Principles of control of Johne's Disease in the dairy herd

Johne's disease, caused by infection with Mycobacterium avium subspecies paratuberculosis (MAP) is a chronic, debilitating disease of ruminants. In cattle the classic clinical signs are profuse diarrhoea and wasting. However such cases represent the 'tip of the iceberg' and it is estimated that for each clinical case there are 10–25 infected animals in the herd, incurring costs associated with subclinical disease and loss of production. This paper describes the diagnosis and strategies to control of Johne's disease in the dairy herd.

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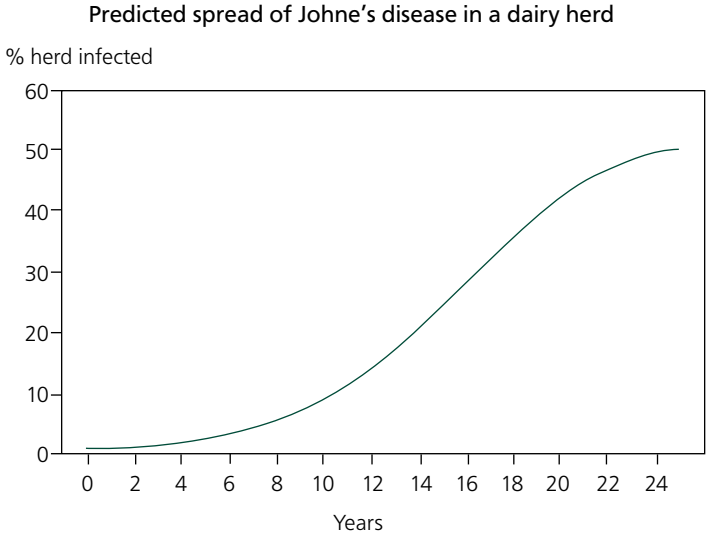

Figure 1. Predicted spread of Johne's disease in a dairy herd; over a period of years the proportion of the herd infected with Johne's is predicted to increase as depicted if commencing with an initially low prevalence of infection (<5% of herd infected) at year 0; after approximately 25 years approximately 50% of the herd is predicted to be infected if control measures are not instigated. The success of preventing this increase in prevalence would depend on the measures used and the diligence with which they are implemented. Image courtesy of www.johnes.org

[Layout - size of 'J']ohne's disease, caused by infection with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a chronic, debilitating disease of ruminants. In cattle the classic clinical signs are profuse diarrhoea and wasting. However such cases represent the 'tip of the iceberg' and it is estimated that for each clinical case there are 10–25 infected animals in the herd, thus it can be seen that the occurrence of clinical cases in a herd is indicative of high levels of infection being present. Left unchecked the disease may spread through the herd as depicted in *Figure 1*.

The fate of an infected animal is dependent on the adequacy of its immune response. The majority (> 80%) of infections with MAP are acquired within the first few weeks of life and a cell-mediated response is mounted resulting in classic granulomatous lesions in the gut wall and associated lymph nodes. In many animals the immune response will be such that infection is limited to these sites and no disease progression ensues. Such animals are infected but not infectious; they do not suffer any detrimental effects of infection and represent no risk to other animals at this time.

In a large subset of animals the cell-mediated immune response will be overcome and the disease process will progress; some of these will eventually succumb to clinical disease. Many animals in this subset will suffer indirect adverse effects associated with infection such as reduced milk yield, increased susceptibility to mastitis and other diseases, infertility, weight loss etc (Villarano and Jordan, 2005). These animals are said to have sub-clinical disease and this is recognised as the chief component of the economic losses associated with MAP infection. As

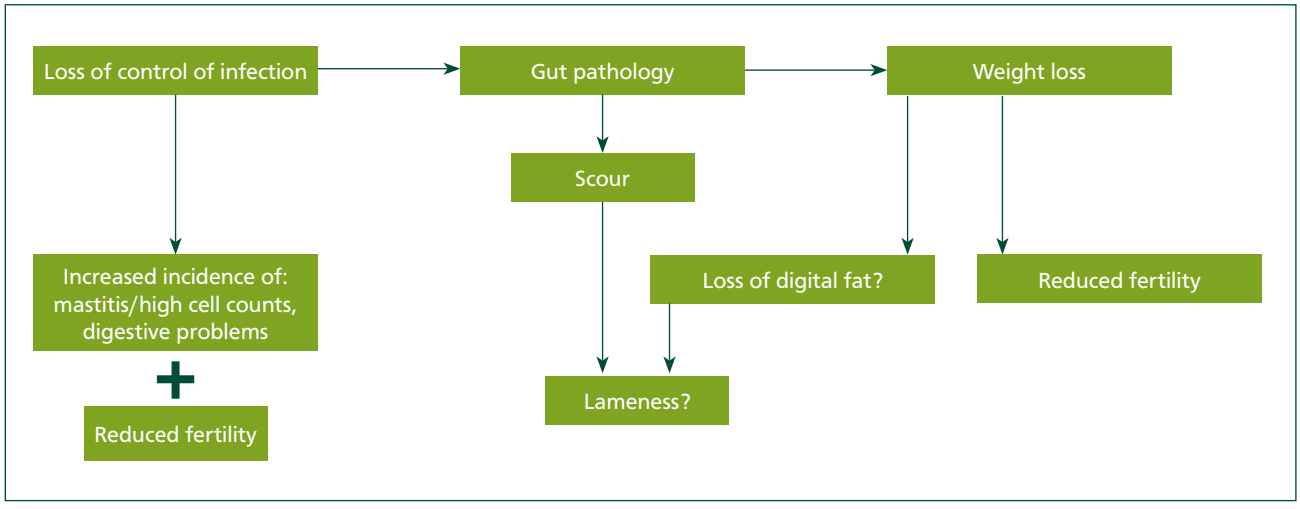

Figure 2 . The potential consequences (some for which there is evidence, others speculative at present) of Johne's disease in the herd.

the disease progresses and the organism proliferates in the intestinal wall the animal will start to shed live bacteria and act as a source of infection to other animals. Such animals are said to be infected and infectious.

It is speculated that loss of the digital fat pad along with other fat reserves may increase prevalence of lameness, as it is also speculated that increase in the quantity of liquid faeces in the cows' environment may contribute to lameness prevalence (Johne's being just one source of increased faecal liquidity). *Figure 2* summarises the potential consequences of Johne's disease.

As the cell-mediated response is overcome by the bacterium, the animal mounts an antibody response. However, this is not protective and the appearance of antibody is associated with further decline in the cell-mediated response (*Figure 3*). Thus presence of antibody is associated with an increased probability that the animal is likely to be shedding bacteria and is suffering from sub-clinical disease. Enzyme-linked immunosorbent assay (ELISA) antibody positivity, therefore, is associated with an increased probability that an infected animal is likely to be infectious

Diagnosis of MAP

There are currently two tests validated and widely used for detection of infection with MAP.

- Faecal culture (FC) which detects the presence of the organism in faeces and thus detects infectious animals. The sensitivity of FC against post-mortem examination (PME) is between 50–60% meaning that it detects approximately half of the infected animals in a population (McNabb et al, 1991; Nielsen and Toft, 2008). Specificity is very high (>98%). By definition, all FC positive animals are infectious.
- ELISA antibody test. This may be performed on blood or milk and detects antibody to MAP. It is measured on a continuous scale of optical density (OD). It detects infected animals that are 'losing the fight' against MAP. Such animals have a high probability of being infectious. The sensitivity of the ELISA against FC is estimated to be between 30–50% (approximately 40% or 0.4) with a specificity of ~98% at the 'standard' quoted cut off (animals over 2 years of age) (Reichel et al, 1999; Eamens, 2000). Since it detects antibody, which is more likely to be present in animals at a relatively advanced stage of infection, its sensitivity in clinical and advanced sub-clinical cases is considerably increased (its sensitivity in clinical cases is estimated at 85%) (Whitlock et al, 2000). Similarly its sensitivity is increased in older animals (since older infected animals are more likely to have antibody than younger infected animals).

As the sensitivity of the ELISA is calculated by reference to FC (which is itself only approximately 50% or 0.5 against PME) the true sensitivity of the ELISA for detection of infected animals is only approximately 0.2 or 20% (true sensitivity = 0.5*0.4

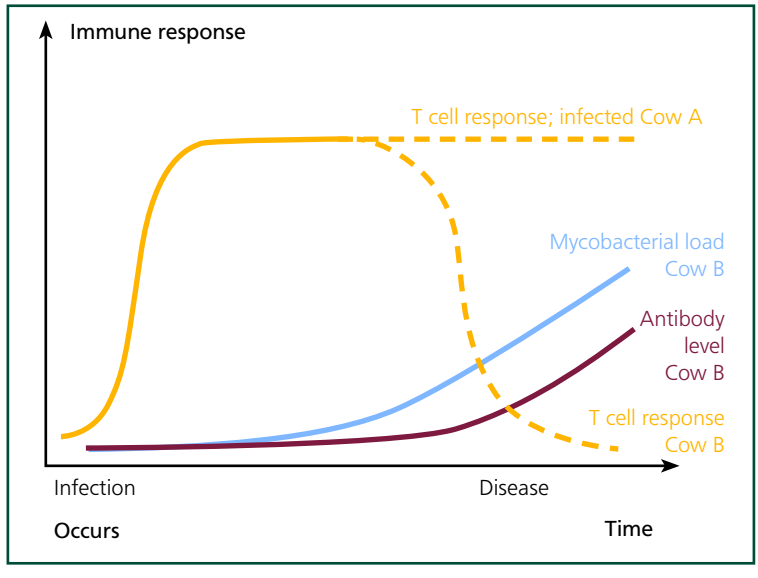

Figure 3. Immune response to Myobacterium avium *(MAP) infection: the yellow line is the initial cell-mediated immunity to MAP ('T cell response') which is protective; its level follows the top horizontal line in a cow that is infected but controlling the infection (Cow A); MAP load will be low and remain so (not shown). If a cow is failing to control the infection (Cow B) her cell-mediated immunity will decline; this coincides with increase in antibody production and increase in load of MAP excreted (red and blue lines respectively). Image courtesy of www.johnes.org with additions for clarification.*

= 0.2), indicating that the approximate true prevalence of infection = (seroprevalence) x 5.

The current paradigm in MAP control schemes is to interpret ELISA test results on a quantitative scale rather than as a simple binary YES/NO. For example, if an OD exceeding 0.3 is taken as indicating that the animal has an 'increased risk of being infectious' compared with an animal with an OD <0.3, then an animal with an OD reading of 0.95 would be regarded as being a higher risk for shedding MAP than one with an OD of 0.2; however, one would not interpret this as the animal with OD 0.2 being 'negative' for MAP infection and the individual with the OD 0.95 as being 'positive'.

In any diagnostic test, such as this, where the result is measured on a continuous scale the sensitivity can be increased by adopting a lower cut-off value albeit there will be a concurrent loss of specificity, i.e. if a lower cut off is adopted, more potentially infectious animals can be detected but the probability of them being a false positive will be increased and we cannot be so sure the animal is truly infectious. Sensitivity can also be increased by repeat testing the same animal at intervals. In part this is believed to be due to 'waxing and waning' of the antibody levels in infected animals that are 'losing the fight' against MAP. Repeat testing has the further advantage of recognising high risk animals earlier, thus allowing management decisions to be instigated earlier.

While the poor sensitivity of the ELISA for detection of infected animals leads many to question its utility saying 'it is a poor test' its value lies in its ability to detect animals at a high risk of being faecal shedders thereby allowing management decisions to be made in order to reduce the probability of that animal transmitting infection to susceptible animals (young calves); fundamentally, the higher the OD score, the greater the antibody response being produced by that individual in response to MAP infection and the greater the chance that the animal will have progressed to faecal shedding of MAP.

Additionally work continues on development and validation of polymerase chain reaction (PCR) for the genetic material of MAP in faeces. According to some authors sensitivity for the direct PCR is comparable to the culture system for high- and moderate-shedding animals (http://vetmed.iastate.edu/departments); it is an evolving technique and may form part of Johne's detection and control programmes.

Faecal smears for the MAP organism is widely regarded as being cheap, easy and specific, but the technique is one of very low sensitivity (resulting in false negatives).

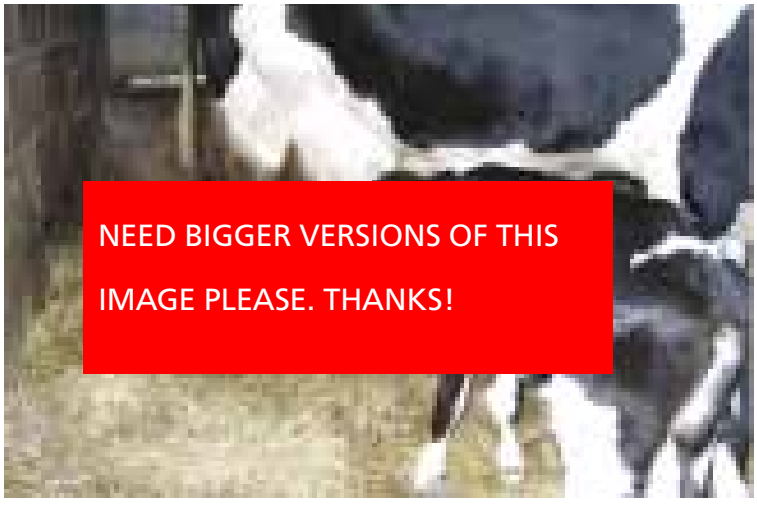

Figure 4. The cow may be contaminated with her own faeces and/or with those of another dam.

Transmission routes of MAP

There is a strong age-associated resistance to infection with MAP with increasing resistance occurring with increasing age; adult animals are generally believed to be at low risk of acquiring infection compared with calves. It is estimated that 80% of infected animals become infected in the first few weeks of life with the greatest risk period being in the first few days after birth, especially in the first few hours after birth. This may be associated with the increased permeability of the newborn calf's intestine which is required for colostral antibody absorption.

Understanding of the transmission routes for MAP and their relative importance is key to development of effective control strategies. While many animals are successful in controlling the infection and are never infectious, a significant number of infected animals (a third according to some estimates) will become infectious at some stage during their life. Increased probability is age associated, in that older infected animals have a greater probability of being infectious compared with younger infected animals.

All infectious animals shed the organism in their faeces and this is, by far, the most important route of transmission. Based on enumeration of FCs, infectious animals may be classified as light, heavy or super shedders. Thus the presence of a shedder, especially a super shedder, will result in heavy environmental contamination bearing in mind that an adult dairy cow produces 30–50 kg of faeces daily. This is of particular importance in animals housed in straw yards during the dry period since this environmental contamination will inevitably result in non-infected animals in the group being passively contaminated and thus acting as fomites (*Figure 4*). This is well illustrated by a recent study from Ohio which found seven of 88 beef cows to be faecal shedders but found heavy contamination of the skin and udder in 33 animals. All the calves of these 33 animals would thus be exposed to MAP within the first few hours of life and it is reasonable to assume a high proportion would become infected since the infective dose is very low.

Cubicle housing, using correctly proportioned cubicle beds and divisions should enable cows to lie on a bedded area and defaecate predominantly into the non-bedded passageway; faecal contamination of other cows from shedders/super shedders is reduced compared with that which occurs in straw yard housing where the faecal contamination of the bedded area is disseminated over a wider communal area.

The other major routes of transmission are via colostrum and milk and it is estimated that approximately third of heavy shedders will also transmit via this route.

A minor transmission route is via in utero transmission but this is only of real significance in late sub-clinical and clinical

cases. However, it must be remembered that cows frequently defaecate during labour thus a calf can be contaminated and infected during the birth process.

Recognition and control of infected herds

Infection with MAP is widespread in the dairy industry globally with studies from other countries suggesting approximately half of dairy herds show evidence of infection (Collins et al, 1994; Neilsen and Toft, 2009; Pillars et al, 2009; Pozzato et al, 2011; Marchetti et al, 2013). A recent UK study carried out by the Veterinary Laboratories Agency (VLA) suggested that 34.7% (95%CI 27.6–42.5) of dairy herds are infected. There are three key steps in establishing a successful Johne's control scheme: risk assessment; demonstration of the presence of disease; and control in the infected herd.

Risk assessment for introduction and spread of the disease

This will identify biosecurity risks for introduction such as purchase of animals and identify the key risks for spread on the farm. Identification of key risks for spread is vital since this in turn will allow the development of a realistic achievable control programme.

Demonstration of the presence of the disease on the farm.

This will involve testing and there are various options, each with its advantages and disadvantages.

- Whole herd ELISA test on milk or blood samples. This is likely to have the highest sensitivity in terms of detection of disease and also allows a crude estimation of the infection prevalence.
- Culture of pooled environmental samples, e.g. two samples each from collecting yard, passageways etc. Sensitivity of 70% has been shown in American studies (Raizman et al, 2004; Berghaus et al, 2006; Lombard et al, 2006; Pillars et al, 2009)
- ELISA '30 cow screen' — this is usually carried out on milk samples. 30 cows are selected for testing based on history, e.g. older cows, cows with high somatic cell counts or other indicators of mastitis, cows that have performed poorly in current lactation, infertile cows, cows with 'health problems' etc. The basis for this protocol is that animals with a high probability of being ELISA positive are selected from the general population for testing. Thus, its utility is completely dependent on the selection of which animals to test.
- Bulk milk ELISA testing — this suffers from a lack of sensitivity in that it will only pick up herds with relatively high levels of infection (>5% of herd infected) (van Weering et al, 2007).
- Irrespective of method employed, a negative result cannot be taken as meaning a herd is conclusively free of MAP infection. In herds that test negative, it is advisable to carry out further testing on more than one occasion to further ascertain infection status. If herds test negative and if adequate biosecurity is present then it is worth considering enrolment in Johne's Disease Certification under the CHeCS schemes.

Figure 5. The communal calving pen. *(AQ13 Can you provide a high res image?)*

Control in the infected dairy herd

The objective of control is to reduce the likelihood of calves becoming infected with MAP thus efforts must be directed to minimising the chief routes of infection namely:

- Oro-faecal infection of the young calf in the peri-natal period
  - From the dam
  - From other cows in the calving area, i.e. cows acting as fomites
  - From the calving environment.
- Oro-faecal infection from other infected young calves during the first few weeks or months of life. This route of infection has been suspected and is now confirmed (Van Roermund et al, 2002; Marcé et al, 2011).
- Infection via ingestion of contaminated colostrum or milk by the young calf.
- Efforts should also be addressed at minimising oro-faecal infection in older calves although the risk is progressively reduced as the calf grows older.

Thus the key physical areas to address are: housing of the dry cow prior to calving; the calving area; the calf housing; and the colostrum and milk.

Housing of the dry cow prior to calving — the objective here is that uninfected or non-shedding cows should not become passively contaminated with MAP acquired from infected shedding cows via a shared environment. Straw yards are a very high risk environment in this respect and this may outweigh the undoubted comfort benefits they have. It may be opportune to consider large sand-based cubicle housing for dry cows in the future. This would likely cut down the contamination risk for cows sharing an environment with an infectious herd mate.

Calving area (*Figure* 5) — often this is the dry cow housing area (although ideally cows should calve in clean individual pens). Adequate cleaning and disinfection between cows should be carried out; in reality it is likely to be cursory at best. One limiting factor is the need for a 'firm footing' for the calving cow — this usually means a bed of manure is present always with

new bedding added to 'top up' — this will not achieve realistic decontamination if MAP is present.

Calf housing — group housing will represent a risk as will inadequately cleaned and disinfected individual pens or hutches.

Prevention of contamination of the dry cow (and calving) areas is almost impossible if a faecal shedder spends any appreciable time in that environment. Thus the current recommendation is risk management of individual cows based on repeated milk ELISA testing. Repeat testing and utilisation of a lower cut off allows identification of animals considered to be at a 'high risk of being infectious' allowing management decisions based on their ELISA positivity to be made. All such cows should be permanently marked as being 'high risk' — this is generally achieved by inserting a red ear tag, and possibly freeze branding 'J' on the rump. Ideally any animal with a high ELISA reading should be culled as soon as is possible taking other factors into account (there is no point culling a farm into bankruptcy!).

All high risk cows should be managed separately during the dry period and at calving. Any calf borne of a red cow and due to be kept must be 'snatch calved' at birth, i.e. delivered into a clean wheelbarrow or similar and allowed no contact with the dam. If it is contaminated at calving with faeces, the calf should be washed clean with a disinfectant solution, e.g. Hibiscrub or similar. Calves borne of 'red cows' should be considered as being of high risk of being infected and if kept should themselves be identified as 'red' and managed as such throughout their lives.

Figure 6. Communal milk feeder; useful if kept scrupulously clean; a risk factor for disease spread if pooled milk and/or colostrum includes that from infectious cows.

Thus a key requirement is a separate dry cow and calving facility for red cows — the so called 'leper colony'. After calving a red cow will be able to return to the milking string, but ideally her calf (if kept) should be tagged 'red' and reared separately from the future dairy replacement calves for at least the first few months of life.

Colostrum and milk (*Figure 6*) — this is a lesser route than the oro-faecal route with about one third of heavy shedders excreting the organism in their colostrum or milk. The route may be controlled relatively easily as follows:

- No waste milk feeding to be carried out.
- Calves only receive their dam's colostrum. If the dam is herself a red cow and her calf is being kept then it should be fed frozen colostrum from another dam (see below).
- Colostrum must never be collected from a red cow.
- Colostrum for freezing should only be collected from heifers and ELISA lifetime test negative cows.
- Pooled colostrum feeding must never be practised
- Colostrum may be pasteurised to reduce (but not abolish) the risk of MAP contamination using purpose built pasteurisers. If this is carried out, all pasteurised colostrum must be fed immediately or stored chilled at 40°C (this is best practice for all colostrum, pasteurised or not).

A particular challenge is the large spring block calving herd; it may be that outdoor calving with different pastures for high risk animals is suitable here. Such herds are often group fed on waste milk and colostrum; this is a recipe for disaster in the infected herd and should be replaced by powdered milk feeding or pasteurisation.

Other risk factors for spread

In essence any practice whereby youngstock are exposed to adult faeces constitutes a risk, albeit a lower risk the older the stock being exposed. Thus practices such as grazing youngstock on fields previously grazed by adult stock, spreading manure on fields destined for youngstock etc all represent a risk. Other risks include drinking from stagnant water courses, sheep and rabbits (Shaughnessy et al, 2013). However the magnitude of these risks is much lower than those associated with the calving cow and the baby calf.

Conclusion

As should be apparent, development of a control programme is farm specific and must be based on a detailed risk assessment carried out by the veterinary surgeon in conjunction with the farmer. Different management systems will throw up different challenges with different solutions.

For successful control it is essential to prioritise the risks then deal with the major risks first and foremost. LS [layout - this should be a solid box]

Acknowledgements for images: http://www.johnes.org and www.myhealthyherd.co.uk/

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KEY POINTS

- Johne's disease is a chronic debilitating disease of cattle which has important clinical consequences and an economic impact on the productivity of the herd.
- Infected cattle become infectious and excrete MAP , becoming 'shedders'.
- Cattle are most vulnerable to infection when they are young, with neonates and calves being the most susceptible.
- Faeces from infected adult cattle are the most significant source of infection to young bovines.
- Control of the disease depends on identification and management of risk factors for spread, as well as identification of infected animals and their removal from the herd.