

JOHNE'S DISEASE

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ABSTRACT

Johne's disease is a chronic, debilitating condition caused by *Mycobacterium avium* subspecies *paratuberculosis* (*Map*). It is characterised by diarrhoea, loss of condition and ultimately death. Economic losses result from premature culling and decreased productivity, which is a feature of the subclinical disease.

In 2001 it was estimated that as many as 20% of UK dairy herds could be endemically infected with *Map*. Data from other countries, from where animals have been imported into the UK, would suggest a herd prevalence of up to 70% indicating that the likely herd prevalence within the UK might be higher than 20%. Since 2000 there has been a significant increase in the number of Johne's disease diagnoses made in Britain (VIDA), a trend indicating that the disease is becoming more prevalent.

Similarities in the pathology of Johne's disease and the human enteric condition Crohn's disease, have long been recognised. A link between these conditions has been suggested following the isolation of *Map* from some Crohn's sufferers. Such a link has yet to be either proved or disproved but following the demonstration of *Map* in pasteurised milk at retail outlets the Food Standards Agency has urged a precautionary approach, advising all parts of the agricultural industry to work to prevent *Map* from entering the food chain.

There are sound financial reasons why heavily infected herds should work towards the control of Johne's disease and why remaining free of infection should be an important objective for those herds yet to experience the disease. Control of Johne's disease, however, on a herd level presents considerable practical difficulties arising from the complex pathogenesis of the disease and the difficulty in identifying infected animals in the preclinical phase.

This paper outlines current knowledge about Johne's disease and discusses measures that can be taken to control the disease.

KEY WORDS: Johne's Disease, *Mycobacterium avium paratuberculosis*, MAP

INTRODUCTION

Johne's disease is a chronic, granulomatous enteritis caused by *Mycobacterium avium* subspecies *paratuberculosis* (*Map*). It is a disease that predominately affects ruminants, but infection with *Map* has been demonstrated in wild rabbits, and a number of other non-ruminant wildlife have been shown to be susceptible to infection (Olsen *et al.* 2002). In cattle, the disease is characterised by chronic diarrhoea, progressive weight loss and decreased production and may cause substantial losses in infected herds. The disease has pathological similarities to the human enteric condition Crohn's disease, and *Map* has been demonstrated in the tissues of Crohn's sufferers, but a causal link with livestock has yet to be demonstrated (see review by Rubery 2001). The large financial losses caused by the disease in heavily infected herds and concern over its possible zoonotic potential have led several countries to adopt national control or eradication programmes.

In the UK there are three voluntary accreditation schemes - Premium Cattle Health Scheme, HI-Health and Herdcare, licensed under the auspices of Cattle Health Certification Standards (CHeCS). Defra has produced education material on the control of Johne's disease in the dairy herd to support the Food Standards Agency strategy for reducing levels of *Map* in milk.

CHARACTERISTICS OF MAP

Mycobacterium avium subspecies *paratuberculosis* belongs to the *M. avium* complex within the genus *Mycobacterium*. The bacterium is very closely related to *M. avium*, with the DNA between the two bacteria 99% homologous. They are therefore considered to be of the same species, hence "subspecies" *paratuberculosis*. *M. avium* are acid fast, aerobic rods. They take 8-16 weeks to produce visible colonies on culture media, though certain strains will take longer. *Mycobacterium avium* subspecies *paratuberculosis* is relatively biochemically inactive, and identification is based on detection of the insertion sequence IS900, by means of polymerase chain reaction, which is the only genetic element found to distinguish *Map* from the other *M. avium* subspecies (Olsen *et al.* 2002). *Map* is very resistant to environmental degradation and can survive for over a year in water and slurry (Sweeney 1996) and up to 47 months in soil (Caldow *et al.* 2001).

Three groups of strains of *Map* have been recognised: bovine, pigmented and small ruminant. The question of strain typing and whether strains of *Map* isolated from different species are the same or different is extremely important, not only to understand whether inter-species transmission has a role within *Map* epidemiology but also to determine any potential causation in relation to Crohn's disease.

Research so far would indicate that specific *Map* strains may be adapted to a particular host species without being host specific (Caldow *et al.* 2001). Molecular tools are now available to take this area of study further.

The close homology of the various strains of *Map* is one of the main problems in developing diagnostic tests with adequate levels of specificity. A major area of research therefore, is the identification and characterisation of immunogenic components of *Map* with the aim of developing diagnostic tests based on cellular or humoral immune responses to such antigens. Several potential proteins and enzymes have been identified and their usefulness is still being evaluated.

PATHOGENESIS

Ingested bacteria enter the intestinal wall through the small intestinal mucosa, specifically via M cells lining the dome areas of the Peyer patches. The bacteria are subsequently phagocytosed by sub-epithelial macrophages. The bacteria are resistant to intracellular degradation and will slowly replicate and stimulate inflammatory and cellular responses. An increased resistance to infection is seen with age, and this may be due to the incomplete development of the immune system in young animals, allowing easier access to the intestinal mucosa via the ileal Peyer patches, which become atrophic in older animals (Sweeney 1996).

Most animals are able to control the infection and only 10-15% of infected animals develop clinical Johne's disease (though subclinical infection will result in significant production losses and hence cost (Olsen *et al.* 2002)). Whether animals can eliminate infection is unclear. The exact immunopathological mechanisms for development of clinical disease are not known.

Gross pathology is primarily seen in the intestine and mesenteric lymph nodes. The intestinal wall is thickened and oedematous and the mucosa demonstrates transverse folds. The ileum is the primarily affected region but lesions can occur throughout the tract. Mesenteric lymph nodes are swollen and oedematous. Occasionally other organs, particularly liver and hepatic lymph nodes will demonstrate lesions. In severe disease, animals may be alopecic, cachectic, anaemic and demonstrate peripheral oedema and serous effusion into body cavities.

Histopathological lesions are characterised by multifocal diffuse granulomatous inflammation of the intestine and mesenteric lymph nodes. In mild lesions there are a few scattered giant cells and a few macrophages in the villi. In severe lesions there are a large number of both cell types infiltrating all layers of the intestinal wall and the lumen of lymphatic vessels.

IMMUNE RESPONSE

Protective immunity against *Map* is conferred by activation of the cellular arm of the immune response, with antibody production having little or no value (Olsen *et al.* 2002). Animals whose cellular immune responses are unable to control the disease will develop a humoral response along with the onset of shedding of bacteria in faeces. This humoral response acts in antagonism to the cellular mediated immunity, further suppressing it and consequently allowing more rapid development of the disease.

Of special importance for protective immunity are the T lymphocytes, in particular a subset of T-helper (Th1) cells via their role in the production of cytokines, interleukins, interferon and tumour necrosis factor.

NATURAL RESERVOIRS AND TRANSMISSION

The natural hosts of *Map* are different species of wild and domestic ruminant. There are no strict host restrictions with cross species infections of *Map* having been documented. Experience in western Europe indicates that the pigmented forms of the organism are principally found in sheep, but that cattle strains also infect sheep. In contrast, in Australia, sheep are not considered a source of infection to cattle.

In addition to ruminants, wild rabbits have been demonstrated to be infected with *Map*. Experimental inoculation of calves with *Map* from a rabbit showing typical lesions resulted in infection in the calves demonstrating that wild animals other than ruminants may contribute to infection. Similarly a number of non-ruminant wild life species have been shown to be capable of harbouring *Map* including stoat, weasel, rook, jackdaw, fox, badger and hare (Beard *et al.* 2001). There is still a fundamental gap in knowledge as to whether wildlife may provide a reservoir of infection for domestic stock or whether infections in these species merely represent spill over from domestic ruminants.

Despite this, the major source of new infection is considered to be infected farm ruminants that shed the bacilli in faeces. The route of infection is usually through ingestion of contaminated milk or food. In the advanced stages of the disease *Map* can be isolated from a variety of organs and secretions, including male and female genital tract, foetus, blood, semen and milk (Sweeney 1996). Vertical transmission may therefore occur, and studies have estimated that between 20-40% of foetuses from clinically infected cows, but only around 9% from asymptomatic infected cows, become infected *in utero* (Sweeney 1996). The chance of *in utero* infection appears to be correlated to the level of faecal shedding: *in utero* infection is unlikely in cows shedding less than 3000 colony forming units per

gram of faeces (Sweeney 1996).

Recent work has questioned the importance of vertical transmission suggesting that it occurs relatively infrequently and is not important in the epidemiology of the disease. Horizontal transmission is considered therefore to be by far the most significant means of spread. The incubation period can be extremely long, up to five years. After this period cows begin to excrete *Map* organisms in their faeces, initially at very low levels but gradually increasing so by the time of the onset of clinical signs a cow may be excreting billions of organisms per day in its faeces. It should be noted that not all cows will follow this pattern and some will demonstrate intermittent shedding from relatively early on in the disease course.

Young calves are considered to be most susceptible, with 80% of infections being acquired within the first month of life. Resistance to *Map* increases up to one year of age when it appears to plateau. Adult animals are still potentially susceptible to infection (Sweeney 1996). The likelihood of infection developing is primarily a function of age and level of exposure to the disease, though genetic factors, condition of the animal and concurrent disease state are all possible predisposing factors. The most likely source of infection to the calf is oral ingestion of contaminated faeces, particularly during the suckling process, very early in life. The "open gut" of the first 24 hours after birth is considered a particularly important period of risk to the neonatal calf. Any management process that allows for faecal contamination of calf or young stock feed must be considered a risk. Research performed in Australia also suggested that nematodes could potentially act as a vector (Lloyd *et al.* 2001).

Even if, however, all steps are taken to prevent such contamination some cows shed *Map* directly in their milk. This is more likely to occur in cows in the advanced stages of the disease; 35% as compared to 19% in subclinical heavy shedders and 3% in subclinical light shedders. Colostrum shedding also occurs and apparently at higher levels than with milk, 36% of subclinical heavy shedders and 9% of subclinical light shedders having been demonstrated to shed *Map* in colostrum (Sweeney 1996). This finding is of particular interest given the widespread practice of feeding pooled colostrum to calves for the control of enteric disease.

Inter-uterine inoculation of *Map* can result in infection and, given the potential for excretion of *Map* in semen, natural service may also be a potential source of infection (Sweeney 1996). No studies have informed of the potential for iatrogenic infection of Johne's disease during rectal examination.

Inter-herd transmission is almost always associated with the introduction of asymptomatic infected animals that act as carriers. Other lapses in biosecurity that potentially introduce contaminated

material to an uninfected farm are possible causes of new herd infections. However, almost all new outbreaks can be traced back to the purchase of infected stock (Sweeney, 1996).

PREVALENCE

The situation in the UK is unclear with no data to estimate prevalence available. Most recent data from the results of a postal survey in 1998 indicated that around 1.5% of dairy farms are affected. This is likely to be an underestimate but even so this is a much lower figure than in the 1950's when Johne's disease was considered the most important disease of adult cattle. Caldow *et al.* in 2001, estimated the likely UK prevalence to be 20% (based on prevalence figures from other countries and limited regional data). A study in the south west of Britain, using polymerase chain reaction to identify *Map* in mesenteric lymph nodes of cull cows, estimated the individual animal prevalence to be 3.5% (Cetinkaya, 1996). A twofold rise in diagnoses at the Veterinary Laboratories Agency and SAC disease surveillance centres in the last 4 years suggests that the incidence of the disease may be increasing (VIDA).

Prevalence studies in other countries that have been conducted *e.g.* Muskens *et al.* (1999); Jakobsen *et al.* (1999), Nielsen *et al.* (1999) have indicated a higher herd prevalence than the UK figures *e.g.* Muskens *et al.* (1999) estimated 31-71% herd prevalence. Consequently some commentators suggest that the UK prevalence figures are likely to be higher than 20%.

CLINICAL SIGNS

The clinical signs of *Map* in cattle are intermittent to chronic diarrhoea, cachexia despite normal appetite and decreased production. Advanced cases demonstrate emaciation, lethargy, submandibular oedema and anaemia. The majority of affected animals become clinically ill at between two and six years of age although the range is 4 months to 15 years (Caldow *et al.* 2001). It has been suggested that in heavily infected dairy herds for every clinical case within the herd there are likely to be 25 further infected animals (Whitlock and Buergelt 1996) though in beef herds this is probably an overestimation.

It has been observed that several factors may precipitate the onset of the clinical phase of disease including inadequate nutrition, concurrent infection (including immunosuppression associated with BVDV), parasitism, parturition and management stress (Downham 1950, Allen *et al.* 1986).

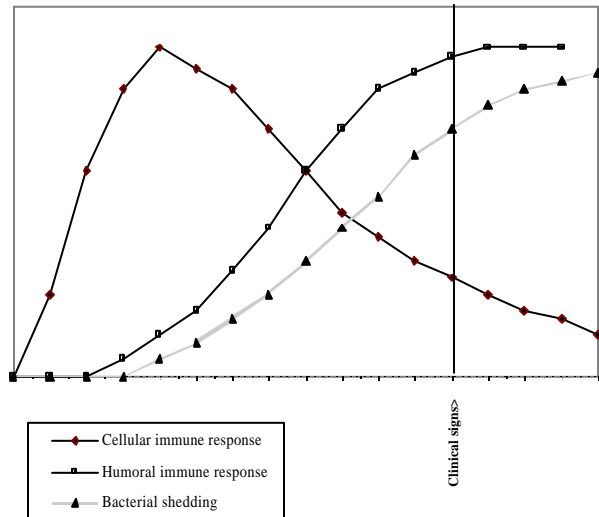
DIAGNOSIS

The diagnosis of Johne's disease is based on clinical signs, immunological reactions and identification of *Map*. Post-mortem diagnosis is based on the finding

of macroscopic and histopathological lesions and isolation of *Map*.

Diagnosis in clinically affected animals is relatively easy and the challenge lies in the identification of subclinically infected animals. These animals only intermittently shed bacteria and will usually be negative on the standard immunological tests that measure antibody production (see figure 1). Subclinically infected animals therefore pose the greatest risk for the spread of disease into new herds.

Figure 1. Schematic Model of Immune response.



DETECTION OF MAP

The most common diagnostic test method for Johne's disease is direct acid fast staining of faeces or tissue. Whilst simple and cheap, the test suffers from low sensitivity and specificity. Culture of *Map* from faeces or tissue, whilst considered the gold standard, also has a low sensitivity, estimated to be at 33% (Whitlock, Wells *et al.* 2000), due to the rigorous decontamination procedure required prior to culture. Furthermore culture techniques are slow, with both radiometric or conventional processes taking at least 7 weeks (Valentin-Weigand 2002).

The use of PCR amplification of *Map* specific IS900 and now ISMav2 (Strommenger, Stevenson *et al.* 2001) has a high sensitivity and specificity. Unfortunately problems with interference of components of the sample mean that the test is not being used in direct probing of biological samples. An alternative approach is to employ immunomagnetic separation in combination with PCR. This has been applied successfully to detect organisms in milk and was used in the recent investigation into whether *Map* survived pasteurisation (Grant *et al.* 2002). The use of PCR as a test for *Map* is now commercially available via the SAC for the detection of *Map* in faeces and bulk milk.

IMMUNODIAGNOSIS

The problems related above with detection of *Map* have led to efforts towards the use of host immune responses for diagnosis. During infection with *Map* animals develop both humoral and cell-mediated immune (CMI) responses, which can be correlated with the stage of disease, see figure 1.

Early immuno-diagnostic testing was limited to the intradermal test ("Johnin test"); however this test suffered from poor specificity. In recent years attempts have been made to detect elevated IFN- γ levels in response to *Map* infection by a bioassay and ELISA, but the tests have to be further optimised to improve specificity (Valentin-Weigand 2002).

Different tests are available to measure antibodies against *Map*, namely complement fixation test (CFT), agar gel immunodiffusion (AGID) test, and ELISA. The latter seems to be the most promising method, especially because of its higher sensitivity, though this is still estimated to be only 25% in the early clinical stage rising to 90% for clinical cases (Sweeney *et al.* 1995). To overcome low test sensitivity the ELISA and faecal culture can be used in parallel, with a resultant estimated increase in sensitivity to 75%. The specificity of ELISA tests is more robust exceeding 97% (Caldow *et al.* 2001).

It must be remembered that the sensitivity of tests like the ELISA are calculated against the gold standard of faecal culture which is itself estimated to only have a sensitivity of 50% (Collins 1996). Consequently when a figure of 50% is quoted as an average for the ELISA sensitivity it is likely that this is an over estimate and the true sensitivity may be only 25%.

Many variations of the ELISA have been established using different antigen preparations. Taken together, the ELISA is a promising diagnostic test, but it has to be appreciated that detection of an infection by ELISA depends on the stage of infection and the antigen used. Therefore, different ELISAs can identify only a subset of any infected population. There is also a possibility that animals infected *in utero* may develop a tolerance, be unable to recognise *Map* and so be unable to mount an immune response.

ZOONOTIC CONSIDERATIONS

There is a question mark over a possible link to Crohn's disease in humans. The Food Standards Agency has reviewed the evidence-linking *Map* to Crohn's disease (Rubery 2001). An EU Scientific committee also examined the evidence linking *Map* and Crohn's disease and published its opinion in March 2000. Both studies concluded that no conclusive link has yet been established.

The latest result of research published by researchers at St George's Hospital Medical School (Bull *et al.* 2003), investigates the incidence of *Map* in 37 patients diagnosed with Crohn's disease and 34

patients diagnosed as suffering from non-inflammatory bowel disease. They concluded that the rate of detection of *Map* in individuals with Crohn's disease is highly significant and implicates this chronic enteric pathogen in disease causation.

Whatever the scientific view the potential for risk has to be considered, particularly in the light of the new attitude to potential food zoonoses post-BSE. The reports of the FSA and the EU Scientific Committee indicated the need to carry out surveillance for *Map*. The FSA established a surveillance programme for the purpose of determining the levels of *Map* in raw milk and also to determine if *Map* survives the pasteurisation treatments currently employed in Irish liquid milk pasteurising dairies (Grant *et al.* 2002). This work concluded, "Viable *Map* is occasionally present at low-levels in commercially pasteurised cows' milk in the UK".

There are a number of possible options for the control of *Map* when pasteurising. One option is to alter the time/temperature combination used to pasteurise milk up to and including the point of peroxidase inactivation. This will undoubtedly result in the destruction of some functional properties of the milk. As clumping is a feature of *Map*, which bestows a level of protection to the organism during heat treatment, breaking up the clumps prior to the pasteurisation may be an option. However, shear force and the speed of reaggregation, if any, are unknown at present.

RISK FACTORS

HERD

The following factors have been demonstrated to be associated with an increased risk (reviewed by Caldwell *et al.* 2001):

- Non-closed herds.
- Large herd size (this may reflect an increased likelihood of bought-in animals).
- Practices that increase the exposure of immature cattle to the faeces of both older cattle and their contemporaries. These factors include: -
 - ◆ Group housing of cattle before and after weaning.
 - ◆ Newborn calf care.
 - ◆ Group calving.
 - ◆ Failure to remove calves from cows immediately after birth.

INDIVIDUAL ANIMAL

- Breed (though in the UK a number of breeds have a reputation for susceptibility to Johne's disease there is no data or information to support a genetic susceptibility to *Map*).
- High yield.

A recent study in Denmark showed that there was an increased probability of a test positive for antibody to *Map* with an increase in parity and within the first month following parturition.

ENVIRONMENT

The following factors are associated with an increased risk:

- Failing to clean maternity pens after each use.
- Increasing soil acidity (results in increased iron availability to the organism).
- Slow moving or stagnant water, or water that has flowed through another farm.
- Spreading of contaminated slurry on grazing pastures.

COST

The estimation of the cost of Johne's disease is difficult, as the cattle that demonstrate clinical disease or death will only form a small proportion of the total number of affected animals. Whitlock and Buergelt (1996) suggest that for every animal with clinical signs originating in the dairy herd 15-20 more are likely to be infected. In beef herds this is likely to be an over estimate.

Economic losses are insidious in nature with infected cattle suffering from: -

- Production losses.
- Health and reproductive losses. Infected cows have been identified by researchers to demonstrate higher levels of mastitis and reduced fertility (Merkal *et al.* 1975, McNab *et al.* 1991, Abbas *et al.* 1983).
- Increases in culling.

Further costs originate from the cost of control, monitoring and diagnosis. Gunn (2004) concluded that the average loss was £26.00 (dairy) and £17.00 (beef) per cow per year. Ott *et al.* (1999) suggested that averaged across all herds Johne's disease cost the US dairy industry \$22-27 per cow annually, with the majority of the losses attributed to lost milk production and higher cow replacement costs.

CONTROL

There is a general lack of data to measure the efficacy of the most frequently proposed farm level control measures and where this has been done conflicting results have been found. Recent papers also question the economic viability of control measures. Gunn (2004) concluded that, based on average losses of £26.00 (dairy) and £17.00 (beef) per cow per year, disease control was unlikely to be a main priority. Groenendaal (2003) produced a model to evaluate and assess the economic and epidemiological effects of different strategies for control. He concluded that test and cull control strategies were not economically favourable and were only effective if used in conjunction with calf hygiene policies. Improving

calf hygiene was found to be the most cost effective method of Johne's disease control. However, there is a lack of reliable epidemiological data on which to base the models, so that they should be used cautiously to make decisions for action on individual units. Individual farm circumstances are more pertinent.

On a national scale there is general consensus that a two-pronged approach is required. Firstly, testing to identify infected herds and individuals and herds free from disease, with subsequent appropriate isolation and culling. Secondly, measures to reduce the risk of spread of infection within and between herds. Treatment is not an option on the grounds of cost and efficacy though the demonstration that monensin sodium can be used to limit the extent of lesions raises the possibility of inexpensive ionophores being used as a prophylactic measure in young cattle (Brumbaugh *et al.* 2000).

TESTING

Culling test-positive animals should be part of any Johne's disease control programme. The frequency of testing and number of different types of tests used is governed by several factors among which are: the type of business in which the animals are used, the estimated herd prevalence of Johne's disease the owner's perception of the importance of Johne's disease to herd productivity, the capacity of the owner to pay for diagnostic tests, the speed with which the owner wants to achieve control of Johne's disease, and whether the goal is control or eradication of the disease (Collins 1996).

For most commercial dairy herds, a herd ELISA on all animals aged 2 years and older is the first step in any control programme. Results are most reliable for herds where infection is confirmed by isolation of *Map* from at least one animal. The percentage of a herd that is ELISA-positive for *Map* (apparent prevalence) should be doubled to get a rough estimate of the true prevalence of Johne's disease (given a test sensitivity of roughly 50%, only half of infected animals test positive). Using the ELISA or any other test for Johne's disease to estimate herd prevalence of *Map* infection is only possible in herds that do not routinely test and cull test-positive animals. A whole herd ELISA will underestimate the prevalence of Johne's disease once the cattle in advanced stages of *Map* infection have been removed.

The poor sensitivity of the ELISA discussed previously may not be, in practical terms, as large a problem in relation to control as might be first considered. This is because of two factors. Firstly, the prime concern in relation to control is to remove the infected animals that are shedding. As an increase in shedding is related to an increased likelihood of detection by ELISA the cattle that present the greatest

risk to the herd should still be detected. Secondly, a test that is 100% sensitive might reveal so many positives that the potential for culling all such animals may not be a viable proposition.

Subsequent herd tests should occur within a year (there is some indication that annual testing may provide little advantage over removing clinically diseased animals) and that six monthly testing is preferable.

NATIONAL STRATEGIES

Countries can be divided into high and low prevalence countries. Low prevalence countries, *e.g.* the Nordic countries, adopt an eradication scheme based on the disease being notifiable. High prevalence countries have adopted a variety of different schemes, the majority of them being voluntary.

There is little evidence to suggest that the identification of herds free from *Map* has led to an improvement in regional or national disease status. This may be because most national strategies are relatively new.

The greatest concern over the value of these programmes rests with the long period of time required to achieve disease free accreditation (theoretically this should be at least 5 years although this is not the case for any of these programmes) and the poor sensitivity of the ELISA. One Dutch study looked at 100 herds assumed free of disease and subjected them to six monthly faecal testing. Only 58 herds remained free by the end of the study.

CONTROL ON INDIVIDUAL FARMS

Given the time required to obtain disease control it is vital that the vet, client and personnel involved understand the complexity of the task. This should include the requirement for control, the problems inherent within any control programme, the impact on the unit that the control measures will bring and the realistic goals that control should achieve.

A control plan will need to be developed that will fit within the management of the unit. It will be worth stressing that there will be spin off benefits in the control of other diseases with some of the management changes required for Johne's disease control. For instance, improving the hygiene of calving boxes would reasonably be expected to have an impact on diseases such as environmental mastitis and infectious diarrhoea. Generally, on most farms, the largest benefits will be realized from changes in calf rearing practices, which is frequently one of the farm management areas given least attention.

Every Johne's disease-control programme should invest in methods to identify and cull infected cows in a herd, in particular those shedding *Map* in the faeces.

TESTING TO DEFINE HERD STATUS

There are two potential methods which could be used alone or in combination to detect infection within the herd, namely, test the environment for the presence of *Map* or test the individual animal(s): -

1. Environmental sampling.

As discussed earlier *Map* is an organism that persists in dung and slurry and can be found in milk. Consequently testing samples from these areas can provide useful information on infection status. Furthermore it has the advantage of being straightforward and does not involve any handling of the animals (and therefore does not disturb the routine management of the herd). Samples can be pooled and can then be tested for the presence of *Map* either by PCR or by culture. An investigation in Minnesota (Raizman *et al.* 2004) found that targeted sampling of cow alleyways and manure storage areas appeared to be a viable strategy for herd screening for Johne's disease status assessment and for estimating herd faecal prevalence. The SAC are now running a scheme using PCR or culture on pooled faecal samples taken from the environment to aid determination of Johne's disease status.

The use of PCR on bulk tank milk as a screen would appear to be the most attractive option of all for the herd screening of dairy cattle. However, this method has a poor sensitivity for presence of infection at herd level (though it may be useful in demonstrating that the milk product is not contaminated by *Map*).

2. Individual Animals.

The most sensitive method to determine herd infection status would be to test all adult (over two years of age) animals in the herd. This is potentially very costly and, while it would be justified if there is an obvious clinical disease problem or the client requested an aggressive approach, in many cases a more targeted approach to screening is required. There are several potential strategies: -

- Test all suspect clinical cases.
- Test the older animals within the herd on the basis that animals in this group are more likely to be positive to an ELISA than younger animals. There is no methodology to the exact numbers that should be tested or the age that should be used as a cut off though obviously the greater the number and the higher the proportion of the herd tested the more the reliable the result would be.
- Test cull animals. Not only do cull cows tend to come from the older section of the herd, if disease is present at a low level, infected cows may be culled because of infertility or low yield as a result of infection before they develop overt clinical signs of Johne's disease. Consequently the use of cull cows would appear to be an obvious sentinel group. Cows can be blood tested before they leave the herd and any blood test positives can be confirmed by faecal PCR or by faecal culture.

It should be noted that none of the above screening protocols, including the whole herd test, prove freedom from infection.

MANAGEMENT OF TEST POSITIVE ANIMALS

- When identified remove clinical and late stage animals immediately or at least segregate them from calving areas and young stock.
- Consider culling or segregating all offspring of infected dams.
- Strongly consider keeping replacement animals only from test-negative cows.
- Have a plan for high and low risk animals, based on test results, that enhances control efforts.

HERD BIOSECURITY

As the purchase of infected stock is considered to be by far the most important means of introduction of infection into an uninfected herd, measures to prevent this should be in place. Ideally this would require the formation of a closed herd. If this is impractical then the following options should be considered in descending order of preference: -

1. Purchase from Johne's disease free accredited herds.
2. Purchase from herds where the owner and veterinarian can document active Johne's disease monitoring and that the herd has had no cases for at least five years. (Confidence in actual Johne's disease prevalence will depend on the extent of the monitoring undertaken).
3. Purchase from herds where the owner and veterinarian can document that passive testing has occurred (*i.e.* suspect clinical cases tested for Johne's disease) and the herd has had no cases for at least five years.
4. Acquire from a herd with low Johne's disease incidence; *i.e.* animals have tested positive for Johne's disease but herd history and test results indicate a low incidence.

Ideally purchased animals should be pre- and post-tested though the limited sensitivity of the test, particularly in younger animals in the early stages of incubating the disease, makes this check test of limited use. A more useful approach may be to perform follow up tests at annual intervals following purchase.

Further biosecurity measures include: -

- Ensuring farm boundaries are secure.
- Avoid co-mingling with other potentially infectious stock *e.g.* deer and rabbits.
- Where there is a suspicion that drinking water could be contaminated supply water from a mains supply.
- Avoid grazing youngstock on known infected pasture.

- On farms where a high level of endemic disease occurs consider reverting to a flying herd (adults only on farm, therefore less susceptibility to disease).

CALF REARING

The calf should be removed from the dam as soon as possible after calving and colostrum feeding. Several studies have looked at this measure and failed to show a significant correlation between Johne's disease prevalence in a herd and the length of time the calf remains with the mother. Despite this, logic would definitely favour reducing the time the calf and dam are together. Dutch models suggest that snatch calving may be sufficient alone to remove Johne's disease from a herd because, although the method is not perfect (for instance, it does not stop placental transfer), the fact that on average only 25% of cows will give birth to a herd replacement ensures that the probabilities work in favour of elimination of infection). Obviously this measure cannot be implemented in beef herds.

2. Feed "low risk" colostrum: -

- Thoroughly clean the udder and teats before suckling or collection to avoid faecal contamination.
- Do not pool colostrum - use colostrum from the calf's dam.
- Recently tested negative cows are the best alternative colostrum source.
- Do not feed waste milk to calves.
- The "safest" feed is milk replacer administered using clean utensils.

ENVIRONMENT

Maintaining a clean environment is vital in reducing the build up of contamination and subsequent challenge to susceptible stock. In essence the measures are common sense and revolve around reducing faecal contamination of the environment as much as possible and protecting feed and water sources.

- Calve in clean and disinfected pens.
- Avoid a manure build up in pastures, housing and corrals where late gestation cows are kept.
- Ensure stocking densities are kept as low as possible.
- Avoid keeping high risk or sick cows in any common calving area.
- Provide clean feed for all cattle.
- Avoid manure contamination of feed by feeding off the ground.
- Use large amounts of clean bedding in young stock accommodation.
- Consider providing creep feeding to prevent infected adults accessing the same feed as young stock.
- Provide hay as soon as possible in the calf diet.

● Avoid using grazing or forage crops for young stock that had fresh manure applied as fertilizer during the current growing season.

- Use separate equipment to handle manure and feed.
- Provide clean water for all stock.
- Restrict access to streams and ponds.
- Divert manure run off from water sources.
- Prevent transporting bacteria to young stock by people, run off and equipment.
- Transport cattle in clean trucks.

VACCINATION

Vaccination will reduce the number of clinical cases by up to 90% (van Schaik *et al.* 1996). However, vaccination does not reduce the number of cattle that are infected in a herd. (One Dutch study suggested that while clinical numbers are reduced following vaccination, the number of subclinically infected cattle found at slaughter increased, which, with the concerns of the possible zoonotic implications, has to be considered a problem (Wentink *et al.* 1994)). Vaccinated animals that are sold to other herds will remain an effective route for the transmission of disease. Vaccination should, therefore, ideally only be considered as a control method in herds that do not sell stock for breeding purposes.

Other potential problems with using vaccination are:

- Expense, though killed vaccines cost much less than live vaccines. Killed vaccines are currently unavailable in the UK (a Dutch study still showed vaccination to be cost effective).
- Self-injection may potentially be very hazardous (causes intense vascular spasm).
- Interferes with TB testing. Vaccinated cattle tend to give reactions to both Avian and Bovine TB and there is reportedly an increased number of "false" IR's.

CONCLUSION

The epidemiology of Johne's disease and the inadequacy of the tests available would suggest that eradication of the disease is not likely to be a viable option and a compulsory national scheme unlikely to be workable. However, the current policy of purely adopting a voluntary approach is, as far as UK agriculture is concerned, at best a gamble. I would argue that a more rigorous approach to identifying infected herds becomes the basic approach and could or should be partly subsidised by the Government.

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