



## ANIMAL HEALTH AND WELFARE

# Diagnosing ovine Johne's disease

Accurate and rapid detection of ovine Johne's disease (OJD) infection, in both individual sheep and flocks, is a major challenge to the sheep industry as it works to control the spread and impact of this disease in Australia.

The disease is caused by a bacterium, *Mycobacterium avium* subsp. *paratuberculosis*, and has a very long incubation period from the time of initial infection until the development of obvious signs of illness. By shedding bacteria in their dung, contaminating pasture, soil and water, infected sheep can spread OJD infection for many months, or even years, before obvious signs of disease are noticed.

In order to develop effective control programs, the ideal diagnostic test for OJD should be able to identify infected sheep or flocks before they start to shed bacteria that exposes other livestock to the infection. A range of flock tests and individual sheep tests are currently available; however, all have benefits and limitations, and none are able to accurately detect early infections. Ongoing research is focusing on the development of new tests that can quickly and accurately detect infected sheep, particularly in the early stages of infection.

## Flock testing

Most of the currently available tests for OJD have a limited capacity to detect individual infected sheep, particularly young animals and those in the early stages of infection. In addition, testing of every sheep in a flock is impractical and uneconomic with the current tests in most situations. Flock testing of a sample of animals is therefore commonly used to detect the presence of OJD within a flock. Existing tests can be used with good accuracy to assess the flock's infection status.

## On-farm flock testing

Two main tests are currently used for on-farm flock screening:

1. **Pooled faecal culture (PFC)** to detect OJD bacteria in dung
2. **Blood tests** to detect antibodies to OJD bacteria in the blood (serology)

## Key points

- Current diagnostic tests for OJD have a limited ability to detect individual infected sheep, particularly in the early stages of infection. However, they can be used with good accuracy to assess the infection status of flocks.
- The most common flock screening test is pooled faecal culture (PFC). PFC is able to detect OJD in 98% of infected flocks if more than 2% of sheep in the flock are infected.
- The AGID blood test is also an official OJD flock screening test. It is less sensitive than PFC. Because of this, more sheep must be tested to provide a similar level of confidence in the results.
- Abattoir surveillance of culled adult sheep is conducted in all states, and provides a practical and cost-effective means of detecting OJD in flocks. Negative abattoir surveillance results can now be used to earn credit points towards the new ABC Score when trading sheep.

## Pooled faecal culture

The most common test used to screen flocks for OJD infection is pooled faecal culture (PFC). A single dung pellet is collected from at least 350 adult sheep in the flock and cultured in 'pools' of 50 for the OJD bacteria.

PFC is sensitive enough to detect infection when only one sheep in a pool of 50 is shedding the bacteria. In flocks where more than 2% of sheep are infected, PFC can detect the infection in at least 98% of cases, meaning a very high chance that infected flocks will be detected. However, in flocks where less than 2% of sheep are infected, the ability of the test to detect the disease falls to around 80% or less, depending on the percentage of infected sheep present.

PFC is cheaper than blood testing, costing around \$1,000–\$1,500 for an average flock of 4,000 sheep. However, as the OJD bacteria grow very slowly, it takes at least three months to obtain a negative result. A positive result from an infected pool of dung samples may be detected sooner, but can take up to five months to be confirmed and reported.

## Blood tests (serology)

Flocks can also be screened for OJD by blood testing a sample of sheep. The blood test traditionally used for flock testing has been the agar-gel immunodiffusion test (AGID) that detects antibodies to the OJD bacteria. When similar numbers of sheep are sampled AGID is less sensitive than PFC, detecting only 85% of flocks with an OJD prevalence of 2% or greater and only 33% of lower prevalence flocks. Due to the lower sensitivity of the AGID test, more sheep (at least 875) must be tested, in comparison to PFC (350 animals).

AGID is a simple and fast test with results available in about one week; however, it is expensive, costing between \$6 and \$15 per sheep, or \$5,000–\$13,000 for a flock test using 875 animals. AGID cannot detect OJD in sheep in the early stages of infection and is only used in animals over two years of age. The use of this test has decreased since the introduction of PFC. AGID can, however, be used to quickly identify individual infected sheep for culling purposes in heavily infected valuable flocks.

## Abattoir surveillance

Abattoir surveillance is a practical and cost effective means of checking for OJD infected flocks across geographical regions. Meat inspectors at abattoirs in all states now inspect lines of adult sheep for visible signs of OJD in the intestines and lymph nodes. Since late 1999, over 18 million sheep have been examined, with less than 4% of lines detected as positive. In areas that have a high prevalence of flocks with long-standing OJD infection, inspectors have been able to detect 90% of infected lines from visual examination of the internal organs. The capability of abattoir surveillance to detect infection in sheep from low prevalence regions, or areas where the disease has only recently been introduced, is expected to be lower because there will be fewer infected animals.

Producers are notified if OJD lesions are found in their sheep and follow-up testing is carried out on the property to confirm the infection. The success of abattoir surveillance relies on the use of effective sheep identification to ensure reliable trace-back to the property of origin and on trained, experienced inspection personnel.

Negative abattoir surveillance results can now be used to earn credit points towards the 'ABC Score' when trading sheep under the new Assurance Based Credit (ABC) Scheme that started on 1 July 2004. The ABC Scheme is a nationally agreed method for assessing the risk of OJD infection in sheep at the flock or mob level. The scheme will help producers to keep the infection out of their flocks, and will also provide a pathway for owners of infected flocks to improve their trading position via on-farm management of the disease.

## Individual sheep testing

### Live sheep

All of the current tests for OJD in live sheep are primarily used as flock tests, as they have a relatively low chance of detecting an individual infected animal, except in advanced cases.

It is now technically possible to use culture of dung from an individual animal to detect OJD-causing bacteria; however, the cost and time needed to obtain a result are major limitations for timely decision-making at the property level. Early infections of less than nine months will not be detected, as faecal shedding will not have commenced. Shedding of the bacteria may also be intermittent in some infected animals, so manure samples may need to be taken on several occasions. A recent study has shown that up to 15% of sheep may recover from early OJD infections, which means that testing of young sheep (eg at around 12 months of age) may not be a reliable predictor of their later disease status. Individual faecal culture is usually reserved for high value animals, such as stud rams.

A skin test, similar to the human Mantoux test for tuberculosis, has been used to detect OJD in individual animals. This test is very labour intensive, as it requires two visits to the property – one to administer the test dose and one to assess the skin reaction to the dose. Results under laboratory conditions did show promise; however, the test has failed to deliver adequate results in on-farm situations and is not currently being used.

### Dead sheep

Routine post-mortem examinations, microscopic examination of intestinal tissues and culture of intestinal tissues and lymph nodes can be carried out on dead sheep to detect OJD infection. These tests are considered to be the 'gold standard' of OJD infection status.

## New tests under evaluation

### Gamma interferon test

The gamma interferon test is a new test that has been under evaluation for some time. The test initially showed promising results for early identification of OJD-infected sheep before they started to shed bacteria in their dung. This blood test detects a cell-mediated immune response, which is the first reaction generated by the immune system in animals exposed to infection with mycobacterium.

Field trials to establish the accuracy of the test are continuing, however the incidence of false positives (uninfected animals that test positive) in older sheep has proven to be high. Additionally, the ability of the test to detect positive sheep has varied considerably, ranging from greater than 50% down to as low as 8%. Therefore, the test cannot currently be considered for approval as an official diagnostic test.

A new research project is attempting to improve the gamma interferon test, to make it more effective at detecting infection and more practical for use in the field. If the test proves able to detect early infections that progress to clinical disease, it may have potential application as a flock-screening test and to identify individual high value animals that have not been exposed to the disease. The test may also allow infected sheep to be culled at an early age before they begin to spread the infection.

## Polymerase chain reaction (PCR) test on faeces

This test on dung samples detects fragments of DNA from OJD bacteria. Pooled or individual dung samples are tested from a number of sheep in a flock. A result is obtained in about a week, compared to up to four months for PFC. However, in its present form, the PCR test has not proved

to be as sensitive as faecal culture and only detects about two-thirds of the positive pools of dung detected by PFC.

As the PCR test relies on the detection of OJD bacteria in the dung it cannot identify early infections before sheep begin to shed the organisms. This test is not yet accepted by animal health regulatory authorities and is not currently used in OJD control programs.

Table 1: OJD tests

Test	Application	Benefits	Limitations
<b>Existing tests</b>			
Clinical signs	<ul style="list-style-type: none"> <li>Individual live animal test</li> </ul>		<ul style="list-style-type: none"> <li>Signs not apparent until late in disease</li> <li>Can get false positives due to other diseases causing similar signs</li> <li>Requires confirmation by other tests</li> <li>Heavy bacterial contamination of the environment may occur before diagnosis is made</li> </ul>
Faecal culture	<ul style="list-style-type: none"> <li>Flock screening test</li> <li>Can be used as an individual animal test</li> </ul>	<ul style="list-style-type: none"> <li>No false positives</li> <li>Less expensive than blood testing as a flock test</li> </ul>	<ul style="list-style-type: none"> <li>Takes several months to get results</li> <li>May get false negatives</li> <li>Can take months or years for infected sheep to test positive as they must be shedding the OJD bacteria in their dung</li> <li>Very expensive if used as an individual animal test</li> </ul>
Blood tests for antibodies	<ul style="list-style-type: none"> <li>Flock screening test</li> </ul>	<ul style="list-style-type: none"> <li>Result obtained in 1–2 weeks</li> </ul>	<ul style="list-style-type: none"> <li>Unreliable in sheep less than two years old</li> <li>Less sensitive than faecal culture so more animals must be tested</li> <li>More expensive than PFC as a flock test</li> </ul>
Gross pathology	<ul style="list-style-type: none"> <li>Dead animal test</li> <li>Abattoir surveillance</li> </ul>	<ul style="list-style-type: none"> <li>Useful flock-screening test for detection of infected flocks (abattoir surveillance)</li> </ul>	<ul style="list-style-type: none"> <li>Not apparent until late in disease</li> <li>Not suitable for live animals</li> <li>Requires confirmation by histopathology or culture</li> </ul>
Microscopic examination of intestinal tissues	<ul style="list-style-type: none"> <li>Dead animals</li> <li>Confirmation of other tests</li> </ul>	<ul style="list-style-type: none"> <li>False positives rare</li> <li>Usually considered the 'gold standard' test</li> </ul>	<ul style="list-style-type: none"> <li>Not suitable for live animals at this stage</li> <li>Can miss recently infected animals</li> </ul>
Culture of intestinal tissues	<ul style="list-style-type: none"> <li>Dead animal test</li> </ul>	<ul style="list-style-type: none"> <li>More sensitive than faecal culture and possibly histopathology</li> <li>Detects infection earlier than faecal or blood tests</li> </ul>	<ul style="list-style-type: none"> <li>Can miss early infections</li> <li>Takes several months to get results</li> </ul>
Skin test	<ul style="list-style-type: none"> <li>Live animal test for early infection</li> </ul>	<ul style="list-style-type: none"> <li>Detects early infections</li> </ul>	<ul style="list-style-type: none"> <li>Has not proven effective in the field</li> <li>Labour intensive</li> <li>Antigen not currently available for use</li> </ul>
<b>New tests</b>			
Gamma interferon test	<ul style="list-style-type: none"> <li>Live animal test for early infection</li> </ul>	<ul style="list-style-type: none"> <li>May be able to detect early infections</li> </ul>	<ul style="list-style-type: none"> <li>High incidence of false positives</li> <li>Ability to detect infected animals has proven very low in some studies</li> <li>Blood samples must arrive at lab within 12–24 hours</li> <li>Not currently used in OJD control programs</li> <li>Likely to be expensive</li> </ul>

Table 1 (cont.): OJD tests

Test	Application	Benefits	Limitations
<b>New tests (cont.)</b>			
Polymerase chain reaction (PCR) faecal test	<ul style="list-style-type: none"> <li>• Live animal test</li> <li>• Can be used as a flock test or an individual animal test</li> </ul>	<ul style="list-style-type: none"> <li>• Faster than faecal culture – results available in days rather than months</li> </ul>	<ul style="list-style-type: none"> <li>• False negatives possible</li> <li>• Cannot detect early infections</li> <li>• Less sensitive than faecal culture</li> <li>• Not currently accepted by regulatory authorities</li> </ul>
<b>Research tests</b>			
Live animal intestinal biopsy	<ul style="list-style-type: none"> <li>• Biopsy and culture of intestinal tissues and lymph nodes of live sheep</li> </ul>	<ul style="list-style-type: none"> <li>• Can detect infection much earlier than blood or faecal tests</li> <li>• More sensitive than faecal culture</li> <li>• Has proved to be the most sensitive individual animal test for live sheep</li> </ul>	<ul style="list-style-type: none"> <li>• Biopsies are time consuming, expensive and invasive</li> <li>• False negatives can occur</li> <li>• Up to 15% of sheep may recover from early OJD infections so may not be a reliable predictor of later disease status</li> <li>• Despite being the most sensitive test available can still only detect 2/3 of infected sheep at three years of age</li> </ul>
Tracer weaners	<ul style="list-style-type: none"> <li>• Use of 'monitor' sheep that undergo a range of tests including post mortem examination and culture to check whether pastures are still infected after destocking</li> </ul>	<ul style="list-style-type: none"> <li>• Can detect infection from six months after exposure to infected pastures</li> </ul>	<ul style="list-style-type: none"> <li>• False negatives can occur so cannot guarantee that a pasture is not infected</li> </ul>

## Research tests

### Live animal intestinal biopsy

Biopsies of the intestinal wall and lymph nodes can be performed in live sheep. The tissue samples collected are then cultured for the presence of OJD bacteria. This technique has been able to detect infection at a much earlier stage than conventional tests, such as faecal culture or blood testing.

Culture of tissues obtained by biopsy has recently been shown to be the most sensitive test for detecting infection in individual live animals. Despite this, biopsy is still only able to detect about two-thirds of infected animals, even those sampled at 36 months of age when the disease is likely to be well established.

Live animal biopsies are time consuming, expensive and invasive to the animal, and are therefore unlikely to be used routinely for detection of OJD infection, other than for research purposes or for very high value individual animals.

### Other diagnostic tests

The University of Sydney has recently started a major new research program to try to develop a new or improved test that can detect early OJD infection and can discriminate between resistant or recovered sheep and those that have a subclinical infection without obvious signs.

A second project in this research program will investigate whether the bacteria causing OJD can be detected in blood samples, as an alternative early diagnostic test. This study will

use new technology to detect bacteria inside white blood cells and differentiate between live and dead organisms.

## The bottom line

A range of tests is available to detect OJD in sheep. All the tests have limitations – particularly in detecting infection in the early stages of the disease in live animals. Unfortunately contamination of the environment with OJD bacteria and transmission of the disease to other animals will occur before the currently used tests can detect infection. Research is continuing to study the events that occur in sheep during the early stages of infection, to try to discover other options that may be useful to diagnose the disease.

Despite the limitations of current testing methods, they do provide vital information on disease incidence and prevalence within flocks. Research is underway to discover new ways to detect OJD infection; however, this is high risk, long-term research. As with other human and animal diseases caused by this group of bacteria, there are significant hurdles to be overcome before practical advances can be made.



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