AXIOM
Veterinary Laboratories Ltd
'The Quality Clinical Pathology Service'

Johne's Disease
– what test should I use and when?

MICROSCOPY:

A ZN smear is made from faeces and examined for acid fast bodies that are typical of *Mycobacterium avium* subsp *paratuberculosis*. If clumps are seen this is consistent with a diagnosis of Johne's disease. If single acid fast bodies typical of MAP are seen then this is considered suspicious of Johne's disease but it is not sufficient to make a diagnosis.

Advantages: A result can be obtained the same day that the sample is received. Relatively low cost

Disadvantages: The test is of low sensitivity in clinical cases (approximately 30-35%).

SEROLOGY:

The sensitivity is about 90% in clinical cases. In healthy animals the test has a relatively low sensitivity as cattle will not develop antibodies until close to the clinical phase. However some cattle can be detected as antibody positive for up to a few years before showing clinical signs but others only develop detectable antibodies shortly before they show clinical signs.

Timing for routine serological screens:

Routine herd screening allows subclinically infected cattle to be identified so that they are removed for culling whilst still in relatively good body condition. Blood testing suckler herds a few months before the start of calving is ideal as it allows the antibody positive cows to be removed from the calving pen and calved in a separate area which reduces the challenge to the calves born into the herd. In suckler herds it may be possible to retain the cow, in isolation with her calf, until it is old enough to be weaned and she can then be culled. The calf should not be retained for breeding as it will be at high risk of having become infected. An infected cow's last two calves would be considered to be at highest risk of being infected. The dung from the pen should preferably be spread on arable ground but if this is not possible it should be stored for at least a year.

In dairy herds blood testing at drying off can identify infected cows allowing them to be managed in a separate calving area and their colostrum and milk should not be fed to calves. These cows can be removed at the end of lactation or sooner if they start to lose condition before this. As some cows develop antibodies only a short time before showing clinical signs, and as signs are most common within a few months of calving, then a pre-service blood test can also be useful (around 30 days in milk). Blood testing is preferable as milk serology has been shown to have a sensitivity of about 80% compared to serum.

Avoid serological testing within two weeks either side of calving as false negatives can occur at this time due to serum antibodies declining just before parturition. This has been shown to occur with antibodies to other agents too. It may be due to sequestration of antibodies in colostrum or it could be due to periparturient relaxation in immunity.

The specificity of the serum ELISA that we use is over 99% so about 1 in a 100 uninfected cows will give a false positive result. False positives tend to be low positive results. Faecal testing can be used to further investigate an animal's status or serology can be repeated after a month if it is a healthy animal.

The specificity of the milk ELISA is about 99% so for a herd with 300 cows in milk about three results could be false positives on average. This is why it is important not to cull on the basis of one positive milk result. As serum testing tends to be more sensitive than milk then cows that were positive on milk testing can be blood tested to investigate their status further. If they then test as negative on blood they should continue to be monitored. Faecal testing can also be done e.g. a PCR or culture however in the early stages of infection faecal shedding can be intermittent or at a low level.

False positives can occur following a TB test. Samples can be collected on the first or second day of a TB test but cross reactions can occur if samples are collected within three months of completion of a TB test. If blood samples do have to be collected within this time period then any positive animals can be retested after a further six weeks to check for declining titres or the animal can have faecal testing done on it instead (though shedding can be intermittent or at a low level initially).

Advantages of serology: Low cost test so useful for herd screening, good sensitivity for clinical cases, quick turnaround time

Disadvantages of serology: Low sensitivity in young, healthy cattle. About 10% of clinical cases will test as antibody negative. False positives possible after a TB test.

Faecal PCR

The PCR test detects *Mycobacterium avium* subsp *paratuberculosis* in faeces samples. It is a very specific test so false positives are very rare. It is useful in clinical cases and can be a useful second line test if a suspected case is seronegative on blood testing. In the earlier stages of infection shedding of MAP in faeces can be intermittent and/or at a low level so infected animals can be missed. It is possible that cattle that are just passaging the organism could test as positive on faeces.

Advantages: Useful for clinical cases, especially if only faeces are available. Useful for seronegative suspected clinical cases. Quicker result and lower cost than faecal culture. Useful if there has been a recent TB test. Can be used as second line test if concern about a potential false positive result on serology

Disadvantages: Low level or intermittent shedders may be missed.

Culture

Faecal culture is the gold standard test in the living animal. It usually involves culture for up to 42 days. It was previously considered to be more sensitive than direct PCR testing of faeces however the sensitivity of the MAP PCR test on faecal samples has improved recently due to a new extraction method. Culture may be negative in the earlier stages of infection due to intermittent or low level shedding. It can be a useful test for bought-in animals.

Advantages: Possibly more sensitive than PCR for faecal shedding detection. Useful for bought in animals in addition to serology. Can be used as second line test if concern about a possible false positive result on serology

Disadvantages: Up to eight weeks for a result. Relatively high cost. Low level or intermittent shedders might not be detected.

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