AXIOM
Veterinary Laboratories Ltd
'The Quality Clinical Pathology Service'

Sampling for Respiratory Disease

Respiratory disease in cattle is a frequently seen clinical entity affecting all age groups under different systems of management. Cases can occur as group problems of acute onset to individual animals with chronic unresponsive disease. A large number of pathogens and other insults can be involved with many cases being multifactorial in nature precipitated by stressful events and adverse environmental conditions all of which must be addressed to resolve the problems-on-farm.

When faced with respiratory disease it is important to gather information on previous farm history, age, management (including any recent changes), vaccination and worming regimes, weather and housing conditions and purchase policy if a non-closed herd. This information should be used in conjunction with the clinical history and findings for the case in hand to help establish a differential diagnosis list.

Establishing a definitive diagnosis is important for treatment, instigating a vaccination program (when appropriate) and wider herd health, as in for example *Mycoplasma bovis* infection. The problem is though that a definitive diagnosis is often not established and it is worthwhile considering some of the reasons why so that you are in a better position to choose what testing would be cost effective.

1. Animals are presented at the wrong stage of the disease process. For example viral antigen becomes increasingly harder to identify the longer the clinical course although new PCR tests will improve this situation.
2. Antibiotic therapy will compromise bacterial culture results.
3. The wrong sample is submitted or it is inappropriately collected, stored and transported.
4. The disease is not primarily respiratory in nature, *Salmonella* Dublin being a case in mind, where a pneumo-enteritis presentation can be seen particularly in young calves.

The following guide provides an overview of sample collection in cases of respiratory disease but bear in mind any results we provide will be based on the samples we receive so consider carefully what you want to achieve and consider the tests available. Broadly you can test for the antigen/insult or the animals' response to it (e.g. culture, PCR, histopathology or serology).

Broadly speaking, sampling early in the course of the disease outbreak, selecting cases presenting with typical signs and submitting material from 2-3 animals will help improve the diagnostic rate (bearing in mind even experienced stockmen may fail to spot early acute stages of disease in typical multifactorial 'enzootic' pneumonia outbreaks).

Live animal sampling

When sampling live animals, naso-pharyngeal swabs and broncho-alveolar lavage (BAL) can be used for virus detection and bacteriology. Naso-pharyngeal swabs are relatively easy to collect but have their limitations. The detection of BHV1 is significant if animals are presenting with

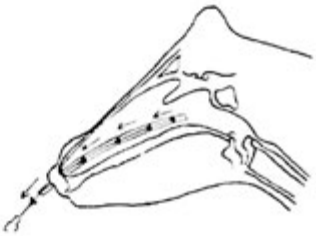
suggestive clinical signs – bear in mind the PCR is very sensitive and recrudescence of infection may occur under periods of stress so interpretation of a positive result needs to take this into account. If RSV is detected it is likely to be significant but as this virus often has a restricted distribution to the lower airways/alveoli, a BAL would be more successful in picking up infection. Bacteriology, likewise, can be problematical as many of the organisms acting either as primary or secondary respiratory pathogens (e.g. *Past. multocida, Mann. haemolytica, Hist. somni, Mycoplasma bovis* etc.) are also normal commensals of the oropharynx. Having said this detection of *Mycoplasma bovis* maybe a significant finding with regards to herd health.

Naso-pharyngeal swabs should ideally be of the guarded variety to avoid contamination from the nostrils and nasal cavity. The diagrams below illustrate their insertion (A), swabbing (B) and removal (C).

Swabs for IBR PCR should have non-wooden stems and ideally should be placed in viral transport medium (NOT bacterial transport medium); alternatively they can be submitted without transport medium. Swabs for bacteriology should be immersed in a suitable bacterial transport medium. All swabs should be couriered to the laboratory on the day they are taken and, with swabs for viral and *M. bovis* PCR testing, ideally avoid submitting swabs at the end of the week due to delays in processing over the weekend.

A. Insert swab inside sheath to avoid contamination

B. Push centre swab out once in naso-pharynx and gently rub on mucasol surface



C. Withdraw swab into outer sheath and then withdraw both from the nostril

BAL is the preferred technique as it is sampling the lower airways and alveoli which in the normal animal are sterile. Results are therefore easier to interpret and more reliable. Samples can be sent for virology, bacteriology and cytology. There is an excellent In Practice article written by George Caldow describing the technique which we would refer you to:

Broncho-alveolar lavage in the investigation of bovine respiratory disease. In Practice (2001) 23:1 41-43

A minimum of 2mls BAL fluid should be submitted in a plain sterile pot and couriered to the laboratory on the day it is taken. The sample can be used for respiratory virus and *M. bovis* PCR testing and bacteriology. Ideally, again avoid submitting BAL samples for PCR at the end of the week due to delays in processing over the weekend.

Serological investigation of respiratory disease can be useful but results need to be interpreted with care and in light of the animal's age, clinical presentation and stage of the disease process. In young calves the presence of maternally derived antibody (MDA) will interfere with the humoral response to infection. Paradoxically antibody levels in paired blood samples can appear to fall after infection leading to confusing results. As MDA can persist up to 4-6 months after birth the value of serological testing of young calves is limited. *Mycoplasma bovis* serology is more useful in detecting herd infection but again caution is required. In older stock the collection of paired samples is useful and a sample collected during the acute phase and a second blood from the same animal 2-3 weeks later should be submitted. Serology of an affected group at 9-10 months of age, once the effects of MDA have passed, can provide historical information on the pathogens involved and in situations where infections might be passed from older to younger stock this can prove useful.

Acute samples can be held at the laboratory pending

receipt of convalescent samples in order that they can be run on the same test run, thus removing between test run variation as a confounding factor in the interpretation of results (please indicate clearly on the submission form that acute samples are to be held).

If the clinical history and presentation is suspicious of lungworm patent infection can be confirmed by Baermanns examination of the faeces, BAL (cytology useful) and serology.

Carcass sampling

Post-mortem examination is a particularly useful tool in diagnostics in respiratory disease outbreaks. Again consideration should be given to the suitability of material in achieving a diagnosis with regards to the stage of disease, as well as factors such as autolysis. Ideally a full post-mortem examination should be undertaken; examining all organ systems, particularly if on initial examination there is little evidence of respiratory tract pathology. On-farm examination can be difficult especially in light of the need to 'sew' carcasses up for disposal purposes. The following technique can be used which maybe of help in the latter circumstance.

Lay the animal in lateral recumbency, make a skin incision along the costal arch continuing along the costochondral junction and up the ventral neck to produce a skin flap. Then reflect the foreleg off the thoracic wall. Cut through the thoracic wall along the costal arch and using garden shears (or a sharp knife) cut through the costochondral junction of each rib. To fully expose the thoracic cavity either bend the ribs dorsally so they snap at the vertebral junction or use the shears to cut the ribs (don't remove the thoracic wall as this can be folded down with the foreleg once the examination has finished and the skin closed).

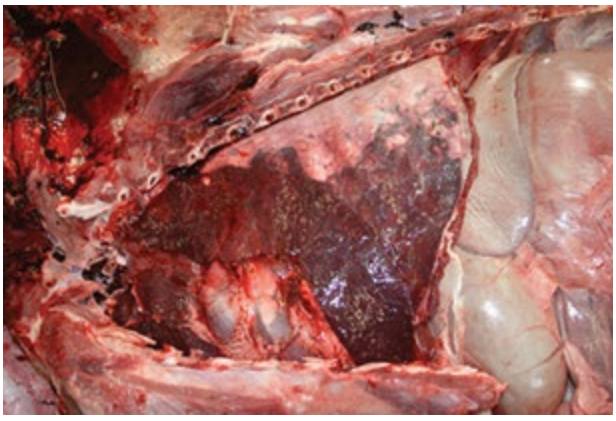


Figure 1. Exposure of thoracic contents in a full post-mortem examination

To remove the pluck cut the trachea at the level of the larynx and 'strip out' the pluck caudally). Figure 1 below shows the exposed chest following a full necropsy to illustrate how the organs can be exposed. The lungs should be palpated and visually assessed

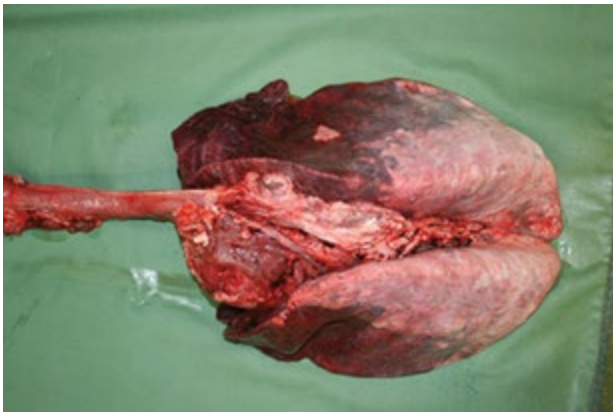


Figure 2. The full pluck has been removed showing a bilateral cranio-ventrally distributed pneumonia typical of an infectious aerogenous insult.

to establish lesion distribution. Cranio-ventral distribution is suggestive of an aerogenous insult, and a multifocal or caudal distribution indicates a systemic or haematogenous insult.

Open the trachea and bronchi looking for evidence of diptheresis, exudate and lungworm etc. For virology swab the tracheal mucosa at the bifurcation and the main bronchi as in Figure 3.

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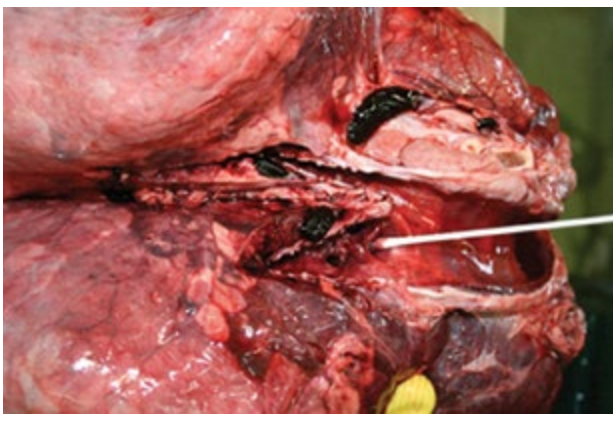

Figure 3. Collection of material for virological examination.

Histological and bacteriological samples should be taken before the lung is serially sectioned. Collect small representative portions of each lung lobe from one affected lung as illustrated in Figures 4 and 5 and place in adequate neutral buffered formalin for fixation. Also collect a sample of the trachea or main bronchus and fix. For bacteriology collect a 2x2x2cm portion of consolidated lung and place in a suitable container for courier dispatch the same day to the laboratory.

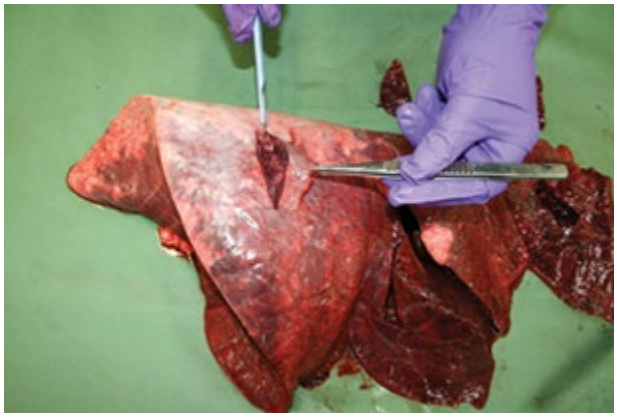

Figure 4. Collecting representative portions of lung for histology.

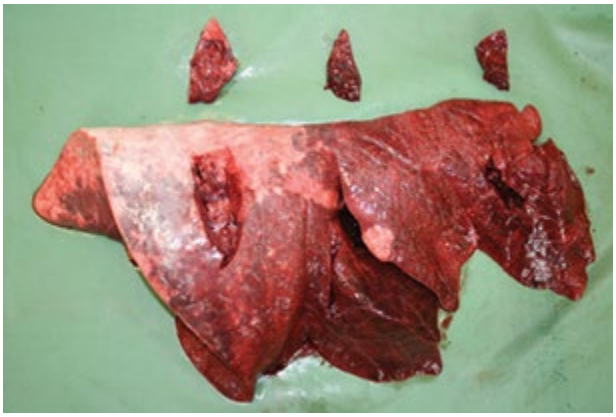

Figure 5. Samples collected from the cranial, middle and caudal lung lobes. The submission of these portions will assist greatly in histological assessment of the lung/pneumonia.

If lungworm is suspected and none seen in the airways the collection of faeces for a Baermanns test will help in establishing if there is a patent infection. Pre- and post-patent infection is better evaluated by histopathology following the above procedure.

In cases of interstitial pneumonia such as fog fever histopathology is the preferred test.

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