep, goats, deer and camels within Great Britain (GB). However, before exploring the costs associated with the disease in small ruminants and deer this chapter seeks to establish the place of sheep, goat, deer and camelid farming within the context of total GB agriculture. This context is particularly relevant in establishing the scale of the problem and the potential scale of administration associated with any control measures that may be recommended. The chapter then continues by appraising the costs of paratuberculosis to the individual sheep, goat and deer sectors of GB agricultural and looks at some economic benefits that may result from a control programme. Figure 1 provides a structure for establishing where the physical characteristics of paratuberculosis impact on the economic performance of the livestock sector and identifies the key issues considered further in this chapter.

### **Sheep, goats, deer and camels in UK agriculture**

Although sheep are the most abundant ruminant species in the UK sheep production is one of the smaller mainstream livestock enterprises in the UK agricultural economy, Table 1. Goat farming, although developing rapidly, is still a small part of the UK agricultural sector and deer farming is smaller again. Camelids cannot really be considered to be an agricultural species within GB.

Although sheep production occurs on the greatest number of holdings of any livestock type, it only accounts for 8% of the total agricultural output (including subsidy income), Table 2. Goat and deer farming combined currently account for around 1% of total agricultural output. The cattle industry, including milk production, in contrast, accounts for almost one-third of total output. The following sections provide an over view of the economic consequences of paratuberculosis in sheep, goats and deer.

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<sup>2</sup> SAC Agricultural and Food Economics Division

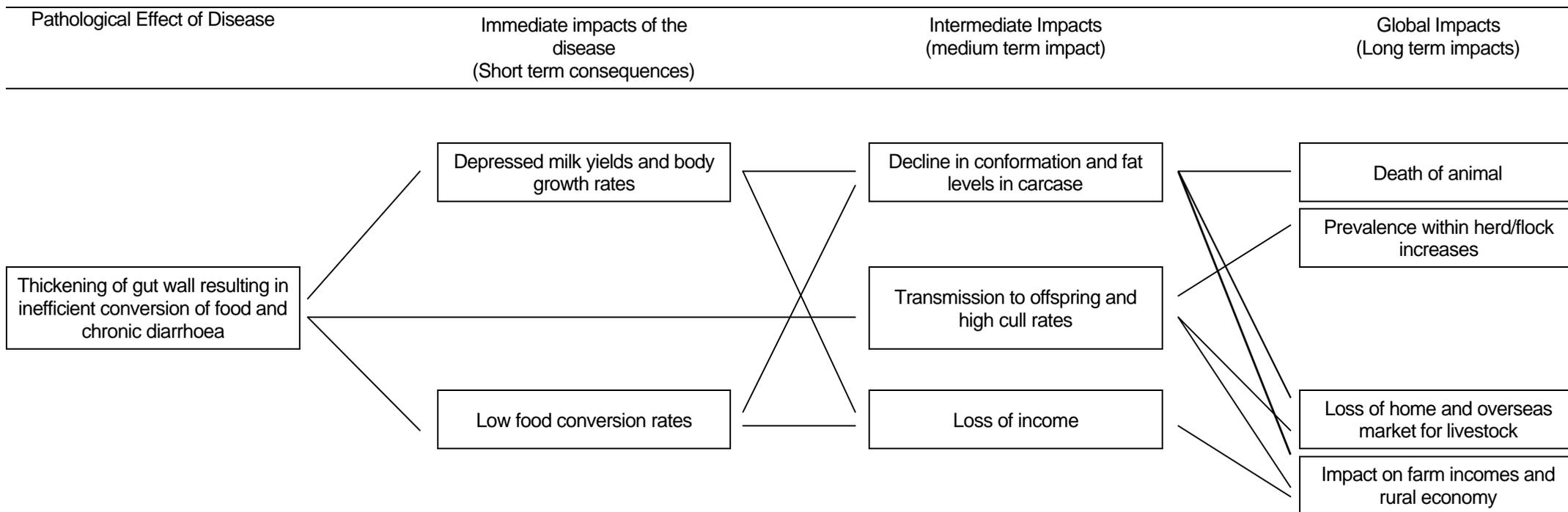
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**Table 1** Number of livestock in the United Kingdom 1998

	Number of animals	Number of holdings
Breeding ewes	21.2 m	82,550
Dairy breeding herd	2.42 m	35,298
Beef breeding herd	1.93 m	70,298
Total goats	0.08 m	n/a
Total farmed deer	0.04 m	n/a

Source: Annual Census (HMSO)

**Figure 1:  
Logic diagram of the impact of Paratuberculosis**





**Table 2**  
**Value of livestock output in the United Kingdom 1998**

	£ million	% of total output
Sheep	1300	8.1
Milk	2720	17.5
Cattle	2300	14.4
Pigs and poultry	2360	16.8
Other livestock	175	1.2

Source: Agriculture in the UK 1999 (HMSO)

### Losses Associated with Paratuberculosis in sheep within Great Britain

Paratuberculosis in sheep appears to be uncommon within GB although it does occur. In a survey of cattle and sheep farms in the South West England reported in 1994 Cetinkaya *and others* reported only 4 paratuberculosis cases in sheep and goats; 2% of a total of 166 cases identified. However, surveillance data is considered to underestimate the extent of this disease, due to the cost of diagnosis relative to the value of an individual sheep.

In Australia and New Zealand annual mortality rates are typically between 0.25% and 5% in infected sheep flocks, (Denholm and others 1994, Brett 1998). However mortality rates as high as 14% have been reported in infected flocks in Australia (Denholm and others 1994). In 1997, 6.4% of all New Zealand flocks were reported to be infected with paratuberculosis. The proportion of infected flocks was higher in the Merino flocks kept primarily for wool production, and with an older age profile, than among dual-purpose (wool/meat) flocks (Brett 1998). Furthermore, because of the difficulty in diagnosing paratuberculosis in sheep, it is considered that the number of flocks confirmed to have paratuberculosis could be a considerable under-estimate and that the true flock prevalence could be as high as 70% (Brett 1998). In many other parts of the world where paratuberculosis has been recorded e.g. European countries, the United States, Canada and South Africa, little information on prevalence and mortality rates is recorded.

### Economic impact of Ovine Paratuberculosis

The long incubation period of paratuberculosis means that the disease has little impact on the quality of lambs produced from a flock although weight gains may be impaired. The economic impact comes instead through having a higher than normal cull rate for older ewes and rams and consequently a higher home reared (or purchased) replacement rate. A secondary impact will arise through poorer wool quality as in extreme cases of paratuberculosis wool slip may occur. A third potential economic impact will arise with regard to the reduced value of breeding stock. This last factor is not a concern in GB, however, because of the limited occurrence or awareness of paratuberculosis. In New Zealand, and particularly in Australia, where paratuberculosis control

programmes are in place significant losses can occur in the value of breeding stock sold from farms associated with paratuberculosis.

Estimates from New Zealand put the cost of the disease, in 1998, at between NZ \$42 and NZ \$75 per unplanned loss of a ewe. The cost of an unplanned loss of a cull ewe under current GB conditions is estimated below.

### **Economic assumptions used to estimate the economic losses associated with Ovine Paratuberculosis**

#### **Cull ewes**

Lightweight ewes sold in the autumn may only realise around £12 while heavy weight ewes sold in the late winter/early spring may realise £35 each (MLC UK Market Surveys). This is a wide-ranging loss reflecting the considerable seasonal variation in cull ewe prices and the physical size of the ewes. At the age when paratuberculosis is likely to become evident most ewes will be culled for slaughter rather than sold for breeding. Nevertheless, £35 is a reasonable estimate of the value for an aged ewe being sold for further breeding.

#### **Wool**

The loss of one fleece from each clinical case of paratuberculosis would add a further £2-£4 to the losses from the unplanned death.

#### **Meat lamb**

The clinical onset of paratuberculosis often occurs at mating and the ewe is likely to die or be culled before lambing. Consequently the number of lambs available for sale from a flock will decline. Assuming an average lamb weaning rate of 1.35 lambs reared per ewe, then the loss of income from a slaughter lamb flock will be in the range £40 - £55 per unplanned death. For store producers with a lower weaning rate, around 1.0 lambs reared per ewe, the loss is likely to be in the range £10 - £25 per ewe lost.

It is considered that the potential loss in value of individual meat lambs produced from a flock with paratuberculosis due to impaired milk production from affected ewes will be negligible.

#### **Breeding sheep**

The consequence of a flock being identified as having paratuberculosis may in the future have considerable impact on the value of younger pedigree male and female animals sold for breeding. This might be true for the small number of flocks specifically targeting female sheep at the breeding market and also in the hill and upland situation where crossbred gimmer lambs are specifically reared for sale to low-ground flocks. These producers may expect a higher value for their gimmer

lambs of perhaps £40 per head while shearlings may make £60 per head. Individual breeders may also sell a limited number of high value rams for breeding purposes. However as the proportion of stock sold in these categories is small, it has not been considered in the industry cost of paratuberculosis discussed below.

## Overall losses

In a GB context the loss of income due to unplanned deaths resulting from paratuberculosis is estimated to be between £24 to £94 per ewe, depending upon the location of the sheep flock and the production system used.

GB has a very distinctive stratified structure to its sheep industry. In the most disadvantaged areas, typically the highest areas of the country, the sheep flock is frequently managed in an extensive way to produce store lambs. Moving down the hill to a more upland environment then the prolificacy of the ewes improves and the ability to sell finished lambs for slaughter as well as store lambs is enhanced. On the most favoured, lowground areas of the country, intensively managed flocks producing finished lambs for slaughter is more common. An assessment of the number of ewes farmed under each system can be gathered from the sheep subsidy system that pays a tiered rate of support to ewes in different categories reflecting the sheep production system and land capability. These categories are known as specially qualified ewes in the most disadvantaged grouping, qualified ewes in the upland rather than hill scenario and lowground ewes in the most favoured zone.

For the purpose of making an estimate of the potential impact of paratuberculosis on the GB flock the following assumptions have been made:

- the economic loss on a Specially Qualified Hill Ewe is £25
- for a qualified hill ewe the loss is £35
- for a lowground ewe the loss is £75
- there is no variation in incidence and prevalence between different sheep systems  
(This is despite evidence from Iceland and Australasia (Denholm *and others* 1994) that there is a higher level of incidence among more intensively managed flocks. In a GB context the more intensively reared flocks are likely to be early lambing lowground flocks)
- incidence and prevalence for GB have been estimated from NZ figures  
(This is because of the lack of data relating to incidence and prevalence for GB. Assumptions relating to these factors have had to be based on New Zealand experience where production systems are broadly similar to those found in GB.)

The cost of ovine paratuberculosis in GB, depending upon assumptions made, is estimated to range from £0.5m to £16.5m, see Tables 3 and 4. The total output of the sheep industry in 1998 is

estimated to have been around £1300m (MAFF, 2000a), thus the cost of paratuberculosis is estimated to be less than 1.5% of the value of sheep output.

**Table 3** Estimated losses due to Ovine Paratuberculosis in Great Britain in 1999

	Number <sup>1</sup>	Flock Prevalence%	Mortality rate %	Loss per ewe (£)	Loss to industry (£)
Specially Qualified ewes (Hill flocks)	9,666,000	5	1	25	120,825
Qualified ewes (Upland flocks)	1,464,000	5	1	35	25,620
Other ewes (Lowground flocks)	7,039,000	5	1	75	263,962
<b>Total</b>					<b>410,407</b>

1. Source: Economic conditions in the hills and uplands of GB - statistical tables MAFF 1999

**Table 4**  
**Sensitivity of industry cost to level of incidence and death rate among infected flocks**

	Prevalence		Mortality rate	
			1%	5%
			£	
5%			410,407	2,052,038
10%			820,815	4,104,075
20%			1,641,630	8,208,150
40%			3,283,260	16,416,300

The estimates made in Tables 3 and 4 assume that no provision for the extra cost of replacing losses within the year of that loss is made. However, this is unlikely to be the case for two reasons. Firstly a high proportion of income to the sheep industry comes in the form of Sheep Annual Premium (a support measure funded by the European Union). This premium requires a specified number of ewes to be retained during a qualifying period. If this number is not maintained and a spot check finds fewer sheep then a loss of support will result. To prevent this occurrence any reduction in flock numbers will be made up quickly. Secondly, to the higher demand for replacements to maintain flock size in a paratuberculosis flock, producers would be likely to organise for a higher number of replacements.

Maintaining flock size could be achieved in two ways. Firstly the replacements could be purchased from outside the flock or alternatively, ewe lambs could be retained which would otherwise have been sold. These two scenarios are examined by extending the method used to establish Table 4. The first scenario considers a full cost of replacing ewe losses and the second considers the

opportunity loss of retaining home reared replacements. The opportunity cost is considered to be the difference between income forgone by not selling the lamb and the extra cost of rearing that lamb until it can be used for breeding. The assumptions used in estimating the impact of these two scenarios are shown in Table 5.

**Table 5**  
**Replacement ewe cost estimates for different farm types**

	Full cost	Opportunity cost
	£	
Specially qualified ewes (hill flocks)	50	35
Qualified ewes (upland flocks)	50	45
Low-ground ewes	60	50

Using the assumptions detailed in Table 5, combined with the production assumptions used in Table 3 the range in the estimated cost of paratuberculosis in GB sheep flocks for three scenarios is shown in Table 6. A considerable variation results in the loss being estimated at between £0.4 million and £36.0 million. At the upper range of the estimate this still only represents about 3% of the total sheep output, including subsidies. If the subsidy payments (estimated to be one third of output in 1998, MAFF 2000) are excluded the estimated cost of paratuberculosis rises to a maximum of around 4.5% of sheep output.

**Table 6**  
**Range of economic losses due to paratuberculosis in the GB sheep flock**

Scenario	Prevalence 5% Mortality Rate 1%	Prevalence 40% Mortality Rate 5%
	£ million	
No replacements in year of loss (Table 5)	0.4	16.4
Full cost replacement of losses	0.9	36.0
Opportunity cost of replacing losses	0.6	31.5

#### Cost / Benefit of control within the sheep flock

Control measures for paratuberculosis involve either a test and cull programme or a preventative vaccination programme. A test and cull programme of an infected flock would require annual blood sampling of the flock and then the slaughter of test positive animals. An alternative would be to test a flock to identify the presence of paratuberculosis and then adopt an annual vaccination programme whereby replacement sheep were vaccinated upon entry to the flock. The experience from New Zealand suggests that a three year vaccination programme would significantly reduce the prevalence of paratuberculosis and after five years paratuberculosis would be almost non-

existent (Brett 1998). This finding confirms the work of Cranwell who reported the successful control of paratuberculosis in a GB sheep flock by vaccination, (Cranwell 1993).

### Test and cull

A test and cull programme suffers from a number of political and economic drawbacks. Firstly there are no reliable tests for paratuberculosis (West 1997 cited in Brett 2000) and those that do exist will only identify clinical cases. Secondly removing clinical cases from the flock is reported not to have a significant impact on overall disease levels (Brett, 2000). While this statement appears counter intuitive it does draw attention to the fact that removing clinical case of paratuberculosis does not prevent sub-clinical cases from shedding the bacteria. These continually re-infect the flock and consequently the prevalence within a flock declines very slowly or may not decline at all. To control paratuberculosis in such circumstance would require replacement stock from paratuberculosis free flocks.

There are no measures in place that would prevent an infected animal from entering the food chain. Thus, any infected animal would have some salvage value and the total cost to the industry would be principally the cost of collecting and testing blood samples at around £5 -£7 per ewe plus the cost of replacing infected ewes with paratuberculosis free stock.

A **full analysis** of this option **has not** been explored and in particular the first year cost of identifying the paratuberculosis infected flocks, which could amount to £100m, has not been considered. For the purposes of this analysis it has also been assumed that the test and cull programme combined with purchase of paratuberculosis free replacements will reduce the prevalence of paratuberculosis from 5% clinical cases to zero over a five year period when the programme would stop. On the basis of this scenario and using “full cost replacements” the estimated cost and pay back of a five year voluntary test, cull and replace with paratuberculosis free stock is shown in Table 7 with a pay back period of eight years, excluding any discounting of future income flows.

**Table 7**  
**Estimated cost and pay back of a five-year test, cull and replace with paratuberculosis free stock programme for the control of paratuberculosis in UK sheep flocks**

Scenario: Full cost of replacing culls	Flock prev. 5% 1st year mort. 5%	Flock prev. 40% 1st year mort. 5%
Net cost of a five year programme	£13.7 m	£109.7 m
No of additional years to pay back	4	4
Total time for pay back (years)	9	9

## Voluntary vaccination programme

Table 8 summarises the estimated pay back period for a voluntary vaccination programme among flocks identified as having paratuberculosis for the UK sheep industry as being five years from the adoption of the programme. The assumptions used in arriving at Table 8 are that the base prevalence of 5% ewe deaths is reduced to zero deaths over a five year period at which point vaccination is suspended. The remaining assumptions are those used above for a flock that retains extra lambs to replace those losses due to paratuberculosis. The cost of the vaccine was assumed to be £6.00 per sheep. However, as with the previous example the initial cost of identifying infected flocks through blood testing has not been considered.

**Table 8**  
**Estimated cost and pay back of a five-year voluntary vaccination of replacement programme for the control of paratuberculosis in UK sheep flocks**

Scenario: Opportunity cost of replacing culls	Incidence 5% 1st year losses 5%	Incidence 40% 1st year losses 5%
Net gain of a five year programme	£1.1m	£8.6m
Year during which pay-back occurs	5	5

Both voluntary control options described above do demonstrate a theoretical possibility of bringing paratuberculosis under control in sheep. Indeed they indicate that eradication might be an option over a reasonable time scale. However, the problem remains one of convincing the sheep farmers that they will benefit from adopting such a programme. MLC flock recording data reveals annual ewe mortality rates of around 5% in both lowground and upland flocks. Recent data on flock mortalities in hill flocks is not readily available. It is unlikely that mortality among hill flocks will be lower than the levels quoted for lowground and upland flocks and in some areas may be 1-2 percentage points higher. At such low levels of mortality it is unlikely that shepherds will identify mortality associated with paratuberculosis in their flocks. Paratuberculosis related deaths are likely to be occurring within the “normal” expectation of ewe mortality. However, if paratuberculosis deaths were to approach 5% then the overall mortality within flocks would be likely to increase to 8-10% at which point shepherds may begin to try to identify the cause.

Table 9 examines the starting paratuberculosis mortality rate for different flock types where the cost of adopting a replacement ewe vaccination programme would just break even over a five year period; assuming a linear decline in deaths. For a hill flock, not replacing deaths immediately, it would only be where mortality was as high as 10% that a shepherd would become interested in a paratuberculosis control programme. In contrast for a lowground flock having to immediately replace ewes (for example, to maintain eligibility for support payments) the break-even point would be 1.7% mortality. An increase in mortality of 10% would clearly attract the shepherd’s eye however, at the lower rates the increased mortality may go un-noticed.

**Table 9**  
**Starting rate of paratuberculosis mortality, which results in five year eradication programme through vaccination breaking even**

	No within year replacement	Full cost replacement	Opportunity cost replacement
	Starting mortality rate (%)		
Specially qualified ewes (hill flocks)	10	3.3	4.5
Qualified ewes (upland flocks)	7	3	4
Lowground ewes	3	1.7	2.4

If the vaccination programme was considered as part of a routine annual control programme, then the annual average reduction in ewe mortality, required to fund such an annual vaccination programme is shown in Table 10. Thus in a lowground situation with home reared replacements the cost of an annual vaccination programme would be covered by a reduction in mortality of 1.6%. For an extensive hill flock an annual reduction in mortality of 3% would fund the cost of an annual vaccination programme if home reared replacements were used to maintain the flock size.

**Table 10**  
**Annual reduction in mortality due to paratuberculosis that would cover the annual cost of a vaccination programme**

	No within year replacement	Full cost replacement	Opportunity cost replacement
	Starting mortality rate (%)		
Specially qualified ewes (hill flocks)	6	2	3
Qualified ewes (upland flocks)	4.25	1.75	2.5
Lowground ewes	2	1.1	1.6

The analysis of control measures for paratuberculosis in GB sheep flocks is shown to be sensitive to the type of sheep production systems and also to the cost of vaccination. At the current cost of vaccination a voluntary vaccination programme for paratuberculosis is unlikely to receive support from the industry. However, if the cost of vaccination can be reduced by 50% then interest may increase.

If the vaccination programme was to cost £3 rather than £6 per animal vaccinated then the industry would see a pay back in the programme within three years and a net benefit to the industry of up to £35.8m (Table 11). Similarly the annual reduction in mortality required to offset the annual cost of a routine vaccination programme is much reduced, Table 12. At these reduced levels of breakeven, lowground flocks in particular may be persuaded to adopt a vaccination programme. However, for

extensive hill flocks an annual reduction in mortality of 3 percentage points from a base of between 5% and 7% may still result in a situation where shepherds are reluctant to incur the extra cost.

**Table 11**  
**Estimated cost and pay back of a five year voluntary vaccination of replacement programme for the control of paratuberculosis in UK sheep flocks at a vaccination cost of £3 per head**

Scenario: Opportunity cost of replacing culls	Incidence 5% 1st year losses 5%	Incidence 40% 1st year losses 5%
	Net gain of a five year programme	£4.5m
Total time for pay back (years)	3	3

**Table 12**  
**Annual reduction in mortality due to paratuberculosis that would cover the annual cost of a vaccination programme at £3 per replacement**

	No within year replacement	Full cost replacement	Opportunity cost replacement
	Starting mortality rate (%)		
Specially qualified ewes (hill flocks)	3	1	1.3
Qualified ewes (upland flocks)	2.1	1	1.2
Lowground ewes	1	0.1	0.5

# Chapter 15 Losses Associated with Paratuberculosis goats, deer and camelids in Great Britain

S Ashworth<sup>2</sup> and G J Gunn<sup>1</sup>

## Introduction

Goat keeping is a small component of agriculture within Great Britain (GB). Census data from 2000 suggests that there are some 8700 holdings with goats in GB with a total goat population of around 77,000 and an average herd size of less than ten animals. Census data indicates that less than 2% of the holdings have more than 100 goats and conversely that more than 90% of the holdings have less than 10 goats. Nevertheless, at a time of low and declining incomes in traditional farming activities, interest in goat farming has increased to the extent that between 1999 and 2000 the annual census indicates that goat numbers in GB have increased by more than 11%. In England the proportion of breeding goats kept for meat production is around 25% of all breeding females. The number of does involved producing kids for meat has declined steadily over the past three years and is now 25% lower than it was in 1997. In contrast the number of breeding females kept for milk production has increased by 14% between 1999 and 2000 (MAFF 2000b) and is almost 25% higher than in June 1997. It is estimated that currently there are some 3000 holdings keeping goats for milk production in GB but that only around 60 of these hold more than 100 breeding does. Dairy goat farming therefore occurs on about one-tenth of the number of holdings on which dairy cow farming takes place.

The small size of the goat sector means there is very little information available on the profitability of these enterprises and the technical performance levels they achieve. On the basis of enterprise performance levels suggested in advisory handbooks e.g. ABC (2000) an overall estimate of the potential impact of paratuberculosis in goats has been made. However, because of the lack of validated epidemiological data the following discussion can only be considered to be a subjective assessment of the potential impact of the disease in the goat sector.

## Paratuberculosis in goats

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Caprine paratuberculosis is reported most frequently as a disease of dairy goat breeds rather than of meat goats (Ellis and others 1998). This difference may be due to the greater numbers of dairy goats.

Weight losses of the order of 3.5 to 5.9 kg over 2-3 months period may occur in affected animals (Singh and others 1997). In addition to weight loss reproductive inefficiency is a problem. In Spain a herd prevalence of 52% has been reported (Reviriego and others 2000) and an annual mortality due to paratuberculosis among Spanish goat flocks is reported to have been as high as 10% (Corpa and others 2000). In Norway 50% of herds are infected by caprine paratuberculosis and among the most heavily infected flocks mortality rates of between 5% and 10% are common. Other Norwegian work suggests that in heavily infected herds that 50% of the flock could be infected with the agent (Fodstad 1980 cited in Singh and others 1997).

Paratuberculosis is known to occur in goat herds in GB. However, because the structure of the sector is small and fragmented prevalence or incidence estimates for the disease are not available. Equally however, because of a growing interest in commercial farming of goats for milk production paratuberculosis is being diagnosed more frequently (Cranwell pers. comm.). This is believed to be the result of buying in goats from many flocks to quickly establish a sizeable herd for milk production.

### **Control**

Controlling paratuberculosis among goats using a test and cull strategy is very difficult because of the poor sensitivity of the majority of diagnostic tests for identifying infected animals. However, vaccination has offered good results in controlling the disease in goats (Sigurdsson 1960, Crowther and others 1976, Saxeggard and Fodstad 1985 cited in Corpa and others 2000).

### **Economic impact of Caprine Paratuberculosis**

Economic losses will occur through increased mortality; increased culling rates; lower prolificacy; slow growth rates and milk yield losses. A qualitative assessment of the potential economic impact is made below using a simple gross margin analysis.

### **Economic assumptions used in estimating the economic cost of caprine paratuberculosis**

#### **Cull does and bucks**

Market information on the selling price of cull does and bucks is not recorded or published on a regular basis. A number of farm management handbooks do record benchmark information for a dairy goat enterprise e.g. ABC 2000 and put the value of cull goats at between £10 and £12 each.

Young does suitable for immediate entry into the milking goat herd are estimated to have a market value of around £175 - £180 (ABC 2000). Goats suitable for entry into a fibre producing enterprise have a lower value.

### **Milk**

No information is available on the milk yield losses associated with paratuberculosis in goats. As fertility is affected milk yield loss can be expected. In the absence of evidence from the goat sector then the losses found among dairy cows of around 15% per infected animal has been assumed. With a typical goat milk yield of 800 litres per year selling at 40 p/litre (ABC 2000) the loss from an infected animal is estimated to be around £48 per year.

### **Fibre and meat**

Demand for goat meat is low within the UK with many kids being sold for negligible sums or incurring a cost for euthanasia. Goats with paratuberculosis are likely to be culled early for poor reproductive performance or because of poor body condition. Therefore the impact of paratuberculosis on meat returns in GB is considered to be negligible. Similarly, although a small number of goats are kept for angora or cashmere fibre production in GB the losses from fibre quality associated with paratuberculosis are considered to be negligible.

Consequently the annual losses to the goat sector from paratuberculosis relate to reduced milk yield in dairy goats and increased mortality or culling across all breeding goats. The cost associated with having to replace deaths and culls with breeding animals is estimated to be £175 per unexpected replacement.

### **Overall losses**

For the purposes of the current exercise and extrapolating from the experience of Norway, Spain and India it is estimated that 25% of the breeding goat population may have clinical or sub-clinical paratuberculosis. Thus 25% of the GB breeding dairy goat population may suffer some milk yield loss. This is estimated at 15% of the annual yield for infected goats. Similarly, drawing from Norwegian and Spanish experience as many as 10% of the goat population in infected herds may die each year as a consequence of paratuberculosis. However, for the purpose of this

assessment, losses of breeding does due to paratuberculosis are fixed at 5% of goats with clinical or sub-clinical disease. That equates with approximately 1.25% of the total breeding doe population. Thus the annual losses to the GB goat industry, if no effort is made to control the disease, have been estimated to be £0.42M (Table I). The most significant cost of the disease is loss of milk revenue. Changing the milk yield loss by plus or minus 5 percentage points changes the estimated losses to the industry by plus or minus £0.11M.

**Table I**  
**Estimated annual losses to the GB goat herd of**  
**paratuberculosis.**

	£
Milk yield losses	325,000
Opportunity costs of deaths and higher culling rates	90,000
<b>Total</b>	<b>415,000</b>

#### Cost-Benefit of Control

A test and cull programme or indeed a test cull and vaccinate programme are impeded by low sensitivity tests. Alternatively the vaccination of replacements has been shown to be effective in a number of countries. A successful vaccination programme can apparently eliminate the disease within a four to ten year period.

If it is assumed that the annual replacement rate in goat herds is 20% per year and that the cost of vaccination is the same as for sheep, £6 per animal, then the annual vaccination cost is around £240,000 per year. Over a five-year programme this would amount to £1.2M. Equally if it is assumed that the effect of a vaccination programme is to create a linear reduction in the annual losses due to paratuberculosis the programme would just break even over a five-year period. If the vaccination programme were extended to a ten-year programme, at a cost of £2.4m, with a linear decline in losses the net cost over ten years would become just £0.2m. Under these assumptions a vaccination programme to eradicate paratuberculosis from the GB goat herd would just break even at 15% yield losses. Clearly reducing the cost of the vaccination programme would improve the cost/benefit for such a programme. Halving the cost of vaccination would create a benefit within five years of £0.6m and over ten years £1.2m.

If it emerged that the true milk yield loss was lower then the benefits from a vaccination programme would consequently also be reduced. With a milk yield loss of only 10% rather than 15%, and with vaccination charges reduced to £3 per head, the benefit after five years would fall to £0.3m

On the basis of this simple cost benefit analysis and using assumptions and not hard data it can be shown that by adopting a vaccination programme to control paratuberculosis a benefit may accrue over a five to ten year period within the GB dairy goat industry. Individual goat herds with a high prevalence of paratuberculosis are likely to be encouraged to adopt a vaccination programme. A programme of voluntarily paratuberculosis eradication for large dairy goat herds in GB might provide a useful model that could later be transferred to the sheep industry.

### **Requirements for further research**

The above analysis has drawn attention to the general lack of information about the incidence a prevalence of paratuberculosis in the GB goat herd. Furthermore it has drawn attention to a lack of information on the technical performance effects of the disease in the goat herd. These deficiencies in the knowledge base are similar to those identified earlier for the GB sheep flock and limit our ability to develop a cost/benefit model. The control of paratuberculosis in the goat herd is, as for sheep, dependent upon developing a lower cost vaccine. Further research work is needed to develop such vaccines.

## **Losses Associated with Paratuberculosis in deer in Great Britain**

### **Introduction**

Within Great Britain there are around 350 holdings with farmed deer (MAFF pers comm., SERAD pers comm) with a total deer stock of around 33,500 animals (SERAD 2000). Within the context of the scale of the British livestock sector farmed deer remain a minor part of the livestock industry. Nevertheless, as the economic down turn continues within British farming then deer farming is increasingly being considered as an alternative enterprise and expansion in both the number of holdings with deer and the total number of deer is occurring. The Deer Commission in Scotland (2000) reported a red deer count of 70,164 from their sample census. This represents the largest population of deer in Britain.

### **Paratuberculosis in deer**

Paratuberculosis in deer has been reported in farmed deer in Ireland, Scotland, the United States and New Zealand (Stehman 1996). Losses due to clinical disease have been reported in animals as young as six months old and poor weight gain has been reported in yearling bucks, (Fawcett and others 1995, Mackintosh and de Lilse 1998 cited in Brett 1998). Mortality rates of up to 12% were reported from New Zealand among young stock (Mackintosh and de Lilse 1998 cited in Brett 1998). In the case reported by Fawcett and others (1995) mortality among yearling stock in the first

year of the outbreak was reported to be 5%. New Zealand researchers are unsure of the extent to which adult deer progress to a clinical stage of the disease. Brett (1998) reported that 4.2% of New Zealand deer herds had paratuberculosis but that this was increasing at around one percentage point per year. However, the level of clinical cases within herds is not known although estimates from New Zealand suggest that the level of clinical infection may on average be little more than 2% in infected herds. Equally however, the identification of paratuberculosis in deer herds in New Zealand occurred relatively recently and the incidence level within a herd may rise quickly if it is not controlled.

### **Control**

As with the other species discussed, control can be achieved by a test and cull programme, a vaccination programme, or a combination of the two. A test and cull programme is constrained, as with cattle, sheep and goats, by the accuracy of the testing mechanism. In New Zealand it was concluded that a test and cull program based on current tests is not a technically feasible option for deer because there is no reliable test for paratuberculosis in sub-clinically infected deer (Brett (1998)). Consequently this option is not explored further.

Fawcett and others (1995) have shown vaccination of Scottish farmed deer to decrease the incidence of paratuberculosis although sub-clinical infection could still be demonstrated.

### **Economic impact of Paratuberculosis in deer**

The economic impact of paratuberculosis in deer is through the loss of yearling stock that would otherwise have been sold for meat or retained for breeding. The evidence from the literature suggests that significant losses occur among young stock as well as older stock. For the purposes of this evaluation it has been assumed that half of the losses occur through mortality of young stock (Brett 1998) and the remainder occur in mature breeding stock.

#### **Yearlings for meat**

Estimated losses through the death of yearlings intended for meat production are £165 per animal lost. Although other animals are likely to suffer from slower growth rates the loss in revenue from this occurrence is considered to be small and ignored in this estimate.

#### **Yearlings for breeding**

Hinds for breeding are estimated to have a value of around £300 each (ABC 2000).

### **Value of cull deer**

Mortality among older hinds is estimated to result in a loss of cull value of £80 per unexpected death. However, the cost of replacing these hinds would involve an additional expense, or loss of revenue if home reared replacements are used, of up to £300 giving at total income loss of up to £380 for each unexpected loss.

### **Overall losses**

To estimate overall losses to the British deer industry the following assumptions have been made (Fawcett 1995):

- 5% of the deer population has clinical or sub-clinical paratuberculosis
- among this infected population mortality is:
  - 5% among yearlings
  - 5% among older animals
- all yearling losses were of animals targeted for meat
- the herd size is maintained by a) retaining young stock that would otherwise have been sold for breeding or b) buying in breeding hinds.

On the basis of these assumptions and the revenue losses identified above it is estimated that the annual cost of paratuberculosis in the British deer herd is £20,000 per year. If mortality among young stock is assumed to be 10% then the estimated cost rises to £25,000 per year. Equally if the level of clinical and sub-clinical infection is considered to be 10% of the population with a 5 % mortality within this population then the annual cost of paratuberculosis in the British domestic deer herd would rise to £40,000.

### **Cost-Benefit of Control**

Vaccination programmes have been shown to provide partial control of paratuberculosis in deer. However, because of the high incidence of the disease among younger animals, two options for a vaccination programme could be considered. Firstly all newborn deer could be vaccinated or alternatively only those intended for replacements could be vaccinated. The deer industry has a low replacement rate and it is not uncommon for deer to be retained in the herd for more than ten year thus it would take ten years before all breeding stock are likely to have been vaccinated against the disease.

If it is assumed that around 12,000 deer are born each year, then to vaccinate the whole population at any cost of more than £3 per animal is likely to accumulate to a higher annual cost than the estimated total annual losses. This control option is unlikely to receive support from the industry. The vaccination of replacement stock, which may amount to 1500 animals per year, may prove

more attractive. Assuming an annual cost of £6 per vaccination, in line with the sheep and goat examples discussed earlier; this would amount to a ten-year cost of £90,000. Assuming a ten-year linear decline in the lower level of losses the net benefit of such a programme would amount to £20,000 but it would take 9 years to achieve a breakeven point. With the higher level of losses over a ten-year period the benefit may accumulate to £130,000 with the break-even occurring during year seven.

### **Requirements for further research**

In reviewing the situation of the British deer herd with regard to paratuberculosis a number of issues are highlighted for further research. These issues are:

- a requirement for a clear audit of the level of prevalence and incidence of cervine paratuberculosis
- an economical and reliable screening test is required
- a low cost vaccine is required.

### **Losses Associated with Paratuberculosis in camels within Great Britain**

It is estimated that there are between 1500 and 2000 camelids in Great Britain (ABC 2000). The majority of these are kept as pets, the remainder may be kept as educational exhibits in zoos or farm parks, as pack animals in tourist ventures or for fibre production. Camelids are not used to produce human food products in GB. As such they are of minor significance to GB agriculture and the impact of paratuberculosis on them and the overall agricultural economy is considered negligible.

Paratuberculosis, however, has been reported in a llama in a British zoo. Paratuberculosis has also been identified in llamas in the United States of America and in Alpacas in Australia (Fowler 1998). The symptoms remain the same as those seen in other animals namely, weight loss and terminal diarrhoea. Where clinical cases of paratuberculosis in camelids differs from that seen in many other species is that clinical cases are seen in much younger animals. These cases occur in animals less than two years old (Fowler 1998). Where paratuberculosis has been identified as occurring in camelids it has been cultured satisfactorily and Fowler (1998) reported it to be the same strain as that found in cattle. Fowler (1998) concluded "*Lamoids do not pose a threat for transmission of Johne's disease to any other animal, according to the scientific literature and statements of knowledgeable scientists*". As a consequence, the economic impact of paratuberculosis on camelids in Great Britain has not been explored further in this report.

# Chapter 16 International Trade Losses Associated with Paratuberculosis

G J Gunn<sup>1</sup> and S Ashworth<sup>2</sup>

## Historical Details on International Trade

Historically Great Britain (GB) has had a small but significant trade in live cattle and sheep. In recent years this trade has been constrained by public perceptions of the welfare of live animals in transit, particularly in relation to calves and sheep. Additionally, control measures to prevent the transmission of BSE have prevented any cattle being exported from the UK since 1996.

Nevertheless, prior to 1996 the UK was exporting some 425,000 cattle per year of which all but around 100 head were exported to Europe, and of which around 99% were calves for veal production. At the same time the UK imported around 14,500 cattle per year of which around 85% were from Ireland. Some of the Irish cattle may have been imported to Northern Ireland for slaughter with the remainder imported to G B for breeding purposes.

Current estimates for exports of live sheep from Great Britain by the MLC (2000) suggest that some 1.1 million sheep may be exported annually. This represents a growth of 250% since the low point in exports that occurred during 1997, at the height of popular protest over the welfare of live animal exports.

## Current EC position on paratuberculosis

European Commission Directives make no provision for trade control measures as part of an eradication or control programme for paratuberculosis in cattle (Council Directive 64/432/EEC as amended in July 1998). Prior to this date it was possible to present a case for paratuberculosis control. Sweden proposed such a measure when they joined the EU in 1994. They asked for the severe restriction of faecal culture to be carried out for four months prior to importation but this was not approved by the European Commission (Batho, *pers comm.*). The economic significance of paratuberculosis was recognised, and the seriousness of the disease was not disputed. It was the

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lack of good diagnostic methods which resulted in the rejection of that proposal (James Moynagh SANCO, *pers comm*). However, Council Directive 91/68/EEC does make provision for Member States to present plans for the control of *M. paratuberculosis* infection in sheep and goats. Plans to control the movement and trade in livestock under a disease eradication programme would have significant implications for the free trade of produce within the European Single Market and must be considered carefully in this respect.

At this current point in time there is no restriction on the trade in live cattle imposed by the likelihood that animals may be infected with *M. paratuberculosis*. None of the significant trading partners of the UK recognise paratuberculosis as a notifiable disease requiring certification of the animal's health status before export. No country has a control programme approved by the European Commission. Nevertheless, a number of countries, such as Sweden, are known to adopt voluntary control measures of differing magnitudes. While these control measures do not prevent the free trade of animals, they may be sufficient, in some cases, to dissuade importers from purchasing breeding livestock.

Thus, Sweden culls the whole herd where any evidence of paratuberculosis is found. In Spain, some Regional Authorities (which have administrative competence for animal health) ask for certificates to guarantee that cattle, sheep and goats imported into the region are free of paratuberculosis (Badiola, *pers. comm.*).

To sum up the current situation: "The Scientific Committee on Animal Health and Animal Welfare completed a report on paratuberculosis in March 2000. This report was specifically in the context of a possible link between this disease in animals and Crohn's disease in man but contains much useful information on paratuberculosis in animals. It is not the Commission's intention to return to the issue in the short term" (James Moynagh SANCO, *pers comm*).

## Future Developments

Given the economic consequences of the disease it is probable, that in the future, individual purchasers of livestock may seek specific guarantees regarding paratuberculosis. They will purchase breeding livestock only from herds that can be shown in some way to be free from paratuberculosis. This is essentially a question of an individual producer's attitude to risk in balancing the economic losses associated with the disease with the economic benefits that may be available through genetic improvement.

It would be possible to envisage a situation where EC trade in livestock would not be possible without having had a blood test result to demonstrate freedom from paratuberculosis. If an animal was subsequently excluded from export it could have significant impact on that animal's value. This

would be particularly the case with regard to breeding livestock. Before such a measure could be envisaged two issues would have to be resolved. Firstly, a quick, inexpensive and reliable test would need to be developed. Secondly the requirement to test animals being traded for slaughter would have to be considered in detail.

To summarise, currently paratuberculosis has minimal impact on the trade in live animals in to or out of Great Britain. This is because no UK trading partners enforce significant barriers to trade relating to paratuberculosis. Such a requirement would conflict with the free movement of goods within both the Single European Market and with international trade. A number of important points would need to be addressed before any specific requirements limiting international trade in live animals could be introduced. The development of quick, economic and reliable tests for paratuberculosis would be essential. The position with regard to breeding livestock and stock intended for slaughter would have to be established. This would be complicated even further where store livestock are traded for further rearing on farms in the importing country before slaughter. It is difficult to see such issues being resolved in the near future.



Assessment of Surveillance and Control of paratuberculosis in farm animals in GB.

Workshop 1 Proceedings.

25<sup>th</sup> & 26<sup>th</sup> May 2000.

### Paratuberculosis

1. Two major bacterial strains have been identified by restriction fragment length polymorphisms (RFLP); the principle type – C17 and another type – C1.
2. The organism can be found in deer, rabbits cattle, sheep and non-ruminant species and there does not appear to be a correlation between the bacterial strain and infected host.
3. The extent of cross species infection is unclear and the role of wildlife reservoir requires considerable study.
4. The expert opinion was that there is a strong host preference for bacterial strains. Opinion was to effectively treat sheep and cattle infections separately – but to recognise that cross infections can occur sporadically.
5. The expert view was that environmental reservoirs of infection can exist and that the bacterium can persist for prolonged periods (18 months) in water, ponds, run-offs.
6. Primary infection of adult animals was considered feasible.
7. In the live animal there is no accepted single “gold-standard” test and multiple tests increase the likelihood of detecting infection.
8. The expert view was that faecal culture is more sensitive than serological testing using the absorbed ELISA in cattle.
9. The absorbed ELISA assay was recommended for herd level testing but for control purposes because of misclassification of single samples testing must be repeated annually.
10. Bulk milk testing by immunomagnetic separation (IMS) is limited by specificity and sensitivity and is not yet validated.
11. For surveillance work the possibility of testing pools of faeces samples by culture should be considered.

12. The diagnostic test for slaughterhouse material could be polymerase chain reaction (PCR) testing of tissues or gross pathology confirmed by histopathology.

### **Surveillance**

13. The current UK passive surveillance data was not considered informative.
14. A major objective should be to establish national herd prevalence and to estimate of the range of within herd prevalence in infected herds.
15. A recommendation was that the blood samples collected from the national beef herd for the purpose of brucellosis surveillance should be used for paratuberculosis surveillance too.
16. It was suggested that the national dairy herd should be surveyed and that the samples to be tested would be blood and faeces.
17. For sheep flocks the examination of blood samples collected for *Mycoplasma agalactiae* surveillance should be explored.
18. The group did not consider abattoir surveillance to be useful for determining prevalence.
19. The major samples available for a surveillance programme would be faeces, blood or milk. The consensus was that the absorbed ELISA upon blood samples with single well testing and no repeat testing was sufficiently robust to allow national and herd prevalence to be determined.
20. It was suggested that vaccinated herds would need to be excluded from any serological testing or considered infected.
21. Cell-mediated assays were not considered sufficiently useful.
22. The consensus view was that agar gel immunodiffusion test was most appropriate serological test for sheep and goats.
23. Summary conclusions for surveillance testing are presented on page 45.

### **Control**

24. The baseline prevalence must be known before embarking upon a control programme,

25. There must be Industry support for any control initiative.
26. The concept of Biosecurity and adoption of control by management must be embraced by the Industry.
27. There must be good communication/education/risk management advice.
28. The programmes(s) should include a concept of awarding herd status after testing.
29. Support, or compensation, mechanisms for infected herds are desirable.
30. Control programmes for Sheep and Other Species were considered difficult because of the inherently low value of these species.
31. There has been no examination of application of the testing programmes from other species to cervines and camelids.
32. There is a lack of robust data on the efficacy of vaccines.
33. The consensus view was that vaccination did not eradicate infection from a herd but it did suppress clinical cases.
34. A low cost vaccine would be useful. Schering-Plough has a low cost vaccine available in Europe but this is not licensed in UK. This is a dead vaccine and is thought to cost approximately £1 per dose.
35. The group considered that recombinant vaccine technology or similar would not offer an early solution.
36. It was envisaged that fundamentally different control programmes could be applied to sheep and cattle. In the former vaccination may be the favoured option because of the inherent disparity in animal costs.
37. Though there is some evidence of a genetic susceptibility in mice to tuberculosis the evidence in ruminant species is not available and is unlikely to be available for some years.
38. It was suggested that The Sale of Goods Act offered the prospect to control the sale of infected animals in UK.

39. It was emphasised that the role of wildlife populations in transmission of infection was unclear.
40. There was concern expressed that introduction of control programmes could lead to the development of bacterial strains that did not cause clinical disease in animals but were dangerous to humans. The considered view was that this was not a real issue.
41. The expert panel expressed the view that Government support was vital for providing expertise and education but that successful control programmes required Industry leadership.
42. Summaries of the control recommendations are given on page 68.

### **Research Required**

The group considered that there is a requirement to further study the strains of *Map* that infect sheep and how these relate to the common strains found in cattle.

The mechanisms for the spread of the disease between cattle and sheep in UK conditions is still to be defined and is of fundamental importance to the control of the disease given the close proximity between the two species that applies on many British farms.

**PARATUBERCULOSIS - THE DISEASE**

**M SHARP , MOREDUN RESEARCH INSTITUTE, MIDLOTHIAN, SCOTLAND.**

Paratuberculosis exists in two forms, the lepromatous or multibacillary and the tuberculoid or paucibacillary. These are indistinguishable clinically. As infection begins, a cell-mediated immune response is important. With time this wanes and the humoral response increases (figure 1).

**Figure 1**

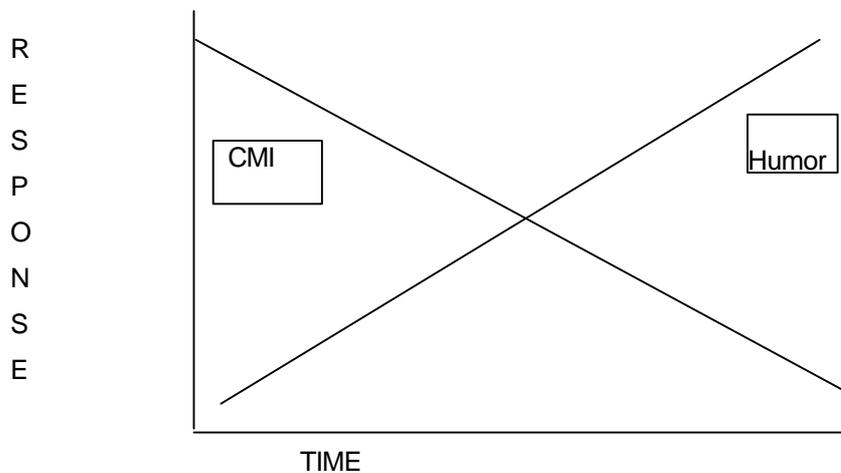
Spectrum of disease

Lepromatous (multibacillary)

Tuberculoid (paucibacillary)

Th-1 type cytokines

Th-2 type cytokines



With time an animal may recover from infection, develop low grade disease or progress into the lepromatous form. As the bacterial load increases due to immune failure in the later stages of the disease there are increased number of bacteria released in the faeces. It is not known what determines the fate of the individual infected animal. This model of the disease is supported by experimental work on the disease in sheep.

## The Organism

The causal organism is a subspecies of the *Mycobacterium avium* complex that includes three subspecies:

- *M avium* subspecies *avium* (*Maa*),
- *M avium* subspecies *silvaticum* (*Mas*),
- *M avium* subspecies *paratuberculosis* (*Map*).

*Map* is distinguishable as it contains insertion sequence IS900. Strain differences within the subspecies exist and can be determined by restriction fragment polymerisation (RFLP). Further differences can be detected by pulsed field gel electrophoresis. There is thought to be 23 different genotypes, but within the UK one genotype is believed to predominate. The available antigens used in diagnostic tests do not allow the differentiation of *Maa*, *Map* or *Mas*. However, there is an antigen specific for *Maa* and this could be used in two stage testing to improve overall test specificity.

## Host Range

A wide range of ruminants, camelids and other herbivores have been shown to develop the disease. Lagomorphs and their predators can become infected and develop lesions. In addition infection has been detected in species of rodents, pig, man and several species of bird.

## Interspecies Transfer

It has been shown that rabbits excrete enough *Map* to initiate infection in ruminants. However it is unclear if these act as reservoirs of infection or whether strains of *Map* show host specificity or host preference. Therefore the risk of interspecies transfer is not known, but nevertheless such spread of infection remains a possibility.

## PARATUBERCULOSIS IN SMALL RUMINANTS

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### Introduction

This paper provides general information on paratuberculosis primarily in sheep but also goats and deer. The main topics are:

- Pathogenesis.
- Bacteriology, cultural and molecular techniques.

- Serology and measurement of CMI.
- Vaccination.
- Interspecies transmission.

Before any action on ovine paratuberculosis is taken in the UK it will be necessary to answer these questions:

- 1 How common is this disease in sheep?
- 2 How good are the available tests to detect infection and the absence of infection?

Other questions of relevance:

- What control methods have been used in other countries and have they been successful?
- What are the sheep industry's views or knowledge of paratuberculosis and their views on control?
- Should any research on paratuberculosis be considered necessary who would fund it?
- What financial impact does the disease have?

## **PATHOGENESIS**

### **Experimental Infection**

Historically, culture of the organism from ovine material has generally been unsuccessful. To follow the course of an experimental infection, McEwen (1939) induced disease in lambs by dosing with minced up infected intestines. Twenty years later workers at the Moredun (Brotherston 1961) could reliably produce the disease by oral dosing of culture-derived bovine and ovine strains. Some animals recovered. They developed a method to quantitatively determine the weight of infection in intestines and associated lymph nodes and thereby showed that by using a killed organism as a vaccine that infection did not progress. They also found that animals producing an innate strong cell-mediated immunity (determined by using intradermal avian tuberculin) and that were subsequently infected, showed a low level of intestinal infection whilst those with a weak response carried numerous organisms within intestinal macrophages. Juste (1994) in Spain also studied experimental oral infection of vaccinated and unvaccinated lambs and commented that early lesions were found in the interfollicular areas of Peyer's patches and that this tissue was greatly reduced in the adult animal. This could provide an explanation for the lower susceptibility that occurs with age.

### **Natural Infections**

Early cases may only show a loss of condition but this usually progresses to more severe emaciation and an inconstant scour. In some animals the wool becomes brittle and is easily pulled

out. There may be a marked reduction in serum albumin and calcium. The likelihood of detecting acid-fast bacilli is higher if the faeces are softer or more fluid than normal.

At necropsy there are collections of clear fluid in the body cavities, emaciation, and atrophy. The walls of the lower small intestine and especially the ileum are thickened. Thickening may extend to involve the jejunum, caecum and colon. The lymphatics are prominent, the mesenteric attachments oedematous and the local lymph nodes swollen. Caseation is most commonly seen in goat lymph nodes. The mucosa is thickened and often granular. A yellowish-orange pigmentation is present in some cases. Some animals may show minimal intestinal changes but exhibit pronounced clinical signs. Huge numbers of organisms are seen in ZN-stained sections of intestines. Lesions, range across a spectrum between two extremes: those where epithelioid macrophage cells are numerous and contain numerous acid-fast organisms (multibacillary or lepromatous), to those cases where there was a profuse lymphocytic contribution with small collections of macrophages and very few organisms (paucibacillary or granulomatous). Acid-fast organisms are more frequently found in faeces from the multibacillary cases and it is also these animals that are detected most reliably by serological tests. Paucibacillary cases have ranged from 60% to 12% of affected animals examined

### **Immunology**

Cell-mediated immunity (CMI) is considered strongest in the earlier stages of infection and humoral antibody increases over time. There is a good correlation with the type of histological response in the intestine: those with a "paucibacillary" type of reaction show a strong CMI whilst those with a "multibacillary" reaction show a weak CMI. This confirms the two extremes of response to this organism – tuberculoid and strong resistance to the pathogen with few or no organisms found and marked local inflammatory responses - and lepromatous, associated with weak CMI, numerous organisms and a diffuse macrophage response.

### **Protection**

Vaccination has generally been successful in the control of clinical disease. Vaccines may be prepared from heat-killed organisms or live avirulent strains. In the UK the latter is used. In the Weybridge vaccine three strains are combined with pumice, olive oil and liquid paraffin. In young lambs half a bovine dose is given subcutaneously in the brisket. In Iceland, the strain of organism that caused severe losses could not be cultured on artificial media and instead two heat-killed bovine strains were used in the vaccine. Vaccinated flocks showed a significant reduction in paratuberculosis. This was despite the fact that lambs were not vaccinated until four or five months old. Vaccine has also been used effectively in both sheep and goats in Cyprus and Spain. However, vaccination does not eradicate the organism and vaccinated animals may carry infection and pass the organism in faeces though possibly only at a low level.

### **Serology**

It is generally accepted that as the extent of the pathological processes occurring within the animal advance cell mediated immunity wanes and humoral antibody increases. This antibody response can be detected by the complement fixation test (CFT); agar gel immunodiffusion test (AGIDT) or an enzyme-linked immunosorbent assay (ELISA). Serological testing performed on clinical cases of paratuberculosis, where infection status was confirmed by histological examination, showed that both the AGID and the ELISA performed well in detecting the multibacillary form. Control sera were all negative by both tests confirming 100% specificity. However, detection of the paucibacillary animals is not nearly so effective with 10% giving a positive ELISA result and 30% a positive AGIDT result

### **Bacterial Culture**

Sheep strains have proved more difficult to isolate than the bovine strain. However *Map* can be isolated from sheep on Herrold's egg yolk medium, Lowenstein-Jensen medium and Middlebrook 7H11. Lowenstein-Jensen medium without sodium pyruvate has also been used for strains from sheep and goats.

Recent work from Australia has shown that colonies of *Map* could be reliably isolated using solid media based on Middlebrook 7H10 and 7H11 agars with mycobactin and egg yolk. Culture was most sensitive in detecting infection in tissues from both multibacillary and paucibacillary cases. However, only 48% of samples of faeces from 31 paucibacillary cases were culture positive. These isolations took longer than using Bactec 12B, which is a more expensive technique.

### **Paratuberculosis in Goats**

The disease occurs world-wide in this species. Clinical signs and pathological findings are similar to those seen in sheep but diarrhoea may be more of a feature in the terminal stages. The only difference from sheep is that necrosis or even calcification is more commonly seen in mesenteric lymph nodes. Diagnosis is commonly made by examination of ZN-stained smears prepared from faeces. The AGIDT was traditionally used to confirm clinical cases and identify infected animals. More recently an ELISA test system has been developed from the bovine test with a quoted specificity of 100%. Vaccination, using the CVL Weybridge product, has been shown to be very effective in reducing the incidence of clinical disease. On occasions adults have been vaccinated as well as kids, to good effect.

## **Interspecies Transmission**

In Iceland outbreaks of ovine paratuberculosis were traced to the importation of 20 Karakul sheep. Depopulation was employed on some farms but was often unsuccessful and this led to the suggestion that infection had spread to cattle. It was later confirmed that paratuberculosis was present in the cattle.

There are several reports of sheep and goats becoming infected after grazing pasture that had been grazed by infected cattle. In general clinical disease and gross intestinal lesions are not a feature of these reports and infection was often identified on culture.

Recent work in the Netherlands reports on the investigation of sheep flocks on farms where paratuberculosis has been confirmed in the cattle. Poor doing sheep and those found positive by serology were examined at post mortem. 8/50 had macroscopic changes consistent with paratuberculosis. Altogether culture confirmed infection in individuals in ten flocks, most likely due to a bovine strain.

In summary, more extensive surveys need to be carried out in wildlife in different parts of the UK to determine the extent of these infections. Molecular studies are needed to determine whether there is a heterogeneity or homogeneity of strains and experimental work is needed to determine how easily strains can establish infection in cattle and sheep. Work would also need to be carried out to determine the persistence of and the bacterial load of infection on pasture. This could be done by using naïve tracer calves or lambs. Advice about the possible control of the disease, and the likelihood of success, should only be given once this further information is available.

(References cited in this paper can be found in the review document reference list).

## **Diagnosis of Paratuberculosis**

### **Diagnostic Tests: Immunological**

**Michael Collins , School Of Veterinary Medicine, University Of Wisconsin, USA.**

### **Introduction**

This presentation concentrated on use of serological tests for the diagnosis of paratuberculosis in cattle. The experience in Wisconsin, USA is that from 1900 to the present paratuberculosis has gone from an uncommon disease to a very common one. The current estimate is 40% to 60% of herds are infected and the diagnostic challenge is to identify animals in the subclinical stages of the disease. Such diagnoses depend on the detection of the organism or on detection of the host

response to the organism. There is enormous variation in the detectable cell-mediated or humoral host responses to infection. Genetic determinants, age at infection, infective dose, and the temporal pattern of organism excretion and immune response as the infection progresses may all be factors of importance in this variation.

### **Multiple Tests Increase Detection**

It has become practice to use faecal culture to confirm the true infection status of cattle with a positive serum antibody response, but this practice is misleading. In a study involving 177 clinically normal, but infected cattle, serology alone identified 10%, culture alone 27%, both culture and ELISA were positive for 34% of cattle and in 29% of infected animals both tests were negative (Sockett et al. 1992). This suggests that by combining tests, or alternating the type of test used in a herd, more infected animals will be identified. It also illustrates that a negative faecal culture on an ELISA-positive animal is not necessarily indicative for the absence of infection.

### **Use of Antibody ELISA**

The absorbed ELISA, where cross-reacting antibodies are removed by preabsorption of sera with whole or lysed *Mycobacterium phlei* cells, has become standard international test. Several companies make test kits and several more have kits under development. The ELISA can be used in a semi-quantitative way: animals with the highest optical density reading in the test are likely to develop the disease more rapidly than those closer to the positive cut-off. The specificity of the IDEXX kit used in the U.S. has been estimated at 98% and the sensitivity is 45-55%.

The prolonged incubation period and slow development of the disease complicate assessing test sensitivity. Hence the sensitivity of the test varies with the age of the population and the time since exposure to infection. In the pre-clinical, pre-patent stage of the disease estimates of the sensitivity are as low as 25%, but are 88% for clinical cases. The prolonged incubation period of the disease hampers the assessment of specificity and populations considered free from the disease may be infected. It only takes one or two infected animals to be present in such a population to reduce the specificity estimate significantly.

In the absorbed ELISA used in the U.S. optical density values for each sample are normalised by comparison, as a ratio to OD values of positive and negative controls provided with the kit. This ratio is called the s/p value. Use of such ratios is common practice in ELISA technology and helps to limit variability in assay performance between days, kit lots, technicians, and laboratories. s/p values at or above the cut-off of 0.25 are positive, the rest negative.

Interpretation of serological tests is more powerful if predictive value concepts are applied. This requires use of the estimated infection prevalence for the test population (herd) in standard epidemiological formulas. The results, as negative or positive predictive values, permit expression

of diagnostic certainty in probability terms. The problem in using this concept is the difficulty in making a reasonable estimate of the herd prevalence of infection, and that after culling of test-positive animals the distribution of infection severity among animals is so perturbed as to render the predictive value concept useless.

An alternative way of expressing diagnostic probability from ELISA results is to use likelihood ratios. The likelihood ratio is defined as the odds that a given level of diagnostic test result (in this case the s/p value) would be expected in an animal with as opposed to without paratuberculosis. This technique allows an assessment of the probability an animal is infected with *M. paratuberculosis* based on the magnitude of the s/p value without knowing the prevalence of the disease in the population under test. Likelihood ratios were established by analysing of ELISA results on 126 cases of subclinical bovine paratuberculosis and 722 paratuberculosis certified-free cattle. A strong, non-linear association was shown between the magnitude of ELISA s/p value and the likelihood the tested cattle were infected. Based on this, a reporting system was established where both the s/p value and a word interpretation are provided to herd owners and their veterinarian. The detail below accompanies all results sent from my laboratory as a further guide and explanation of the meaning of ELISA results (Table 1).

**Table 1. Guide to the interpretation of absorbed ELISA results.**

<b>S/P ratio</b>	<b>Interpretation</b>	<b>Explanation &amp; recommendation:</b>
<b>0 - .09</b>	<b>Negative</b>	Antibodies to <i>M. paratuberculosis</i> were not detected. Cattle classified as ELISA-negative are either not infected or not producing antibodies. Retesting in 6-12 months will increase confidence the animal is free of infection.
<b>.10 - .24</b>	<b>Suspect</b>	Cattle with ELISA results in this range may be in the early stages of the infection. They are roughly 15 times more likely to be <i>M. paratuberculosis</i> -infected than the ELISA-negative animals. Retesting these cattle in 3 to 6 months is recommended.
<b>.25 - .39</b>	<b>Low positive</b>	Cattle with ELISA results in this range are >30 times more likely to be infected than ELISA-negative cattle. These cattle may be in early stages of infection and not yet faecal culture-positive.
<b>.40 - .99</b>	<b>Positive</b>	Cattle testing in this range are likely to be shedding <i>M. paratuberculosis</i> in faeces and milk and their foetuses may be exposed to infection <i>in utero</i> . Such cattle should be culled from the herd and sold for slaughter only.
<b>1.00 - 10.00</b>	<b>Strong positive</b>	Cattle testing in this range are in advanced stages of paratuberculosis, have a generalised infection with exposure of foetuses to infection <i>in utero</i> , are likely shedding the bacterium in faeces and milk, and probably will soon develop clinical signs of disease. Such cattle should be culled immediately and sold for slaughter only.

Note: Occasionally cattle with paratuberculosis will test negative, even if showing clinical signs of paratuberculosis. The reason for this is not known but it may be the result of *in utero* infection. ELISA results should not replace sound clinical judgement, and use of ancillary diagnostic tests like faecal culture.

## Use of the Test in Populations

The test is most powerful when used at the herd level and will allow infected and non-infected herds to be distinguished with good reliability. As the test is imperfect, annual re-testing is required.

## Conclusions

- Confidence that a herd is free of paratuberculosis increases with the number of annual herd tests performed.
- Tests of higher sensitivity give more confidence of herd freedom from infection.
- Existing tests are more than adequate for herd surveillance and herd infection classification.
- A national strategy is required to slow if not halt the spread of *M. paratuberculosis* among cattle, sheep and goat herds.

## DIAGNOSTIC TESTS: BACTERIOLOGICAL

**DOUWE BAKKER, INSTITUUT VOOR DIERHOUDERIJ EN DIERGEXONDHEID, LELYSTAD, NETHERLANDS.**

## Introduction

Faecal culture for the diagnosis of bovine paratuberculosis has been used as the “gold” standard in the Netherlands for several decades. This paper covers the experiences with the culture methods and the use of the polymerase chain reaction.

## Culture of Map

Decontamination is an important step where faeces are cultured. The slow growth of the organism means that long incubation periods are necessary. Samples are routinely cultured for up to six months and around 10% of eventual positives are still negative after 4 months. There are two basic methods in use world-wide: 1. Decontamination by successive incubation with oxalic acid and NaOH, then culture to egg yolk media, such as Lowenstein-Jensen or 2. Decontamination with hexadecylpyridinium chloride (HPC) and culture using Herrod's egg yolk medium. Decontamination makes the processing of samples very time consuming, therefore expensive, and limits the number of samples that can be processed in the laboratory.

Nevertheless, despite the elaborate decontamination of the samples, in the Netherlands around 30% of cultures are lost due to overgrowth with either fungi or Gram-positive spore-forming bacteria or both.

As has been reported for *Mycobacterium tuberculosis*, a single bacterium does not seem to produce good growth and aggregates of the organism are required for successful culture. Enumeration of Map in samples e.g. milk samples can be difficult since not all the bacteria will form colonies.

Culture of Map from clinical cases, that are shedding large numbers of bacteria, is straightforward but samples with low numbers of bacteria may yield false negative results.

As the majority of non-clinical, infected animals produce only 2 - 5 colony forming units per gram of faeces and the organisms are not homogeneously distributed, repeatability of culture may be poor. The lack of standardisation of the methods between laboratories means that results can be difficult to compare. Even where culture systems are optimised some strains of the organism (e.g. those of ovine origin) remain very difficult to culture.

### **Sensitivity of Faecal Culture**

Various sensitivity estimates appear in the literature, ranging from 33% to 55%.

(As the sensitivities of the absorbed ELISAs are sensitivities relative to culture, their reported sensitivities of 30-40% are in fact only 15-25% absolute sensitivities). A complete long-term evaluation of culture has yet to be performed.

### **Automated Culture Systems**

To overcome the high labour requirement and to reduce incubation times automated culture systems have been developed. Of these the Bactec 460 is the most widely used in veterinary laboratories, where in the past decades it has been used for the culture of *M. bovis*. The system has never been implemented in the Netherlands as bovine tuberculosis is absent from the country. In addition, this system employs radioactive isotopes and as such contravenes safety legislation for routine tests in use by the Animal Health Service in the Netherlands. For the same reason most of the users of this equipment from the human medical field will switch to non-radioactive systems and in the future the manufacturer may no longer provide technical support. Non-radioactive systems like the MGIT, MBBACT and BACTEC 9000 are being evaluated in several labs but reliable data on their sensitivity are not available.

## **Polymerase Chain Reaction (PCR)**

Polymerase Chain Reaction (PCR) is a rapid methodology, and in theory allows high throughput when compared to faecal culture. Depending on the primers and probe used the test is also highly specific. However, processing to remove inhibiting substances from the sample that interfere with the polymerase reaction is difficult and limits the number of samples that can be processed. In general, the test is insensitive on faeces but very sensitive when used on tissues. In addition, cross contamination is a severe problem in all diagnostic labs using PCR-methods, especially when large numbers of samples need to be processed.

## **Conclusions**

- Culture is the most sensitive method available, but methods require standardisation,
- Culture is the “gold” standard to which other tests e.g. the absorbed ELISA are compared, and the low sensitivity causes an overestimation of the sensitivity for the other tests,
- Long term evaluation to get better estimates of sensitivity and specificity is necessary,

PCR is a highly sensitive and specific test for use to confirm the identity of the isolate, or for detection of Map in tissues, but not for routine examination of faecal samples.

## **Panel Discussion on the Biology and Diagnosis of the Disease**

### **Paratuberculosis**

In sheep two forms of the disease are observed that are indistinguishable on clinical grounds: Lepromatous (multibacillary) with many bacteria and predominately a macrophage response and Tuberculoid (paucibacillary) with few bacteria present and a mainly lymphocytic response. This differentiation was not considered relevant to the disease in cattle. Additionally, in sheep there are infections caused by “pigmented” strains that are not seen in cattle.

The organism can be found in deer, rabbits cattle, sheep and non-ruminant species and there does not appear to be a correlation between the bacterial strain and infected host. The extent of cross species infection is unclear and the role of wildlife reservoir requires considerable study. Two major bacterial strains have been identified by restriction fragment length polymorphisms (RFLP); the principle type – C17 and another type – C1.

### **Host Preference & Environmental Factors**

The expert opinion was that there is a strong host preference for bacterial strains and certainly in Australia there was a 20-year gap between identifying infection in cattle and recognising the disease in sheep. However, there are examples of cattle becoming infected from sheep in Australia and evidence from Iceland indicates that cross infection can occasionally occur. Quantified evidence is lacking. Thus the opinion must be to effectively treat sheep and cattle infections separately – but to recognise that cross infections can occur sporadically.

The expert view was that environmental reservoirs of infection can exist and that the bacterium can persist for prolonged periods (18 months) in water, ponds, run-offs. It was stated that some Australian research is in progress into this aspect of the disease.

### **Primary Infection in Adults/Transmission:**

The group were of the opinion that primary infection in adults can occur although the majority of infections arise from infection of neonates. It was stated that there are records of bulls introducing infections in adult animals in herds and Australian evidence was that 30% of infected young could arise from non-infected dams. Bulls may be of more importance for introducing infection into suckler herds than dairy herds as in the former bulls are exposed to the herd for longer.

### **Diagnostic Tests**

In the live animal there is no accepted single “gold-standard” test and multiple tests increase the likelihood of detecting infection. However, there are estimates of sensitivity and specificity and the expert view was that faecal culture is more sensitive than absorbed ELISA in cattle. It was recognised that culture of sheep strains of the organism may be prolonged and difficult. Dr Collins stated that histopathology and culture of multiple tissues would be the “gold standard” but this type of work is prohibitively expensive. The consensus view was that the specificity of faecal culture is high and that detection of passive carriage is unlikely.

For the absorbed ELISA the assay signal was considered proportional to the degree of infection and that it was feasible to fix multiple cut-off levels. The assay was recommended for herd level testing but through misclassification of single samples testing must be repeated annually.

The microbiological culture assays are more sensitive than absorbed ELISA. However, the number of colonies per sample varies from 1 - >100 per 3g faeces, and since 25% have only 1 colony these may be easily undetected. The Netherlands experience is that because of the need to maintain cultures for long periods many are lost through overgrowth of contaminant fungi and other organisms.

### **Other Diagnostic Tests**

Bulk milk testing by immunomagnetic separation (IMS) is limited by specificity and sensitivity and is not yet validated.

For surveillance work the most interesting prospect was the possibility of testing pools of faeces samples by culture.

The diagnostic test for slaughterhouse material could be polymerase chain reaction (PCR) testing of tissues or gross pathology confirmed by histopathology.

## SURVEILLANCE

### Surveillance Strategies In The UK

**G GUNN , SAC VSD, INVERNESS, INVERNESS, SCOTLAND.**

#### **Introduction**

The major problem facing surveillance for paratuberculosis is the poor sensitivity of the serum antibody test and the cost and long incubation period for culture. Considering these concerns eight main approaches to surveillance in the UK may be considered.

#### **Passive**

The term passive is used as usually no action is taken to initiate collection of the material forming the basis of the surveillance. Material may be obtained from:

- Veterinary Investigation Diagnosis Analysis (VIDA),
- Health Schemes,
- Meat Inspection data.

- **Active**

- Surveys at farm level,
- Surveys at slaughterhouse,
- Bulk milk testing,
- Sentinel Farms,
- Postal Surveys

Passive surveillance suffers from several important sources of bias. Firstly, the denominator is never known and the likelihood of a sample being submitted reflects the value of the affected animal. Recent trends in diagnostic data show an increase in the number of diagnoses made. This may be real or artificial. For example the absorbed ELISA has been introduced in the past few years and has resulted in more serum samples being examined and in the past 3 years the number of samples submitted for diagnosis has doubled. However the impression remains that there is a true increase associated with certain geographical areas and an apparent high prevalence in some cattle breeds. VIDA data are presented in Table 1.

## **Health Schemes**

Formal programmes have been in existence for 3 years. The information is confidential and access to the data cannot be guaranteed.

## **Slaughterhouse Data**

In the course of routine meat inspection there is no examination for paratuberculosis and no records kept. Clinical cases that enter a slaughterhouse might conceivably be condemned because of emaciation or ascites but these are non-specific findings.

## **Farm Level Surveys**

Serological Surveys: The absorbed ELISA is the assay of choice. The objectives of the survey would determine the design in terms of numbers of herds surveyed and the number and age of animals within each herd that were tested. Blood samples are already collected for brucellosis monitoring in beef herds and these would offer access to samples where the collection had been paid for.

Faecal Surveys: The high cost of faecal culture precludes the use of this method for surveillance.

Bulk Milk Samples: Milk antibody ELISA test kits are not yet available. Detection of *Map* in milk using immunomagnetic separation (IMS) with PCR for confirmation is a test system that is also currently being examined. These tests may allow the examination of bulk milk and therefore offer a low cost sampling system.

Tissue Samples Collected at Slaughterhouse: PCR is a test of high sensitivity and specificity when used on tissues such as mesenteric lymph node. Both the slaughter generation and the over thirty months animals could be sampled in this way.

Sentinel Farms: Longitudinal surveys allow the rate of new infections to be assessed. Health Scheme herds could provide these data, but selection of herds through a random process would be of greater value.

Postal Surveys: These surveys are cheap to carry out but can suffer from poor responses and the data cannot easily be verified.

## **Future Options**

- Random survey,

- Serological,
- Faecal culture,
- Slaughterhouse Survey of old or young animals,
- Longitudinal Studies.

## 1. SURVEILLANCE STRATEGIES IN AUSTRALIA

**DAVID KENNEDY, AUSTRALIAN ANIMAL HEALTH SERVICES PTY LTD, ORANGE, AUSTRALIA.**

### **Introduction**

In Australia surveillance of paratuberculosis is carried out to achieve the following objectives:

- To understand and identify changes in epidemiology,
- To estimate prevalence or incidence of the disease,
- To detect disease and so enable intervention,
- To underpin disease control planning,
- To monitor effectiveness of control programs.

### **Background**

The disease in both sheep and cattle is notifiable in Australia. These surveillance exercises are carried out against a population of 3.16 million dairy cows on 14,000 farms; 22.7 million beef cattle on 60,000 farms and 117 million sheep on 43,450 farms. Forty seven percent of farms with beef cattle also run sheep.

### **Considerations**

- The degree of heterogeneity in the reference population dictates the scale of the surveillance,
- Disease characteristics: animals showing clinical paratuberculosis are fewer than those that are subclinically affected, but detectable using the available diagnostic tests and fewer than those that are infected, but cannot be detected by the diagnostic tests,
- Likely prevalence: If the disease is expected to be of low prevalence the surveillance effort required will be greater than where prevalence is high. Some estimate of prevalence is required at the planning stages,
- Selection Method bias: this can apply at both herd and animal level. Care is required to minimise the source of bias,

- Tools available: the sensitivity and specificity of the tests used in surveillance must be known both for planning, but also for interpreting the resultant data,
- Use of data: at the planning stage the purpose for the surveillance should be clear,
- Cost effectiveness: surveillance is expensive and the costs of the various approaches must be examined carefully against their limitations and the expected quality of the results.

### **Social Considerations**

The costs and benefits of the disease must be clear to the herd owners. Regulatory action is taken at the animal, herd and regional level. Education programmes have been employed to overcome the adverse social and market implications of a positive diagnosis. This is further supported by financial assistance to infected herds and there needs to be incentives for herd owners to participate in the surveillance.

### **Opportunistic/Passive Surveillance**

As the disease is notifiable suspicious cases are detected and investigated. A standard diagnostic approach is followed employing set diagnostic techniques and defined test kits. In addition the herd accreditation programme provides data: only 3 of 1000 disease free herds have broken down. Tests are also performed on animals moving to a disease free herd, although this is of limited surveillance value.

### **Positive/Active Surveillance**

All positive diagnoses detected by passive surveillance are traced to identify a possible source of infection. Abattoir surveillance is also used and involves gross pathology, histopathology and serology. This work is targeted to make most efficient use of resources.

Random herd selection is essential for the field surveys carried out, but animals are targeted within herds. The test should be designed to be 100% sensitive at the herd level. This may allow part herd tests to be used. Assumptions have to be made on the minimum within herd prevalence and the sensitivity of the test to detect infection in the individual animal.

In sheep pooled faecal samples have been used as the test sample. Sheep over 2 years are sampled and 50 sheep are included in each pool. The Bactec culture system is used and the identity of the isolates are confirmed using IS900 PCR. By taking 7 pools per flock there is a 95% probability that flocks with a within flock prevalence of 2% or greater will be detected.

### **National Ovine Johne's Disease Programme**

There is tracing and investigation of high-risk flocks - these are the flocks that either neighbour the outbreak or can be linked through animal movement. Abattoir inspection is employed with histological confirmation sought. An accreditation programme exists and animal movement is subject to testing.

As at March 2000 there was a national flock prevalence of 0.6%. There were 8,730 flocks in the free zone; 69,300 in the control zone with a flock prevalence of 5.4% and 84,360 in the Residual zone where 0.6% of flocks were infected.

### **National Bovine Johne's Disease Evaluation**

The recommendations following evaluation of the bovine Johne's disease Programme were to investigate high risk dairy herds and to use sera collected at the abattoir for antibody testing. Consideration should be given to the possibility of using gross pathology at the abattoir. A survey of beef herds is required. Herds should seek accreditation through the programme and animals moving should be tested.

At May 2000 the overall herd prevalence in the dairy herds was 11% (Table 2).

### **Conclusions**

Surveillance for paratuberculosis in Australia is considered necessary to:

- Support free and protected zoning (within the country),
- Identify infection for regulatory action,
- Estimate herd/flock prevalence and geographic distribution,
- For planning,
- For easing trading restrictions.

**Table 2. Dairy herd prevalence of paratuberculosis.**

N <sup>o</sup> of Herds	Prevalence (%)
450	0
2842	0.9
9663	14.9
735	2.7
13705	11

**DOUWE BAKKER**

**INSTITUUT VOOR DIERHOUDERIJ EN DIERGEXONDHEID, LELYSTAD, NETHERLANDS.**

### **Introduction**

Concerns in the Netherlands over the possible relationship between paratuberculosis and Crohn's, the reputation of the dairy industry in relation to the export market and the economic effects of paratuberculosis led the Government, farmers organisations and the dairy industry to consider disease eradication. The disease was not notifiable and no reliable data on the number of affected herds existed. An initial questionnaire in the 80's estimated that 20% of the dairy herds were affected. However, for a more precise estimate so as to monitor the effect of an eradication programme it was decided that a prevalence study was required.

### **Study Design**

The Dutch Animal Health Service and other parties in the Netherlands carried out the study. There are 28,500 dairy herds in the country. The study used the commercially available "absorbed" serum antibody ELISA. Herds with more than 20 dairy cows were selected. These were taken from across the country to minimise the effect of regional bias on national prevalence. (The "north" was traditionally thought to have a higher prevalence and to be the source of paratuberculosis in the Netherlands). Samples were collected only from cows over 3 years of age in an effort to maximise test sensitivity. A total no of 15,822 cows belonging to 378 herds were sampled and at the time of sample collection a questionnaire on the herd management was completed.

### **Results**

Using the absorbed ELISA, 55% of the tested herds had one or more serologically positive cows. As there is no "gold standard" the true sensitivity and specificity of the antibody ELISA are unknown. To overcome this different test sensitivity and specificity combinations for the survey results were used and show that the true herd prevalence could range from 36% to 71% (Table 3). The majority of these herds (74%) had only one or two serologically positive animals. These high prevalence data was confirmed in the experimental certification programme in which a significant proportion of the participating farms were shown to be infected.

**Table 3. Estimated true prevalence at animal and herd level.**

Sensitivity + specificity of ELISA	True prevalence herd level
30% + 98,5%	35.8% - 3.65%
30% + 99,5%	70.6% - 6.92%
40% + 98,5%	31.3% - 2.70%
40% + 99,5%	61.7% - 5.16%

Herds that vaccinated against paratuberculosis were considered positive and had higher number of seropositive animals (23.3%) than non-vaccinated herds (2.5%).

### **Reservoirs of *Mycobacterium avium* subsp. *paratuberculosis***

It is estimated that more than 90% of the goat herds in the Netherlands are infected, but because of the high costs of a nation-wide survey and the poor economics for the goat industry true prevalence data is not available.

The disease was not thought to be a problem in “native” Dutch sheep as the first clinical case was only recognised in the mid 1990's. Apparently, the infection was present but the disease rarely detected. A further 50 sheep from Map infected dairy herds were examined. Ten proved to be positive by culture of Map from lymph nodes and a further 7 by histological examination, but no Map was isolated from intestinal contents.

It remains unknown if sheep act as a reservoir of Map for dairy cattle. However, clinical cases with heavy shedding of Map do occur and it is important to remove sheep from dairy herds participating in a certification programme.

### **Prevalence In Other Mainland European Countries**

In Belgium 511 herds were surveyed using the serum antibody ELISA and 18% of herds had evidence of infection. In Denmark bulk milks were tested by ELISA for the presence of antibody and evidence of infection was found in 70% of the herds.

### **Conclusions**

- Surveillance is necessary to determine the baseline before deciding upon feasible control strategies.
- The true prevalence of infection is significantly higher than that identified based on the detection of clinical cases. Surveys should be repeated regularly. The Netherlands

plans to repeat the study every 5 years.

- Alternative techniques such as histology (sheep), PCR or bacterial culture on slaughterhouse material should be considered. However, these techniques are significantly more expensive and therefore not yet realistic options for routine use on a large scale (certainly not for goat or sheep herds).

#### Panel Discussion of Surveillance Presentations.

Currently in UK there is passive surveillance and the questions are: are these data meaningful and can they be improved?

The current passive surveillance was not considered useful, as it does not include an indication of the proportion of the population affected though it may identify trends or changes. The major objectives should be to establish national herd prevalence and to estimate of the range of within herd prevalence in infected herds.

One of the objectives of this MAFF funded study is to cost the impact of paratuberculosis in UK. It was agreed that these costs must include measures of losses from subclinical infections and premature culling but should not include an account for potential human infections. The assumption from the meeting was that costs of disease are significant, these do not simply include losses from clinical cases, and there is a requirement to obtain more data through surveillance.

#### **Consideration of Improved Surveillance and Longitudinal Studies**

It was agreed that a primary objective should be establishment of the national prevalence of paratuberculosis.

Currently there is national testing for brucellosis and EBL. It was suggested that to achieve compliance paratuberculosis should not be notifiable, but for statutory monitoring any testing and reporting must be compulsory. A recommendation was that the blood samples collected from the national beef herd for the purpose of brucellosis surveillance could be used for paratuberculosis surveillance too. A proportion of herds could be selected and tested by the absorbed ELISA. In the dairy herd it was recognised that no relevant samples were collected and it was agreed that testing of milk samples, by ELISA or PCR, required considerable development. It was therefore suggested that the national dairy herd should be surveyed and that the samples to be tested would be bloods and faeces. For sheep flocks the use of samples collected for *Mycoplasma agalactiae* surveillance should be explored.

## **Abattoir Surveillance**

The group identified concerns regarding the practicalities of abattoir surveillance as sheep are only identified to the level of the original holding. However, there is a cattle movement record system and electronic tagging is to be introduced. The expert view was that the significant prevalence measure is that of herd or flock prevalence. It was considered that histopathology is not a useful tool for prevalence surveillance although PCR performed on mesenteric lymph nodes is of proven value. Importantly examination of culls gives no indication of the prevalence of infected herds. The preference was therefore for surveys to be directed at herds.

## **Tests To Apply in Surveillance Programmes**

The major samples available for a surveillance programme would be faeces, blood or milk. The advantages and disadvantages for each sample and the most relevant tests for a surveillance programme were considered.

Faeces allow the recovery of bacterial isolates that may be maintained indefinitely at  $-80^{\circ}\text{C}$  and used for other research or surveillance purposes, but the costs of culture are high. The view of the experts was that PCR detection in faeces samples still required development, the costs were as high as culture and that there was no currently accepted PCR method for direct screening of faecal material. There was discussion of the specificity of the absorbed ELISA test, particularly false positive results from exposure to Maa. The expert view was that this was not an issue and there is certainly no documented evidence for failure due to Maa. The consensus was that the absorbed ELISA with single well testing of samples and no repeat testing was sufficiently robust to allow national and herd prevalence to be determined. It was noted that vaccination did cause a serological response that could not be predicted and vaccinated herds would need to be excluded from the study. David Kennedy stated that the reliability of the absorbed ELISA was being examined and a review was underway in Australia.

The experience of the group was that cell-mediated assays were not useful and it was the view that no reliable specific cell-mediated assay existed. The consensus view was that agar gel immunodiffusion test was most appropriate serological test for sheep and goats.

## **Alternative Surveillance**

Postal questionnaires were not considered accurate, as results are likely to underestimate the prevalence of paratuberculosis.

It was considered feasible to examine the data on the number of farms using Johne's vaccine as an indicator of prevalence but the view was that this information was subject to other influences and prevalence estimates would be inaccurate.

## Summary

- Test Beef Cattle Using Brucella Blood Samples.
- Select Herds At Random.
- Test Animals Over 2 Years.
- Absorbed ELISA Satisfactory.
- Test Samples Singly.
- Test Sheep & Goats Through *M. agalactiae* Samples.
- Samples Are 20 Per Flock/Herd Selected Randomly.
- Samples Collected Annually.
- Apply AGID Test.

Dairy herds to be tested using a similar survey design to that used in beef cattle, but samples would have to be collected specifically for the purpose.

OVERVIEW OF CONTROL PROGRAMMES

GEORGE CALDOW , SAC VSD, ST BOSWELLS, SCOTLAND.

**Introduction**

The objective of a control programme is to reduce losses from a specific disease, or where the disease is absent or of low prevalence to put in place measures that will prevent the introduction or spread of the disease. Control programmes therefore include elements of risk management as well as loss reduction. Infectious diseases that are amenable to such control programmes are usually caused by obligate pathogens and not by organisms that are part of the healthy animals' normal flora.

**Sources of loss**

The losses can be real financial costs to the individual producer and the livestock industry or represent costs to society:

- Where the presence of disease has a direct effect on the physical output of the individual producer. *Through mortality; infertility; reduced growth rates or food conversion efficiency; or reduced milk production.*
- Where the presence of disease restricts the markets available to the infected herd. *For animals that will be slaughtered after a further rearing period or for breeding stock. It can be within national boundaries or affecting international trade. It rarely affects markets at the point of slaughter.*
- Where the presence of disease in infected herds results in disease in the human population. *This may be through direct or indirect contact with animals in the infected herds or when infected material enters the food chain.*
- Where the disease is perceived by society to cause an adverse effect on the welfare of infected animals. *Most disease by definition has an impact on the welfare of the diseased animal and it is the goal of most modern humane societies to keep domesticated animals in conditions that do not compromise the welfare of the animals. Where this is not achieved it is a loss to society.*

In managing the risk of new infection entering either a herd or a country it is necessary to provide estimates of the potential range of the losses and to provide an estimate of the risk of that infection entering the herd or country. The previous discussion on surveillance has emphasised how difficult it is to provide these data. For paratuberculosis there is no merit in considering the question of controlling the entry of disease to GB. However the ability of a control programme to provide disease free stock is essential to the success of most mature cattle industries. Therefore a major objective is to provide breeding stock for sale that can be accredited free of infection. This provides a means to reduce the spread of infection between herds.

It is also clear that control programmes must have a positive cost benefit at the level of the infected herd. This may not be the case, if benefits accrue at the level of the national herd or produce measurable benefits for society such as advances in human health or perceived improvement of animal welfare.

### **Fundamental Requirements for a Control Programme**

In general the following must be available or achievable:

- A good understanding of the biology of the disease. *This does not need to be absolute. There are many records of successful disease control programmes where the understanding of the disease was rudimentary. Nevertheless gaps in understanding should be identified and either research carried out to provide the missing information or alternative strategies developed.*
- The diseased (infected) animal must be recognisable. *Though for many diseases the clinical signs of infection are sufficiently distinctive to render laboratory tests of second importance for paratuberculosis the detection of the infected state must include laboratory methods. Laboratory tests should be inexpensive and of adequate sensitivity to allow the majority of infected and subclinically infected animals to be detected. Where disease prevalence is low then a highly sensitive test is likely to detect a high proportion of false positive results and for individual high value animals the tests should not sacrifice specificity. In most situations controls depend upon a battery of tests used in different ways and at different stages of the control programme. The collection of samples must be as cheap and as practical as possible.*
- Reservoirs of infection out with the target population must be identifiable and the risk of reinfection from such sources should be manageable. *The simplest case is where the infection causes similar disease in other domesticated species. Alternatively infection may persist in the environment, be maintained by intermediate hosts or occur in but*

*cause no disease in other animal species. Maintenance of infection by wild animal populations makes determining the presence of infection and effecting control of the disease difficult or it may conflict with the interests of non-farming sectors of society.*

- *The farming industry must be amenable to change. Control programmes will often require changes in farming practices. Acceptance of control programmes will be related to the level of education within the industry; the industries attitude to change generally; the degree of pressure that the disease exerts on individual businesses or the perceived commercial opportunities that may follow either control or demonstration of freedom from the disease.*
- *The farming industry or the Government must recognise that the disease causes sufficient actual or potential financial loss to merit control. This may be related to a high prevalence of the disease within the country or dissemination of information on severe problems due to the disease in other countries. High disease prevalence within a country may lead to inertia as owners of individual infected herds see little incentive in adopting a control programme that carries with it an implicit acknowledgement of infection. Conversely where the disease is of low prevalence owners may regard efforts to demonstrate freedom from infection as a low risk strategy that carries with it potential benefits. This is further favoured if the cost of the control programme is low.*

### **Control Options**

Treatment, vaccination and test and cull is often the natural progression for control programmes as infection moves from high prevalence to low prevalence. Both treatment and vaccination can be used to reduce transmission rates and to slow the infection rate down to the level that a test and cull programme is affordable. Clearly where the prevalence of infected herds is high and within herd prevalence is high a test and cull programme may be prohibitively expensive.

### **Requirements for an effective vaccination programme**

- *Cost. Vaccines must be affordable both in unit cost and in administration cost.*
- *Efficacy. A minimum requirement is that they reduce the expression of both clinical and subclinical effects of the disease. They should reduce the transmission rate of the disease. It is unrealistic to expect vaccines to be 100% effective in protecting against new infections.*
- *Safety. Side effects must be minimal. They must carry low risk of injury following accidental self-injection.*

- Vaccines should not result in reactions that can be confused with the host response to natural infection. *This may then lead to a positive result in a diagnostic test and preclude the use of such tests for diagnostic or disease control purposes. Interference with the diagnosis of other diseases of economic significance is clearly undesirable.*
- Vaccines should not mask the presence of natural infection. *In some cases vaccines may prevent clinical disease, but fail to clear the infection from the individual.*

These last two points are only of importance where either eradication of disease is the end goal or trading in disease free animals is of importance.

#### **Requirements of a Test and Cull Programme**

- Within herd disease prevalence must be low. *Where the prevalence of infection is high the costs of removal of all infected animals over a short period of time may be prohibitive despite the long-term benefits. This argument applies across herds too when there may be insufficient disease free herds from which to provide clean breeding animals.*
- Test(s) must be of adequate sensitivity and specificity.
- Tests must be affordable.
- Samples must be easily obtained.
- Appropriate biosecurity measures must be implemented.

#### **How Do These Arguments Relate to the Control of Paratuberculosis in Cattle in Britain?**

##### **Sources of Loss**

- Economic Losses: *Surveillance data indicates that the disease is present in the country and of sufficient importance for farmers to pay for examinations and application of diagnostic tests. The existence of a small number of farmers that are prepared to pay for a vaccination programme or a test and cull programme is of relevance, but does not allow us to estimate the overall importance of the disease.*
- Public Health: *There is concern that M. avium subspp. paratuberculosis is a zoonosis but it is unclear if Map has a causal relationship with Crohn's disease.*
- Animal Welfare: *There is no evidence available to indicate that society considers paratuberculosis of importance.*

Only a number of farmers and veterinary surgeons have demonstrated their belief that the control of paratuberculosis is worthy of pursuit.

### **Fundamental Requirements for a Paratuberculosis Control Programme**

- A good understanding of the biology of the disease. *There are gaps in our understanding of the pathogenesis and epidemiology of the disease. Are these gaps sufficient to prevent the design of an adequate control programme or can alternative strategies be adopted to overcome these gaps? Experience from other countries is necessary to guide us in the short term, but there is an apparent need for targeted research to overcome the gaps in our understanding.*
- Reservoirs of infection out with the target population must be identifiable and the risk of infection from such sources should be manageable. *Paratuberculosis may spread between domesticated ruminants and the extent that wild life maintains a reservoir of infection is unknown. However, techniques exist to allow the identification of infection in these populations and population reduction can be employed in the case of rabbits without contravening wildlife protection laws.*
- The farming industry must be amenable to change. *The livestock industry is traditionally conservative, but the pressures that have been operating in recent years have encouraged a more responsive attitude. At the same time there are frequent reports of intolerable administration loads within these businesses and a drive to minimise costs.*
- The farming industry or the Government must recognise that the disease causes sufficient actual or potential financial loss to merit control. *Without adequate data on the current prevalence and associated losses it is difficult to convince the industry as a whole for the need for a control programme.*

### **Control Options**

Treatment for paratuberculosis can be excluded as a current option for control as no currently effective treatments exist. Control options therefore have to be based around vaccination or test and cull.

### **Can Vaccination be Used to Control Paratuberculosis?**

Vaccination may be used in place of a structured control programme or it can be used at the start of a control programme. This latter approach can be used at the herd level or be part of a national programme. In GB vaccination against paratuberculosis is only applied at farm

level. The qualities of the vaccines, licensed and unlicensed in GB, must be considered if these are to be applied for paratuberculosis control.

- *Cost. As the vaccine is given once in the life of the animal despite the high cost per dose it remains a relatively cheap vaccine. At the present time the cost per dose of the Weybridge vaccine is £10.00.*
- *Efficacy. Impressions of the efficacy of the Weybridge vaccine are that it reduces the numbers of clinical cases within the herd and increases the average culling age of the cows. It does not remove infection and in herds where the prevalence is high or hygiene poor it may have little impact.*
- *Safety. The Weybridge vaccine produces granulomas in vaccinated calves and in humans if self-injection occurs.*
- *Vaccination should not lead to a reaction that can be confused with the host response to natural infection. As the vaccine is only used in infected herds it is difficult to determine if vaccination results in a significant serological response. The Weybridge vaccine does interfere with the interpretation of the intradermal comparative test for tuberculosis.*
- *Vaccines should not mask the presence of natural infection. Vaccination effectively masks the presence of clinical disease in the herd.*

As with many vaccines used in cattle there is little data to either support the use of a vaccine against paratuberculosis or to allow a categorical statement that vaccination has a limited role in the control of paratuberculosis. Given the length of the incubation period and the multitude of factors that determine within herd transmission the lack of such data is not surprising. The conclusion must be that the vaccine currently available may have a role in heavily infected herds, but in general it is an unsuitable tool for control.

### **Can a Test and Cull Programme be Used to Control Paratuberculosis?**

Prior to implementation of test and cull programmes for paratuberculosis consideration should be given to the following requirements.

- *Within herd disease prevalence must be low. There is a lack of data but it is thought that there may be sufficient herds of high genetic merit (both dairy and beef) where the disease is absent or of low enough prevalence to allow a test and cull programme to be employed.*

- Test(s) must be of adequate sensitivity and specificity. *Specificity, according to most reports, is not a problem. Second tests exist that can be used to confirm a diagnosis. The limiting factor is the poor sensitivity of both culture and serology. This is a function of the pathogenesis of disease in the animal rather than the failure of the detection systems. However, by removing animals as soon as possible, the amount of infection in the environment may be reduced and so perhaps the rate of infection. Removing offspring of known infected female has been advocated.*
- Tests must be affordable. *The currently available serological tests are no more expensive than similar tests for other cattle diseases. (Currently this is around £3.60 for high volume testing.) Culture of the organism is time consuming and demanding on technical expertise and therefore often costs around 5 times that of the serological tests. Confirmatory tests such as PCR are of a similar cost to culture. As the testing programmes will last many years and the majority of animals in the herd must be tested on an annual basis testing programmes are expensive.*
- Samples must be readily obtainable. *The collection of blood samples is simple and relatively cheap. Faeces sampling is more demanding and introduces safety considerations due to possible human infection with organisms such as E. coli O157.*
- Appropriate biosecurity measures must be implemented. *The biggest difficulty is the absence of a test of adequate sensitivity to allow added animals to be tested prior to introduction to the herd. Therefore the addition of an animal to the herd carries an unknown risk and will do so until such times as a significant population of herds are accredited free from disease. On many farms surface water run-off and faecal contamination from neighbouring herds can only be controlled with difficulty. Improving within herd hygiene will also be difficult for the majority of beef herds and the high number of cows managed per man in the dairy herd will also compromise hygiene around the time of calving. Since the importance of wildlife reservoirs is unknown there is a potential hazard from other external sources.*

In conclusion the control of paratuberculosis by a test and cull programme on a voluntary basis is likely to be difficult, but it may have a role in individual herds. A programme of testing could also be used to demonstrate freedom from infection and may have a role in reducing the spread of infection between herds.

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### **History**

A control scheme based on the complement fixation serological assay was run in the 1970's and 1980's. The programme failed as the rules for participation were not well defined, and the sensitivity of the test was poor. Vaccination using a heat-killed vaccine has been used to help the financial survival of heavily infected farms. However, all paratuberculosis vaccines, presently available interfere with the monitoring of the bovine tuberculosis status of vaccinated animals. In addition vaccination interferes with the paratuberculosis diagnosis: animals become serologically positive and detection of infection by faecal culture is more difficult because of the reduction of the faecal shedding. Therefore, registration of vaccinated animals is crucial. Vaccination leads to a rapid reduction in clinical cases by 90%; reduced faecal shedding and a reduction in infection pressure. Since all the available diagnostic methods, especially faecal culture, are expensive, vaccination is considered to be the only satisfactory way to control the disease in heavily infected goat herds.

From the beginning of the programme the use of a live vaccine was not considered to be an option: it was not acceptable to use living bacteria that could be involved in the aetiology of Crohn's disease in humans. The cost of the heat-killed vaccine, which is produced in several countries, is around one tenth that of the UK (live) Weybridge vaccine.

### **Why have a Paratuberculosis Programme?**

In the Netherlands the high economic cost of the disease and the apparent increasing prevalence were major factors, but the high prevalence of the disease was also considered to be undesirable for a country exporting dairy livestock and their products. Even though there is no conclusive evidence for a link between Crohn's Disease and the presence of *Map* in milk, the point of view was that livestock as well as dairy products should be free of *Map*

### **Economic Impact of Paratuberculosis**

The disease reduces milk production, reduces slaughterhouse value and leads to impaired fertility and increased susceptibility to other diseases. Several years ago these losses in infected herds were estimated per clinical case at 250-400 ECU's for production; 260-350 ECU's for reduced slaughter value and 450 ECU's for early replacement. These are rough estimates since the actual losses per infected animal are difficult to assess and the "true" number of infected animals in an

infected herd is not known, because of the limitations of the available diagnostic tools. In addition, in dairy producing countries, like Denmark and the Netherlands, both with a high prevalence of paratuberculosis, the average lifespan of cattle is very short (2.2 lactations). It is likely that the number of clinical cases of paratuberculosis is greatly reduced by the short lifespan caused by a strong selection of animals based on milk yield. At present it is not known, whether paratuberculosis itself, is a primary or contributory factor in this short lifespan. This complexity makes the collection of reliable data from the field and therefore (economic) modelling very difficult.

### **Pilot Programme**

The Dutch Animal Health Service designed an experimental certification programme using faecal culture. One hundred and twenty five herds entered the project, with the claim that there had been no case of paratuberculosis in the last 5 years. However, checking the records of the Animal Health Service, only one hundred and thirteen of these had no 5 year history of the disease. Herds were tested every 6 months using faecal culture and at each test positive herds were detected. After five rounds of testing (24 months) only 58 herds remained clear of infection. Two conclusions can be drawn from this. Firstly, the true prevalence of paratuberculosis is significantly higher than was thought based on the "prevalence" of clinical cases. Secondly, faecal culture, regarded as the "gold" standard, is not sensitive enough to detect all infected animals in one round. In fact, the project is still continuing and at 42 months more cases are detected. Also there is the problem of farmers that were not aware of having the disease and had a productive herd were suddenly labelled "infected". As a result the highly motivated farmers that participated in the programme felt that they were ill rewarded.

### **Certification Programme**

In 1997 plans were made for a mandatory programme, but by 1999 support for this had fallen for several reasons. The problems with implementation of the mandatory IBR programme, using a vaccine contaminated with BVD, resulted in a loss of interest of the Dutch farmers. The high prevalence of infection, which became apparent and the uncertainty of the situation of the farmers with herds, where the infection was detected (another 10-15 year programme before reaching the start of a certification programme again, a possible ban on trading, etc) made participation unattractive. In addition as 30% of the faecal samples were 'lost' due to fungal contamination, resampling of the farm and the resultant increased costs made streamlining of the programme very difficult.

This led to the decision to drop the mandatory programme and instead use voluntary participation. At present work on a trial basis has started on 250 farms. The programme employs annual testing of adult cattle. Faecal samples are pooled in batches of 5 and the antibody ELISA is used individually. Pooling of faecal samples is done to reduce costs. Pooling leads to a loss of sensitivity and will result in a reduction in the speed of progress of eradication.

Status is based on negative faecal culture. Positive antibody results decrease status, when confirmed by faecal culture. When a pooled faeces sample is positive the cows that made up the pool are then individually screened.

Changing management in order to control the disease is regarded to be essential for success. Herd management advice and control is one of priorities of the Animal Health Service at the moment.

Management rules are aimed at reducing the spread of infection to young calves. Pooled colostrum must not be used; cows should calve separately in a clean calving box etc. There are strict rules governing the purchase of animals - they must be from herds with an equivalent or higher status; faecal culture positive animals are culled; goat and sheep cannot share grazing and vaccinated cattle are excluded from the programme.

### **Support for Positive Herds**

For the success of the programme, indeed to convince farmers that they should participate, it is essential that there is a programme for infected herds. This ensures that farmers are not punished for participating. Once a herd is labelled "infected", it can take 5-10 years of repetitive testing at high costs before a farmer can re-enter the certification programme again. Therefore a programme has been designed and funds set aside to help these farms.

### **Conclusions**

- Before starting a programme the prevalence of infected herds in the country must be known, in order to know what the options are for controlling or eradicating the disease.
- Producers must be motivated and informed.
- Research is need to develop a safe vaccine that does not interfere with diagnostic tests. It is estimated that this may be 10 to 15 years away. For infected goat herds and sheep flocks vaccination may be the only option.

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### Background

In Australia, the distribution of both sheep and cattle strains of *M. avium* subsp. *paratuberculosis* (*Map*) is restricted and movement restrictions have been used for many years to protect free and low prevalence areas.

### Objectives of Control Programmes

The objectives are to protect the status of free regions and non-infected herds and flocks; to contain infection and to control infection where it is present. Providing assistance to infected herds is a further objective.

### Protecting “Free” Regions

Paratuberculosis disease zoning was formally introduced in 1999.

- Residual zone – disease endemic and/or little control enforced,
- Control zone - moderate prevalence and control enforced,
- Protected zone - sporadic or very low prevalence,
- Free Zone - no endemic infection.

Control measures are tighter in higher status zones, so that incursion of infection is stamped out in Free zones and in some Protected zones. A similar approach is used for both cattle and sheep although the distributions of the diseases are different and they are managed as epidemiologically different infections.

Vaccination is being used on a trial basis in sheep, but has not been in cattle since the mid 1990's.

### Protecting Non-Infected Herds and Flocks

Market assurance programmes (MAP) are promoted as the most appropriate means of demonstrating a low risk or negative herd status in Control and Residual zones. This is supported by vendor declaration where the vendor declares in writing to potential buyers the status of the herd as assessed under a range of options from MAP to low prevalence within a disease control program.

As most herds are still non-assessed, communication emphasises that this means there is no assurance of the status of their animals. Purchasers can then make an informed decision the history of the herd or flock.

#### Containing Infection in Infected and Suspect Herd or Flock

There are both formal and voluntary quarantine measures. Animals from infected and suspect herds and flocks are usually not sold for breeding and move directly or indirectly to slaughter. Means of effective management of the risk associated with effluent and manure are being considered

#### **Controlling the Effects of Disease in Infected Flocks and Providing Equitable Assistance**

On farm control programs are funded by the industry and individual farmer with technical input from official veterinary services. Measures to reduce the within herd prevalence include improved calf rearing management and test and cull in dairy herds; pasture management and vaccination in sheep flocks. Currently eradication by destocking of infected sheep flocks followed by 15-month decontamination of pasture is being evaluated.

#### **Australian Approach**

The control of paratuberculosis has been largely driven by the livestock industries that also provide a large part of the funding. Government animal health authorities provide in-kind support in program management and technical advice and also funding, particularly for research and development. Governments provide technical input to the program through the National Committee of Chief Veterinary Officers.

National co-ordination also involves the National Farmers Federation and its livestock member councils, Animal Health Australia (i.e. Australian Animal Health Council Ltd) and National Programme Advisory Committees.

The National Johne's Disease Control Programme is funded to A\$0.5 million per annum (approximately equivalent at October 2000 to £200,000). This is used to co-ordinate a national approach including defining national Standard Definitions and Rules for disease control, Market Assurance Programmes and diagnostic tests standards. Communications are also an important part of the remit and information on the disease and, programmes, lists of MAP herds and flocks and associated matters are maintained on a web site.

National paratuberculosis control and evaluation programme is funded to A\$40 million (over 6 years) and the National BJD programme is funded to A\$0.35 million (1 year), (approximately equivalent at October 2000 to £16,000,000 and £140,000 respectively).

### **State Johne's Disease Control Programmes**

Some of these preceded the national approach. They comply with the national standards, but vary with the States' resources.

### **Market Assurance Programmes (MAPs)**

These began life as the National Johne's Disease MAP for Cattle in 1996, the sheep programme followed in 1997. Revision and re-badging to CattleMAP etc took place in 1998 and there are now also MAPs for goats and alpaca. New sheep and cattle MAPs have been produced in 2000.

Herds progress through levels of assurance on the basis of annual negative herd tests from Monitored Negative 1 (MN1) to Monitored Negative 3 (MN3), the highest level. Auditing procedures are used. Herds can opt to stay at a level by carrying out a maintenance test every 2 years where a maximum of 50-100 animals is tested. An audit of herd and flock management is carried out annually by the supervising approved veterinarian.

A check test is where a limited number (50-100) of usually older animals are tested negative to support a vendor's declaration, but this does not give MAP status.

There has been a linear increase in herds participating in CattleMAP from 1996 to around 1000 herds in 2000. The SheepMAP has reached a plateau at just below 700 members, partly because of changed perceptions of risk in large parts of south-eastern Australia, changes to testing requirements, and the lack of financial support for infected sheep flocks.

Demonstrating benefits and providing incentives and equitable assistance remains a significant challenge to successful control of paratuberculosis in Australia.

## **PANEL DISCUSSION OF CONTROL PRESENTATIONS**

### **ESTABLISHMENT OF SUCCESSFUL CONTROL PROGRAMMES**

The external experts summarised their views on the most important points in establishing successful control programmes for dairy and beef cattle.

#### **Primary Issues were Considered**

The baseline prevalence must be known before embarking upon a control programme,

- There must be Industry support for the initiative,
- The concept of Biosecurity must be embraced by the Industry,
- There must be good communication/education/risk management advice,
- The scheme(s) should include a concept of awarding herd status after testing,
- Support, or compensation, mechanisms for infected herds are desirable.

The experience in the USA, Australia and the Netherlands was that the prevalence in beef herds was much lower than in dairy herds and because of this there have been difficulties in establishing control programmes in beef herds. This all most certainly does not apply to the UK situation where certain Beef Breed Societies are very aware that they have a problem that needs to be tackled. Currently the Welsh Black Society has a control programme in place. However herds without a recognised problem have yet to show an interest in demonstrating freedom from the disease. This emphasised the value of good education and risk assessment methods that could be transferred to the Industry. The experts view was that early implementation of control programmes was worthwhile. It was considered unlikely that Government would pay for a scheme unless infection was proven to be zoonotic but surveillance was a legitimate expenditure for Government.

The group considered it possible that Industry was showing a renewed interest in control programmes. It was perceived that the milk buyers and consumers could force the issue. The view was expressed that with education and with an increased demand for paratuberculosis free animals that there would be increasing awareness and a requirement to implement controls.

### **Control Programmes for Sheep and Other Species**

Major issues for implementing control programmes were the relatively low value of sheep or goats against the costs of the controls. Additionally, there has been no examination of application of the testing programmes from other species to cervines and camelids.

### **Control by Vaccination**

There is a lack of robust data on the efficacy of vaccines but some information may be available through product registration of licensed products. The consensus view was that vaccination did not eradicate infection from a herd but it did offer a suppression of clinical cases. The opinion was unanimous that if a vaccination programme is implemented all ages should be vaccinated. One of the major issues is that herd owners practicing vaccination programmes ignore other important elements of biosecurity. The group considered some extreme cases could only be tackled by initial vaccination. A low cost vaccine would be useful. Schering-Plough has a low cost vaccine available in Europe but this is not licensed in UK. This is a dead vaccine and is thought to cost

approximately £1 per dose. (In UK the only available vaccine is the live attenuated “Weybridge” vaccine from MAFF VLA). A major issue in cattle is the interference in tuberculosis testing programmes following vaccination but in UK the use of the vaccine in cattle has been deregulated. The evidence presented was that the dead vaccine was likely to be as effective as the currently available live vaccine.

The group considered that recombinant vaccine technology or similar would not offer an early solution.

It was envisaged that fundamentally different control programmes could be applied to sheep and cattle. In the former vaccination may be the favoured option because of the inherent disparity in animal costs.

### **Control by Genetic Selection**

Though there is some evidence of a genetic susceptibility in mice to tuberculosis the evidence in ruminant species is not available and is unlikely to be available for some years.

## **OVERVIEW OF CONTROL PROGRAMMES: IMPORTANT ASPECTS**

### **Dairy:**

- The group considered that eradication was possible on a farm.
- That this was dependent upon a test and cull programme with altered herd management.
- It was considered that too little emphasis is placed upon the importance of management controls.
- It is vital to remove positives and the remainder of the family line.
- Testing should be based upon herd testing of all animals older than 2 years of age.
- There is some evidence for infection by artificial insemination and bull studs should be established free of infection.

### **Beef:**

- Prolonged exposure of Calves to infection is probable and this is in contrast to dairy herds.
- It is important to consider early weaning; splitting the herd into age groups/peer groups and determining where the infection is and getting rid of high-risk animals.

- Test and cull programmes have been conducted in Australia (very expensive) and have been applied to recently infected range cattle herds.
- In a heavily infected herd control can take 10 years if there's a 30% prevalence of infection.
- Of primary importance is the provision of clean drinking water.
- Infection is also increased where herds have a concentrated calving pattern and there has been some move towards a more spread out calving.
- Vaccine is typically used in heavily infected herds.
- It is vital to tailor the control programme to the individual herd requirements.

#### **Sheep:**

- The mode of transmission may be different in sheep due to differences in faecal consistency.
- There were fundamental epidemiological details still to establish, in particular the role of *in utero* infection, milk transmission etc. and maternal/daughter transmission.
- Depopulating and repopulating flocks and hopefully decontaminating the environment was being attempted in Australia, but no accredited free source is readily available to UK farmers. It was noted that there was a clear advantage from the immediate benefits to be obtained within 12 months in contrast to the likely control programme lasting 84 months.

#### **Mixed Enterprises**

It was noted that grazing cattle and sheep together did offer potential for cross infection. However, the group admitted to a lack of knowledge in this area but considered that in UK situations it was not likely to present as a major issue.

There was discussion of the lack of legal controls in UK on the sale of infected stock; this is not the situation in USA or Australia. It was suggested that The Sale of Goods Act offered the prospect to control the sale of infected animals in UK.

It was emphasised that the role of wildlife populations in transmission of infection was unclear though Scottish data did suggest a correlation between rabbits and bovine paratuberculosis. This reinforced the need to carry out transmission dynamics and modelling studies.

#### **Driving Forces in Control Programmes**

Two major drivers for the application of control programmes were identified.

- Firstly, farm economics - income/expenditure.
- Secondly, pressures through controls arising because of the zoonotic potential of infection.

There was concern expressed that introduction of control programmes could lead to the development of bacterial strains that did not cause clinical disease in animals but were dangerous to humans. The considered view was that this was not a real issue.

### **Financial Support for Control Programmes**

The expert panel expressed the view that Government support was vital for providing expertise and education but that successful control programmes required Industry leadership.

**In Australia:** There is a hierarchical structure that enables communication and allows taxes to be used for surveillance etc. This provides about A\$10,000,000 financial support for the various control programmes.

**In Netherlands:** The direct costs of the control programme are funded by: Farmers, Dairy Processor and Animal Health Service. The farmers pay via a levy per litre of milk. There is 50% Government support.

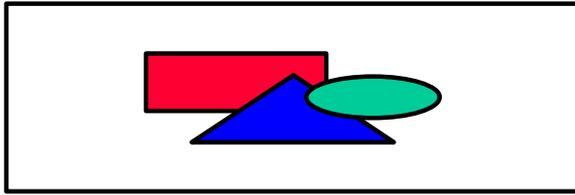
**In UK:** It was considered that Government support for a robust Industry led proposal could be explored. It was recognised that joint initiatives dealing with all species may be impractical and that a species-specific initiative might be appropriate. If there is a need for legislation then that's something that only Government can do but it requires pressure to bring it about.

### **Control of the Food-chain**

The group debated the ethical question of control on infected animals entering the food chain. It was considered most appropriate for Food Standards Agency to consider the options but it was stressed that there is no policy to prevent the consumption of meat from tuberculosis or paratuberculosis infected animals. Clinically ill animals should not be able to enter the food chain.

### **Research Required**

The group considered that there is a requirement to further study the strains of *Map* that infect sheep and how these relate to the common strains found in cattle. The mechanisms for the spread of the disease between cattle and sheep in UK conditions is still to be defined and is of fundamental importance to the control of the disease given the close proximity between the two species that applies on many British farms.



United States Animal Health  
Association

## 1998 Committee Reports--Johne's Disease

# U.S. Voluntary Johne's Disease Herd Status Program for Cattle

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## Preface

Johne's disease (paratuberculosis) is a growing concern to U.S. cattle industries. Years of research effort has produced several new diagnostic tests to detect *Mycobacterium paratuberculosis*-infected cattle. These tests have been evaluated independently by several research groups and been found sufficiently accurate to reliably be used in a program to certify cattle herds as having negligible risk of infection.

In 1993 a task force of the Johne's disease committee of USAHA drafted a model Johne's disease herd certification program (see 1993 USAHA Proceedings). Some states modified their Johne's disease certification programs to conform to this model. However, relatively few herd owners have elected to pursue herd certification citing the amount of testing required and the associated costs as the main deterrent. Consequently, in 1997 the USAHA National Johne's Working Group (NJWG) appointed a committee to try to design a more affordable and yet scientifically sound herd certification program.

Multiple meetings were held to design this program and input was solicited from experts and all stake holders. The program was submitted for discussion at NJWG meetings held in conjunction with the Livestock Conservation Institute's meeting March 1998 and the National Cattlemen's Beef Association meeting in July 1998. In August 1998, the program was sent to all state veterinarians, area-veterinarians-in-charge, veterinary associations, cattle breed associations, and interested industry groups for comment. The program was adopted by USAHA in October 1998.

The program presented in this document is intended as a model. The guidelines are considered minimal requirements for operation of a scientifically sound program to identify herds of low risk of *M. paratuberculosis* infection (Johne's disease). This model program was developed to assist State Veterinarians and Johne's disease advisory committees, or their equivalent, in each state as they consider implementation of Johne's disease herd certification programs. It is hoped that the model program will promote greater similarity and equity among different state programs.

NJWG Herd Certification Subcommittee members:

Leslie Bulaga, USDA-APHIS-Veterinary Services, Co-chair  
Michael Collins, University of Wisconsin School of Veterinary Medicine, Co-chair  
Ian Gardner, University of California, School of Veterinary Medicine  
William Hartmann, Minnesota Board of Animal Health

Karen Jordan, National Milk Producers Federation  
Richard Keene, National Holstein Association  
Lee McPhail, Ohio Department of Agriculture  
Ken Olson, American Farm Bureau Federation  
Boyd Parr, American Farm Bureau Federation  
William Rotenberger, Steele Veterinary Clinic and Chair USAHA Johne's  
Committee  
Joseph VanTiem, USDA-APHIS-Veterinary Services  
Gary Weber, National Cattlemen's and Beef Association  
Scott Wells, USDA-APHIS-Veterinary Services  
Robert Whitlock, University of Pennsylvania, College of Veterinary Medicine

## **U.S. Voluntary Johne's Disease Herd Status Program for Cattle**

**October 1998**

### **Definitions:**

**Herd:** a group of cattle managed as a separate and discrete unit not commingled with other groups of susceptible species.

All cattle on two or more premises geographically separated but on which cattle have been interchanged or where there has been contact between the premises is considered one herd. Contact of animals between separated premises under common management is assumed to have occurred unless complete separation and biosecurity measures between premises can be established by the herd owner or manager.

**Herd member:** any susceptible species of animal that is commingled with the herd.

**Commingling:** physical contact or exposure to manure or raw milk of susceptible species. For example, all cattle and other susceptible species grazed together or on the same area of a property or farm, at any time during any 12-month period, are considered to be commingled. Susceptible species include domestic and exotic ruminants such as sheep, goats, cervids and camelids. Exposure to manure via contaminated water or feed sources is also considered commingling.

**Biosecurity:** animal husbandry and hygiene practices designed to limit opportunities for exposure to *M. paratuberculosis*.

**Animal identification:** all cattle in a Program herd must be permanently and individually identified using an identification method approved by the State Johne's Advisory Committee. However, Level 4 herds must individually identify all cattle using a USDA approved official identification system.

**Accredited Veterinarian:** a veterinarian approved by the Deputy Administrator of USDA, APHIS, VS to perform functions required by State-Federal-Industry cooperative programs. For the Voluntary Johne's Disease Herd Status Program (VJDHSP) these duties include annual herd visits, animal testing, and producer education. All samples for Program testing must be collected by an accredited veterinarian or State or Federal animal health official

**Accredited Laboratory:** a laboratory that has passed an annual check test for Johne's disease administered by the National Veterinary Services Laboratories. All program testing must be conducted by a laboratory approved for the specific test being used.

**Johne's Epidemiologist:** a State or Federal regulatory health official who has demonstrated the knowledge and ability to perform the functions specified by the VJDHSP. The Johne's epidemiologist should be selected in consultation with the State Johne's Advisory Committee, State animal health official, Area-Veterinarian-in-Charge, and the National Program Coordinator.

**National Program Coordinator:** a USDA staff veterinarian who will assist State Johne's epidemiologists, State Johne's Advisory Committees, and the USAHA Johne's Committee with the administration and review of the Voluntary Johne's Disease Herd Status Program.

**Herd Status Levels:** herds may achieve status Levels of 1, 2, 3 or 4. Each level of increase indicates higher confidence in the Johne's disease free status of the herd. (Percentages shown on the flow diagrams in Appendix II represent a mathematical estimate of the probability herds at each level of certification are free of Johne's disease based on serial testing.)

**Level achievement year:** the year in which a herd met Program standards to be granted a specific status Level. For example, a herd completing Level 2 testing in 1998 which elects to remain at Level 2 would have Level 2 1998 status. Level achievement year is noted because continued monitoring increases confidence the herd is not infected.

**ELISA or Fecal Culture Statistical subset:** an ELISA test on or fecal culture of a statistically determined number of animals. The table in Appendix I shows the number of animals to include in Program ELISA and fecal culture statistical subset testing.

**State Johne's Advisory Committee:** an appointed committee which is the authority responsible for overseeing and coordinating the State's Voluntary Johne's Disease Herd Status program. The Committee may be comprised of any or all of the following:

Dairy producers - purebred, commercial and commodity groups

Beef producers - purebred, commercial and commodity groups

University Extension - beef and dairy

Veterinary practitioners - beef and dairy

Regulatory veterinary medicine - state, federal, and/or field services

Responsibilities of the committee should include, but are not limited to:

- 1) informing and educating the industry regarding Johne's disease
- 2) overseeing financial needs for state Johne's disease activities
- 3) recommending state policies with approval of the appropriate State authority on operating a Johne's disease program which enhances a Johne's disease herd status program, reduces the spread of Johne's disease and assists infected herds in managing or controlling the infection
- 4) setting standards for release of information on Program herd status
- 5) overseeing appeals of Program herd status
- 6) providing input to the USAHA Johne's Committee National Johne's Working Group for evaluation and revision of the VJDHS Program.

The duties of the Johne's Advisory Committee could be assumed by an existing animal health committee in the State.

**Program Protocol:**

**Confidentiality**

Within the limits of each state's laws, it is important to maintain as much confidentiality of testing results as possible. At the same time, to promote the program, stimulate the market place to assign added value to animals from program herds and demonstrate the benefits of buying cattle from John's disease status program herds, it is desirable for owners of status level 1 - 4 herds to disclose their herd status. When possible, program herd owners should have the option of publicly withholding or promoting their herds' status level.

#### **Entry to the Program:**

Herds may enter the VJDHSP by two methods -Standard and Fast Track. All samples for Program testing must be collected by an accredited veterinarian or State or Federal animal health official and submitted to an accredited laboratory.

It is recommended that a farm or herd risk evaluation be done before completing herd testing for Level 1 Standard Track or Level 2 Fast Track. This evaluation would inform producers entering the program of existing herd risk factors for the spread of John's disease. A farm risk checklist may also be used as a yearly reminder of existing herd risk factors.

Additionally, Program herds should be encouraged to implement Best Management Practices as provided by the National John's Educational package to prevent the introduction and spread of John's disease in their herd.

#### **Standard Track -**

The herd must meet identification and commingling requirements described in the definitions. Herds enter the Standard Track Program by John's ELISA testing 30<sup>1</sup> second lactation or higher animals. No declaration of prior disease freedom is required. Negative test results on this initial test qualifies the herd for Level 1 status.

#### **Fast Track -**

The herd must meet identification and commingling requirements described in the definitions. Additionally, the herd owner must submit a signed statement that:

- 1) I am fully aware of the management and disease history of the herd and the property during the past five years.
- 2) John's disease is not known or suspected to have existed in the herd for the past five years or on the property during the past twelve months.
- 3) Cattle are not known to have been introduced from known infected herds during the past five years.

The above written statement and a negative ELISA test on a statistical subset of second or higher lactation animals qualifies the herd for Level 2 status. States may additionally require the herd veterinarian of record to co-sign the owner statement.

#### **Previously infected herds -**

Cattle herds previously culture positive for *M. paratuberculosis* may enter the program by completing Standard Track entry requirements. Infected (positive on an organism detection test) and/or test positive (positive on any John's test) animals must be removed from the herd before Program entry.

#### **John's Disease Vaccinated herds -**

Herds previously vaccinated for John's disease may enter the Program once vaccination has been discontinued. These herds must utilize fecal culture as the only test until enough non-vaccinated natural additions qualify for ELISA testing. The number of animals to test at each level remains the same.

## Herds tested negative prior to implementation of this program -

Herds Johne's disease tested negative prior to the implementation of this program may be entered at an assigned Program Level determined after a review by the Johne's epidemiologist. That review must include:

- 1) verification that the herd meets the minimum standards for testing (fecal culture results can be used in place of ELISA results). Future Program tests must be performed in accredited laboratories.
- 2) risk assessment of the number, source, and testing history of herd additions made after the first qualifying test.

Additionally, producers wishing to use the Fast Track must make the required written statements for the time period (five years) prior to the first qualifying test.

**Animals to test:** Random sampling will give the most confidence and should be used to select animals for testing when feasible. When possible, the same animals should not be tested in consecutive testing rounds. Animals should be selected to be representative of the herd population. This program uses second or higher lactation animals as the most obvious indication of animal age. Detailed requirements for sample handling and submission must be provided by the testing laboratory.

**Maintaining a Status Level :** Producers may elect to remain at any level of confidence in either Track by conforming with the program standards and performing an ELISA test on 30 randomly selected animals of second or higher lactation every 10 - 14 months. A level achievement year for each herd should also be noted as continued monitoring increases confidence the herd is not infected.

**Testing Intervals:** testing intervals are every 10 - 14 months from the date the test samples are taken. Herds will be removed from the program if the testing interval requirements are not met, unless an extension has been received from the Johne's Epidemiologist and/or State Cattle Committee.

## Biosecurity

A program herd must have biosecurity measures in place in order to avoid exposure to manure or milk from ruminants of unknown Johne's disease status. These measures include:

- 1) pooled milk from cows of unknown Johne's disease status should not be used to feed baby calves;
- 2) manure from Embryo Transfer donors or other "visiting" cows (e.g. transport cows that lay over at program farms for rest or to be milked) should not be allowed to come in contact with the program herd and this manure should not be disposed of on pastures or in a manner which would contaminate pastures or animal feed.
- 3) exhibition cows and calves (especially under 6 months old) should be hauled in cleaned and disinfected trailers and avoid commingling; (Animal exhibition, consignment sales and transport are considered situations of low *M. paratuberculosis* infection transmission risk. However prudent care and diligence about biosecurity is recommended),
- 4) a program herd must not be commingled with or grazed behind susceptible species, (e.g. sheep, goats, farmed deer, camelids, non-program cattle.)

## Program Levels

Flow diagrams depicting progression through each status level appears as Appendix II. Percentages on the diagrams represent mathematical estimates of the probability herds at each level or certification are free of Johne's disease infection based on serial testing. This program does not certify animals free of Johne's disease. Owners may elect for their herd to remain at any status level by ELISA testing 30 second or higher lactation animals every 10 - 14 months. Maintenance of Level 4 status gives the producer a high level of certainty that their herd is free of infection. With continual maintenance of Level 4 status, it could be assumed that there is negligible risk of infection from Level 4 herds.

All samples for Program testing must be collected by an accredited veterinarian or State or Federal animal health official and submitted to an accredited laboratory. If an animal is removed from the herd while ELISA results are pending, a fecal culture should be collected and submitted on hold to the laboratory. This will allow, if the owner wishes, an appeal of herd status to be made if the animal tests ELISA positive. (See Appeal Process, page 9).

### Standard Track

The standard track is designed to allow entry to the program with a minimal investment of funds and gradually increases the producer's investment in the program. The standard track will require at least three years and four tests to reach Level 4.

**Level 1** - program entry requirements met, negative ELISA on 30 second or higher lactation animals. A sample size of thirty was selected to optimize herd sensitivity and herd specificity and maintain a fixed cost for all herds entering the program.

**Level 2** - met requirements for Level 1, and negative ELISA on a statistical subset of second or higher lactation animals. (See Appendix I for the Herd Subset Testing chart.) The Level 2 testing must be completed within 10 - 14 months of any Level 1 testing.

**Level 3** - met requirements for Level 2 and have negative fecal culture results on a statistical subset of second and higher lactation herd members. Bulls two years of age and older must be included in this testing. (See Appendix I for the Herd Subset Testing Chart.) The fecal culture must be collected within 10 - 14 months of any Level 2 testing.

**Level 4** - met requirements for Level 3 and have a negative ELISA on a statistical subset of second or higher lactation animals. Level 4 testing must be completed within 10 - 14 months of any Level 3 testing. Level 4 status is maintained by achieving negative ELISA results on 30 second or higher lactation animals every 10 - 14 months.

### Fast Track

The fast track allows producers to proceed to a higher status level of confidence more quickly than the standard track, and requires greater financial investment at program entry. The fast track will allow herds to reach Level 4 in two years with three tests.

**Level 2** - program entry requirements for Fast Track met, negative ELISA statistical subset test of second or higher lactation animals. (See Appendix I for the Herd Subset Testing chart.)

**Level 3** - met requirements for Level 2 Fast Track and have negative fecal culture results on 30 second or higher lactation animals.<sup>2</sup> Level 3 testing must be completed within 10 - 14 months of any Level 2 testing.

**Level 4** - met requirements for Fast Track Level 3 and have negative ELISA results on a statistical subset test of second or higher lactation animals. Level 4 testing must be completed within 10 -14 months of any Level 3 testing. Level 4 status is maintained by achieving negative ELISA results on 30 second or higher lactation animals every 10 -14 months.

### Herd Additions

**Heifers that have not calved and bulls less than 2 years of age:**

from herds of equal or higher program levels **-or-**

from program herds which are one level below the purchasing herd.

Animals in this category may not be added to Program herds from non-program herds.

**First and higher lactation cows, bulls greater than or equal to 2 years of age**

:

**Level 1 - 3 herds** - from herds of equal or higher level **-or-**  
from any other herd as follows:

1. ELISA test of addition(s) in the herd of origin within 30 days prior to entry to the program herd **-and -**
2. Submission of fecal culture from addition(s) within 30 days of arrival **-and-**
3. Testing of herd addition(s) in addition to required animal sampling numbers for the next required annual testing. Herd additions are not granted the same status as the receiving herd until this additional testing is negative.

Additional risk of infection is incurred when animals are purchased from non-program herds. Non-program herds should be encouraged to ELISA test 30 second or higher lactation animals before a Program herd will purchase from them (i.e. non-program herds should be encouraged to achieve Level 1 status.) When possible, additions from non-program herds should be isolated from the program herd and biosecurity maintained until fecal culture results are reported negative.

**Level 4 herds** - from herds of equal level **-or-**

from Level 2 or 3 program herds as follows:

1. ELISA test of addition(s) in the herd of origin within 30 days prior to entry to the program herd **-and-**
2. Submission of fecal culture from addition(s) within 30 days of arrival **-and-**
3. Testing of herd addition(s) in addition to required animal sampling numbers for the next required annual testing. Herd additions are not granted the same status as the receiving herd until this additional testing is negative.

Additional risk of infection is incurred when animals are purchased from herds of lower status.

**Replacements raised elsewhere:** Replacements may only be raised with animals from equivalent status Level herds. Replacements must not be commingled with lesser status level animals or herds.

**Embryo Transfer / Artificial Insemination:** Program herds may utilize semen and embryos from any other cattle herds. However, embryos must be processed according to International Embryo Transfer Society protocols. Embryo transfer recipient cows must meet herd addition requirements.

The risk of transmission of *M. paratuberculosis* from semen is unknown. AI centers are encouraged to routinely test their bulls for Johne's disease and to remove for sale all semen from bulls found to be infected. All semen used in program herds must be processed according to Certified Semen Services standards.

**Changes in Herd Status**

**Herd additions** : The status of the herd will revert to the status of the lowest animal if Program herd addition requirements are not met.

**Change of ownership**: herd status is determined by the status of the lowest herd member. Therefore, when ownership of a herd or part of the herd changes and no new animals are added to the herd, the herd level remains the same. When ownership of a herd changes and the herd is commingled with another herd or herds, the resulting new herd shall be assigned the lowest level and achievement year of the herds combined to make the new herd.

## Appeal Process

**Appealing ELISA positive results**: status of herds with ELISA positive test(s) is "suspended pending confirmation". Producers may elect to either leave the program or confirm the test results.

Confirmation of ELISA-positive cattle will be done by fecal culture. Fecal culture must be submitted within 45 days of notification of ELISA results. If found to be culture-negative, the herd may advance or retain its status Level. Additionally, the animal(s) must be included in the next round of Program testing if still resident in the program herd.

**Appealing Fecal culture positive results**: fecal culture-positive cattle will be assumed to be infected with *M. paratuberculosis* and the herd will have its status revoked. Herds may reenter the program at Level 1 by following program standards.

If the animal owner wishes to appeal this decision, the herd is assigned a status of "suspended pending appeal". Appeal may be made using one of three means of proving the animal in question is not infected:

1. Necropsy of the animal with culture and histopathology of at least the ileum, mesenteric lymph node and ileocecal lymph node **-or-**
2. Biopsy of the ileum (full thickness) and mesenteric or ileocecal lymph node with histopathology and culture of the tissues and culture of a fecal sample taken at the time of biopsy **-or-**
3. Six separate fecal cultures from the animal on samples collected not less than 30 days and not more than 45 days apart.

The herd Johne's disease status will be suspended until all testing is completed. Only negative results on all tests on all samples will allow the herd to advance or retain its Program Level.

**Appeal of status for other reasons**: herd owners may appeal any decision or discuss extenuating circumstances that prevent compliance with the program rules to the State Johne's Advisory Committee.

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<sup>1</sup> A sample size of 30 was selected to reduce cost while maintaining acceptable accuracy. [Return to text.](#)

<sup>2</sup> A history of Johne's disease freedom for five years prior to program entry adds sufficient confidence to allow Fast Track herds to test 30 animals rather than the statistical subset used in the Standard Track at to obtain Level 3 status. [Return to text.](#)

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## Appendix I Herd Subset Sampling

PLEASE NOTE - The sample numbers below have been calculated based on the following assumptions:

- The cattle to be tested are in 2nd or higher lactation
- For these calculations, 25-percent test sensitivity of the ELISA and 40- percent test sensitivity of the fecal culture were assumed (this were the consensus estimates of the Herd Status Committee for subclinically infected cows in first of higher lactation, and no changes were made for the older population sampled)
- For these calculations, 100-percent test specificity of the ELISA and fecal culture was assumed (given follow-up of all ELISA positives with fecal culture)
- The confidence of detecting infection (at least 1 test-positive cow), if present at a true prevalence of 2 percent, is 95 percent
- Sampling without replacement (hypergeometric distribution)

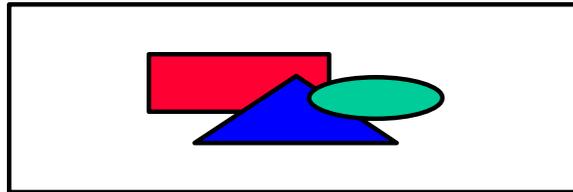
No. cows in herd of 2nd or higher lactation	No. cattle to sample (2nd lactation or higher)	
	ELISA	Fecal culture
< 300	test all	test all
400	test all	313
500	test all	324
600	531	332
700	540	338
800	547	342
900	552	345
1000	580	360

Note: In smaller herds, all cattle second or higher lactation must be tested. In herds with fewer than 30 second and higher lactation animals, first lactation animals must also be tested.

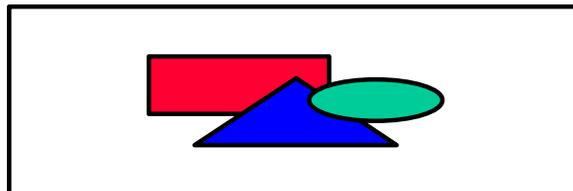
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### Appendix II

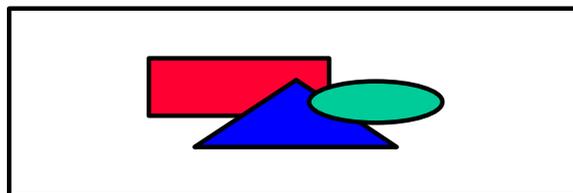
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Go to [key to charts](#)

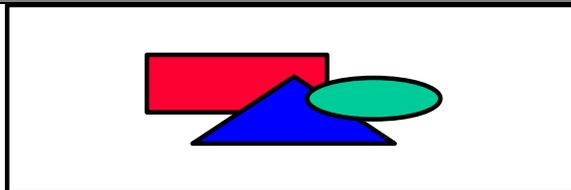


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- 
- [1998 Report](#) of the Committee on Johne's Disease.
  - [Members](#) of the Committee on Johne's Disease.
  - [Resolution No. 28](#) of the Committee on Johne's Disease regarding ELISA test for Johne's disease.
  - [Resolution No. 29](#) of the Committee on Johne's Disease regarding ELISA test for Johne's disease.

- [Resolution No. 30](#) of the Committee on Johne's Disease regarding Johne's disease pilot project.
- [Resolution No. 31](#) of the Committee on Johne's Disease regarding Johne's disease strategic plan.
- [Resolution No. 32](#) of the Committee on Johne's Disease regarding Johne's disease educational plan.
- [Resolution No. 33](#) of the Committee on Johne's Disease regarding Johne's disease herd-status program.
- [Resolution No. 34](#) of the Committee on Johne's Disease regarding Johne's disease pathogen-free embryos.
- [Resolution No. 36](#) of the Committee on Johne's Disease regarding Johne's disease funding for laboratories.
- [Resolution No. 37](#) of the Committee on Johne's Disease regarding Johne's disease funding.

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Appendix 2 (b)

# **National Johne's Disease Program**

## **Standard Definitions and Rules**

**for**

**Cattle**

and for goats, deer and camelids  
infected with cattle strains of *Mycobacterium paratuberculosis*

Standing Committee on Agriculture and Resource Management

**Second Edition**

July 1999

ISBN 1876714 00 X

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## INTRODUCTION

These Standard Definitions and Rules (SDRs) comprise nationally accepted standards and practices upon which the States and Territories formulate disease control programs to suit their circumstances, and have been prepared by the Standing Committee on Agriculture and Resource Management (SCARM) through the Veterinary Committee (VC).

The SDRs are designed to assist disease control in a nationally coordinated manner. They complement the Rules and Guidelines of the Australian Johne's Disease Market Assurance Programs for Cattle (CattleMAP) and other eligible species, which have been designed to provide a degree of assurance for the sale and movement of animals from herds which have a low risk of infection. State programs are designed to control disease in known infected herds. The SDRs also outline criteria for control of movements between zones with regards to JD status.

These SDRs refer to other authoritative documents including the Australian Standard Diagnostic Techniques (ASDTs) and the CattleMAP, AlpacaMAP and GoatMAP. The relevant definitions used in the MAPs are consistent with the SDRs.

Detailed operating procedures developed for implementation of JD control programs are the responsibility of the animal health authorities in each state and territory who can provide advice as to the interpretation of this document.

It is intended these definitions and rules will be progressively reviewed in the light of anticipated progress with existing State/Territory Johne's disease (JD) control programs and the MAPs, and in response to improvements in scientific knowledge and understanding of the disease in the various susceptible species.

### **Note:**

1. These SDRs relate only to animals, herds and properties infected or suspected to be infected with the **bovine** strains of *M. paratuberculosis*. Bovine Johne's disease is believed to be a different disease entity to that in sheep under Australian grazing conditions. A review of the cross-infectivity of strains of *M. paratuberculosis* was recently conducted by Veterinary Committee, which concluded that cross infection between sheep and cattle in Australia is considered to be a rare event on the basis of current information. However where JD is suspected in any animal species in direct or indirect contact with another species that is known to be infected, the case(s) should be carefully investigated and typing undertaken to determine the strain of *M. paratuberculosis* causing the infection. Separate SDRs are in place for ovine Johne's disease.
2. For the purposes of these SDRs, species susceptible to infection with bovine strains of *M. paratuberculosis* are cattle, goats, deer and camelids.
3. The traditional certification that JD is not known or suspected to have occurred in a herd for 5 years has been abandoned as a basis for defining a non-assessed herd. However, because of the variable availability of records on which to base historical certification, it has been agreed as an interim measure that 1 January 1991 be used as a basis for time limiting some aspects of the criteria for determining herd status.

## LIST OF ACRONYMS

AAHC	Australian Animal Health Council Ltd
AlpacaMAP	Australian Johne's Disease Market Assurance Program for Alpaca
AQIS	Australian Quarantine and Inspection Service
AGID	Agar Gel Immuno-Diffusion Test
ASDTs	Australian Standard Diagnostic Techniques
BJD	Bovine Johne's disease
CattleMAP	Australian Johne's Disease Market Assurance Program for Cattle
CVO	Chief Veterinary Officer
DB	Disbanded
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immuno-sorbent Assay
GoatMAP	Australian Johne's disease Market Assurance Program for Goats
HC	Health Certificate
IN	Infected
IS900	Insertion Sequence 900
JD	Johne's Disease
MN	Monitored Negative
MN1,2	Monitored Negative in a numbered series
NA	Non Assessed
OJD	Ovine Johne's disease
PDEP	Property Disease Eradication Plan
PCR	Polymerase Chain Reaction
RD (1,2)	Restricted (no. of consecutive negative herd tests)
SCARM	Standing Committee on Agriculture and Resource Management
SDRs	Standard Definitions and Rules
SU	Suspect
VVD	Voluntary Vendor Declaration
CVD	Compulsory Vendor Declaration

## PART 1: DEFINITIONS

### 1.1 Miscellaneous

#### 1.1.1 Johne's disease (JD)

For the purposes of this document, unless otherwise stated, "Johne's disease" is deemed to be caused by infection with bovine strains of the organism *Mycobacterium paratuberculosis*

#### 1.1.2 Infected animal

A "Johne's disease infected animal" is one confirmed as infected by histopathological examination, culture of faeces or tissues or other definitive tests conducted in accordance with the ASDTs for bovine Johne's disease, and in accordance with any provisions in the relevant appendix for that species.

Where infection is detected in a herd for the first time, a range of definitive tests may be applied and interpretation based on the results of all tests conducted.

In a known infected herd, a reactor or an animal showing clinical signs consistent with JD may be deemed by the CVO to be infected without further testing.

#### 1.1.3 Suspect animal

An animal may be classified as suspect if it has:

- Clinical signs consistent with a diagnosis of JD, which remain uninvestigated;
- Gross post mortem lesions consistent with JD;
- Been in direct contact with an infected animal at a susceptible age;
- Been run on contaminated land at a susceptible age;
- Reacted to a test but not been subject to a follow-up definitive test in accordance with these SDRs; or
- Originated from an IN or SU herd.

#### 1.1.4 High risk animals

Animals in an infected herd which the CVO considers on epidemiological grounds to be at particular risk of being infected, and which should be preferentially culled. The following animals may be considered as high risk animals:

- Dam, progeny and maternal siblings of clinical cases;
- Dam and maternal siblings of infected animals;
- Peers of clinical cases (ie cohorts reared with infected animals);
- Animals exposed at a susceptible age to clinical cases or to highly contaminated land;
- Animals introduced from the same source as the infected animal/s;
- Animals, which when at a susceptible age, cannot or could not be prevented from grazing contaminated land;
- Groups or classes of animals in a herd which have been identified as high risk through the results of herd testing.

Note: it is likely that most animals in endemically infected herds would initially be considered as high risk animals, depending on the time that BJD was introduced into the herd. They may be subsequently reclassified after further investigations."

### **1.1.5 Progeny**

Progeny is an animal physically born of a dam. Dam includes a surrogate mother, ie embryo recipient, but excludes an embryo donor.

### **1.1.6 Contaminated land**

Land, including yards, cattle sheds, loading ramps etc, contaminated with the faeces of an infected animal or herd, and which has not been decontaminated.

### **1.1.7 Decontaminated land**

Contaminated land which has been decontaminated according to the procedures described in section 2.10.2.

### **1.1.8 Approved veterinarian**

A veterinarian approved by the CVO to carry out JD tests and to manage JD programs in herds. This approval will involve specific duties described under specific programs following appropriate training and accreditation.

### **1.1.9 Approved laboratory**

A veterinary laboratory approved by the CVO to carry out diagnostic tests for the identification of JD in livestock.

### **1.1.10 Approved Control Program**

An official control program developed with the owner, and approved by the CVO, to prevent or minimise the spread of infection within the herd and to other herds. As a minimum, approved control programs must be based on the implementation of herd management procedures addressing all of the following issues :

- preventing spread of infection to other farms;
- identification of animals at high risk, for preferential culling for slaughter;
- husbandry and herd management to prevent infection of replacements and introductions;
- control of dairy effluent discharges (where applicable);
- maintenance of accurate breeding records and permanent cattle identification.

### **1.1.11 Approved Test and Control Program**

An official control program, approved by the CVO, which incorporates all the elements of an *approved control program*, with the addition of a whole herd testing and culling program, and controls over introduction of susceptible species.

### **1.1.12 Approved property disease eradication program (PDEP)**

A program to eradicate Johne's disease from a property, which is approved by the CVO and is based on either an official, audited herd testing and management program (in accordance with the Appendix for that species), or destocking all susceptible species in an infected herd with subsequent management of the land to ensure it is decontaminated (in accordance with section 2.10)

### **1.1.13 Approved monitoring program**

An approved monitoring program is one approved by Veterinary Committee, and will vary in nature and extent in different zones. (see 2.14.1)

**1.1.14 Approved fence**

A fence or other physical feature approved by the CVO as providing assurance that all cattle or other susceptible species in the herd are under effective control and are maintained as a discrete unit.

**1.1.15 Approved barrier**

A physical separation approved by the CVO which minimises the risk of environmental spread of infection.

**1.1.16 Susceptible species**

For the purpose of these SDRs, cattle, deer, goats and camelids are susceptible to infection with bovine strains of *Mycobacterium paratuberculosis*.

**1.1.17 Susceptible animals**

Cattle are usually infected as calves and, for the purposes of these SDRs and the CattleMAP, cattle over the age of 12 months are considered to be at very low risk of becoming infected. Deer, goats and camelids are considered to be susceptible at any age.

#### **1.1.18 Herd**

A herd is a group of animals of a susceptible species which is maintained as a separate and discrete unit in terms of physical contact with other susceptible species by an approved fence or barrier to the satisfaction of the CVO.

#### **1.1.19 Breakdown**

The occurrence of JD infection in any herd of MN1/TN1 status or better under the CattleMAP, or any herd in a Protected or Free Zone.

#### **1.1.20 CVO**

The person appointed as the Chief Veterinary Officer, or Chief Inspector of Stock or other equivalent title as the case may be, under legislation for the control of Johne's disease in that State or Territory, or the person for the time being having the delegated authority of that office.

#### **1.1.21 Quarantine**

An order or written undertaking empowered by legislation restricting susceptible species to a certain location and requiring authorisation for movement to and from that location.

#### **1.1.22 Traceback**

The identification of the property or properties of origin of animals.

#### **1.1.23 Traceforward**

The identification of the place of destination of animals.

#### **1.1.24 Notification**

Advice by persons in charge of cattle and other susceptible species, meat inspectors, veterinarians, or approved laboratories of JD infection or suspicion of JD infection in accordance with the legislative requirements of the State or Territory concerned.

#### **1.1.25 Reactor**

An animal which has a positive reaction to an approved immunological test for Johne's disease.

### **1.2 Zones**

Areas declared by legislative or administrative action to enable the exclusion, control or eradication of JD infection.

### **1.3 Approved Tests**

Approved tests for JD are the techniques recommended and documented, and as modified from time to time, in ASDTs approved by SCARM and Veterinary Committee or in special circumstances approved by Veterinary Committee.

Approved Tests for diagnosing bovine JD include:

#### **1.3.1 Clinical examination**

Assessment of the history and clinical features necessary to make a presumptive diagnosis or a possible differential diagnosis.

### **1.3.2 Post-mortem examination**

Post-mortem is the examination of a carcass for JD as prescribed in the ASDTs.

### **1.3.3 Histopathology Examination**

Histopathology examination consists of microscopic examination of tissue samples as prescribed in the ASDTs.

### **1.3.4 Bacteriological methods**

Culture of faeces or tissues using bacteriological methods as prescribed in the ASDTs.

### **1.3.5 DNA detection using polymerase chain reaction (PCR)**

Examination of bacterial culture media, faeces, tissues, blood, milk or other material to detect the presence of the DNA insertion sequence according to methods as prescribed in the ASDTs.

### **1.3.6 Immunological tests**

Approved tests for the immunological diagnosis of JD are detailed in the appendix for each species and may include the absorbed Enzyme-Linked Immuno-sorbent Assay (Absorbed ELISA), Agar Gel Immuno-diffusion (AGID) test or any other approved test.

## **1.4 Screening and definitive tests**

### **1.4.1 Screening Test**

A test that is used, mainly on a large number of animals, to identify animals that are to be tested by a definitive test. An approved screening test may detect immunological, bacteriological or molecular evidence of infection.

### **1.4.2 Definitive Test**

A test which provides a definitive diagnosis of JD infection. The definitive tests for bovine JD are histopathological examination of tissues, bacteriological culture of tissues and/or faeces, molecular tests such as DNA detection by PCR, conducted in accordance with the ASDTs, or any other test approved as a definitive test by Veterinary Committee.

## **1.5 Herd test**

Test of all, or a statistically valid sample of, animals in a herd that have reached the eligible age for that species and for the test being used (see Appendices).

Where a herd test comprises a screening test, the herd test is not complete until the reactors have been followed up using a definitive test.

### **1.5.1 Herd Test for infected herds**

A test of all animals of eligible age for the test being used in an infected herd.

### **1.5.2 Market Assurance Program Test**

A test of all, or a statistically valid sample of, animals in a herd in accordance with the requirements of the CattleMAP, or other relevant Market Assurance Program.

### **1.5.3 Vendor Declaration Test**

A veterinary assessment of a herd using clinical, immunological and/or histopathological examinations, conducted for the purposes of supporting a Vendor Declaration, and approved by Veterinary Committee.

**1.6 Movement test**

A test of individual animals within a prescribed period prior to movement of those animals between zones of different status.

The period may be prescribed by the State/Territory or zone of destination, or will otherwise be 30 days.

**1.7 Miscellaneous test**

Testing of some or all susceptible animals in a herd for purposes not primarily related to the conduct of either a disease control or surveillance program or a Market Assurance Program (eg. for export, introduction, show, sale, etc).

**1.8 Diagnostic test**

Testing of one or more animals in a herd for JD in connection with the investigation of a disease problem.

**1.9 Positive herd test**

This is a herd test at which one or more infected animals are detected.

**1.10 Negative herd test**

A herd test at which no infected animal is detected.

**1.11 Vaccine**

A vaccine for Johne's disease approved by Veterinary Committee, AQIS and the National Registration Authority.

**1.12 Herd status**

**1.12.1 Assessed status**

A herd status, based on an objective assessment, assigned under the Rules and Guidelines of a Market Assurance Program (MAP) for the species concerned.

**1.12.2 Monitored Negative (MN) and Tested Negative (TN1)**

Monitored Negative 1, 2 or 3 (MN1, MN2 or MN3) are assessed herd status under the CattleMAP. Tested Negative 1, 2 or 3 (TN1, TN2 or TN3) are assessed herd status under the original NJDMAP, now superseded by the CattleMAP. TN and MN herds of the same level are of equivalent status.

**1.12.3 Non-Assessed (NA)**

A non-assessed herd is one

- with no history of JD or where any suspicion of infection has been resolved to the satisfaction of the CVO; or

- in which the last confirmed case was prior to 1 January 1991 and has been the subject of an official *approved control program* to the satisfaction of the CVO, and which has not been assessed under an approved MAP for that species. A herd status of NA should not be taken to imply freedom from JD.

#### **1.12.4 Suspect (SU)**

A suspect herd is one where the CVO determines that there is **sufficient epidemiological evidence** to classify the herd as Suspect such as where:

- a herd has a history of infection since 1 January 1991, but more than 5 years ago, and has been subject to an *approved control program* to the satisfaction of the CVO; or
- a herd had its last confirmed case of JD prior to 1 January 1991 and has not been subject to, or has not satisfactorily complied with, an *approved control program*; or
- a herd containing susceptible animals has been grazed on contaminated land; or
- there is traceback or traceforward evidence of contact with an infected herd, or
- reactors have been detected in a herd or movement test but have not been investigated; or
- a herd contains animals with clinical signs consistent with Johne's disease that remain uninvestigated; or
- eradication of past infection has been attempted, but not under an approved property disease eradication program; or
- an infected animal has been introduced and the CVO is satisfied that there has been little or no potential for transmission of infection to the herd, or all exposed susceptible animals have been culled for slaughter ; or
- a herd contains infected cattle or camelids in which sheep strains of *M. paratuberculosis* have been identified, and there is no evidence of spread to other susceptible species in the herd or investigation of the herd is incomplete.

Suspect status can be resolved by the CVO obtaining evidence to remove the suspicion of infection from the herd. One or more herd tests may be a necessary component of the process to remove suspicion.

#### **1.12.5 Infected (IN)**

An infected herd is one in which, since 1 January 1991:

- an infected home-bred animal has been found, or
- an infected animal has been introduced, and there has been potential for transmission of infection within the herd or the potential for transmission cannot be ruled out, and all high risk animals cannot or have not been identified and isolated from the herd, or
- infection by sheep strains of *M. paratuberculosis* has been identified and there is evidence of spread of such infection among cattle or other susceptible species in the herd, and which has not been the subject of an approved property disease eradication program since the most recent detection of infection.

#### **1.12.6 Restricted (RD1, 2)**

A restricted herd is a previously infected (IN) herd which is undertaking an *approved test and control program* (in accordance with the relevant appendix), and which has achieved one or more negative herd tests commencing at least 12 months after the last known infected animal was removed from the herd. The RD number refers to the number of consecutive negative herd tests which have been achieved.

#### **1.12.7 Disbanded (DB)**

A herd for which records are held but the herd no longer exists.

## PART 2: RULES

### 2.1 Declaration of zones

The Veterinary Committee on the recommendation of a CVO may declare a Residual, Control or Protected Zone if satisfied that all requirements for that zone status have been met. These zones are presented below in an order which represents a decreasing risk of JD infection being present within, or spreading from, a zone. To the maximum extent possible, zones should be declared and implemented in a nationally coordinated and orderly manner to ensure artificial and unnecessary barriers to trade are not imposed on industry.

#### 2.1.1 Residual zone

- JD infection is endemic.
- No or minimal regulatory measures are enforced.
- No restrictions on movement into zone.
- Vendor/owner declarations may be used for voluntary movement controls.
- Vaccination may be approved by the CVO.

#### 2.1.2 Control zone

- JD is notifiable and tracing of high risk animals is undertaken.
- An approved monitoring program is in operation.
- There may be restrictions on movement into the zone from residual zones.
- All IN and RD herds are subject to official control measures to minimise spread of infection within and between herds.
- Vendor/owner declarations are encouraged for voluntary movement controls.
- Vaccination may be approved by the CVO.

#### 2.1.3 Protected zone

- JD occurs only sporadically.
- JD is notifiable.
- An approved monitoring program is in place and operating, and there is ongoing evidence to justify the zone's status.
- Reports of activities and outcomes are presented annually (or as otherwise agreed) to Veterinary Committee.
- Thorough tracing of all movements from IN and RD herds, and investigation of all known or suspected infection is undertaken.
- All IN, RD or SU herds are placed in quarantine. Approved eradication or control measures are enforced in IN and RD herds, and SU herds are actively investigated to determine whether infection is present.
- Movements into the zone from zones of lower status must meet prescribed health standards for JD.
- An advisory program is in place to ensure there is producer awareness about the disease and its prevention, and that there are movement requirements for introducing cattle into the zone.
- There are no vaccinated animals, and vaccination is not permitted.

#### 2.1.4 Free zone

A Free Zone may be declared by SCARM on the recommendation of Veterinary Committee, when:

- No herds are IN, RD or SU and the population of susceptible species in the zone has been assessed epidemiologically for the absence of JD to the satisfaction of Veterinary Committee.
- JD is notifiable.
- An approved monitoring program is in place and operating.
- There is ongoing evidence to justify the zone's status.
- Reports of activities and outcomes are presented annually (or as otherwise agreed) to Veterinary Committee.
- Thorough tracing and investigation of all known or suspected infection is undertaken;
- All Breakdowns and SU herds are placed in quarantine. Eradication measures are enforced in IN and RD herds, and SU herds are actively investigated to determine whether infection is present.
- Movement from zones of lower status must meet prescribed health standards for JD.
- An advisory program is in place to ensure there is producer awareness about the disease and its prevention, and that there are requirements for introducing cattle into the zone.
- There are no vaccinated animals, and vaccination is not permitted.

Discovery of infection in a Free Zone will not initially effect its classification.

## **2.2 Testing for JD**

### **2.2.1 Performance of tests**

Laboratory testing must be performed at an approved laboratory.

An approved laboratory is required to keep the records of all testing carried out for JD for a minimum period of 5 years and to provide information to an authorised officer as required by the audit process.

### **2.2.2 Reporting of tests**

Interpretation and reporting of tests will be done according to the ASDTs.

All testing for JD must be reported to the CVO of the State/Territory in which the herd is located.

### **2.2.3 Retesting of reactors**

Retesting of reactors with the same immunological test is **only** permitted when:

- the laboratory reports inconclusive results;
- when a further sample is specifically requested by the laboratory;
- when conducted in association with follow-up definitive testing of the reactor; or
- to clarify the identity of reactors.

### **2.2.4 Initial infection**

When an animal is being slaughtered to establish a diagnosis in a herd in which JD has not been previously confirmed, attempts should be made, in addition to histopathology, to culture the causative organism from tissues.

## **2.3 Fate of reactors**

The fate of reactors depends on:

- conditions set for JD control in that species and the zone;
- whether or not the herd is actively testing in a market assurance program;

- the previous history of infection in the herd.

Failure to meet the conditions for follow-up of reactors may affect the status of the herd or the action taken in the herd, and may affect the status of the Zone.

The appropriate appendix for each species provides specific details.

## **2.4 Equivalence of status**

### **2.4.1 Herd status**

Herds of the same assessed status will be regarded as equivalent.

Herds with a TN status will be regarded as equivalent to herds with an MN status of the same level. When a herd with a TN status progresses it will change to the next highest MN status (eg MN2 or MN3)

Herds in a Protected Zone which have no history of JD infection have a status equivalent to MN1 for the purposes of movement to other herds or zones. Progression beyond this status requires formal entry to the CattleMAP (or other relevant MAP for the species concerned) and compliance with the rules and guidelines for herd status progression under that program.

In a Protected Zone, herds already assessed, or becoming assessed, under a MAP will be granted one status credit (ie accelerated progression in the MAP) for the zoning of their region as Protected, ie. assessed herds in Protected Zones will have an initial status of MN2.

Herds in a Free Zone will have status equivalent to the highest status in the CattleMAP, ie MN3

## **2.5 Movement of susceptible species**

Certification and/or vendor declarations should progressively move to being based on an assessed zone and/or herd status.

### **2.5.1 For the purposes of movement**

Where movement of animals is based on a history of no knowledge or suspicion of JD, the certifying authority must consider all properties on which the animal(s) have resided or grazed whilst at a susceptible age. Due recognition must be given to properties which have completed an approved property disease eradication program as defined by these SDRs when the history of properties is required to be certified.

### **2.5.2 Movement for finishing and/or slaughter and/or short term grazing**

The CVO in the state of destination of a proposed movement not permitted under these rules may specify conditions to allow movements for

- immediate slaughter,
- to a feedlot approved for that purpose and subsequent slaughter,
- to allow the entry of desexed cattle from NA herds,
- for short term grazing where there are no other susceptible species on the land,
- routine movements between properties in common ownership where the movement is considered to present a low risk of spreading infection,
- temporary relocation of animals under exceptional circumstances such as flood or fire with appropriate restrictions placed on subsequent movements, or

- other movements considered by the Chief Veterinary Officers of the States concerned to present a low risk of spreading infection.

**2.5.3 Phased introduction**

For movement of animals to Protected or Free Zones, Veterinary Committee may decide that, when sufficient herds in other zones have been assigned an MN/TN or other assessed status, animals must only originate from herds with an assessed status.

**2.6 Restrictions on movement within zones**

**2.6.1 Free or Protected Zone**

The movement of susceptible species from an IN, RD or SU herd will be restricted by quarantine, and all such animals will be moved for slaughter only.

**2.6.2 Control Zones**

Movements of animals from IN and RD herds will be subject to control as determined by the CVO. High risk animals from IN and RD herds will be moved for slaughter only. (See 2.8)

**2.6.3 Residual Zones**

Regulated movements within a Residual Zone may be imposed under the direction of the CVO.

**2.7 Movement between zones**

Movement of animals between zones is permitted according to the following schedule:

STATUS OF ZONE OF ORIGIN	STATUS OF ZONE OF DESTINATION			
	Residual	Control	Protected	Free
Residual	VVD	VVD or CVD	CVD or HC	HC
Control	VVD	VVD or CVD*	CVD or HC	HC
Protected	VVD	VVD	VVD	CVD or HC
Free	VVD	VVD	VVD	VVD

- VVD is the Voluntary use of a Vendor or owner Declaration.
- CVD is the Compulsory use of a Vendor or owner Declaration, as defined under State/Territory legislation.
- CVD\* denotes use of a CVD only with the agreement of the CVOs of both Control Zones
- HC is a Health Certificate defined under state or territory legislation, certified by a person authorised by the CVO.

For movement into a Protected or Free Zone, a CVD or HC shall require:

- No knowledge or suspicion of JD (NA herd), plus an approved movement test, OR,
- TN/MN or other assessed status under the CattleMAP or other relevant MAP for the species.

Herds in a Protected or Free Zone which do not have a history of disease will be considered to have a status equivalent to MN1 or MN3 respectively for the purposes of movement to other herds or zones.

## **2.8 Movement of animals from infected herds**

In Control Zones, high risk animals shall not be moved from IN and RD herds except for direct consignment for slaughter at an abattoir, slaughterhouse or knackery, or to approved slaughter-only sales. The CVO may also approve movement of such animals to other IN or RD herds.

In Protected and Free zones, quarantine shall be imposed on IN and RD herds and no animals shall be moved from such herds except for slaughter.

## **2.9 Movement from suspect herds**

Movement controls over animals from suspect herds in Residual or Control zones may be applied at the discretion of the CVO. In Protected and Free zones, quarantine shall be imposed on SU herds.

## **2.10 Destocking and decontamination of land**

### **2.10.1 Destocking**

All susceptible species in the infected herd must be removed from the land.

### **2.10.2 Decontamination of land**

Land will be deemed to be no longer contaminated if it remains destocked of all susceptible species for 12 months, or any alternative period determined by the Veterinary Committee.

The CVO may, however, allow grazing of susceptible species which are not regarded as shedding infection during the decontamination period. Susceptible animals must not be grazed for >12 months (or with CVO approval in low contamination situations, up to 18 months for cattle), and then must be consigned directly for slaughter at the completion of the grazing period.

Land may be also decontaminated by one or more of the following procedures:

- physical or chemical treatment to the satisfaction of the CVO;
- completion to the satisfaction of the CVO of an approved disease eradication program based on herd management and testing.

Restocking for unrestricted grazing with a new herd of susceptible species may occur once the land has been decontaminated. The new herd shall assume a status of the originating herd that has the lowest status. A new herd may be allocated an assessed status in line with the minimum status of contributing herds, provided all the requirements for establishment of a new herd under the CattleMAP or other MAP for that species are met.

## **2.11 Register of herd status**

In zones where JD is notifiable, the CVO will ensure that a register of IN, RD and SU herd status and changes in those status is maintained.

## **2.12 Disease notification and tracing**

### **2.12.1 Suspicion of infection**

Suspicion or knowledge of infection must be notified to the CVO in accordance with the statutory disease notification requirements in force for that State/Territory.

#### **2.12.2 Interstate notification**

Where on the basis of tracing, JD is suspected to occur in, or may have spread to, another State/Territory, the CVO of that state/territory must be notified in writing within 30 days of its being known.

#### **2.12.3 Notification to owner**

Where a herd has been determined to have a status of Infected, Restricted or Suspect, the CVO will ensure that the owner is notified in writing of the herd status, and of changes in the herd status, within 30 days of that determination.

### **2.13 Animal identification**

#### **2.13.1 Herds in approved test and control programs**

All animals in a herd in an approved test and control program must be permanently and uniquely identifiable in such a manner that their identity can be easily verified by an official or approved veterinarian. The identification system is to be approved by the CVO.

#### **2.13.2 Tested Animals**

Any animal subject to a test for JD must be individually identified at the time of sample collection, and until all testing is completed.

#### **2.13.3 Infected animals**

Infected animals must be permanently identified in such a manner that their identity can be easily verified by animal health officials or approved veterinarians at all times prior to their disposal.

#### **2.13.4 Vaccinated animals**

Vaccinated animals must be permanently identified in a manner approved by Veterinary Committee.

### **2.14 Monitoring for infection**

#### **2.14.1 Approved monitoring program**

An Approved Monitoring Program is used to detect infected herds in a defined area or zone.

The methods and intensity of monitoring to be applied in a particular zone will be determined by the Veterinary Committee.

An Approved Monitoring Program may include:

- tracing from infected herds;
- investigating high risk herds;
- investigating suspect clinical cases using Australian Standard Diagnostic Techniques that are subject to quality assurance programs;
- abattoir or knackery surveillance, or other program to monitor a broad cross section of a zone's herds;
- a targeted advisory program on JD detection; and
- using a data recording system from which tracing information relevant to infected herds can be retrieved efficiently.

An Approved Monitoring Program should give due consideration to the movement of animals into the zone, the degree of risk, zone status, past history, and should be capable of detecting infected herds. In Protected and Free Zones, an Approved Monitoring Program should include a active mechanism to monitor or screen a broad cross section of the zone's herds over time, independent of any tracing activity associated with known infection.

## **2.15 Breakdowns in a Free Zone**

### **2.15.1 Reporting of breakdowns**

All breakdowns in Free Zones are to be reported formally to the Veterinary Committee within 7 days of being confirmed.

### **2.15.2 Infection found in a Free Zone**

Detection of infection in a Free Zone will not initially effect its classification. The CVO shall notify and consult Veterinary Committee on action being taken to eradicate the infection.

In a Free Zone, a herd will be quarantined if the herd status is Infected (IN), Restricted (RD) or Suspect (SU).

Quarantine will be released when the herd status is no longer IN, RD or SU.

The Infected herd shall be subjected to an approved property disease eradication program.

Zone classification will be re-assessed by the Veterinary Committee if the number of Infected or Suspect herds remains unacceptable.

## **2.16 Breakdowns in a Protected Zone**

### **2.16.1 Reporting of breakdowns**

Breakdowns in Protected Zones are to be reported through the National Animal Health Information System, with a report to Veterinary Committee bi-annually.

### **2.16.2 Infection found in a Protected Zone**

Detection of infection in a Protected Zone will not initially affect its classification.

In a Protected Zone, a herd will be quarantined if the herd status is Infected (IN), Restricted (RD) or Suspect (SU). Quarantine will be released when the herd status is no longer IN, RD or SU.

The infected herd shall be subjected to an approved property disease control or eradication program.

Zone classification will be re-assessed by the Veterinary Committee if the number of Infected or Suspect herds remains unacceptable.

## **2.17 Procedure for investigating breakdowns in Protected and Free zones**

The initial investigation should aim to:

- determine the source(s) of infection;

- determine whether infection has spread to other animals in the herd or remains only in introduced animals.

Full traceback and traceforward are to be carried out.

Where it is determined that the suspect or infected animals were born or reared on another property, further tracing shall occur to determine, if possible, the other properties on which the animal(s) had been depastured. Appropriate action should then be taken to determine the status of these herds.

To identify the direct contacts of all infected animals, tracing may have to go back for the full life time of the infected animal(s). If unable to identify the source of the infection, tracing should go back ten (10) years if possible.

Herds which have received animals from the breakdown herd since the infected animals were introduced to it, are to be initially classed as SU.

Traceback herds may be classified as SU until their status, or the source of the breakdown infection, is established.

Where a herd is classified as Suspect, the initial investigation should aim to confirm whether or not infection exists on the property.

Infected and Restricted herds in Free Zones must be subject to an approved property disease eradication program, based either on destocking or an official test and control program. The latter program will be pursued as outlined in section 5.4 of Appendix 1 (for cattle) or in accordance with the relevant appendix for that species until the herd achieves a status equivalent to MN1 status.

#### **2.18 Establishing a new herd**

Where a new herd is assembled from other herds, it shall adopt the status of the originating herd which has the lowest status.

A new herd may be allocated an *assessed* status in line with the minimum status of contributing assessed herds, provided all the requirements for establishment of a new herd under the relevant Market Assurance Program are met.

## PART 3: IMPORTATION OF ANIMALS INTO AUSTRALIA

The negotiation and development of animal importation protocols is the responsibility of the Commonwealth Government through AQIS. The conditions detailed below are considered necessary to support the National Johne's Disease Program, consistent with these Standard Definitions and Rules. The control program is based on a scientific approach and import requirements need to reflect this approach.

### **3.1 Considerations**

Epidemiologically different strains of *M. paratuberculosis* exist in other countries. Introduction of these strains into Australia may seriously hinder the control of ovine and bovine Johne's disease under the current National Johne's Disease Program. Cattle and other susceptible species must be subjected to appropriate importation requirements so as to minimise the risk of introducing further strains of *M. paratuberculosis*.

All susceptible species imported into Australia should be derived from populations or herds where the status of JD has been objectively assessed.

Serological, cell-mediated immune assays and other approved diagnostic procedures alone should not be presumed to accurately define the JD status of an individual animal. Herd and regional status should be used as an indicator of risk.

### **3.2 Conditions for imports**

#### **3.2.1 Status of area or herd of origin**

JD susceptible species shall not be imported for unrestricted entry into the national herd in Australia unless

- the country or area of origin is certified by the national authority to have a status equivalent to Australian requirements for a Protected Zone; or
- the status of the area in which the herd of origin is located and of the herd from which animals will be imported have been assessed by the national authority, and the animals are certified to have met requirements equivalent to those required for entry into a Protected Zone under clause 2.7 of these; or
- the herd of origin is certified by the national authority to have a status equivalent to MN1, MN2 or MN3 under the CattleMAP or an equivalent program.

#### **3.2.2 Vaccination status**

Importation of animals vaccinated against Johne's disease shall not be permitted.

## APPENDIX 1 - CATTLE

### 1. Interpretation of Tests

Decisions on the JD status of an individual animal or of a herd requires consideration of the herd history and local conditions. The results of diagnostic procedures will be reported as described by the ASDTs.

Where JD is suspected in any cattle in direct or indirect contact with another species that is known to be infected, the case(s) should be carefully investigated and typing undertaken to determine the strain of *M. paratuberculosis* causing the infection.

### 2. Herd Test Criteria

#### 2.1 Age eligibility for testing

For the purposes of these definitions, eligible animals for immunological testing are cattle over 24 months of age. There is no age restriction on animals subject to other tests.

#### 2.2 Assessed Herd Status

Assessed herd status are those described in the Rules and Guidelines of the CattleMAP.

### 3. Fate of reactors and effect on herd status

All reactors to JD immunological tests are to be investigated according to the following protocol.

#### 3.1 Fate of Reactors in Protected or Free Zones

A history of the herd should be taken, including any clinical signs suggestive of JD, and the previous movement history of animals into the herd, (in particular the reactors), and in particular introductions from Control or Residual Zones.

##### 3.1.1 Herds with no history

In herds with a history suggestive that the herd is **unlikely** to be infected with JD:

- reactors must be investigated by a **single** faecal culture or by post-mortem and histopathological investigation; and
- the herd's status will not change pending the outcome of the investigation of reactors. However if the reactors are not investigated within one month the herd will be quarantined.

##### 3.1.2 Herds with a history suggestive of infection or exposure

In herds with a history that suggests they **may be infected** with JD (for example, a clinical case suggestive of JD, tracing information suggests that JD may have been introduced, or, according to recognised statistical methods, the proportion of the herd reacting to a herd or sample test suggests that infection is likely):

- All reactors should be examined by faecal culture and/or post-mortem examination and tissue examination as follows:
  - faecal cultured **twice** at an interval of three (3) to six (6) months, or
  - slaughtered with full post-mortem examination and culture and histopathological examination of tissues.
- The herd will be quarantined until the status of reactors is resolved.

### 3.1.3 Herd with a history of infection.

A herd with a history of past infection that has not been resolved will be quarantined until the reactor's status is resolved.

Reactors in such a herd are assumed to be infected unless full post-mortem examination with histopathological and culture examination of tissues is conducted.

## 3.2 Fate of Reactors in Residual or Control Zones

### 3.2.1 Herds with no previous history

The fate of reactors in a herd with no previous history or suspicion of JD depends on whether or not the herd is participating in the CattleMAP

#### (a) Herd not in CattleMAP

Where reactors are detected in a herd in which there is no previous history or suspicion of infection, and which is not undertaking regular herd or sample testing as part of the CattleMAP, the following applies:

All reactors should be examined by faecal culture and/or post-mortem examination and tissue examination as follows:

- faecal cultured **twice** at an interval of three to six months, or
- slaughtered within three months with full post-mortem examination and culture and histopathological examination of tissues.

The status of the herd will be SU until the infection status of the reactors is clarified.

#### (b) Herd in CattleMAP

In the CattleMAP, because the whole herd is being regularly assessed, reactors may be dealt with differently. These principles may be applied at the discretion of the CVO where reactors are detected at whole herd tests and the epidemiological assessment indicates that infection is unlikely.

Where reactors are detected in a herd which has a herd status in the CattleMAP of NA, TN1 or MN1 or better:

- reactors must be investigated but may be examined by a **single** faecal culture or by post-mortem and histopathological examination; and
- the status of the herd in the CattleMAP will be **maintained** pending the outcome of the investigation of the reactors.

The time period within which investigation of reactors is to be undertaken is specified in the CattleMAP.

### **3.2.2 Herds with a previous history or unresolved suspicion of infection**

In a herd with a previous history or unresolved suspicion of infection, faecal culture is not sufficient to determine the status of reactors. Full post-mortem examination with histopathology and culture of tissues must be undertaken on all reactors in such herds.

### **3.2.3 Herds in contact with sheep**

Where the reactors have a history of running with infected sheep, investigations must be capable of detecting and differentiating infection with sheep strains of JD.

If infection with sheep strains is confirmed in any species, the status of sheep running on the same land should be considered Suspect, and investigated in accordance with the SDRs for ovine Johne's disease.

Cattle herds in which sheep strains of *M paratuberculosis* are identified should be assigned a Suspect status, be subject to movement restrictions and investigated to establish the extent of infection and likely epidemiology of the infection in that herd. Action should be taken to control and eradicate the cross-infection.

## **4. Disposal of reactors**

Provided that the necessary samples can be obtained for follow-up testing, reactors may be disposed of by slaughter by:

- destruction on the property under supervision;
- consignment direct to an abattoir for supervised slaughter; or
- consignment to other place approved by the CVO (eg knackery).

## **5. Managing infected or suspect herds**

### **5.1 Epidemiological assessment**

When a herd is detected to be infected, an epidemiological assessment will be undertaken to determine:

- the source of the infection,
- how long it may have been infected,
- which animals or groups of animals are infected,
- which animals or groups of animals are at high risk of being infected,
- what spread may have occurred to other herds.

This assessment may include a herd test of all eligible animals in the herd, including goats, alpaca and deer.

Where the infected animal/s have a history of running with infected sheep, investigations must be capable of detecting and differentiating infection with sheep strains of JD.

### **5.2 Approved Control Programs**

A control program, approved by the CVO, will be developed with the owner to minimise the spread of infection within the herd and to other herds. As a minimum, approved control programs must be based on the implementation by the herd owner/manager of herd management procedures addressing all of the following issues :

- preventing spread of infection to other farms;
- identification of animals at high risk, for preferential culling for slaughter;
- calf husbandry and herd management;
- control of dairy effluent discharges (where applicable);
- maintenance of accurate breeding records and permanent cattle identification.

Management based control programs may include one or more herd tests conducted to assist with the identification of infected or high risk animals and/or groups of animals.

Animals and groups of animals identified as infected and at high risk of being infected will be preferentially culled from the herd for slaughter as agreed under the control program.

### **5.3 Approved Test and Control Programs**

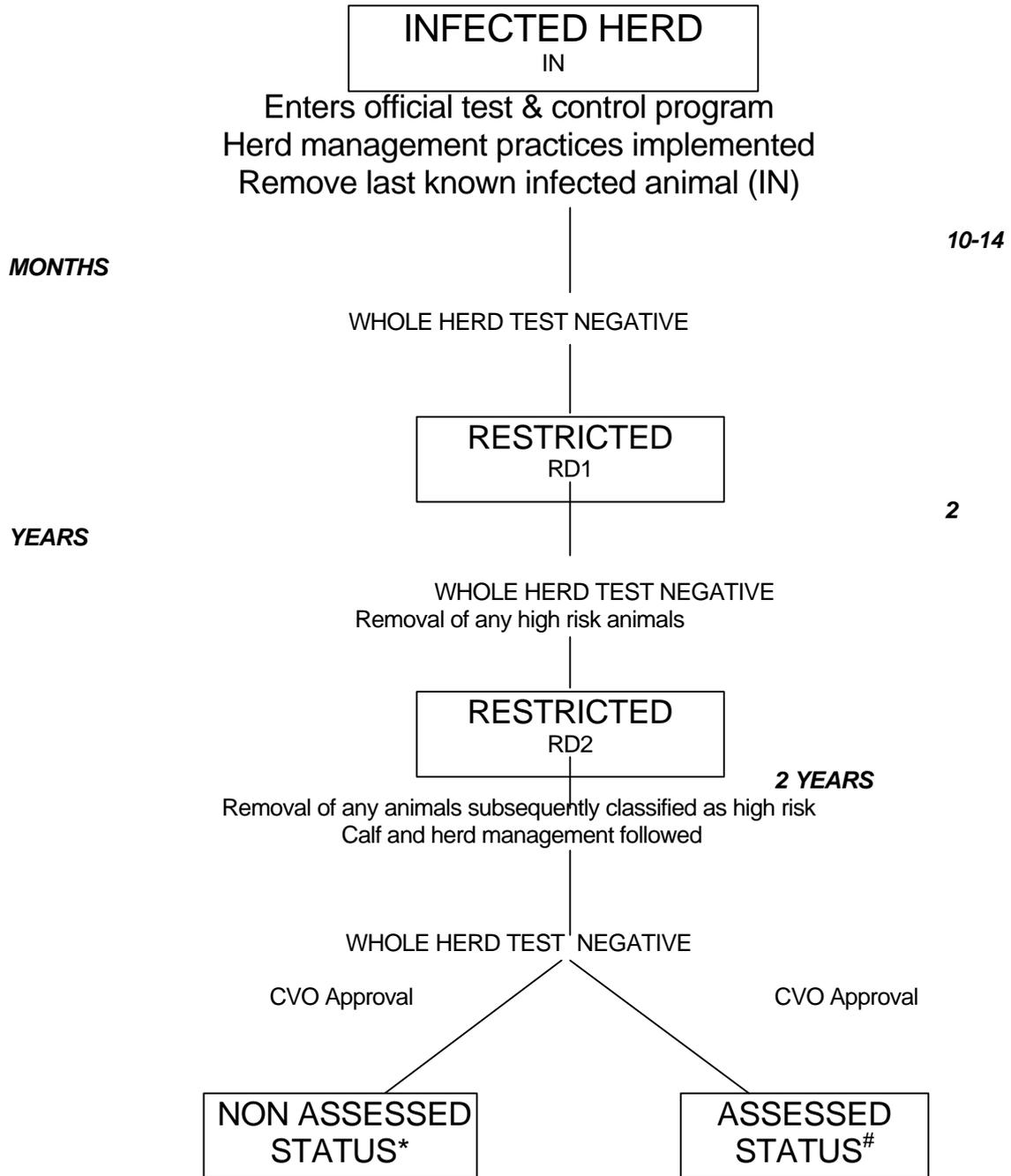
Test and Control programs for infected herds incorporate all the elements of an Approved Control Program as detailed under 5.2, with the addition of a regular whole herd testing and culling program, and controls over introducing cattle. This type of program provides a means to progress the status of participating herds provided the program is under official control and audit. An approved test and control program may form the basis of an approved *property disease eradication program* for JD.

### **5.4 Progression of status for Infected herds**

An Infected herd must undertake an approved test and control program and achieve three (3) negative herd tests at two (2) year intervals after the last known infected animal is removed from the herd before progression to NA or an assessed status will be considered by the CVO for approval. Infected herds undertaking approved test and control programs may progress to Restricted (RD) status after at least one negative herd test of all eligible animals in the herd which will be undertaken not sooner than 12 months after the last known infected animals are removed from the herd.

The following chart summarises the requirements for IN herds to progress through RD status to NA or to an assessed status under a market assurance program.

**HERD STATUS PROGRESSION FOR INFECTED CATTLE HERDS**



\* If no ongoing disease risk management market assurance program in place

# If enterprise enters a program

## APPENDIX 2 - DEER

### Important Note:

There is no evidence of JD in deer in Australia. All species of deer are considered susceptible to infection by bovine strains of *M paratuberculosis*.

Some deer species have been infected with ovine strains of *M paratuberculosis* overseas. Infection of fallow deer with sheep strains has not been recorded. When deer are run in contact with an infected sheep flock, they should be subject to investigation and surveillance in order to determine their infection status and to improve understanding of their role in the epidemiology of OJD. Should cases of JD in deer be found to be a result of infection with ovine strains of *M paratuberculosis*, actions should be taken in accordance with the SDRs for ovine JD.

Where JD is suspected in any animal species in direct or indirect contact with another species that is known to be infected, the case(s) should be carefully investigated and typing undertaken to determine the strain of *M. paratuberculosis* causing the infection.

### **Testing**

The approved laboratory tests for JD, are histopathology, bacteriology, DNA detection and serology.

The tests for detecting disease in deer are one or more of the following.

### **Serology**

The immunological tests approved for the immunological diagnosis of JD in deer are the agar gel immuno-diffusion test and the complement fixation test.

### **Herd Test**

Herd tests of deer consist of bacteriological examination of faeces at minimum intervals of 6 months

### **Animals eligible for Johne's disease testing**

For the purposes of these definitions, eligible animals are all deer over 6 months\* of age except for post mortem examination, bacteriology and histopathological examination where no age restrictions on testing are imposed.

*\*Note : This minimum age is only a suggestion and species-specific market assurance programs, once developed, should be consulted.*

## APPENDIX 3 - GOATS

NB: Goats may be infected with *bovine* or *ovine* strains of *M. paratuberculosis*. In cases where JD in goats is found to be a result of infection with *ovine* strains of *M. paratuberculosis*, actions should be taken in accordance with the SDRs for *ovine* JD.

Where JD is suspected in any animal species in direct or indirect contact with another species that is known to be infected, the case(s) should be carefully investigated and typing undertaken to determine the strain of *M. paratuberculosis* causing the infection.

### **1. Infected animals**

An Infected animal is an animal confirmed as infected by histopathological and/or bacteriological examination.

### **2. Serology**

The approved test for the immunological diagnosis of JD in goats is the agar gel immuno-diffusion (AGID) test.

An absorbed ELISA test for goats is under evaluation and will be an approved test if and when approved by Veterinary Committee.

### **3. Eligible Animals for testing**

For the purposes of these definitions, animals eligible for testing are all goats over 12 months of age\* except for post mortem examination, bacteriology and histopathological examination where no age restrictions on testing are imposed.

*\*Note : This is the minimum age for testing in the current draft market assurance program for goats.*

### **4. Pathways for Change in Herd Status**

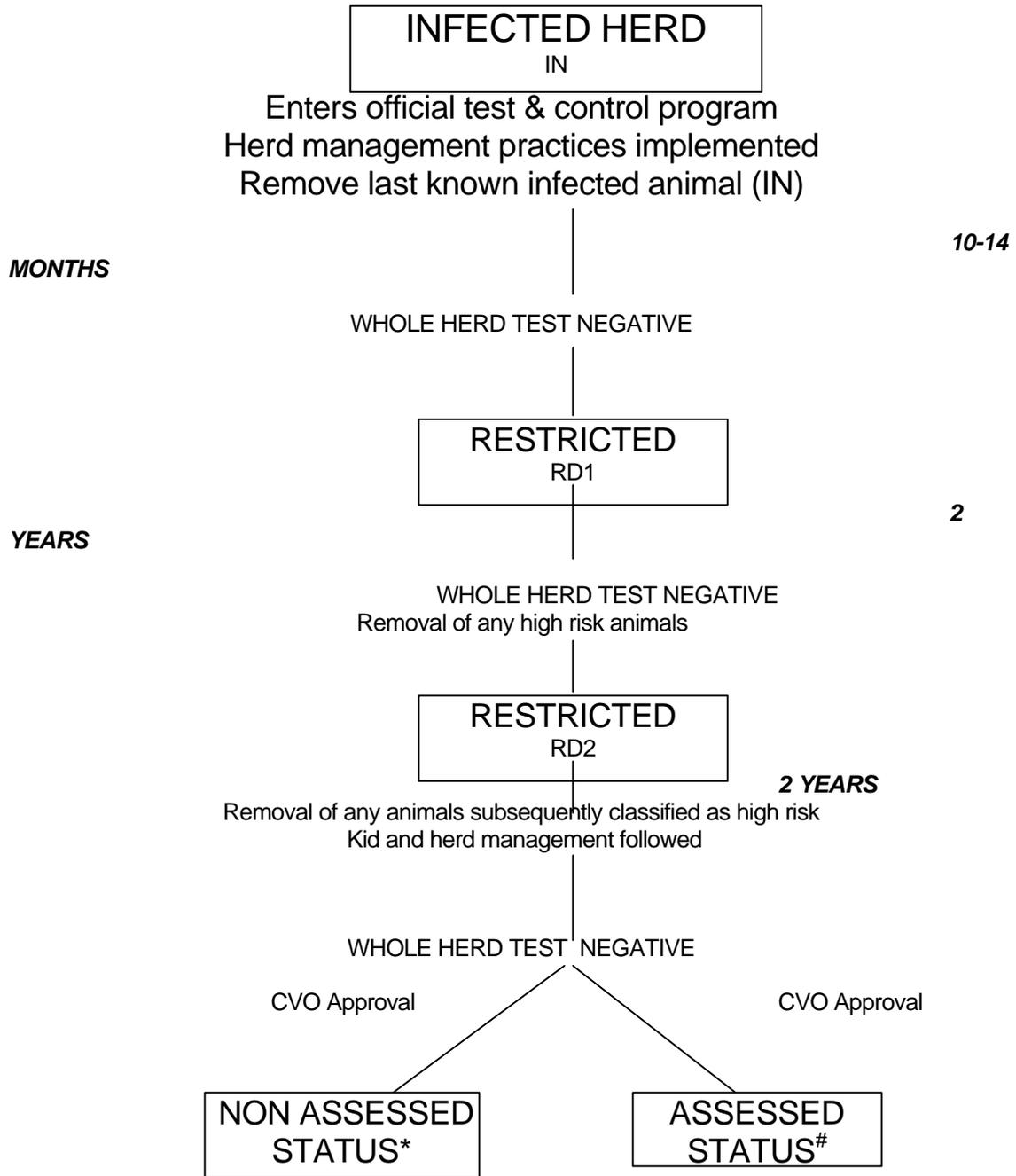
These will be detailed in the Australian JD Market Assurance Program for Goats (GoatMAP).

### **5. Progression of status for Infected herds**

An Infected herd must undertake an approved test and control program and achieve three (3) negative herd tests at two (2) year intervals after the last known infected animal is removed from the herd before progression to NA or an assessed status will be considered by the CVO for approval. Infected herds undertaking approved test and control programs may progress to Restricted (RD) status after at least one negative herd test of all eligible animals in the herd which will be undertaken not sooner than 12 months after the last known infected animals are removed from the herd.

The following chart summarises the requirements for IN herds to progress through RD status to NA or to an assessed status under a market assurance program.

**HERD STATUS PROGRESSION FOR INFECTED GOAT HERDS**



\* If no ongoing disease risk management market assurance program in place

# If enterprise enters a program

## APPENDIX 4 - SOUTH AMERICAN CAMELIDS (ALPACA, LLAMA)

**NB:** JD in South American camelids in Australia to date has been associated with infection by bovine strains of *M. paratuberculosis*. Infection of camelids with sheep strains has not been recorded, and the susceptibility of these species to sheep strains is unknown. When camelids are run in contact with an infected sheep flock, they should be subject to investigation and surveillance in order to determine their infection status and to improve understanding of their role in the epidemiology of OJD. Should cases of JD in camelids be found to be a result of infection with ovine strains of *M. paratuberculosis*, actions should be taken in accordance with the SDRs for ovine JD.

Where JD is suspected in any animal species in direct or indirect contact with another species that is known to be infected, the case(s) should be carefully investigated and typing undertaken to determine the strain of *M. paratuberculosis* causing the infection.

### **1. Infected Animals**

An Infected animal is an animal confirmed as infected by histopathological and/or bacteriological examination.

In a herd undergoing testing in accordance with the AlpacaMAP, and where there is no suspicion of infection with JD or of mis-identification of the animal/s, sample/s or the bacteria, an animal that tests positive to a faecal culture test is classified as infected unless, with CVO approval, it is slaughtered and tested negative by histopathology and tissue culture as prescribed in the ASDTs. However, where infection in a herd is detected for the first time by faecal culture, DNA analysis may be undertaken to confirm the identity of the bacteria in the first instance.

### **2. Herd test (or sample test)**

Testing of all (or a statistically valid sample of all) eligible animals over 12 months of age in the herd by faecal culture or other approved test, as described in the Rules and Guidelines of the AlpacaMAP.

### **3. Eligible Animals**

For the purposes of these definitions, no age restrictions on testing are imposed for post mortem examination, bacteriology and histopathological examination.

### **4. Pathways for Change in Herd Status**

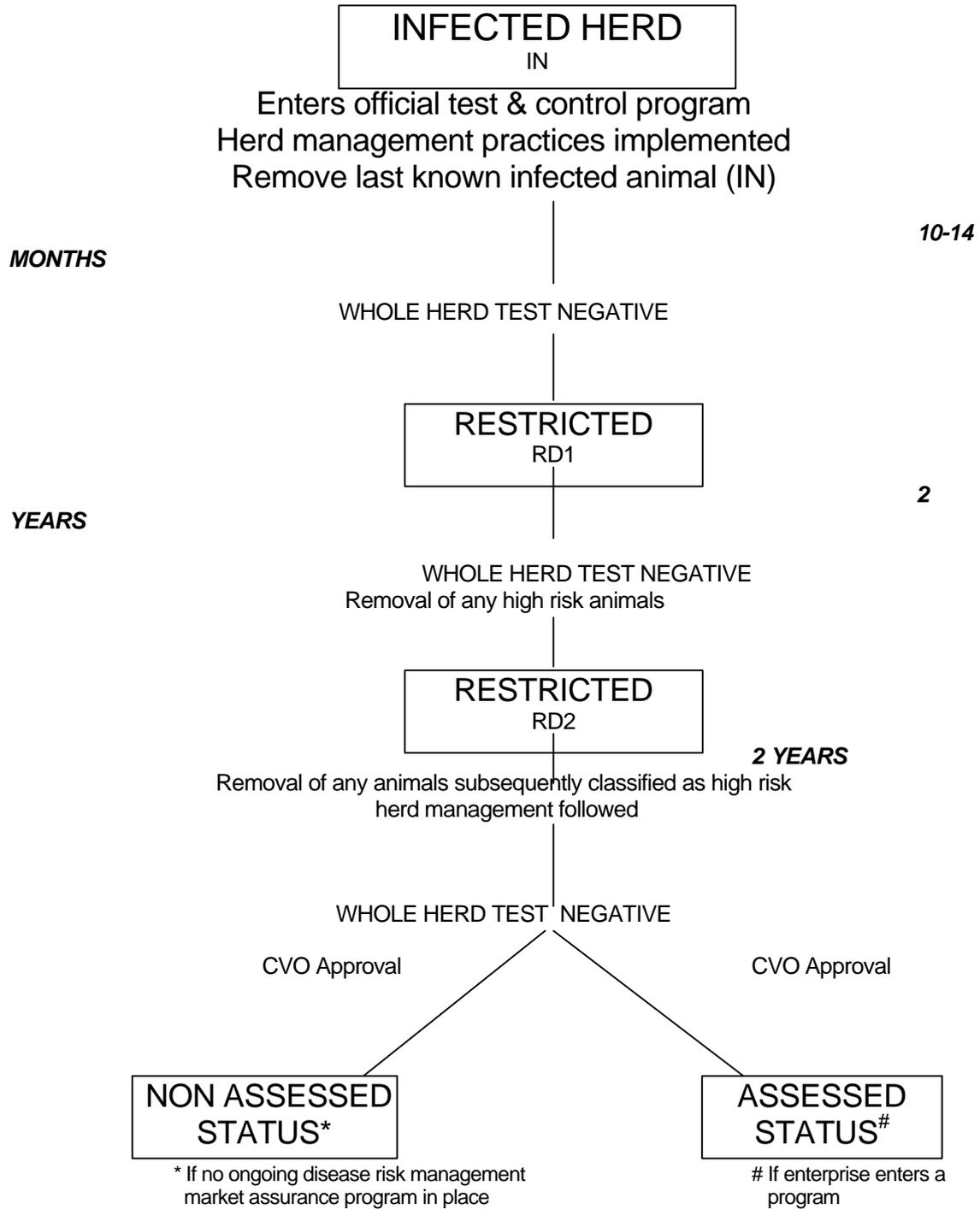
These are detailed in the Australian JD Market Assurance Program for Alpaca (AlpacaMAP)

### **5. Progression of status for Infected herds**

An Infected herd must undertake an approved test and control program and achieve three (3) negative herd tests at two (2) year intervals after the last known infected animal is removed from the herd before progression to NA or an assessed status will be considered by the CVO for approval. Infected herds undertaking approved test and control programs may progress to Restricted (RD) status after at least one negative herd test of all eligible animals in the herd which will be undertaken not sooner than 12 months after the last known infected animals are removed from the herd.

The following chart summarises the requirements for IN herds to progress through RD status to NA or to an assessed status under a market assurance program.

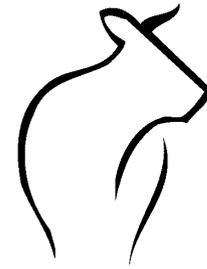
**HERD STATUS PROGRESSION FOR INFECTED ALPACA HERDS**



## APPENDIX 5 MODEL VENDOR DECLARATION

(Under development)

Appendix 2(b)



# **Premium Cattle Health Scheme**

## **Technical Document**

## **THE DISEASES**

### **Johne's Disease**

This disease is a chronic, progressive, wasting condition that affects ruminants and is caused by the organism *Mycobacterium avium* subspecies *paratuberculosis*. Strains of the organism appear to be specific to the different species of ruminants and if cross-infection does occur, it does so rarely. The infectious agent is shed in large numbers in faeces and can be found in colostrum. Animals are infected by ingesting the agent and young animals are considered to be the most susceptible to infection. However, clinical signs of diarrhoea and weight loss usually do not occur until some time after 18 months of age. In heavily infected herds, this leads to a high rate of wastage in cattle at two to four years of age. Infection is nearly always introduced to a herd by purchasing infected replacement breeding stock, including bulls.

Tests carried out on blood samples to detect antibodies and the culture of the bacterium from faeces are both valuable tests for the diagnosis of Johne's Disease. However, they can only be used to detect infected animals in the later stages of the disease, when clinical disease has become apparent, or in the short period prior to this.

This means a simple test and cull programme is not sufficient. It must be supplemented by the removal of offspring of any positive dam from the breeding herd, in an effort to exclude animals before they show signs of the disease.

Because of the difficulties with testing and because the infection can survive in the environment for a limited time, control and eradication is more difficult than for the other diseases in the scheme. However, the on-going losses due to the disease, and the risk to herds purchasing cattle from infected herds, mean that an effort should be made to eradicate the disease from an infected herd.

Vaccination is useful in heavily infected herds to reduce the number of cases and therefore to reduce the amount of infection in the environment. While vaccination will not remove the infection from the herd, it is an aid in the control of Johne's Disease.

### **Rules of The Premium Cattle Health Scheme**

The Premium Cattle Health Scheme (PCHS) seeks to identify herds free from certain diseases and to offer control programmes for those herds in which those diseases have been identified. These rules are mandatory for herds in the Accreditation programme and the Screening & Eradication programme. They do not apply to herds in the Monitoring programme, although they are advisable as good practice for those herds.

### **Herd Health Security**

1. Herd health security is explained and the general principles are detailed under the note on Herd Health Security. Herd owners, managers and veterinary surgeons participating in the scheme must be familiar with this document and seek to achieve the standards set wherever possible.
2. For the purposes of this scheme, a herd is defined as cattle that are under a unified management system not necessarily on one premises.
3. Farm boundaries must be such as to prevent cattle from straying off or onto the farm and must prevent nose to nose contact over fences or walls. Installation of double fencing, with a gap of 3 metres, between their cattle and any neighbouring cattle, is essential where members are following the IBR and/or BVD programmes. It is also a useful standard to adopt for all health control programmes.
4. Because there is a risk of cattle becoming infected with Leptospirosis and/or Johne's Disease from water courses, where members are following the programmes for those diseases, it is preferable, but not essential, that their cattle do not have access to watercourses that have other cattle or sheep grazing upstream or that have flowed through another farm.

5. Accredited status is specific to each disease. Where two or more herds are accredited for different diseases, the rules for movement and contact between herds are those that apply in relation to non-accredited herds.
6. Cattle must not be added to a PCHS herd unless they are of similar or superior health status within the scheme. Otherwise, they must be placed in isolation for the required period and tested by the appropriate test(s) for the disease(s) in question.
7. Cattle from health scheme herds must not contact non-health scheme cattle or health scheme cattle of a lower status; otherwise they will lose their status within the scheme. To re-introduce them to the herd, they must be regarded as non-accredited added animals and must be placed in isolation for the required period and tested by the appropriate test(s) for the disease(s) in question.
8. Colostrum from non-health scheme herds, or from health scheme herds of a lower status, must not be brought in to a health scheme herd.
9. Equipment such as drenching guns, surgical instruments and hypodermic needles must not be shared with cattle from another herd. Veterinary surgical instruments must be sterilised before use in the herd. Hypodermic needles must be used in one animal only and then disposed of.
10. Equipment, livestock trailers and handling facilities that are shared between health scheme cattle and other livestock must be cleaned and disinfected before use by health scheme cattle. For herds in the Johne's Disease programme, a MAFF approved product at the dilution recommended for tuberculosis control should be used. (A list of approved disinfectants can be obtained from your local Animal Health Office).
11. Suitable isolation facilities, in the form of pens or paddocks that do not allow contact with other farm livestock, must be available for cattle coming into the herd. These facilities must conform to the Herd Health Security document.
12. A defined isolation period must be observed for all additions to a health scheme herd and appropriate testing carried out as required for the particular disease programme being adopted. It is only when both the isolation period and the requisite tests have been completed, with results indicating freedom from infection, that those animals can enter the herd.
13. It is a member's obligation to inform the health scheme's supervising veterinary surgeon of any changes that could affect herd health security.

#### **Herd Rules**

14. At the time of collecting, all samples must be identified in order to allow blood, milk or faeces samples to be unequivocally matched with the individuals tested.
15. For the Accreditation and the Screening & Eradication Programmes, samples can only be collected by: -
  - A veterinary surgeon.
  - Someone designated by the veterinary surgeon who is neither the owner of the cattle nor an employee of the owner.
  - In the case of milk samples, by the milk recorder.

For the Monitoring Programme, bulk milk samples collected by the owner of the herd or his representative are acceptable.

Where the herd uses more than one bulk tank, representative samples must be collected from each tank and tested separately.

16. Notwithstanding any records required to be kept by law, the following records must be kept for all PCHS cattle as follows: -
- Identification.
  - Breed.
  - Sex.
  - Date of birth.
  - Identity of dam.

### **Testing Programme**

17. Where it is intended to establish a Johne's Disease, BVD, IBR or Leptospirosis accredited herd by acquiring cattle accredited free of the particular disease, the premises should be inspected by a veterinary surgeon in advance. Provided that the herd health security is satisfactory, the herd immediately achieves accredited status for the particular disease. The first Annual Herd Test, as specified for each disease programme, must be carried out six months after establishing the herd.
18. The testing programme for each disease is detailed in the relevant section and must be followed.

### **Suspicion and Confirmation of Target Disease**

19. Any disease condition which might be attributable to a disease that is the target of the scheme must be investigated by the owner's own veterinary surgeon. If the veterinary surgeon is satisfied that the condition is not the target disease, no further action need be taken. If the veterinary surgeon cannot rule out the target disease, the requisite samples as detailed in the programme must be collected from each affected animal. The affected animals should be isolated from the herd until the results of the laboratory tests are known.
20. After the target disease has been confirmed in a herd, the herd will not be eligible for accredited status until all known reactors have been removed for slaughter, and the herd has passed the requisite tests, as detailed in the specific programme, following removal of the last reactor from the herd.

### **Shows, Sales etc**

21. It should be recognised that any contact with other stock puts the status of the herd at risk. As there are no PCHS accredited sections at cattle shows and sales, any accredited cattle attending a show or sale will be deemed to have lost their accredited status. On being returned to the herd of origin, such cattle must be treated as non-accredited added animals, and must be isolated and tested according to the requirements of the individual disease programmes. The one exception to this rule is Johne's Disease, in which case providing cattle are not in direct contact with other cattle and have been away at a sale or show for a period not exceeding 7 days they can enter the herd without further testing for Johne's Disease.
22. Accredited cattle leaving their home herd for any other purpose must be located, unloaded, transported and accommodated separately from non-accredited cattle. No direct or indirect contact between accredited and non-accredited cattle must be allowed to occur at any time. If these conditions are met, then accredited cattle may return to their herd of origin, or to another accredited herd, without any isolation or testing.
23. Accredited cattle that have come into contact with non-accredited cattle must be treated on their return to the farm as non-accredited added animals. The isolation and testing programmes as required by the particular disease programme(s) must then be carried out. Failure to observe this condition will result in the loss of accredited status.

### **Certification**

24. Certificates are issued by PCHS and are accredited by the Cattle Health Certification Standards. Only herds with valid certificates are deemed accredited for the disease(s) for which they have been tested. The certificates will be valid for one year from date of issue and will not be renewed until the Annual Herd Test(s) have been carried out, with negative results, for the disease(s) in question.
25. Certification by a PCHS veterinary surgeon is based upon: -
- Owner's declaration of compliance with the rules.
  - Inspection of the herd by the practising veterinary surgeon.
  - Collection of the appropriate samples by the practising veterinary surgeon or his appointed deputy.
  - Appropriate laboratory tests carried out at a PCHS laboratory, with results indicating freedom from infection.
26. Certificates may be used as proof that acquired stock, or stock for sale, are accredited for the particular disease.

### **Veterinary Surgeons**

PCHS strongly recommends that members' veterinary surgeons are members of the British Cattle Veterinary Association and have received appropriate training on the PCHS scheme and the target diseases.

### **Johne's Disease Programme**

For Johne's Disease, the scheme offers two programmes. (There is no Monitoring programme for Johne's Disease as there is no test available for screening bulk milk samples.)

#### **Monitoring Programme**

Not available for Johne's Disease.

#### **Screening & Eradication Programme**

Objective: To implement a control programme to reduce the detrimental effects on herd productivity caused by this disease and to allow the sale of animals of known status. The long-term goal is to achieve freedom from the disease, but it should be recognised that this is a lengthy procedure that might take many years.

#### **Monitored Free Programme**

Objective: To demonstrate the herd is free from Johne's Disease; to maintain freedom from Johne's Disease; and to allow the sale of stock as monitored free of Johne's Disease.

### **General points**

(Apply to both programmes).

1. Herd Health Security must be satisfactory as detailed in the Rules of PCHS.
2. All samples must be collected in accordance with the Rules of PCHS (see Rules 14 and 15).  
  
Blood samples should be either clotted or heparinised. Faeces samples of at least 5gms should be submitted in a sample pot designed for the purpose.
3. Any animal that tests positive by the blood test must be placed in isolation and retained there until further testing has been carried out and the results known. (In herds where infection has already been confirmed, the positive blood test result defines the animal as a reactor and isolation plus further testing are not required).

The further tests required are: -

Repeat blood test.

Examination for the infective organism in faeces, by culture.

Alternatively, if the animal concerned is sent for slaughter, examination for the infective organism should be carried out by histological assessment of the ileocaecal junction and drainage lymph node.

If the animal is confirmed as shedding the infective organism by culture from faeces or by finding typical histological lesions in the intestine, that animal is defined as a reactor.

If the infective organism is not found by culture of the faeces, a further blood test must be carried out. If that is still positive, the animal is defined as a reactor.

6. Clinical Disease: Any disease condition in an animal of 6 months or older in a qualifying or Johne's Disease accredited herd that might be attributable to Johne's Disease must be investigated by the supervising veterinary surgeon. This includes all animals that may have diarrhoea or weight loss or both. If the veterinary surgeon is satisfied that the condition is not Johne's Disease, then no further action need be taken. If the veterinary surgeon cannot rule out Johne's Disease, then a blood sample and a faeces sample should be collected from each affected animal. The affected animals should be isolated from the herd until the results of the laboratory tests are known.
7. Added Animals - Accredited: These can enter the herd directly, provided the supervising veterinary surgeon is satisfied that there has been no opportunity for infection of the purchased animal during transit i.e. there has been no contact with non-accredited stock or transport which has not been disinfected in accordance with the Rules of PCHS.
8. Added Animals - Non accredited: These animals always constitute a risk of infection and therefore should not be added to the herd if at all possible. Where this cannot be avoided, the general PCHS rules on isolation and testing apply. These animals must be maintained in isolation for a period of 28 days and tested for Johne's Disease using both blood and faeces samples. Only when the results are negative can the animals be introduced to the herd. Note that the time required to test for Johne's Disease by faecal culture can be at least 4 months and these animals may therefore have to be isolated for up to 5 months. In addition, they must also be re-tested every 12 months, notwithstanding any annual or biennial herd screening programme (see para 21).
9. Shows, Sales etc – Rule 7 of the Rules of PCHS requires that where cattle from a health scheme herd have contacted non-health scheme cattle or health scheme cattle of a lower status, they lose their status within the scheme. To re-introduce them to the herd, they must be regarded as non-accredited added animals and must be isolated and tested as required for that particular disease programme (see para 8 above).

However, because animals normally require prolonged exposure to large doses of the Johne's Disease organism before becoming infected, at the discretion of the supervising veterinary surgeon this Rule can be relaxed for cattle in the Johne's Disease programme that have been away from the herd for a limited period and have had minimal contact with other cattle.

Therefore, if Johne's Disease accredited cattle have been away from the herd at a show for a period not exceeding 7 days and have been prevented from having direct contact with other cattle at the show, particularly their dung and soiled bedding, Rule 7 will not apply and the accredited cattle can rejoin their herd of origin without the need for isolation or testing.

### **Screening & Eradication Programme**

10. Initial herd screen: all animals of 2 years of age or older should be blood sampled. This must be carried out before any vaccination programme has been started.

11. If all samples in the initial herd screen are negative, then this is the first qualifying test for accreditation (see para 19).
12. In a known infected herd, it is not a requirement to have antibody positive animals further tested by faecal examination, except where herds are vaccinated (see para 17).
13. Routinely blood test all cattle to be removed from the herd because of either weight loss or diarrhoea, or both.
14. Blood test all adult breeding cattle to be removed from the herd.
15. Cull all reactors. The offspring of any reactor cow should not be retained for breeding and should be removed from the herd as soon, as is practical.
16. If positive animals constituted less than 5%\* of the herd, routine annual testing (in accordance with the initial screen) continues and management procedures to reduce the exposure of cattle to infection are implemented. After two clear tests and if no further evidence of infection is found, the herd becomes monitored free.
17. Vaccinated: If positive animals constitute more than 5%\* of the herd, all calves are vaccinated in the first week of life and management procedures to reduce the exposure of cattle to infection are implemented. Vaccination continues until no clinical Johne's Disease occurs for a period of at least 2 years. At this point, vaccination can cease and progression towards Johne's Disease monitored free status can begin. This can only be achieved when there are no vaccinated animals in the herd and there have been two clear herd blood tests at least 12 months apart.

Once a vaccination programme has been implemented, the blood test cannot be used as a test in vaccinated cattle. Therefore, adult cattle leaving the herd are not required to be blood tested. However, the clinical disease routine must still be applied, but without the need to blood sample (see para 6). Diagnosis requires the demonstration of either the organism in the faeces or microscopic lesions in the intestinal tract.

18. Johne's Screening & Eradication programme herds can be categorised as:
  - a) Johne's Screening non-vaccinated
  - or
  - b) Johne's Screening vaccinated

*\*This figure can vary depending on the impact of the numbers of cows to be culled on herd profitability ie some herds may be willing to cull all reactors even if they constitute 10% of the herd.*

#### **Monitored Free Programme.**

19. **Qualifying Tests:** Two herd tests are carried out on all animals of 2 years of age or older at an interval of at least 12 months and not more than 24 months. (The first blood test may have been carried out in the Initial herd screen of the Screening and Eradication programme – see para 10).
20. **Monitored Free Status:** A herd is Monitored Free if two clear qualifying tests at an interval of 12 months have been achieved without any reactor being detected nor any reactor being detected by other tests. The date the herd first achieved Monitored free status will be included on the Certificate of Accreditation.
21. **Annual or Biennial Herd Tests:**

Annual - following Accreditation, all animals 2 years of age or older must be tested every 12 months.

Biennial – if a Cull Screen programme can be followed (see para 22 below), all homebred animals 2 years of age or older must be tested every 24 months. However, all non homebred animals 2 years of age or older from non-accredited herds must still be tested every 12 months.

22. Cull Screen: Blood and faeces should be collected from all animals 2 years of age or older prior to removal from the herd. Where this can be complied with, biennial herd testing will suffice; where it cannot be complied with, annual herd testing must be carried out (see para 21 above).
23. Clinical Disease Tests: Any adult cattle that show signs of ill health, from which they may die, must have blood and faeces samples collected. The blood sample is screened first and if it is positive, the faeces sample is then cultured. Animals that die before blood or faeces samples are collected must have the carcass or the intestinal tract or faeces examined at a participating laboratory.
24. Where reactors are found, herds are considered to be infected and have the option to enter the Johne's Disease Screening & Eradication Programme.
25. PCHS operates under licence from the Cattle Health Certification Standards (CHeCS) and cattle with PCHS Johne's Disease Monitored Free status are of equivalent health status to CHeCS Johne's Disease Accredited Free.

### **Herd Health Security for Cattle**

Herd Health Security means the measures taken to reduce the risk of introducing to a herd of cattle infectious agents that may cause specific diseases in those cattle.

Any cattle from herds of unknown disease status must be assumed to be a potential source of disease.

The risk of introducing disease to a herd is directly related to the extent of the preventive measures taken and the degree to which they are applied.

However, Herd Health Security can never be absolute, and, although the advice contained in this document is derived from the best technical knowledge available, it does not offer an absolute guarantee of prevention of new infection.

### **General Measures**

These are measures applicable to the control of most infectious diseases. They represent good husbandry standards and all health conscious cattle farmers should attempt adoption.

1. Where it is necessary to purchase replacement stock, including bulls, avoid potentially infected cattle by acquiring them from herds certified free of specific diseases.
2. If replacement cattle cannot be purchased from disease-free herds, they should be isolated and health tested before being brought to the farm.
3. Bulls should not be hired under any circumstances.
4. Isolation facilities should be provided for all added animals. These should prevent contact with other stock. A dedicated building separate from other cattle buildings is ideal, but a separate paddock that prevents contact with other stock may also suffice. No air space, drainage or dung storage should be shared with other cattle. Dung may only be removed from the dedicated storage area, to be spread on land or added to the main dung store, when all animals in the isolation facility have passed the required health tests and been added to the herd.

You should discuss with your vet any health tests that must be carried out during the isolation period and ensure that animals are inspected regularly for signs of disease. The isolation period is defined by the post-entry testing protocols for each individual programme.

5. Adverse test results – if two or more animals are isolated as a group, either housed or in paddocks, and one or more of the group give an adverse test result, the whole group should be regarded as at risk and kept in isolation and re-tested until the problem is resolved.

If isolated animals are confirmed as reactors, dung from the isolation facility should not be disposed of onto pasture that is to be grazed by cattle within 12 months. Where paddocks have been used to isolate these positive animals, or for the quarantining of disease breakdown cattle, other cattle must not be allowed to graze them for at least 2 months for the BVD, IBR and Leptospirosis programmes. For the Johne's Disease programme, this period must be extended to 12 months.

6. Avoid all contact, either direct or indirect (e.g. dung or urine), with non-health scheme cattle or health scheme cattle of a lower health status. Where contact has occurred, the cattle lose their status within the scheme. To rejoin the herd, they must be placed in isolation for the required period and tested by the appropriate test(s) for the disease(s) in question.
7. Limit farm access to only those people deemed essential.
8. Take precautions to avoid the introduction of infection on clothing or footwear (e.g. AI technician, vet, foot trimmer, and neighbours) or on equipment (e.g. vehicles, crushes, dosing equipment). The safest option is to have dedicated clothing, footwear and equipment for your particular farm. A less secure though acceptable alternative is thorough cleaning and disinfection of clothing, footwear and equipment before use on the farm at risk. Single use disposable overalls and disposable foot covers should be provided for occasional visitors. Particular efforts should be made to clean and disinfect any equipment likely to be contaminated with blood (e.g. hoof knives, instruments for castrating, disbudding or dehorning). Injection equipment should never be shared between farms. Veterinary equipment must not be shared between farms unless it is sterilised before use.
9. Limit and control access of vehicles to the farm, particularly those used for moving cattle. Delivery and pick up points should be at a site isolated from other cattle on the farm. Vehicles must be cleaned and disinfected with an appropriate disinfectant before they are used for moving cattle. Where possible, the driver should remain in his cab and should certainly never assist in removing cattle from pens unless using farm dedicated overalls and footwear.
10. Use reputable suppliers for purchased feed and bedding to reduce the risk of introducing infection.
11. Prevent access of vermin and wildlife to feed and bedding stores and to the cattle whenever possible.
12. Use piped mains water rather than natural water sources whenever possible.
13. Embryos and semen should be from donors free of IBR and BVD.

Sheep can harbour some of the diseases that affect cattle and therefore contact between cattle and sheep should be avoided. This is particularly important at housing when cattle should not share the same building with sheep. Cattle and sheep should not graze together, and where cattle follow sheep onto grazing land, there should be a minimum interval of 2 months. The dung from sheep sheds should not be spread onto fields to be used for cattle.

15. Because there is a risk of cattle becoming infected with Leptospirosis and/or Johne's Disease from watercourses, it is preferable that they do not have access to watercourses that have other cattle or sheep grazing upstream.

## Measures to prevent introduction of infectious agents causing specific diseases

If the introduction of specific diseases is to be prevented, such as in a health scheme, the herd health security measures must be tailored to these diseases. Although most of the measures will be included in the general section above, different diseases have different routes of spread. For example, airborne spread is much more likely with IBR than Johne's Disease. There is therefore a requirement for 3 metre spaced double fencing bordering neighbouring farms for IBR.

Added animals must be retained in isolation until post-entry health tests have been carried out. Unless specified differently in the individual disease programmes, these tests should be carried out not less than 28 days after arrival, and the animals must not be released into the herd until test results indicating freedom from infection have been received.

## PREMIUM CATTLE HEALTH SCHEME – ADDED ANIMAL PROCEDURES

For **Johne's** and **L. hardjo** particularly it should be noted that negative laboratory test results do not necessarily mean an individual animal is uninfected.

**Non accredited cattle added animal procedure:** Retain and test in quarantine all non-accredited cattle added to the herd until they are shown not to be a *reactor*, as detailed below.

- (2) **Contact of health scheme cattle with non-accredited cattle (e.g. at shows or accidental):** The procedures in (1) above should be adopted for **BVD, IBR and L. hardjo** health scheme cattle. For cattle in the **Johne's** scheme quarantine and testing for **Johne's disease** may not be necessary but veterinary advice must be sought specific to the herds individual circumstances.

	BVD	IBR	Johne's Disease	Lepto. hardjo
<b>Minimum Quarantine Period</b>	4 weeks	4 weeks	<b>4 months</b> (negative faecal culture results take up to 4 months)	4 weeks
<b>On Arrival</b>	Blood sample (hep.) for antibody & virus	**	Blood sample (serum / hep.) for antibody & faecal culture	**
<b>28 Days Or More After Arrival</b>	Blood sample (hep.) for antibody & virus (>21 days after 1st sample)	Blood sample (serum / hep.) for antibody		Blood sample (serum / hep.) for antibody
<b>Annually After Joining The Herd (optional for animals under (2) above)</b>		Blood sample (serum / hep.) for antibody	Blood sample (serum/hep.) for antibody & faecal culture	
<b>Definition of A Reactor</b>	One or more of: 1) virus positive* 2) rise in antibody	Antibody positive <sup>▲</sup>	Antibody or faecal culture positive. <sup>▲</sup>	Antibody positive <sup>▲</sup>

	<b>BVD</b>	<b>IBR</b>	<b>Johne's Disease</b>	<b>Lepto. hardjo</b>
	between tests* 3) antibody positive & pregnant.*			



No action required

- \*\* There is the option to additionally test animals for antibody to IBR and L. hardjo on arrival to allow exclusion of antibody positive animals at an early stage. If animals test antibody negative on arrival they still require to be tested after 4 weeks in quarantine  
Animal must not enter the herd (in calves where the antibody could be maternal in origin veterinary advice should be sought).
- \* Take veterinary advice as to how to proceed

An Opinion on the Potential of Testing Bulk Tank Milk samples for Surveillance of Johne's disease in Dairy Herds

Written Submission from Dr Irene Grant, Dept of Food Science (Microbiology), Queens University of Belfast, Newforge Lane, Belfast

There is a certain amount of potential in testing bulk tank milk samples from individual herds for surveillance purposes. *Mycobacterium paratuberculosis* infection of milk is derived, in the main, from faecal contamination during the milking process. Shedding of the organism within the udder of infected animals does occur (2-8 CFU per 50 ml of milk has been reported in the literature) but the contribution from this direct route of infection is much less than via faecal contamination. If infected animals in a herd are shedding the organism in large numbers ( $\geq 10^8$  per g) in their faeces there is the distinct possibility that their milk will be contaminated with fairly substantial numbers of *M paratuberculosis*. Even when this milk is diluted with milk from other cows in the herd that is not infected there may still be detectable levels of *M. Paratuberculosis* present in the bulk tank milk. Certainly, in our experience it is possible to detect and isolate *M paratuberculosis* from bulk tank milk from individual dairy farms.

The main problem with testing bulk tank milk samples is that *M paratuberculosis*, if present, is going to occur in milk at much lower levels than in the faeces of the same infected animals. Consequently, methods used to test milk for the presence of *M paratuberculosis* need to be much more sensitive than methods used for testing faeces. We have spent time developing and maximising the sensitivity of a novel immunomagnetic PCR (IMS-PCR) method for testing milk. This assay has been shown to be capable of detecting natural *M paratuberculosis* infection in milk from sheep, goats and cattle. Recently, on the basis of the results of two milk surveys we have been able to make a preliminary assessment of the performance of the IMS-PCR for testing milk in terms of test sensitivity, test specificity and predictive value. Comparing the IMS-PCR results to the 'gold standard' culture results (after decontamination of the milk with 0.75% cetyl pyridinium chloride for 5 h) the following estimates of test sensitivity, specificity and predictive value were obtained.

Test Attribute	IMS-PCR applied to:	
	Survey 1: Bulk tank milk from individual farms (n=208)	Survey 2: Bulk raw milk from dairy processing plants (n=76)
Sensitivity (%)	30.0	66.6
Specificity (%)	91.5	79.5
Predictive value (-) (%)	88.6	98.3
Predictive value (+) (%)	37.5	11.8

Based on my experiences of testing milk in our laboratory it is my firm belief that decontamination and culture is not 100% efficient at recovering viable *M. paratuberculosis* from milk samples. We experience all sorts of problems with mixed cultures containing acid-fast cells and subcultures of suspect acid/fast colonies that don't grow. Consequently, some of the false positive IMS-PCR (ie culture negative but IMS-PCR positive) results obtained during the surveys may have been infected milk samples in reality. However, the existence of these false positive results has led to lower estimates of both test specificity and sensitivity than was hoped. At best, given the comparatively high predictive values for *M. paratuberculosis* negative milk samples in both milk surveys, the IMS-PCR assay could have potential to be used as an initial screening test for bulk tank milk. Only those milk samples which tested IMS-PCR positive would need to be cultured to confirm the presence of viable *M. paratuberculosis*.

It must be realised that there are certain complicating factors in relation to testing milk for the presence of *M. paratuberculosis*.

1. There is some evidence of seasonality of shedding of *M. paratuberculosis* in milk. A study published in 1996 (Miller and others, App. Env. Microbiol. 62, 3446-3452) suggested that there were peak periods of *M. paratuberculosis* infection of milk in March/April and Oct/Nov for pasteurised milk from the south of England.
2. The IMS-PCR test at the moment is labour intensive and this limits the number of milk samples which can be tested at any one time. However, the advantage is that an indication of the *M. paratuberculosis* infection status of the milk sample is obtained within 48 h as opposed to 18 weeks when culture is used.
3. As large a volume of milk as possible should be tested in order to increase the chances of detecting infection. However, milk volume tested will be limited in practice by the capacity of available centrifuges. We have been testing 50 ml of milk because that is the maximum volume of milk that will fit in our centrifuge.

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