New perspectives on positive results to ELISA testing

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How should we regard animals that have Positive Johne's disease results by an ELISA test and Negative by a PCR test?

This is a relatively common scenario in the Irish Johne's Control Programme, under which all animals aged two years or more are required to be tested annually by ELISA using blood or milk samples and animals with an ELISA result of Positive or Inconclusive are generally required to be tested by PCR using dung samples ('ancillary test'). Almost all of these animals (in the order of 95%) will return a negative PCR result, despite already having had a Positive or Inconclusive ELISA result.

The main difference between the two tests is that the ELISA test looks for the presence of antibodies (the animal's immune system response to the presence of infection) in the blood or milk sample; whereas the PCR test looks for the presence of DNA from the causative bacteria (MAP) in the faecal sample. The nature of MAP infection (which often progresses slowly whilst hidden from the immune system) means that animals are typically infected for years before either test will detect the respective antibodies or DNA, and typically the detection of antibodies precedes the detection of DNA.

In the programme, the PCR test is being used to answer two different questions.

Is MAP present in the herd?

- A positive PCR test demonstrates the presence of MAP, and thus Johne's disease, on the farm. That is why, once a herd has a Positive result from a PCR test that confirms infection in the herd, the programme does not require or fund further PCR testing. Once infection in the herd is confirmed, all animals with subsequent ELISA results of Positive or Inconclusive can reasonably be interpreted as being infected, irrespective of whether they have a PCR test and the result of any such PCR test.

Are ELISA-positive animals infected?

- In herds with no previous evidence of Johne's disease, the PCR test is being used to assist in clarifying the infection status of those animals that previously tested positive to the ELISA test. This step is important because a small proportion (approximately 2%) of non-infected animals will test positive to the ELISA test (so-called 'false positive' reactions). Two results are possible following a PCR test in an animal that previously tested positive to the ELISA test:
  - » **The PCR result is positive**, which provides confirmation of infection. That is, it can be assumed that both the animal and the herd are infected with MAP.
  - » **The PCR result is negative** which, in contrast, does not prove that this animal is uninfected. A potential reason for this relates to the biology of MAP infection: if an animal were infected with MAP, but the disease had not progressed, antibodies may be present in the blood or milk, but DNA is not yet present in the faeces. These animals are considered 'suspect', pending their test outcome the following year.

What should be done to those animals that have a Positive ELISA result but a subsequent PCR test with a Negative result?

It is important that the assessment of the likelihood of infection in these animals has veterinary input, so the programme provides for an approved veterinary practitioner to support each herd.

Factors to be considered in assessing the likelihood of infection under these circumstances include:

- The number of animals in the herd with Positive or Inconclusive ELISA results.
- The strength of the positive ELISA result (known as the S/P value), with higher values associated with higher likelihood of the animal being infected.
- Test results from previous years at both herd level and for the animals with an ELISA positive or inconclusive result.
- Disease history for the herd, especially whether there has been a prior diagnosis of Johne's disease or un-diagnosed clinical disease of profound wasting and diarrhoea.
- The history of animal movements into the herd, including number of animals, number of source herds, and risk factors for those source herds (infection status, test results, trading history).

The likelihood of infection being present is only one element of a risk assessment for Johne's disease; the assessment should also consider the consequences of infection, if present. We now understand that not every

infected animal that is ELISA-Positive for Johne's disease will necessarily progress to clinical disease or even shedding infectious MAP. Progression of infection to disease is triggered and accelerated by stress, influenced by other factors such as genetics.

ELISA-Positive animals may progress to shedding of infectious MAP and clinical disease, and one of the key planks to reducing the risk of spread of Johne's disease within a herd is removing high-risk animals before they spread infection. Whether, and how soon, ELISA-positive and suspect animals are removed from the herd depends on where the herd wants to balance the costs and benefits of controlling risk of spread.

But in the programme we observe that some ELISA-Positive animals are retained and return recurring annual test results of ELISA-Positive and PCR-Negative. The trends in S/P values for these animals over time are interesting: there is generally some fluctuation but for many there is an incremental rise in the ELISA S/P value, indicating a stronger immune response over time and higher likelihood of being infected and progressing to shedding and clinical disease.

For a few animals, the S/P values decline over time for the test result to become Negative. This may be due to the animal not being infected (false-positive ELISA result); containing (but unlikely to totally eliminate) infection, and thus recovering from infection either temporarily or permanently; or a failure of the immune system to sustain antibody production such as in advanced disease.

Remember that just test-and-cull is not a sufficient strategy to effectively control spread of Johne's disease. Protecting calves from exposure to infection through critical attention to hygiene at calving, in calf pens and in the pens and pastures of replacement breeding stock is also essential. Again, your approved veterinary practitioner can advise on this aspect.

In summary, the PCR test is not a better test than the ELISA test; it has a different role. As highlighted above, the PCR test is used to demonstrate the presence of MAP and thus Johne's disease on the farm. Further, in herds with no previous evidence of Johne's disease, the PCR test is also being used to assist in clarifying the infection status of those animals that previously tested positive to the ELISA test. In these latter herds, any animal with a positive ELISA test result should be suspected of being infected pending further testing and veterinary assessment.