INFORMATION

NSW DEPARTMENT OF
PRIMARY INDUSTRIES

Division of Biosecurity and Compliance
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OVINE JOHNE'S DISEASE (OJD)

RISK ASSESSMENT, FIELD INVESTIGATION AND DIAGNOSIS INFORMATION

This circular replaces circulars AI 2004/029 and AI 2004/030.

1 Risk assessment

Risk assessment is the assessment of the risk of a sheep, flock or property:

- being or becoming infected, or
- transmitting infection to another sheep, flock or property.

A person who conducts a risk assessment gathers and documents objective information regarding the risk of OJD.
The risk assessment may include any of the following:

- relevant OJD history, including tracing and testing history,
- property size, general layout, condition of boundary fences,
- other properties under the same ownership,
- shared facilities, including roads,
- relevant information about neighbouring properties, e.g. straying,
- watercourses and run-off,
- schedule of all sheep, including classification by breed and mob,
- general sheep management, especially shearing and lambing times,
- sheep movements, especially introductions,
- agistment on/off,
- mortality rates,
- history of unexplained losses or ill-thrift.

An inspection of the property and stock may be necessary to complete a risk assessment.

On-going risk assessment may be required:

- following altered circumstances,
- as a follow up to the initial risk assessment,
- to fulfil a different purpose.

Ongoing risk assessments can be documented as above to provide an objective risk assessment at any point of time. Where risk assessments are used on an on-going basis, it is essential that they be dated appropriately.

2 Field investigation

2.1 Flock examination

Flock examination should involve a clinical assessment of the whole flock, targeting higher risk mobs, and documentation of results. This would normally entail:

- paddock inspection of the various mobs,
- yarding of appropriate mobs,
- condition-scoring of sheep, especially those in full wool, to identify any in poorer condition.

2.2 Examination of individual animals

Sheep with ill-thrift should be examined and if necessary sampled to rule out other likely or possible causes of their condition. Post-mortem examination should include carcass examination for evidence of emaciation, as well as detailed examination of the intestine, particularly the lower jejunum and ileum, and associated lymph nodes.

2.3 Sample collection – post-mortem

Whenever a post-mortem is performed and OJD is considered as a differential diagnosis, a full range of samples should be taken. Any gross pathological changes indicative of OJD should be noted, recorded, and where possible demonstrated to the owner/manager. Samples for histopathology are to be sealed in containers of buffered formalin and the containers are to be clearly labelled. Inclusion of the ear tag is an optional added precaution for identification. The following preserved tissues should be included for histopathology:

- a section of terminal ileum adjacent to the ileo-caecal valve,
- a section of the caudal jejunal lymph node,
- any other site with suggestive lesions, particularly from the ileum or adjacent lymph nodes.

In addition, the following fresh samples should be taken and submitted, chilled, to an approved laboratory within 48 hours of collection:

- one blood sample (collected in 10 mL plain vacutainer tube for testing of serum),
- 5 cm segment of terminal ileum, in a sterile container,
- faecal sample (12 pellets) in a sterile container.

2.4 Sample collection – faeces

Animals to be faecal sampled should be kept on feed until just prior to collection.

Faecal samples should be collected directly from the rectum, and should not be contaminated with other matter.

Gloves should be worn and changed between each pool or individual sample.

To avoid possible cross contamination, gloves should also be changed when a sample cannot be collected and an animal is subsequently excluded from a pool.

Where there are difficulties collecting faecal samples, ‘empty’ animals may need to be segregated and fed a small amount of roughage, e.g. lucerne hay. Faecal collection should be possible shortly afterwards.

Container lids are to be sealed, and containers are to be clearly labelled and enclosed in a sealed plastic bag separate from accompanying paperwork.

Samples need to be submitted, chilled, to arrive at an approved laboratory within 48 hours of each collection.

All sampled animals should be clearly identified.

All faecal containers should be clearly labelled and cross-coded to ear tag numbers, and where appropriate to blood tubes, on the *Specimen Submission Key List.*

2.4.1 Individual faecal collection

A sample of 12 pellets (equivalent weight 6–12 grams) should be collected from each animal and placed in a separate **sterile** container.

2.4.2 Pooled faecal collection

A single pellet (equivalent weight 0.5 to 1 gram) should be collected from each of up to 50 adult animals and pooled into a 70 mL **sterile** plastic container. If the container size is inadequate, a pool can be split between two containers, clearly labelled, e.g. Pool 1A and Pool 1B, and indicated as such on the Specimen Advice Form.

2.4.3 Serial faecal collection

Samples should be collected into **sterile** containers on three occasions at 10 to 14 day intervals. The three collections will be combined for culture at the laboratory. Samples from up to 10 animals can be pooled at each collection. Several pellets should be collected from each animal. Each sample container should be clearly labelled as collection 1, 2 or 3 of a serial faecal collection and cross-coded to the contributing animals.

Submitters should not store serial faecal culture samples. Each serial collection should be submitted, chilled, to arrive at the approved laboratory within 48 hours of collection. The receiving laboratory is responsible for storing serial samples pending completion of the series of collections

2.5 Sample collection – biopsy

Samples may be collected only by sterile surgical technique from a suitably restrained and anaesthetised animal. Sample collection is subject to a Standard Operating Procedure approved by a NSW Agriculture Ethics Committee.

Biopsy samples should include a resected segment of terminal ileum and a nearby mesenteric lymph node.

Samples should be divided, with a small segment of each being placed in a container of buffered formalin. The balance of the samples should be collected into a sterile container and submitted, chilled, to arrive at an approved laboratory within 48 hours of collection.

2.6 Sample collection – blood

Where a flock is being blood tested, selection of sheep for sampling should be in accordance with Element L6 of the SheepMAP, and/or biased towards any higher risk mobs. In either case, the selection criteria should be entered on the *OJD Specimen Advice Form* under the section marked Flock Information.

Sampled animals should be permanently identified with consecutively numbered self-piercing ear tags.

Tubes should to be clearly identified, numbered sequentially and cross-coded with ear tag numbers on the *Specimen Submission Key List.*

An *OJD Specimen Advice Form* and a *Specimen Submission Key List* should be submitted with the blood samples to the appropriate Regional Veterinary Laboratory.

2.6.1 Sampling for ELISA or AGID

Samples are to be collected in 10 mL plain vacutainer tubes for testing of serum.

Samples should be held at ambient temperature (22° +/– 5°C) until the serum separates, then chilled to 4°C in a refrigerator.

Samples should be submitted, chilled, to an approved laboratory within 72 hours of collection.

2.6.2 Sampling for Gamma Interferon testing

Samples for the gamma interferon test (a minimum volume 6.0 mL of blood) are to be collected into a 10 mL vacutainer containing heparin as anticoagulant and the blood gently mixed by inversion several times to dissolve the heparin.

Samples should be transported to a laboratory which performs gamma interferon testing at ambient temperature (22° +/– 5°C) and put into culture within 12 hours of collection. Do not store in the refrigerator.

2.7 Laboratory requirements for all sample submissions

With regard to all sample collections and submissions:

- All samples are to be accompanied by a properly completed *OJD Specimen Advice Form,* including the RLPB/ID and/or the PIC for the property.
- Samples from different properties and different species are to be submitted on separate *Specimen Advice Forms*.

Where sample submission, or accompanying documentation, is not considered satisfactory by the receiving laboratory, payment to the approved veterinarian may be withheld, and/or approved practitioner accreditation may be rescinded.

3 Diagnosis

OJD is diagnosed by approved laboratory tests.

Approved laboratory tests for OJD are tests that are approved by the Chief, Division of Animal Industries on the recommendation of the Chief Veterinary Officer (CVO).

Tests are approved only if conducted in a manner that is consistent with the techniques that are described in the Australia and New Zealand Standard Diagnostic Protocols, and performed at a laboratory approved by the CVO to carry out diagnostic tests for OJD.

Tests that are approved to diagnose OJD include:

- histopathology – i.e. the presence of histopathological changes consistent with OJD, and the demonstration of one or more acid-fast organisms consistent with *Mycobacterium paratuberculosis*,
- culture – i.e. demonstration of *Mycobacterium paratuberculosis* in the faeces or tissue of the animal by bacterial culture

3.1 Histopathology

Histopathology is reported as positive if in any one histopathological section, one or more single giant cells and/or one or more accumulations of three epithelioid macrophages are observed in the intestinal lamina propria and/or lymph node cortex with the presence of at least one acid-fast bacillus morphologically consistent with *Mycobacterium paratuberculosis.*

Histopathology is reported as suggestive if in any one histopathological section, two single giant cells and/or two accumulations of three epithelioid macrophages in the intestinal lamina propria and/or lymph node cortex are observed without the detection of an acid-fast bacillus.

3.2 Culture

Samples suitable for culture include individual faecal samples, pooled (a single pellet from each of up to 50 animals) faecal samples, serial faecal samples, fresh terminal ileum or other tissue samples, and pooled tissue samples.

On delivery, samples may be prepared immediately for culture, or stored at –80ºC pending preparation and culture.

Samples are normally set up within a week of receipt at the laboratory, and are reported progressively. Negative radiometric cultures will not be reported until approximately 13 weeks following the start of the radiometric (BACTEC) incubation period.

Bacterial culture for OJD consists of three distinct testing procedures:

- **Radiometric (BACTEC) culture -** Radiometric culture for the sheep strain of *Mycobacterium paratuberculosis* comprises initial homogenisation and decontamination by sedimentation/centrifugation of the sample, followed by inoculation of a radiometric liquid culture medium (BACTEC). Cultures are incubated for up to 12 weeks. When growth is detected in radiometric culture, the culture is

incubated until a maximal growth index is reached, and the culture is then submitted for further testing as described below. Growth in BACTEC medium is not necessarily *Mycobacterium paratuberculosis,* so confirmatory testing is critical in reaching a diagnosis.

- **Solid medium subculture -** Solid medium subculture is the subculture of organisms grown in BACTEC medium, onto modified 7H10 solid medium (with and without mycobactin supplementation) for a further 10 weeks. This is also known as mycobactin dependency testing.

- **Polymerase Chain Reaction and Restriction Endonuclease Analysis (PCR/REA) -** PCR/REA is testing for the insertion sequence IS*900* on BACTEC growth. These tests can be applied to solid medium growth in special circumstances (for example in the rare event when Bactec growth is found negative by PCR but typical mycobactin-dependent colonies are produced on subculture).

3.2.1 Culture reporting

A sample with no growth in radiometric (BACTEC) culture at 12 weeks is reported with a conclusion of being 'Culture negative'.

A sample with growth in radiometric (BACTEC) culture and positive for IS*900* on PCR/REA testing, is reported as 'having DNA consistent with *M. paratuberculosis'*, and is concluded to have 'Culture findings consistent with *M. paratuberculosis'.*

Similarly, a sample which produces mycobactin-dependent colonies on solid medium with morphology typical of *Mycobacterium paratuberculosis* is concluded to have 'Culture findings consistent with *M. paratuberculosis'.*

A sample which:
- is shown to contain IS*900* on PCR/REA testing on either radiometric or solid medium growth, and
- produces mycobactin-dependent colonies with typical colony morphology on solid medium,

is reported with a conclusion of 'Culture positive for *Mycobacterium paratuberculosis'.*

3.2.2 Strain typing

Strain typing differentiates between sheep and cattle strains of *Mycobacterium paratuberculosis*. Typing is routinely available from Bactec growth and is based on PCR/REA that targets the IS*1311* sequence.

3.3 Tests to assist in the assessment and investigation of OJD

3.3.1 Direct – Polymerase Chain Reaction (D-PCR)

D-PCR is approved as a rapid means of:
- screening faecal samples for risk assessment and management purposes, and
- detecting groups of sheep at high risk of shedding large numbers of *Mycobacterium paratuberculosis'*

D-PCR tests for the insertion sequence IS900 in faecal samples. REA testing is applied to D-PCR product to confirm that any detected IS900 is consistent with *Mycobacterium paratuberculosis'.*

D-PCR may be used on individual, pooled or serial faecal samples. Results are normally available within two weeks and are reported as positive or negative.

3.3.2 Agar Gel Immunodiffusion Test (AGID)

The AGID test has been used as a screening test for OJD. It is highly specific (> 99%) and has a variable but generally low sensitivity (< 30%). This means that the test will miss a large number of sheep which are actually infected, but of those that it identifies, most are likely to be infected. Results are given as 1+, 2+ and 3+ and inconclusive. A positive AGID result does not confirm infection.

Where the AGID is being used as a screening test that is equivalent to pooled faecal culture of samples from 350 head over 2 years of age, the recommended sampling size is 875 head.

3.3.3 Absorbed Enzyme Linked Immunosorbent Assay (ELISA)

The ELISA test has also been used as a screening test for OJD. At a 99% specificity (using a cut-off of 3.6 OD units), this test has a similar sensitivity to the AGID test (≤ 30%). At a specificity of 95% (using a lower cut-off of 2.4 OD units) the sensitivity is increased to 50%. This means that while more true positive animals will be detected, there will also be more false positives. A positive ELISA result does not confirm infection.

3.3.4 Gamma Interferon

Gamma interferon testing is not an approved test, but may be used to assist in risk assessment. Field trials are currently being undertaken to establish the specificity and sensitivity of the test in unexposed and infected sheep flocks.

4 Resolution of suspicion

The following should be considered when investigating a flock/property with the aim of resolving suspicion of infection.

- Assessment of the risk of infection in the suspect animals, and the risk of spread to other susceptible stock on the property, or other properties.
- Assessment of the likelihood of shedding if the animals are infected.
- Time since last contact with known infection.

  Note. The onset of detectable shedding may not occur until two or more years after exposure. Hence negative test results for shedding that are obtained within two years of the last contact with infection may not resolve suspicion.

- Proportion of suspect sheep still available for investigation, including sheep belonging to a pool that has reacted to a pooled faecal culture test.

- Opportunity to investigate sheep that have had two or more years' contact with the suspected sheep.

- Presence of lambs that are the progeny of suspected sheep.

  Note. Lambs under 9 months are not considered likely to shed infective OJD bacteria.

- Isolation of suspected sheep.

- Availability of serial faecal culture to investigate small numbers of suspected sheep.

- The time since sheep that have been found infected elsewhere, including by abattoir surveillance, left the property being investigated.

  Note. Where this time is greater than 12 months, careful assessment needs to be made regarding the source of infection.

- Risk of infection in neighbours, which will vary significantly according to:
  - the apparent prevalence, within-flock distribution and duration of infection on the neighbouring infected property,
  - the standard of fencing,
  - the presence of any barriers,
  - the likely or known occurrences of straying sheep,
  - the source, direction, volume and ultimate destination of any run-off,
  - any other factors relating to the potential exposure of the flock/land/facilities to faecal material from the neighbouring infected property.

5 Assessing risk for a particular purpose

Assessing the likelihood that a particular sheep or group of sheep may:
- have been exposed to an infective dose of *Mycobacterium paratuberculosis* bacteria,
- have been infected by exposure to an infective dose,
- shed infective organisms following exposure and infection,

is required in a number of circumstances, including:
- resolution of suspicion,
- certification of adult approved vaccinates,
- certification of assessed low-risk sheep.

5.1 Assessing likelihood of exposure

The following factors should be included when assessing likelihood of exposure:
- known/estimated prevalence, particularly of multibacillary shedders, within the flock, the neighbouring flocks and the locality,
- known/possible grazing of contaminated land,
- known/possible use of contaminated land/facilities,
- environmental factors affecting survival of bacteria on pasture or in water.

5.2 Assessing likelihood of infection

The following factors should be included when assessing likelihood of infection in sheep that may have been exposed:

- known/estimated likelihood of exposure (as above),
- degree of exposure (particularly to multibacillary shedding),
- breed (all sheep are susceptible but merino sheep appear to be particularly susceptible),
- age at time of exposure (all age sheep are susceptible but younger sheep appear to be particularly susceptible),
- stress factors at time of exposure (young stressed sheep are considered highly susceptible).

5.3 Assessing likelihood of shedding

The following factors should be included when assessing likelihood of shedding in sheep that may have been exposed and may be infected:

- vaccination status (vaccination prior to exposure significantly reduces the likelihood of shedding),
- stress factors (factors such as movement to another location, nutritional stress, lambing often appear to trigger onset of clinical disease and shedding).

5.4 Investigation to assist in the assessment of risk for a particular purpose

Where the above assessment of risk is not conclusive, further investigation should be undertaken. In most circumstances such investigation will include testing.

Tests that may be used for risk assessment include:

- culture of individual and pooled faecal and tissue samples,
- direct-PCR of individual and pooled faecal and tissue samples,
- post-mortem and histopathology,
- abattoir inspection,
- serology using AGID, ELISA or and gamma interferon.

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