

Johne's disease – a diagnostic breakthrough

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The dairy industries of the world suffer major economic impacts from Johne's disease.

According to a study conducted by the National Animal Health Monitoring Systems (NAHMS) in 2007, around 68% of all US dairy operations were infected with *Mycobacterium avium* paratuberculosis (MAP), the pathogen that causes Johne's disease. It is estimated to cost the US dairy industry around \$250 million each year as a direct result of reduced dairy production, extra care needed for calves, additional veterinary expenses, etc.

The indirect loss, however, could be much higher: consumers are increasingly worried by reports which seem to confirm a link between Johne's disease and Crohn's disease, a human chronic enteritis. If this hypothesis is ever confirmed, consumer confidence in dairy and beef products could decline dramatically. In this case the economic consequences for dairymen in the US and around the world would be impossible to forecast.

In many countries, MAP infection is merely 'notifiable', which means that rehabilitation measures are undertaken on a voluntary basis. However, in the past, this has proven difficult for the cattle owner and veterinarian due to the lack of a prompt and reliable diagnostic tool.

Since Johne's disease is an incurable disease without any prospect of an effective treatment, or a reliable vaccination, diagnosis remains the basis for the only means to manage the disease: good hygiene and herd management.

The tip of the iceberg

It is very clear that a correctly diagnosed clinically diseased cow represents only the tip of the iceberg for a livestock farmer. According to scientific projections, for every one clinically diseased cow that has tested positive and that excretes pathogen, there are approximately 15-25 additional infected cattle. Infection with MAP occurs either in

Stage	Disease status	Livestock affected
IV	Progressed clinical signs of diseases	1
III	Clinical disease (first signs of disease, recurring diarrhoea and weight loss, excretion of pathogen with faeces and, potentially, milk; diagnosis mostly correctly positive)	1-2
II	Subclinical disease (no signs of disease, intermittent excretion of pathogen with faeces, diagnostic tests rightly positive or falsely negative)	4-8
I	Silent infection (no signs of disease, no excretion of pathogens, conventional tests falsely negative)	10-14

Table 1. The iceberg effect of MAP infection.

utero (10% of infections) or during the calf's first six months of life. After six months, pathogen challenge needs to be significantly higher to infect a cow.

Infection occurs via colostrum or whole milk from infected mother cows, faeces carrying pathogens or contaminated fodder.

The orally absorbed pathogen gets into the intestinal mucosa, duplicates there and retires to the os ilium and mesentery lymph nodes. This is known as the silent infection stage.

After three to five months the next stage starts: MAP is excreted intermittently; however, the animal does not show any symptoms, and diagnostics remain mostly negative. In this second stage, which can last for a very long time, very high economic losses occur.

Correct diagnosis happens mostly in the third (clinical) stage, or in the fourth (pro-

gressed clinical) stage; respectively, the animal shows incurable diarrhoea and, as a consequence, an excessive loss of weight and, potentially, throat oedema.

The cow enters this stage often during its second or third gestation. It is only then that the animal is correctly tested positive and can be eliminated from the stock – only after it could have infected many new calves.

Combating Johne's disease

The increasing prevalence and cost of Johne's disease in many countries has led Life Technologies, a company that specialises in molecular diagnostics, to develop a real-time PCR (Polymerase Chain Reaction) test as a significantly improved diagnostic tool for identifying MAP. The test has recently become the first of its type to be validated and registered by the Friedrich Loeffler Institut (FLI) in Germany – and is therefore likely to be widely used by veterinarians in that country and others in Europe.

Unlike previous diagnostic tests, PCR can detect the presence of very small amounts of MAP DNA in faeces in the early stages of infection. Thus, it has the potential to be used for screening herds and as the basis for eradication programs.

A reliable and effective diagnostic tool that can identify infected animals at a stage when they are not yet clinically diseased is crucial for effective management and rehabilitation of infected stock.

It is worth remembering that such rehabili-

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MAP and Crohn's disease

Johne's disease is caused by *Mycobacterium avium* ssp. *Paratuberculosis* (MAP). Infected cattle excrete it mainly in faeces.

Clinically diseased animals, however, also excrete it in the milk. MAP is very robust and can survive up to nine months in bovine faeces. Moreover, MAP can also be found in pasteurised milk, which is therefore a source of infection for humans.

Currently, the scientific debate focuses on whether the existence of MAP in the intestine of Crohn's disease patients constitutes mere coincidence or causality.

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tation requires intensive and costly hygiene management procedures that will severely affect the farmer's day-to-day work.

Basically, the hygiene management requires that young cattle of up to two years must not be in touch with the rest of the stock; any infected calf will be separated from its mother immediately; good colostrum management, with no pooling of the milk for the calves; separate fodder for old and young cattle and separate pasture management; changing of clothes/overalls for all employees and the veterinarian when entering the young animals' area; and much more!

The fight against Johne's disease requires a

combination of hygiene management, diagnosis, elimination of excretors, status definition and rules for trading in dairy cattle.

Therefore, it is more complex than other eradication programmes for epizootics; it requires a tailor-made strategy for every farm and this cannot be designed from the desktop.

Improved diagnosis

Indirect pathogenic proof, ie the presence of antibodies in the blood, unfortunately lags significantly behind the infection due to the long incubation period of MAP. A mere 15% of the animals that excrete few pathogens

(stages II and III) tested positive in a study using ELISA, as compared to 87% of the animals in stages III and IV. That is equivalent to an average sensitivity of 45% for the ELISA test.

Direct pathogenic proof – samples usually being taken from faeces – depends on the disease stage of the animal. With high and regular excretion, ie at a progressed stage of the disease, MAP can be instantly verified by microscopy with a particular staining.

However, if there are only few mycobacteria in the blood even this test can give a false negative result. In that case, a very time consuming faecal culture has to be undertaken.

This is designed to allow the pathogen to multiply and therefore enable its identification, but it can take up to 16 weeks – a period of time during which the infected animal will cause economic loss and remain active as a source of further infection.

This extremely long period of time can be reduced to a few hours thanks to testing based on real-time PCR. This molecular technology has the potential to revolutionise the rehabilitation of livestock infected with MAP.

Real-time PCR has been part of the Dutch national control program for Johne's disease over the last three years and has been very effective. The relevant veterinarian has to take faecal samples if there are indications of infection and send them to the laboratory.

The presence of MAP DNA can be confirmed (or refuted) by the laboratory after only a few hours.

Managing Johne's disease has been very difficult in the past because there has been no quick, reliable test which can identify infected animals at an early stage.

The real-time PCR test from Life Technologies means that farmers and veterinarians now have a proven tool which can identify paratuberculosis (MAP) infection much earlier, and thus take appropriate action before animals start to spread the infection around the herd.

The test can detect small amounts of MAP DNA in faecal samples from infected animals within a matter of hours. Because it is based on DNA identification, it is very accurate and has a low level of false positives and false negatives.

So, the farmer can make good decisions with confidence. This is potentially a very useful tool to help farmers deal with this difficult disease.

The Life Technologies PCR test provides a way of checking individual animals, such as new additions to a herd, and also a means of screening herds for the presence of the disease. If the base of the 'iceberg', i.e. the 15-20 infected cattle, can be identified more quickly, then rehabilitation measures can be implemented quickly and effectively.

As such, the PCR test provides a good basis for the veterinarian and the cattle-owner to develop an effective strategy to combat this costly and potentially dangerous disease. ■