Livestock Diseases

Bovine Herpes Virus Infections

Introduction

Bovine herpes viruses belong to the family Herpesviridae. Bovine herpes virus 1 (BHV-1) is of world wide distribution and causes a wide range of diseases in cattle namely respiratory disease, keratoconjunctivitis and reproductive diseases including infectious pustular vulvovaginitis (IPV) in cows, infectious pustular balonoposthitis (IPB) in bulls and abortions. DNA studies of BHV-1 have revealed differences between the BHV-1 virus causing respiratory disease and abortion (BHV-1.1) to those causing IPV/IPB (BHV-1.2). With infection of the foetus abortion is the consequence, while in older animals neonatal gastrointestinal ulceration, rhinotracheitis, vulvovaginitis, pneumonia and drop in milk production are described. Abortion generally follows the respiratory form of the disease or use of modified live IBR vaccine in pregnant previously unvaccinated animals. Bovine herpesvirus 5 (BHV-5) is more frequently associated with fatal meningoencephalitis in cattle than BHV-1. Common to all herpesviruses is latent infection (carrier state) and reactivation thereof may play a role in maintaining the virus in a herd.

BHV-1 Antibody Detection - Serology

ELISA / Virus Neutralisation / Indirect Immunofluorescence

These assays demonstrate the presence of antibodies against bovine herpes viruses. Serum samples collected in a red or yellow stopper tube are required. Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should be centrifuged and the serum poured off. An acute serum sample collected as early as possible during the outbreak and a convalescent sample collected two to three weeks after the outbreak are the preferred samples. Acute centrifuged and separated sera are stored at -20°C until the convalescent sera arrive. Both acute and convalescent sera are tested concurrently. All serum samples are transported at 4°C to the laboratory.

A four-fold or greater increase in specific antibody titres is suggestive of BHV-1 exposure and probable infection. ELISA tests are more routinely used due to ease, cost and high sensitivity and specificity of these assays. Serological assays are widely employed for identifying herds with potential latently infected animals. Animals testing serologically positive (on acute and convalescent sera), should be considered as possible latent carrier of the virus. There are currently marker vaccines available which enable distinction of vaccine immunity from latent infection on serological assays.
BHV-1 Antigen Detection

Virus isolation

Samples of choice include:

- **Live calves / adults** – swabs of nasal, ocular, or genital secretions (vaginal discharge, semen) at the peak of infection. With semen samples, care must be taken to avoid non-specific cellular toxicity.
- **Dead calves / adults** – nasal mucosa, trachea, lung, GIT, vaginal / preputial mucosa.
- **Aborted foetuses** - lung, liver, spleen, kidneys and placental tissues.

Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. (See Reference Ranges – Section 3). Specimens are stored and transported at 4°C to the laboratory. Alternatively samples may be frozen at -70°C and stored for testing at a later date.

*Note*: storage in a regular freezer (-20°C) will inactivate the virus.

Direct Immunofluorescent Antibody Test

Viral antigens may be demonstrated in swabs of nasal, ocular and genital scrapings, or in fresh or frozen tissue samples. Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. (See Reference Ranges – Section 3). Specimens are stored and transported at 4°C to the laboratory.
Polymerase chain reaction (PCR)

PCR techniques are extremely sensitive, even allowing for detection of BHV-1 in latently infected ganglia and semen. Preferred samples are swabs from nasal, ocular or genital discharges in PBS or semen. In animals surviving primary respiratory or genital infection, the virus invariably establishes a latent state in the trigeminal or sacral ganglia, respectively. As with other herpesviruses reactivation of latent infection can play a role in maintaining the virus in a herd.

Immunohistochemistry (IHC) and Histopathology

Confirmation of the diagnosis of BHV-1 infection in post mortal tissues is by IHC demonstration of BHV-1 viral antigen within histological lesions. IHC staining for BHV-1 is highly specific and shows greater sensitivity and specificity than virus isolation and PCR. These techniques are particularly useful for the diagnosis of BHV-1 associated abortions, even in tissues with advanced autolysis. In the image above-left of an autolysed bovine foetal liver with routine HE stain reveals the classic small foci of hepatic necrosis (arrow). The image above-right is IHC staining of a similar autolysed foetal liver with haematoxylin counterstain, showing similar foci of hepatic necrosis, but containing abundant granular brown black staining BHV viral antigen within the lesions.

In respiratory infections with lesions of necrotising rhinitis or rhinotracheitis, pharyngitis / laryngitis and necrotising pneumonia, then nasal mucosa, trachea, pharynx/larynx and lung should be collected into 10% buffered formalin.
In **genital infections** vesicles, pustules and erosions or ulcers of the vulva / vagina or penis / prepuce should be collected into 10% buffered formalin.

In abortions foetal lung, liver, spleen, kidneys, adrenal gland, brain and placenta should be collected into 10% buffered formalin.

**Further reading**

Bovine Malignant Catarrhal Fever (MCF)

Introduction

Malignant Catarrhal Fever (MCF) is a highly fatal, sporadic disease affecting several ruminant species including all bovinæ, bison and all species of Cervidae except fallow deer. Clinical disease has also rarely been described in pigs, giraffe, African buffalo and some species of antelope. This condition results from infection by closely related gammaherpesviruses namely alcelaphine herpesvirus 1 (AHV-1), AHV-2, ovine herpesvirus 2 (OHV-2), caprine herpesvirus 2 (CHV-2) and hippotragine HV (roan antelope), with AHV-1 and OHV-2 accounting for most infections. MCF due to OHV-2 is the sheep associated form encountered in the USA and other parts of the world, whereas AHV-1 causes the wildebeest-associated form of the disease in Africa and zoos throughout the world.

Sample Guide

**MCF Antibody detection – Serology**

Virus neutralisation (VN), immunoblotting, competitive inhibition enzyme-linked immunosorbent assay (CI-ELISA) and immunofluorescence (IFA)

Serological analyses particularly with wildebeest associated disease (AHV-1) is limited as cattle do not produce neutralising antibodies. The competitive inhibition ELISA is becoming the serological test of choice, although for both wildebeest and sheep-associated assays, AHV-1 is used as the source antigen. IFA assays are complicated by cross-reactions with bovine herpesvirus 4. Antibody detection of sheep associated MCF is limited to the CI-ELISA, but sensitivity is poor.

Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should ideally be centrifuged and the serum poured off. Serum or un-spun clotted blood should then be transported at 4°C to the laboratory.

*Note: Antigen detection assays have largely replaced serology in MCF diagnostics.*
MCF Antigen detection systems

Polymerase chain reaction (PCR) – Wildebeest Associated (AHV-1) + Sheep Associated (OHV-2)

Due to the low levels of cell free infective virus PCR assays, which are capable of amplifying DNA, have become the diagnostic assays of choice in MCF diagnostics. Apparently the level of circulating virus in peripheral blood can be extremely variable, particularly with the wildebeest strain, and animals with MCF can test negative on EDTA blood samples. Therefore, a negative PCR test on EDTA blood cannot exclude a diagnosis of MCF. To try and improve the sensitivity of the test on blood, submission of both EDTA samples and clotted blood should be considered. If the EDTA tests negative the PCR is then run on the blood clot. However, this is not infallible, as some animals with MCF will still test negative. Post mortal tissues, particularly spleen and lymphoid tissues, are the ideal samples for the PCR assay, although the assay may also be run on brain, liver and kidney.

Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. Specimens must be stored and transported to the lab at 4°C.

Virus isolation

AIHV-1 virus can only be recovered from viable cells and therefore tissue samples should be collected within a few hours of death. Virus isolation for AHV-1 is often unsuccessful due to the low levels of cell free virus present in infected cattle. OHV-2 has to date not been isolated in culture, with PCR detection of genetic DNA material being the diagnostic assay of choice.

Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. Specimens must be stored and transported to the lab at 4°C.
Histopathology

MCF, irrespective of the virus involved, produces characteristic histopathological changes. Necrotising vasculitis of predominantly medium sized arteries of multiple organs and hyperplasia of lymphoid tissues with accumulations of lymphoid cells in non-lymphoid organs are classic findings. The brain, kidneys and liver are usually fairly consistently involved. However, a full set of organ tissue samples (brain, lung, heart, liver, spleen, kidneys, lymph nodes, gastrointestinal sections, muscle) should be collected in 10% buffered formalin to search for characteristic lesions at common and unusual sites, as well as to rule in or rule out other disease conditions.

The image above-left is of a MCF necrotising vasculitis in the lung of a bovine, while the image on the right demonstrates dense interstitial lymphocytic accumulates in the kidney of an African Buffalo with AHV-1 associated MCF.

Further Reading

Bovine Viral Diarrhoea (BVD) and Mucosal Disease

Introduction

Bovine viral diarrhoea virus (BVDV), a member of the Pestivirus genus of the family Flaviviridae is the cause of bovine viral diarrhoea (BVD) and mucosal disease (MD) in cattle. Two genotypes, type 1 and type 2 are known and in both of these genotypes cytopathic (c-BVDV) and non-cytopathic (nc-BVDV) biotypes occur. Currently, three species of pestiviruses are recognized namely BVDV, the related border disease virus of sheep and classical swine fever/hog cholera virus of pigs.

In utero and congenital infections: The embryo or foetus is a primary target of BVDV. It is only during the viraemia of acute infection or persistent infection in seronegative dams that the virus invades the placenta and replicates in the trophoblast before spreading to the foetus. Early embryonic death, infertility and repeat breeder cows are common sequelae of Pestivirus infections. All foetuses born to PI dams become persistently infected. Although this vertical transmission from PI infected dams is 100%, this route of infection only accounts for 7% of PI calves in a herd, the other 93% arise from acute infection of seronegative dams in early pregnancy. Bovine pestivirus is capable of transplacental infection of the foetus within 4 days from a primary viraemia in the absence of maternal immunity.

The outcome of foetal infections depends on the age of the foetus at time of exposure and the biotype of the infecting virus.

- Infection during first trimester (0 - 110 days) abortion, congenital damage or birth of PI calves.
- During second trimester (111 - 200 days) congenital damage or foetal loss.
- During third trimester, the foetus is immuno-competent and able to mount an active immune response with infection usually being unapparent.
- Biotype responsible for in utero infection is nc-BVDV.

Acute BVDV infections: This is a common syndrome of acute transient infection in immuno-competent calves and adults, with pyrexia and leukopaenia developing about 3 - 7 days post infection. Under certain circumstances these acute infections can cause severe clinical disease in immuno-competent animals, particularly with the BVDV type-2 biotype.

Mucosal disease: MD is a fatal condition, mainly of young cattle aged 6 - 18 months, characterised by an erosive oral / intestinal syndrome. Only PI animals (nc-BVDV) are susceptible to this condition and it occurs when these PI animals are super-infected with a c-BVDV biotype, which has mutated from a nc-BVDV biotype. The clinical condition is typically rapid in onset, although chronic debilitating forms are known to occur.

The identification and elimination of persistently infected (PI) animals forms an important cornerstone of any BVD control and / or eradication scheme.
Sample Guide

BVDV Antigen Detection Assays

Identification of Persistently Infected animals

The two ear skin notch assays namely the BVD Antigen Capture ELISA (ACE) and Immunohistochemistry (IHC) have been specifically designed for the detection of BVDV persistently infected (PI) animals. The basis of these assays are that PI animals have extremely high levels of circulating virus, resulting in heavy viral loads in the digestive tract, pancreas, mammary glands, adrenal glands, liver, lung, kidney, reproductive tracts, CNS and fairly uniquely, the skin. Such high cutaneous BVDV viral concentrations are generally not observed in acute transient infections or in animals vaccinated with modified live (MLV) BVD vaccines. A small number of transiently infected animals may test positive on ear-notch ACE and IHC, therefore any positive test must be repeated with another test method.

Ear notch BVD Antigen Capture ELISA (ACE)

- Ear notch samples (fresh or frozen) are collected with an ear-notcher / sterile surgical scissors and placed into individual labelled envelopes / tubes with the animal identification on the tube / envelope.
- Although BVDV in ear notch skin may remain viable at room temperature for a week, if kept moist, the samples should be maintained and transported to the laboratory at 4°C.
- If ear notches are to be tested at a later date, they should be frozen at -70°C for storage prior to testing.
- In various diagnostic-test comparative surveys the ACE performed on skin provided the greatest consistency in detecting positive samples and a perfect level of agreement among laboratories.

Note: Todd *et al* (2005) describe a sensitivity of 100%, a specificity of 98.4%, a positive predictive value of 88.1% and a negative predictive value of 100% for the antigen capture ELISA to detect PI animals.
Ear notch Immunohistochemistry (IHC)

- Ear skin notches are collected with sterile surgical scissors or an ear-notcher.
- Each individual notch is placed in a serum tube containing 10% buffered formalin and clearly labeled with the animal’s identification details. Do not use un-buffered formalin as acid haematin pigment deposits can interfere with the ability to interpret the IHC staining pattern.
- Samples can be transported at ambient temperature to the laboratory.
- Skin biopsies can be held in formalin for 2-3 weeks without loss of reactivity. However, after this the positive skin biopsies start to lose their staining intensity dramatically. Therefore, skin specimens should be trimmed into multi-channel cassettes, passed through the histology processor and embedded in wax as soon as possible by the laboratory, as once tissues are wax-embedded they no longer lose any of their positive staining reactivity.
- In skin biopsies from BVD PI animals there is positive IHC staining in the epidermis, follicular epithelium, follicular adnexae and hair bulbs (image above-right)

Note: Todd et al (2005) describe a sensitivity of 100%, a specificity of 98.8%, a positive predictive value of 90.81% and a negative predictive value of 100% for IHC to detect PI animals.

Facts to consider when testing animals for BVD PI status:

- Although most PI animals are antibody negative and antigen positive, a few PI animals will test seropositive for BVD antibody.
- **Never rely on a single test** or one test method only. To confirm an animals status, make use of more than one test method (eg. antigen capture ELISA and IHC staining).
- If an animal tests positive on ear notch assay (ACE/IHC), repeat the test in 4-6 weeks because a small percentage of acutely infected animals can test positive with both these techniques, thus mimicking a PI animal. Hence the less-than 100% specificities and positive predictive values reported by Todd et al (2005).
**BVDV Antigen Diagnostics**

**Antigen Capture Elisa (ACE)**

*Live animals:* BVD ACE can also be performed on plasma (EDTA) or serum to detect BVD viraemia and so has diagnostic applications for identifying acute transient BVD infections and mucosal disease / PI. When used in conjunction with antibody serology:

- acute transient infections = antigen positive + / antibody positive +
- mucosal disease / PI = antigen positive + / antibody negative —

*Aborted foetuses:* the BVD ACE is run on foetal thoracic fluid or heart blood as well as skin. A positive result on thoracic fluid / heart blood would confirm that the foetus is viraemic. BVD ACE testing of skin samples collected from aborted foetuses and stillborn calves found in the field, may represent a practical surveillance method for BVDV-induced reproductive disease.

**Immunohistochemistry (IHC) and Histopathology**

**IHC distribution** of BVDV antigen in **PI animals** has been documented in epithelial cells of the skin (epidermis, follicular epithelium and hair bulb), digestive tract, respiratory system (epithelium, glands, vessels, nerves, cartilage of nasal conchae), pancreas, mammary glands, adrenal glands, liver, lung, kidney, reproductive tracts and CNS (thalamus, hippocampus,
basal nuclei, pyriform cortex). Macrophages / histiocytes and vascular smooth muscle cells are positive in all of these locations.

**IHC distribution** of BVDV antigen in **acutely infected** animals is predominantly in lymphoid tissues with marked lymphoid depletion and in the muscular walls of large arteries showing vasculitis (particularly myocardium and ileum, but also sometimes muscular arteries in other tissues). The images above are of a large muscular artery of the myocardium. The HE stained section, above-left, reveals lymphocytic vasculitis while the IHC stain of the same artery, above-right, demonstrates positive viral antigen staining of vascular smooth muscle and lymphocytes. Using histopathology in conjunction with IHC staining for BVD viral antigen enables confirmation of BVD infection.

**IHC distribution** of BVDV in **aborted foetuses** can vary depending on the stage of foetal infection. Infection in the first trimester (persistent infection) and second trimester (acute transient infection) can be associated with positive BVD viral antigen staining. Brain, skin, heart, liver, lung, spleen, adrenal gland and eye are the sites most frequently involved with vasculopathy with positive staining of vessel walls being common at these various sites.
Polymerase Chain Reaction (PCR)

The PCR can be performed on a wide range of samples to diagnose acute transient and persistent infections.

- In **live animals** blood collected in EDTA, serum, nasal swabs, semen, milk and skin tissue (unfixed).
- In **dead animals** and **aborted foetuses**, samples of the lymphoid organs (spleen, mesenteric lymph nodes, thymus, Peyer’s patches) thoracic fluid, pericardial fluid, abdominal fluid, and other tissues are suitable.
- Specimens stored for testing at a later stage, must be frozen at -70°C.
- Although BVDV antigen may remain viable at room temperature for longer than a week, if kept moist, samples should preferably be transported to the laboratory at 4°C as soon as possible.
- Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996.
- The PCR has also been adapted for use on formalin fixed samples and in bulk milk tank samples.

Virus isolation

- **Live animals**: EDTA blood, serum and body secretions (nasal, semen, vaginal).
- **Dead animals / aborted foetuses**: lymphoid organs (spleen, mesenteric lymph nodes, thymus, Peyer’s patches).
- Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. Specimens are stored and transported at 4°C to the laboratory.
BVDV Antibody Detection - Serology

Virus Neutralisation (VN) / Indirect Enzyme-Linked Immunosorbent Assay (ELISA)

These assays demonstrate the presence of BVDV antibodies against the virus in serum samples. Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should ideally be centrifuged and the serum poured off. Serum or un-spun clotted blood should then be transported at 4°C to the laboratory.

Acute and convalescent serum samples collected 3 weeks apart provide the most useful diagnostic information. A four-fold, or greater, increase in antibody titres in convalescent samples indicate viral exposure. A small percentage of PI animals may indeed test seropositive and so seropositive status does not altogether exclude PI. Interpretation of titres results should take into account endemic prevalence, herd history, vaccination status and the presence, or absence, of any forms of clinical disease in a herd.

Further reading:


NOTES
Enzootic Bovine Leukosis

Introduction

Enzootic Bovine Leukosis is an infectious lymphoproliferative disease of cattle with worldwide distribution. This disease is caused by an exogenous C-type retrovirus (Bovine Leukaemia Virus) and is distinct from sporadic bovine leucosis (SBL). There is a brief viraemia following infection in susceptible animals, followed by a long incubation period. The virus establishes a persistent infection in a sub-population of B lymphocytes by integration of proviral DNA at a number of sites on the cellular DNA. The majority of infected cattle remain healthy for life, although approximately 30% develop persistent lymphocytosis and up to 10% lymphoma, usually in animals older than 3 years of age. Infection can result in 3 different clinical states namely; asymptomatic aleukaemic (AL), persistent lymphocytosis (PL) and development of disseminated lymphoid tumours.

Sample Guide

EBL Antibody detection – Serology

Agar immunodiffusion gel (AGID) test and monoclonal antibody enzyme linked immunosorbent assay (ELISA)

These assays demonstrate the presence of antibodies against the virus in serum samples, individual or pooled. These techniques detect antibodies directed against the gp51, gp30 and gp24 antigens of the virus. Most AGID tests and ELISA’s in routine use detect antibodies to the glycoprotein gp51, as this monoclonal group of antibodies appears earlier, enabling earlier detection of infected animals. The sensitivity of the AGID test is limiting and some infected animals fail to produce a detectable AGID antibody response. Monoclonal ELISA assays targeting the major envelope glycoprotein gp51 are currently the preferred serological assays.

Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should ideally be centrifuged and the serum poured off. Serum or un-spun clotted blood should then be transported at 4°C to the laboratory.

- Maternally derived antibodies may persist for up to 6 or 7 months.
- Since BLV infection is for life, the presence of gp51 monoclonal BLV antibodies in animals older than 7 months of age is diagnostic for infection.
- All newly acquired animals should be tested prior to arrival on the farm and any positives must not be allowed to enter the herd.
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- Newly arrived negative animals should be quarantined and should re-test negative 45-90 days later prior to being allowed into the herd.
- Eradication programmes are based on identification and elimination of infected animals, which is achieved by detection of specific BLV gp51 antibodies in serum. Eradication could be achieved by serological screening and removal of infected animals on a 6 monthly basis. In some herds sero-positivity is so high that culling becomes impractical. In this situation, separate the herd into positive and negative animals. Positive animals then monitored on haematology and differential counts on a monthly basis and any abnormals are culled. Entire herd is tested serologically on a 6 month basis.

Monoclonal ELISA milk samples – individual or pooled.

Milk samples may be screened for the presence of BLV group specific antibodies utilising the monoclonal antibody ELISA assays. The AGID test is unsuitable for milk samples because of a lack of specificity and sensitivity. Bulk milk ELISA assay is a useful screening test for identifying possible BLV negative herds, positive bulk milk tank tests should be followed up by individual animal serum ELISA assays.

*Note: Heifers, bulls, dry cows and calves are not included in the bulk tank screen and these animals would need to be individually tested before the herd can be declared free of the virus.
EBL – Antigen detection systems

Polymerase chain reaction (PCR)

This is a sensitive and specific assay for the direct diagnosis of BLV genetic material in peripheral blood lymphocytes (EDTA blood), milk and lymphoid tissues of individual animals. The BLV-PCR is useful for the early detection of BLV infection before antibodies are present and is more sensitive than the serological antibody assays where the prevalence of infection is less than 5%.

The BLV-PCR has a very practical application in the identification of infected calves irrespective of their colostral immunity status. It detects proviral DNA in lymphocytes (EDTA blood) of calves born to infected dams and so enables detection of infected calves at birth allowing for immediate removal of virus sources from the herd. This assay therefore distinguishes uninfected newborn calves with colostral immunity from BLV infected calves, identifying the virus in the presence of antibody.

Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. Specimens are stored and transported at 4°C to the laboratory.

Virus isolation

Virus can be isolated from tumour tissue, peripheral lymphocytes in blood samples, taken in EDTA and body fluids (nasal and bronchial fluids, saliva and milk). However, virus isolation has largely been superseded by the BLV-PCR and is rarely performed.
Histopathology and Immunohistochemistry

In cases of lymphoma gross and histological lesions can provide useful diagnostic information and enables exclusion or inclusion of other diseases. Tissues to be collected into 10% buffered formalin should include lymph nodes, kidney, bone marrow, liver, spleen, myocardium, abomasum and uterus. Ensure that containers have adequate volumes of formalin and that the tissue : formalin ratio of 1 part tissue to 9 parts formalin is maintained.

Immunohistochemical tumour marker stains can be utilised to determine the cell lineage (B / T lymphocyte) of lymphomas. Enzootic Bovine Leukosis tends to be of B cell origin while SBL may be of B or T cell lineage.

The images above demonstrate lymphomatous infiltrates in the myocardium of an adult bovine with Enzootic Bovine Leukosis.

Further Reading

Bovine Ephemeral Fever (Three Day Stiff sickness)

Introduction

Bovine Ephemeral Fever (Three Day Stiff sickness) is a vector-borne viral disease of cattle and domestic water buffalo extending over tropical and subtropical zones of Africa, Australia and Asia. The virus, belonging to the family *Rhabdoviridae*, is transmitted via insect vectors, namely: *Culicoides* and *Anopheles*. Distinct strains of the virus have now been identified and this could adversely affect the response to vaccination. Economic losses are related to deaths, abortion, loss of milk production plus cost of treatment and vaccination. Clinical signs are characterised by fever, stiffness and a reluctance to move around. Atypical cases may exhibit severe respiratory distress with severe pulmonary and subcutaneous emphysema. Clinical signs are usually of short duration with recovery seen in most cases.

Sample Guide

**BEF Antibody Detection – Serology**

**Blocking ELISA / Virus Neutralization (VN) /Agar Gel Immunodiffusion (AGID) / Complement Fixation (CFT)**

Blocking ELISA and serum neutralization tests are preferred over CFT and AGID tests for diagnostic use. ELISA tests are more routinely being used due to ease, cost and high reported sensitivity and specificity.

Confirmation of BEF exposure is aided by running acute and convalescent sera 2-3 weeks apart, with the blocking ELISA and VN techniques being most frequently employed. A four-fold or greater increase in titre is suggestive of possible exposure. Serum samples, collected in a red or yellow stopper tubes, are required. Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should ideally be centrifuged and the serum poured off. Serum or un-spun clotted blood should then be transported at 4°C to the laboratory.

Factors which complicate interpretation of serological results include:

- Cross reactions between other antigenically related *Rhabdoviridae*
- An anamnestic response which can result in antibody levels reaching a plateau as early as five days post infection.
**BHF – Antigen Detection**

**Virus isolation**

Specimens suitable for virus isolation include post mortal tissues (spleen, synovial membranes, lung and pericardium) as well as heparin blood collected from live animals during the febrile reaction. Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. Specimens are transported at 4°C to the laboratory. If samples are to be stored for testing at a later date, then they should be frozen at -70°C.

**Molecular DNA testing**

Polymerase chain reaction (PCR) technology allows for detection of the virus in clinical (blood or synovial fluid in EDTA) and post mortem tissues including lungs, synovial membranes, tendon sheaths and muscle. Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. Specimens are transported and stored at 4°C to the laboratory.
Pathology

Gross and histological lesions can be suggestive but should be correlated with PCR and virus isolation. A full set of organ samples should be collected in 10% buffered formalin to confirm or eliminate the presence of lesions consistent with BEF and rule in / rule out other differentials as possibilities. Tissue samples of particular interest in cases of BEF include lungs, synovial membranes, tendon sheaths and skeletal muscle. Skeletal muscles of special importance include the quadriceps group and larger muscle masses of the shoulder and back.

Further reading


NOTES
Livestock Diseases

Bluetongue and Epizootic Haemorrhagic Disease

Introduction

Bluetongue (BT) is an infectious, non-contagious, insect-borne viral disease of sheep, goats, cattle, deer, bighorn sheep, most species of African antelope and various other Artiodactyla. It is caused by the bluetongue virus (BTV), belonging to the genus Orbivirus, family Reoviridae. It is probably economically most important in sheep although infection may be unapparent in most infected animals and only fatal in some clinically affected animals. In the non-ovine species infection is usually subclinical or mild. Exposure to the virus (natural or vaccination) may, under the correct circumstances, be teratogenic. BT is a notifiable disease in South Africa.

Note: Please see section on Controlled and Notifiable Animal Diseases for procedures regarding result reporting.

Epizootic haemorrhagic disease virus (EHDV) is an Orbivirus closely related to Bluetongue and is also insect transmitted being spread by Culicoides, causing a similar disease in deer and cattle (Epizootic haemorrhagic disease – EHD). Clinical symptoms associated with this virus include; oral ulcerations, facial swelling, necrosis of epithelium of the muzzle, difficulty in swallowing, swelling of the tongue, muscle stiffness and laminitis. On post mortem ulceration of the abomasum is common while the oesophagus and forestomachs are less commonly involved. There is frequently necrosis of striated muscle of the tongue, pharynx, larynx and oesophagus

Sample Guide

BT/EHD Antibody Detection – Serology

Agar Gel Immunodiffusion (AGID) / Indirect Enzyme-Linked Immunosorbent Assay (ELISA) / Virus Neutralization (VN)

These assays demonstrate the presence of antibodies against these 2 viruses. A serum sample, collected in a red or yellow stopper tube, is required. Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should ideally be centrifuged and the serum poured off. Serum or un-spun clotted blood should then be transported at 4°C to the laboratory.

Serological interpretation is complicated by cross reactions of BTV antibodies and EHDV antibodies. Monoclonal antibody-based competitive ELISA more specifically detects anti-BTV antibodies. Type specific antibodies (serogroups) are determined by virus neutralisation tests.
Antibodies may appear 7-14 days after BTV / EHDV infection and these antibody responses usually persist for long periods. The exposure to multiple serotypes, heterotypic antibody responses, serotype cross reactions, and cross reactions between different orbiviruses (Palyam virus, Ibaraki disease virus) can complicate interpretation of some serological results.

**BT/EHD Antigen Detection**

**Virus isolation**

In **live** animals blood (10 to 20ml) collected as early as possible during the febrile reaction, in an anticoagulant (heparin / EDTA / sodium citrate) is the preferred specimen for virus isolation.

In **dead** animals tissue samples (spleen, lymph node and bone marrow) should be aseptically collected as soon as possible after death.

Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. Specimens are stored and transported at 4°C to the laboratory.

Alternatively if samples are to be stored prior to testing, specimens may be frozen at -70°C for storage.

Rapid detection of virus in insect tissues and blood of infected sheep has also been achieved using an antigen capture ELISA without prior virus isolation.
Polymerase chain reaction (PCR)

Molecular DNA testing of Orbiviruses are rapidly expanding procedures especially following the recent emergence of Bluetongue across Europe. This technology allows for rapid amplification of Orbiviral RNA in clinical specimens and provides information on the virus serogroup and serotype.

Blood samples in EDTA and tissue samples (spleen, lymph node, bone marrow, lung) on ice are the preferred diagnostic specimens. Tissue swabs should be transported in PBS / viral transport medium and not re-inserted into the bacteriology gel. Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. Specimens are stored and transported at 4°C to the laboratory.

Pathology and Immunohistochemistry (IHC)

Gross and histological lesions may be highly suggestive but are not confirmatory alone and need to be interpreted in conjunction with IHC and antigen testing. A full set of organ samples including the target organs of tongue, pharynx, larynx, oesophagus, skeletal muscle (neck), heart, pulmonary artery, lung, abomasums, GIT, liver, kidney, brain, adrenals, should be collected into 10% buffered formalin. IHC staining enables visualisation of viral antigen with histological lesions.
Further reading


NOTES
Palyam Serogroup Orbivirus Infections

Introduction

The Palyam serogroup of orbiviruses are associated with abortion and teratology in cattle and possibly also other ruminants. These are arthropod-borne (mostly Culicoides sp midges) viruses that occur on the African, Asian and Australian continents. Infection usually only becomes apparent when abortion or foetal teratology present. Abortion or teratology can be sporadic or may occur in epidemics, involving one or more herds over a period of one to two weeks up to several months. Fifteen viruses are recognised as members of this serogroup, of which five serotypes (Abadina, Nyabira, Gweru, Marondera and Apies river viruses) are known to occur in Southern Africa. Clinical symptoms that have been described with this group of viruses include corneal opacitiy, blindness, nystagmus, cerebellar ataxia, opisthotonus and tremors.

Sample Guide

**Palyam Antibody Detection – Serology**

Blood is collected into serum tubes with clot activator (yellow stopper) or without (red stopper). Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should ideally be centrifuged and the serum poured off. Serum or un-spun clotted blood should then be transported at 4°C to the laboratory.

- Most dams are likely to have antibody titres by the time abortion occurs.
- Demonstrating rising titres (> 4 fold increase) in paired sera taken at the time of abortion and two to four weeks later provides more definitive evidence of exposure.
- Screening for antibody with a group-specific test is advisable.
- Demonstration of antibody in foetal heart blood/thoracic fluid or pre-colostral calf serum indicates *in utero* exposure.
Palyam Antigen Detection

Virus isolation

Gross pathological lesions that have been described in foetuses infected with this group of viruses include hydranencephaly, hydrocephalus, microencephaly and cerebellar hypoplasia.

- Gross detection of hydranencephaly, hydrocephalus, microencephaly / cerebellar hypoplasia during foetal post mortem should raise concern for possible palyam virus involvement.
- Unfortunately, as with most teratogenic viral infections culture of tissues from affected calves or lambs for viruses is usually negative.
- Target tissues for virus isolation include brain, spleen, liver, kidney, lung and placenta.
- Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996, stored and transported on ice at 4°C.

Further reading:

Livestock Diseases

Akabane and related Simbu-group viruses

Introduction

Infection with viruses in the *Simbu* group of the family *Bunyaviridae* are known to be able to cause congenital defects, mainly arthrogryposis and hydranencephaly plus abortions and stillbirths in cattle, sheep and goats. Although “Akabane disease” has become a commonly used term, it is however, a misnomer as Akabane virus is only one member of the Simbu group of viruses, of which several viruses may produce similar congenital defects (Aino, Tinaroo Peaton virus, Cache Valley virus, Douglas, Thimiri and Fafcey’s Paddock viruses). Generally infected pregnant animals develop in-apparent infections, and the nature of foetal lesions produced depends on the stage of foetal development at the time of infection. These viruses are insect transmitted with *Culicoides* midges and mosquitoes being the most common vectors.

Sample Guide

**Akabane Antibody Detection – Serology**

**Serum Virus Neutralization / ELISA**

Blood is collected into serum tubes with clot activator (yellow stopper) or without (red stopper). Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should ideally be centrifuged and the serum poured off. Serum or un-spun clotted blood should then be transported at 4°C to the laboratory.

- Serum is collected from heart blood of foetuses or from affected calves or lambs prior to suckling.
- Both IgG and IgM antibodies to Akabane virus are produced by immunocompetant foetuses.
- The presence of Akabane antibody in pre-colostral serum is evidence that the calf or lamb was infected *in utero*.
- Paired serum samples from the dam to evaluate for seroconversion (>4 fold increase in antibody titre) is also useful to demonstrate exposure.
Virus isolation

Congenital defects which have been described in bovine, ovine or caprine foetuses exposed to these viruses include microcephaly, porencephaly, hydranencephaly, hydrocephalus, hydromyelia, arthrogryposis, kyphosis, lordosis scoliosis, brachygnathia, and muscle atrophy. In addition in lambs spina bifida, cyclops, Arnold-Chiari syndrome and anal atresia have been described. The wide range of central nervous system lesions described with *in utero* infection with the Akabane / Simbu group of viruses, is thought to be related to foetal age at infection. The image above left is of the opened skull of a bovine foetus with severe hydranencephaly. The lamb in the image above right demonstrates multiple congenital defects including arthrogryposis, spinal kyphosis and brachygnathia.

- Gross detection of any or multiple of the above listed congenital abnormalities in bovine / ovine / caprine foetuses or neonates, should raise the suspicion for Akabane or related Simbu group virus infection.
- Unfortunately, as with most teratogenic viral infections, culture of tissues from affected calves or lambs for viruses is usually negative.
- Virus isolation can be attempted from most organs although the brain, spinal cord, cerebrospinal fluid, skeletal muscle, chorioallantois and amnion provide the best chance for isolation.
- Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996, stored and transported on ice at 4ºC.

Further reading:

Rotavirus Infections

Introduction

Rotavirus is ubiquitous and frequently causes small intestinal disease in many mammal and bird species, usually neonatal animals. They are now classified in the family Reoviridae, genus Rotavirus and are named after the species in which they occur eg: bovine rotavirus, porcine rotavirus, canine rotavirus etc. Considerable antigenic diversity occurs amongst rotaviruses and they have been divided into groups A-G. These viruses have a worldwide distribution and most commonly cause clinical disease in calves, lambs, piglets and foals.

Sample Guide

Rotavirus Antigen Detection Tests

Faecal examination

Sample collection - in live animals fresh faeces collected per rectum, where possible, is preferred. Where per rectum collection of faecal material is not possible, collection of faeces immediately after observing defaecation is advised. In dead animals portions of the affected distal small intestine should be submitted, tied-off at both ends. All samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996, stored and transported to the laboratory at 4°C.

Transmission electron microscopy (TEM) of negatively stained faeces or intestinal contents is a commonly employed diagnostic procedure, due to the fact that rotavirus may reach very high concentrations in clinical cases.

Faecal antigen ELISA - as all rotaviruses share, group-specific antigens, human rotavirus enzyme-linked immunosorbent assay (ELISA) kits, available commercially, can be used to screen faecal specimens for the presence of rotaviral antigen.

Direct or indirect fluorescent antibody tests - are used to demonstrate antigen in faecal smears.

Polymerase Chain Reaction (PCR) – used most commonly in epidemiological studies to differentiate between viral isolates and rapidly detect atypical rotaviruses in faecal specimens.

Virus isolation - the virus is ubiquitous and other drawbacks are long turnaround times, and high costs - it is therefore not routinely attempted.
Histopathology must be used in conjunction with other antigen tests or TEM to confirm the significance of the pathological changes. Various sections of the small intestine are collected into 10% buffered formalin as per the guidelines in the Gastrointestinal section of Organ Pathology.

**Rotavirus Antibody Detection – Serology**

ELISA may be used for serological screening, but as the virus is ubiquitous with high antibody prevalence in most populations, the diagnostic value of serology is therefore limited. Following collection samples are allowed to stand for 20 minutes at room temperature to enable good clot formation. Blood specimens are then preferably centrifuged and the serum poured off. Centrifuged serum or clotted whole blood is then transported at 4°C to the laboratory. Separated serum samples can be frozen at -18°C for testing at a later stage.

**Further Reading**

Bovine Corona Virus (BCV) Infection

Introduction

Bovine coronavirus (BCV) infection is known to cause neonatal calf diarrhoea, winter dysentery in adult dairy cattle and respiratory infections of cattle of all ages. Bovine coronavirus can be identified in most adult cattle worldwide, and also observed in some wild ruminant species.

Sample Guide

BCV Virus Isolation and Antigen Detection

Neonatal calf diarrhoea / Winter dysentry

- Fresh faeces collected per rectum, intestinal contents, or swabs of rectal fluid from live animals.
- This material should preferably be collected within one to three days of the onset of diarrhea.
- Sections of intestine including distal jejunum, ileum, cecocolonic junction and colon from dead animals.
- Antigens may be detected in epithelial cells of the distal small intestine, usually the distal jejunum, ileum and colon by direct or indirect immunofluorescence (IF) staining, antigen capture ELISA, polymerase chain reaction (PCR), electron microscopy (EM) or virus isolation.

Respiratory infections

- Nasal flushes, bronchoalveolar lavages and trans-tracheal aspirates are the preferred samples from live animals.
- Lung tissues are the preferred post mortal sample.
- Bovine coronaviral antigen is detected in nasal flushes, bronchoalveolar lavages, trans-tracheal aspirates and lung tissue by antigen ELISA, PCR, EM or virus isolation.

All samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996, stored and transported on ice at 4°C to the laboratory.
Histopathology and Immunohistochemistry (IHC)

- **Diarrhoea syndromes**: target tissues include distal jejunum, ileum, cecocolonic junction and colon, collected into 10% buffered formalin.
- **Respiratory syndromes**: target tissues include nasal mucosa with turbinates, trachea and lung collected into 10% buffered formalin.
- Running IHC in conjunction with histopathology enables demonstration of viral antigen within histological lesions.

**BCV Antibody Detection – Serology**

**Virus Neutralisation / ELISA / Haemagglutination Inhibition**

Blood is collected into serum tubes with clot activator (yellow stopper) or without (red stopper). Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should ideally be centrifuged and the serum poured off. Serum or un-spun clotted blood should then be transported at 4°C to the laboratory.

- Due to the ubiquitous nature of the virus, BCV antibodies are commonly detected in most cattle.
- Demonstrating rising titres (> 4 fold increase) in paired sera taken 2-4 weeks apart, provide more definitive evidence of exposure.
- Maternal antibody interference adversely affects the use of serology in calves.

**Further reading:**

Bovine Respiratory Syncytial Virus Infection

Introduction

*Bovine respiratory syncytial virus* (BRSV) is a member of the genus *Pneumovirus* in the family *Paramyxoviridae*. Although infection with BRSV is unapparent in most animals, it may cause mild to severe respiratory tract disease with clinical signs of fever, coughing, serous nasal discharges, ocular discharges, dyspnoea and sometimes subcutaneous emphysema as consequence. BRSV is an important component of the bovine respiratory disease complex / bovine enzootic pneumonia. It mainly affects young cattle and predisposes to secondary infections like *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somnii*, *Arcanobacterium pyogenes* and *Escherichia coli*. This virus is capable of initiating outbreaks of respiratory disease alone and sometimes is fatal independently. Respiratory syncytial virus may also cause rhinitis in sheep, and infect goats. A respiratory syncytial virus (RSV) affecting horses has been reported.

Sample Guide

**BRSV Antigen Detection**

**Fluorescent Antibody Test / Immunoperoxidase**

- Preferred samples are nasal swabs / flushes.
- Assays are performed on smears prepared from these specimens.
- Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996, stored and transported at 4°C to the laboratory.
Virus isolation

- As this virus is labile a sucrose containing transport medium should be used and time delays on reaching the laboratory should be minimal.
- In live animals specimens of choice are nasopharyngeal swabs, or tracheal washes and bronchoalveolar lavages, while in dead animals fresh lungs, trachea and their associated lymph nodes are preferred.
- Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996, stored and transported at 4°C to the laboratory.

Molecular biology

- Nasal flushes / nasal swabs, tracheal washes or bronchoalveolar lavages are the preferred samples in live animals.
- Post mortem samples should include nasal mucosa, trachea, lung and bronchial lymph node collected into sterile containers.
- Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996, stored and transported at 4°C to the laboratory.
Histopathology and Immunohistochemistry (IHC)

Target tissues are upper respiratory tract, lung and mediastinal lymph nodes. Utilizing IHC in conjunction with histopathology enables the demonstration of viral antigen within the histological lesions, thereby confirming the diagnosis.

BRStV Antibody Detection – Serology

Direct Immunofluorescence / Virus Neutralisation / Compliment Fixation Tests

- Serum samples should be collected in a red or yellow stopper tube, stored at 4°C and transported to the laboratory as quickly as possible on ice.
- Due to the high prevalence of infection in the cattle population, results should be interpreted with caution before attributing respiratory disease in cattle to BRStV infection based on serology alone.
- Demonstrating seroconversion (> 4 fold increase in antibody titre) in acute and convalescent sera collected 3-4 weeks apart, provides more definitive evidence of exposure.

Further reading

Bovine Parainfluenza Type 3 (B-PI3)

Introduction

Parainfluenza viruses are grouped in the genus Paramyxovirus and the family Paramyxoviridae. Parainfluenza type 3 (PI-3) virus infection has been reported in cattle, sheep and goats. Respiratory infection is usually asymptomatic. However, B-PI3 infections in association with other viral and bacterial pathogens are important in the “shipping fever” / enzootic pneumonia and the Bovine respiratory disease complex (BRD) syndromes. The virus infects ciliated and non-ciliated epithelial cells of the upper and lower respiratory tract, alveolar macrophages, type II pneumocytes and lymphocytes.

Sample Guide

B-PI3 Antigen Detection

Virus isolation

- Live animals: nasopharyngeal swabs or bronchoalveolar lavages and transtracheal aspirates.
- Dead Animals: biopsies of cranioventral lobes of the lungs, trachea and their associated lymph nodes collected into individual sterile containers.
- All samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996 and transported at 4°C to the laboratory. (See Reference Ranges – Section 3).

Histopathology and Immunohistochemistry

- Target tissues are trachea, lung and bronchial lymph nodes. Utilizing IHC in conjunction with histopathology enables the demonstration of viral antigen within the histological lesions, thereby confirming the diagnosis.
- IHC is most effective in acute cases. In the more chronic complicating bacterial pneumonias, viral antigen may be cleared long before the bacterial pneumonia is fatal.
• In the more chronic cases with complicating bacterial pneumonia, histopathology needs to be interpreted in conjunction with acute and convalescent serology.

Molecular biology

• Nasal flushes / nasal swabs, tracheal washes or bronchoalveolar lavages are the preferred samples in live animals.
• Post mortem samples should include nasal mucosa, trachea, lung and bronchial lymph node collected into sterile containers.
• Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996, stored and transported at 4°C to the laboratory.

B-PI3 Antibody Detection – Serology

Haemagglutination Inhibition / Virus Neutralisation / Plaque Reduction Tests

• Blood is collected into serum tubes with clot activator (yellow stopper) or without (red stopper). Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should ideally be centrifuged and the serum poured off. Serum or un-spun clotted blood should then be transported at 4°C to the laboratory.
• The prevalence of positive B-PI3 antibody titres can be fairly high in some cattle populations.
• Demonstrating seroconversion (> 4 fold increase in antibody titres) in acute and convalescent sera provides more definitive evidence of exposure.
• A high level of seroconversion is usually observed a month after animals arrive at a feedlot.
• There is variable association between seroconversion to B-PI3 and the development of pneumonia.
Serological techniques such as Haemagglutination Inhibition, Virus Neutralisation and Plaque Reduction Tests, have been described to demonstrate the presence of antibodies. As serum antibodies may be present in most cattle, paired serum samples (i.e. acute and convalescent collected 4 weeks apart) should be taken to demonstrate of seroconversion.

Further reading


NOTES
Jaagsiekte

Introduction

Jaagsiekte (JS), a contagious bronchiolo-alveolar adenocarcinoma of the lungs of sheep is caused by the jaagskiete sheep retrovirus (JSRV), belonging to the family *Retroviridae*. Merino sheep are highly susceptible. This transmissible neoplasm grows by local expansion and infiltration, but metastasis to regional lymph nodes is uncommon. JSRV targets type II alveolar epithelial cells with these virally-transformed cells undergoing proliferation which spills into airways and compresses and invades adjacent pulmonary parenchyma. Secondary bacterial pneumonia is common. Incubation period is usually long (months to 2 years). JSRV infection is also considered fairly common in domestic goats.

It has been established that JSRV is found in two closely related but molecularly distinct forms namely an infectious exogenous retrovirus (exJSRV) and as a group of endogenous retroviral loci (enJSRV). The exJSRV form is the aetiological agent of jaagsiekte and is transmitted horizontally between animals. The enJSRV’s by themselves are not associated with disease. Clinical disease is characterized by a history of a long-standing, progressively worsening respiratory disease syndrome in a flock.

Sample Guide

Molecular biology

- PCR techniques have been described for the detection of viral nucleic acids.
- Live animals: nasal swabs / flushes, tracheal washes and bronchoalveolar lavages.
- Dead animals: fresh lung and bronchial lymph nodes.
- Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. (See Reference Ranges – Section 3). Specimens are stored and transported at 4°C to the laboratory.
Histopathology

- JSRV produces fairly characteristic pulmonary histological lesions of well-differentiated adenocarcinoma.
- Secondary bacterial pneumonia is common and may obscure the classic histological lesions of JSRV, therefore several specimens, from different locations in the diseased lung, should be collected into 10% buffered formalin.
- Pulmonary histopathology must be correlated to the flock history, clinical symptomatology and PCR findings.

Virus isolation
The diagnosis of jaagsiekte by means of virus isolation is not possible due to a lack of in vitro-culture systems for the virus.

Serology
Circulating antibodies against JRSV infection are not produced by the immune system, and their absence excludes serology as a diagnostic procedure.

Further reading
Maedi – Visna

Introduction

Maedi-visna (MV) is caused by a non-oncogenic retrovirus of the subfamily *Lentivirinae*. It is caused by the group of related strains of maedi-visna viruses which belong to the lentivirus subgroup of retroviruses which cause changes in antigen processing and immunological systems. The two most common forms of the disease seen are Maedi (dyspnœa) characterised by a progressive interstitial pneumonia, and Visna (wasting) - a chronic encephalitic form. Mastitis and arthritis may also be seen. A number of different lentiviruses have been isolated from sheep in different regions across the world. The virulent Icelandic MVV isolate is the prototype and the isolates of ovine progressive pneumonia virus (OPPV) in the USA, the South African ovine maedi-visna virus (SA-OMVV) and caprine arthritis-encephalitis virus (CAEV), are all serologically related, although variation in their biological properties and pathogenicity is seen. Infections with these viruses are persistent and life long and most infections may be subclinical.

Chronic progressive pneumonia in sheep and goats (lymphoid interstitial pneumonia) is a slow virus infection of the ovine lung characterized by gradual progressive interstitial pneumonia. Other clinical features include emaciation, paralysis, lameness and indurated udders due to lymphoproliferative and degenerative processes. Concurrent infections with "Jaagsiekte" are extremely common in South African flocks.

Sample Guide

**MVV Antigen Detection**

**Virus isolation**

- Virus isolation can be confirmative in typical clinical cases, but in many cases may only be supportive evidence of subclinical infection.
- Samples to be collected for virus isolation from **live animals** would be blood in heparin or milk.
- From **dead animals**: fresh lung, brain (choroid plexus), synovial membrane or udder are collected into sterile containers.
- Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. (See Reference Ranges – Section 3). Specimens are stored and transported at 4°C to the laboratory.
**Histopathology and Immunohistochemistry (IHC)**

- Target tissues for formalin-fixation include lungs, brain, joints, and mammary glands.
- Histological lesions can be masked by secondary bacterial pneumonia and/or concurrent Jaagsiekte.
- Utilizing IHC in conjunction with histopathology enables demonstration of intralesional viral antigen.

**MVV Antibody detection - Serology**

**Virus Neutralisation / Complement Fixation / Immunofluorescence / AGID / ELISA**

- Virus neutralising antibodies only develop late in the infection cycle, plus they are strain specific and therefore not suitable for general diagnostic purposes.
- AGID and more lately ELISA have more practical diagnostic applications.
- Antibody responses may be persistent for life.

**Further reading**

Rift Valley Fever

Introduction

Rift Valley Fever virus is a mosquito-transmitted disease of livestock and humans, which has historically been associated with large epidemics of devastating, severe disease throughout Africa and, more recently, the Arabian Peninsula. This is a notifiable disease in South Africa. The recent 2009 outbreaks in Kwazulu-Natal and the Northern Cape have highlighted the changing face of Rift Valley Fever in South Africa, alerting us to the probable emergence of enzootic RVF foci in the country. Against this background there is greater need to be more vigilant about the safe handling of samples.

Note: Please see section on Controlled and Notifiable Animal Diseases for procedures regarding result reporting.

Sample Guide

Post mortem procedure

- Wear protective clothing (overalls and gum boots), protective eye-ware, surgical mask, surgical gloves and post mortem gloves.
- Post mortem utensils stored in an antiviral solution (eg: F10, Virkon).
- Collect all the necessary samples for confirmatory tests (see below).
- Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996.
- Transport chilled at 4°C.
- Place ‘Infectious Substance’ and ‘UN2814’ labels on outer sample container.
- On completion, disinfect all instruments used, protective clothing and necropsy area with anti-viral solution.
- Safe carcase disposal.
Histopathology

- Samples collected into 10% buffered formalin, virus is rapidly inactivated in formalin, therefore **minimal zoonotic risk** to people handling samples.
- Organs of choice: liver, lymph nodes, spleen, lung, kidney (adults and neonates) plus adrenal gland and placenta (aborted foetuses).
- Tissues are fixed in formalin, therefore safe to handle and transport (no zoonotic risk).
- Formalin-fixed samples should not be packed together with fresh tissue samples or blood specimens as formalin fumes could lead to viral inactivation, adversely affecting sensitivity and specificity of other diagnostic assays.

Immunohistochemistry (IHC)

- IHC performed on formalin-fixed tissues, therefore, ambient temperatures and time taken for samples to reach the diagnostic laboratory are not issues.
- Do not package together with fresh tissues or blood.
- Tissue sections counterstained with haematoxylin following incubation with the RVF antibody, enabling visualisation of RVF viral antigen within histological lesions, giving the pathologist confidence in the diagnosis.
- Good correlation between IHC on formalin-fixed tissues and PCR on blood / tissue.
Reverse transcriptase PCR (RT-PCR)

- Samples of choice are fresh tissues (liver and spleen) and blood (EDTA).
- Zoonotic risk: take precautions, all samples processed in a biohazard cabinet
- Liver and spleen, virus detectable for up to 3 weeks post infection.
- RVF viraemia is short (2-5 days). PCR on blood (EDTA) only positive with recent infection.
- Blood (EDTA) and tissue (liver and spleen) must be packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996.
- Blood and tissue samples must be packed separately to the formalin-fixed tissues.
- RVF is an RNA virus and therefore a copy of DNA needs to be made from the viral RNA (reverse transcriptase) before the PCR can be run. This procedure is temperature dependent.
- Transit time to the laboratory and maintenance of the cold chain are critical.

Antigen ELISA

- Detects viral antigen in tissue (Liver and spleen).
- Zoonotic risk: take precautions, all samples processed in a biohazard cabinet.
- Test with high sensitivity even in decomposed tissues.
- Tissue (liver and spleen must be packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996 and separately to the formalin-fixed tissues.
Livestock Diseases

Virus isolation

- Tissues (liver and spleen) are preferred to blood or thoracic fluid in heparin.
- Zoonotic risk: take precautions, all samples processed in a biohazard cabinet.
- Used as an ancillary confirmatory test for RVF infections confirmed by the rapid diagnostic tests of RT-PCR, antigen ELISA or IHC.
- Tissue (liver and spleen) and blood (heparin) must be packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996 and separately to the formalin-fixed tissues.
- Time consuming and success affected by sample freshness and transit time to the laboratory.

Antibody detection - Serology

- Sample: serum (serum stored at 4°C can retain infectivity for several weeks).
- Not a true diagnostic test, it merely indicates exposure.
- Serology interpretation complicated in vaccinated herds.
- Zoonotic risk: take precautions, all samples processed in a biohazard cabinet.
- Blood (serum) must be packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996 and separately to the formalin-fixed tissues.
- Two serological assays available:
  - Total RVF specific immunoglobulins (IgG + IgM), however can not distinguish between recent or past exposure.
  - RVF IgM immunoglobulin assay used to confirm recent exposure (IgM antibodies detectable for 60-90 days post exposure).
Further Reading


NOTES
Lumpy Skin Disease (LSD)

Introduction

Lumpy skin disease (LSD) is a notifiable disease caused by the Lumpy Skin Disease virus (LSDV) belonging to the family Poxviridae, with the Neethling strain the prototype. Lumpy skin disease is a presumed insect-transmitted, capripox viral infection of cattle, largely restricted to sub-Saharan Africa. Confirmed natural infection has only been reported in cattle, while giraffe and impala are highly susceptible to experimental infection. LSD is caused by strains of the virus which are indistinguishable from strains causing sheep pox and goat pox. However, LSD has a different geographic distribution to sheep and goat pox, suggesting that these LSD cattle strains do not infect and transmit between sheep and goats.

Sample Guide

LSD Antibody detection – Serology

Virus neutralisation (VN), agar immunodiffusion gel (AGID) test, Western blot analysis and enzyme linked immunosorent assay (ELISA)

These assays demonstrate the presence of antibodies against the virus. A serum sample, collected in a red or yellow stopper tube, is required. Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should ideally be centrifuged and the serum poured off. Serum or un-spun clotted blood should then be transported at 4°C to the laboratory.

VN is the most specific method but not sensitive enough to detect animals with low levels of neutralising antibodies.

The AGID and indirect immunofluorescent antibody tests (IFAT) are fraught with cross reactions with antibodies to bovine papular stomatitis and pseudocowpox viruses (AGID); and with orf (contagious pustular dermatitis of sheep virus), bovine papular stomatitis and perhaps other poxviruses (IFAT) and are therefore less specific methods.

Western blotting is specific and sensitive.
LSD - Antigen detection systems

Virus isolation

Virus can be isolated from fresh skin nodules, lung, spleen and lymph node tissues collected into sterile sealed containers/envelopes. Virus can be also isolated from theuffy coat in blood samples, taken in heparin. Virus isolation is most successful in the early clinical stages of the disease (< 1 week), before the development of neutralising antibodies. Blood and tissue samples should be stored and transported at 4°C to the laboratory. Tissue may be frozen at -20°C for longer storage for delayed testing. This is the assay of choice if infectivity of the particular LSDV isolate is to be determined.

Note: The virus is remarkably stable and it can survive in skin nodules kept at –80°C for ten years and from infected tissue culture fluid stored at 4°C for six months. In necrotic skin nodules the virus may persist for up to 33 days although this period may be much longer.

Immunohistochemical staining (IHC)

IHC staining has been successfully applied to formalin-fixed tissue sections and used in conjunction with histopathology to demonstrate LSD viral antigen within typical histological lesions. A full set organ samples (brain, lung, heart, liver, spleen, kidneys, lymph nodes, gastrointestinal sections, skins nodules) should be collected in 10% buffered formalin.

Note: Use of un-buffered formalin can result in artifactual haematin staining confusing possible positive viral antigen staining.
Polymerase chain reaction (PCR)

PCR techniques have been developed to detect LSD virus in tissue samples and EDTA blood samples. Samples are stored and transported at 4°C to the laboratory in sealed containers. Do not transport in the same container as the formalin-fixed samples as formalin fumes can compromise viral genetic material. The PCR is the preferred assay for the detection of viral material in blood and skin and appears to be able to detect virus for longer periods post infection than virus isolation or TEM.

Transmission Electron microscopy (TEM)

The virions of orthopoxviruses may be demonstrated by direct transmission electron microscopy in fresh biopsy samples, in the desiccated crusts of skin lesions as well as in formalin-fixed or glutaraldehyde-fixed tissue samples. LSDV is usually only detectable by TEM for about a month post infection.
Antigen capture ELISA assay

The highly antigenic capripoxvirus structural protein P32 has been cloned for preparation of monospecific polyclonal antiserum and the production of monoclonal antibodies (Mabs) for the use in an antigen detection enzyme-linked immunosorbent assay (ELISA) and this assay is highly specific.

Further reading


NOTES
Bovine Anaplasmosis

Introduction

Bovine anaplasmosis is a tick borne disease caused by infection with the rickettsial bacteria *Anaplasma marginale* (most commonly) and *A. centrale* (rarely). *Anaplasma marginale* is responsible for most outbreaks of clinical disease whilst *A. centrale* generally produces much milder disease and is used as vaccine stain. Cross immunity exists between these two organisms.

Sample Guide

Blood smear examination

In live animals thin blood films prepared from peripheral blood (collected from the ear / tail) or central blood (collected by venipuncture of the jugular vein) in EDTA and stained with Diff-Quik® or Giemsa stains are examined under the microscope utilising the 100X oil objective. From dead animals, blood and / or organ (liver, kidney, heart, lung) smears can be prepared and stained in a similar fashion for examination.

When submitting smears to the laboratory for confirmation, always submit 2 unstained, air-dried, thin blood / organ smears and 1 stained smear. Dried smears should be placed in a sealed cytology slide holder and transported to the laboratory at ambient temperature. EDTA blood submitted to the laboratory for parasite identification should be transported cooled at 4°C to the laboratory.
Parasitaemia varies with the severity and stage of the clinical disease. Maximally *A. marginale* parasitaemias may reach 50%. Parasites can be detected 2-6 weeks after infection, and the level of parasitaemia may double each day, for period of 10 days, after which it decreases at a similar rate.

Blood smear examination may be complicated by the following factors:

- In cases with severe anaemia parasites can be hard to find.
- When infected red blood cells are removed from the circulation and no longer present in blood smears.
- Presence of basophilic stippling in reticulocytes and Howell-Jolly bodies may confuse parasite identification.
- The subclinical carriers have extremely low parasitaemia.
- When smears are prepared on dirty glass slides or dirty smears with skin debris, may mask the presence of parasites.

**Anaplasma spp antibody detection – Serology**

**Complement fixation (CF) / Card agglutination / ELISA** – blood specimens are collected into serum tubes with / without clot activator. Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should ideally be centrifuged and the serum poured off. Serum or un-spun clotted blood should then be transported at 4°C to the laboratory.

Serology finds its best application in the identification of sub-clinical carrier animals (regulatory measures, disease control programmes, chemotherapeutic eradication programmes and epidemiological studies). Infection may be persistent for life in some animals and cross reactions between *Anaplasma marginale* and *A. centrale* can complicate interpretation of results.

Complement fixation (CF) and the card agglutination tests are most commonly used, although they have poor sensitivity for identification of persistently infected cattle, and are considered unreliable assays for disease certification of individual animals. A competitive enzyme-linked immunosorbent assay (C-ELISA) has been demonstrated to have higher sensitivity (96%) in acute and chronic infections and is able to detect carrier animals.
Anaplasma spp antigen detection

Polymerase chain reaction (PCR)

Various PCR techniques including nested, real time, duplex real time and TaqMan have been applied for the diagnosis of clinical anaplasmosis and identification of carrier animals in EDTA blood samples. Some of these techniques are reported to be specific, without cross reactions with other parasites, allowing for identification and quantification of A. marginale and A. centrale parasite load, even in mixed infections. Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. Specimens are stored and transported at 4°C to the laboratory.

Note: it is advised to contact the laboratory prior to sample submission for PCR, to confirm whether this assay is in fact suitable for the disease investigation proposed.

Pathology

The pathology caused by bovine anaplasmosis is suggestive and should be interpreted in conjunction with the demonstration of anaplasma parasites in blood and other organ smears. The image above is of a liver from a bovine that died of anaplasmosis, note the marked cholestasis. A full set of tissue samples (brain, lung, heart, liver, spleen, kidneys, lymph nodes, gastrointestinal sections) should be collected in 10% buffered-formalin to confirm the presence of lesions consistent with anaplasmosis or rule in or out other disease conditions.

Further Reading


**NOTES**
Livestock Diseases

Bovine Babesiosis

Introduction

Infection with protozoan parasites of the genus *Babesia*, order *Piroplasmida*, phylum *Apicomplexa* may cause bovine babesiosis. *Babesia bigemina* (African redwater) and *Babesia bovis* (European / cerebral redwater) are the two important babesial species affecting bovines in Africa. *Babesia divergens* is economically important in some parts of Europe.

Sample Guide

Peripheral blood smears

For the diagnosis of babesiosis in live animals, thick and thin films of peripheral capillary blood collected from the tip of the tail, or the ear are prepared, stained with Diff-Quik® or Giemsa stains and examined under the microscope utilising the 100X oil objective. A blood sample of the general circulation (collected by venipuncture from the jugular vein) is only reliable for the detection of *Babesia bigemina* infections as these babesias are usually numerous, parasitised cells are evenly distributed throughout the blood circulation and are usually easy to detect. Venipuncture central blood is however less suitable for detection of *Babesia bovis*, as parasite concentrations may be extremely low increasing the risk of a false negative smear result.

When submitting smears to the laboratory for confirmation, always submit 2 unstained, air-dried, thin blood / organ smears and 1 stained smear. Dried smears should be placed in a sealed cytology slide holder and transported to the laboratory at ambient temperature. EDTA blood submitted to the laboratory for parasite identification should be transported cooled at 4°C to the laboratory.

Factors which may affect the ability to detect *Babesia* spp parasites in blood films include:

- With low levels parasitaemias, for example in asymptomatic carrier animals.
Following prior treatment with a babesiacide, as parasites may degenerate and disappear, often within 24 hours post-treatment.

- With exposure of the blood films to moisture or formalin fumes during transit.
- When smears are prepared on dirty glass slides or dirty smears with skin debris, may mask the presence of parasites.

In dead animals, smears of peripheral blood, brain (important in cases of suspected cerebral redwater), kidney, liver, spleen and bone marrow are examined for parasites. Bone marrow smears provide the best chance of success in decomposed animals.

**Babesia spp antibody detection – Serology**

Indirect fluorescent antibody (IFA) / Enzyme-linked immunosorbent assay (ELISA) tests - blood specimens are collected into serum tubes with / without clot activator. Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should ideally be centrifuged and the serum poured off. Serum or un-spun clotted blood should then be transported at 4°C to the laboratory.

IFA - is used to detect antibodies to *B. bovis* and *B. divergens*. This assay has poor specificity for the detection of *B. bigemina* antibodies. Serological cross-reactions make species diagnosis difficult, especially in geographical areas where both *B. bovis* and *B. bigemina* co-exist. Disadvantages of this technique are low sample throughput and subjectivity.

ELISA – internationally validated tests for the sero-diagnosis of *B. bovis* are available. Current ELISA tests for *B. bigemina* tend to be less specific.

**Babesia spp – Antigen detection**

Polymerase Chain Reaction (PCR)

PCR techniques are assays with very high sensitivity and specificity for the detection and differentiation species of the various species of Babesia in EDTA blood samples. Therefore,
these procedures are best suited for the determination of carrier status or confirmation of clinical infection in individual animals, but are not ideal for large-scale herd testing, which currently remains the domain of serology.

Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. (See Reference Ranges – Section 3) Specimens are stored and transported at 4°C to the laboratory.

Note: it is advised to contact the laboratory prior to sample submission for PCR, to confirm whether this assay is in fact suitable for the disease investigation proposed.

**Pathology**

The gross pathology caused by bovine babesiosis is suggestive and should be interpreted in conjunction with the demonstration of babesia parasites in blood and other organ smears. A full set of tissue samples (brain, lung, heart, liver, spleen, kidneys, lymph nodes, gastrointestinal sections and muscle) should be collected in 10% buffered-formalin to confirm the presence of lesions consistent with babesiosis or rule in or out other disease conditions.

*Babesia bovis* infections associated with cerebral babesiosis induce quite characteristic changes of cerebral congestion with stark contrast between the grey and white matter (image above-left). This pathology develops as a result of sludging of parasitised erythrocytes in cerebral capillaries due to cytoadherence of red blood cells to one another, as well as adhesive interactions between erythrocytes and receptors on the vascular endothelial cells of cerebral capillaries. Brain smears from such cases reveal blood vessels filled with erythrocytes containing small *Babesia bovis* parasites (image above-right).

**Further Reading**

Bovine Neosporosis

Introduction

*Neospora caninum* is a protozoan parasite that is a major pathogen of cattle and dogs, but occasionally causes clinical infections in goats, sheep and deer plus there are individual reports in rhinoceros and hyena. Dogs and more recently coyotes have been confirmed as definitive hosts and dogs may also be intermediate hosts. All the other species are intermediate hosts. The protozoal organism in all these species is one and the same. *N. hughesi* is a new species of neospora which is associated with myeloencephalitis and more recently abortion in horses. Cattle and other intermediate hosts can be infected through ingestion of oocysts shed in the faeces of acutely infected dogs. In cattle following infection both vertical and horizontal transmission are thought to occur. Vertical transmission is of major importance in the spread of neosporosis and abortion is the most common clinical presentation of the disease in cattle. Congenital infection with neurological deficits is most common in dogs. Following foetal infection foetuses may die *in utero*, be resorbed, mummified, autolysed, stillborn, born alive with neurological deficits or born clinically normal but chronically infected. Occasionally neospora has been associated with birth defects (hydrocephalus, narrowing of the spinal cord).

External infections through oocysts (ingestion) are the likely cause of abortion storms (epidemic), while increase in abortion rate (endemic) is probably the consequence of vertical and horizontal transmission. Potential sources of horizontal transmission include colostrum or milk from infected cows, infected placentae and foetal fluids, or oocyst contaminated feed.

Sample Guide

*Neospora Antibody Detection – Serology*

**Enzyme-Linked Immunosorbent Assay (ELISA) / Indirect Fluorescent Antibody Test (IFAT) / Direct Agglutination Test.**

Serological tests to detect *N. caninum* antibodies are performed on serum or foetal thoracic fluid collected into a serum tube with / without clot activator. Following collection samples are allowed to stand for 20 minutes at room temperature to enable good clot formation. Blood specimens are then preferably centrifuged and the serum poured off. Centrifuged serum or clotted whole blood is then transported at 4°C to the laboratory. Separated serum samples can be frozen at -18°C for testing at a later stage.

The presence of serum antibodies in a cow that has aborted only indicates exposure to *N. caninum* and does not confirm infection. Demonstrating a 4 fold or greater increase in
antibody titres in acute and convalescent serum samples collected 3-4 weeks apart; provide more convincing evidence of infection.

- Dams from herds with sporadic *N. caninum*-associated endemic abortion had significantly higher ELISA indices than those from herds with *N. caninum*-associated abortion storms (epidemic).
- Seropositive animals are at greater risk of abortion than seronegative animals and there is a rise in antibody levels 4 to 5 months prior to parturition, implicating reactivation of latent infection.
- Demonstrating *N. caninum* antibody in foetal serum or pre-colostral calf serum indicates possible infection.
- Antibody production in the foetus is determined by the stage of gestation, level of exposure, and the time between infection and abortion.
- Antibody titre levels in an aborted foetus are frequently lower than those in the dam.
- In acute abortion storms in naïve herds, foetal and dam serology is often negative, as abortion occurs prior to the development of serum titres.

**Neospora Antigen Assays**

**Immunohistochemistry and Histopathology**

IHC in conjunction with histopathology is considered the diagnostic test of choice for confirmation of neosporosis in post mortem tissue and enables confirmation of the diagnosis. Foetal pathology is characterized by a non-suppurative meningoencephalitis, mononuclear interstitial myocarditis and multifocal necrotising hepatitis. IHC enables the visualisation of protozoal tachyzoites in histological lesions as well as demonstrating protozoal tissue cysts in nervous and skeletal/cardiac muscle.

- **Foetal tissues** in 10% buffered formalin– brain, heart, liver and placenta.
- **Calf tissues** in 10% buffered formalin– brain, spinal cord, heart and skeletal muscle.
Polymerase chain reaction

Polymerase chain reaction (PCR) methods detect *N. caninum* DNA. Post mortem tissues (brain, heart and liver) are all considered suitable. Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996, stored and transported at 4°C. (See Reference Ranges – Section 3).

Further Reading

Heartwater (*Ehrlichia ruminantium*)

**Introduction**

Heartwater is caused by the endotheliotropic organism *Ehrlichia (Cowdria) ruminantium* classified in the family *Anaplasmataceae*, order *Rickettsiales*. It infects cattle, sheep, goats and some wild ruminants. The disease is transmitted by multiple *Amblyomma* species of ticks found in sub-Saharan Africa including Madagascar (*A. hebraeum*) and the Caribbean Islands (*A. variegatum*). Heartwater is infectious but not contagious and therefore, introduction of infected ticks or subclinically infected animals are the most likely mechanisms for introducing heartwater into a new geographical area. *E. ruminantium* has shown great genetic variability, with differences in virulence and pathogenicity; and the existence of immunogenic strains providing varying degrees of cross-immunity between one another.

Peracute and acute disease with high mortality rates are common in naïve susceptible animals. Clinical symptoms and mortality rates are variable dependant on the virulence of the organism and host factors (species, breed and age of animal). Zebu breeds are more resistant than European breeds, sheep are more susceptible than cattle and Angora goats are particularly susceptible. Calves less than 3 weeks of age and lambs less than 1 week of age are innately resistant to disease and this resistance is not related to maternal immunity.

**Sample Guide**

**Cytological examination**

A diagnosis of heartwater may be confirmed post mortally following the demonstration of colonies of *E. ruminantium* in capillary endothelial cells of the brain. Preferably the entire brain should be removed and tissue collected from the hippocampus for brain smear preparation, as the hippocampus is rich in capillary vessels.
Brain tissue collected is then macerated between two, clean glass slides until a smooth pulp is obtained. This brain pulp is then alternately spread thinly and more thickly, in a single cell layer by drawing one slide across the other. Brain smears are then air-dried, fixed with methanol and stained with Diff Quik® or Giemsa. If smears are being submitted to the laboratory for a pathologist’s opinion always submit 2 unstained, air-dried brain smears and one stained brain smear. Dried smears should be placed in a sealed cytology slide holder and transported to the laboratory at ambient temperature.

**More than one brain smear** must be prepared and examined, as the organisms may be scanty and difficult to find, particularly in peracute cases. However, they should always be present in the brain of an animal that has succumbed to heartwater. The colonies may still be visible for a period of two days after death provided the brain has been stored at tolerable room temperature (<22°C) and up to 34 days in a brain that has been stored in a refrigerator (4°C).

Factors which may affect the ability to detect *E. ruminantium* parasites in brain smears include:

- When the animal has been treated with effective heartwater drugs (48 – 60 hours prior to smear preparation).
- In peracute clinical cases.

**Heartwater Antibody detection – Serology**

**Indirect Fluorescent Antibody Tests / Enzyme-Linked Immunosorbent Assays (ELISA) / Western Blotting**

Blood specimens are collected into serum tubes with / without clot activator. Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after
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which they should ideally be centrifuged and the serum poured off. Serum or un-spun clotted blood should then be transported at 4°C to the laboratory.

Serological tests are complicated by cross-reactions with other *Ehrlichia* spp including *E. canis*, *E. chaffeensis* and an unidentified *Ehrlichia* sp infecting white-tailed deer in the southeastern USA. Serology is useful for monitoring experimental infections, checking the immune status of immunised animals, and for screening animals prior to importation. In cattle antibody levels against *E. ruminantium* can be very low in heartwater endemic areas, even in vaccinated cattle or animals continuously naturally challenged by infected ticks. Cattle may become seronegative 14 to 33 weeks after initial exposure.

**Heartwater Antigen Detection**

**Molecular DNA tests**

PCR assays are useful as confirmatory tests in individual animals as well as some other specific instances such as regulatory testing and research on the *Ehrlichia* genome. These molecular DNA tests are generally less suitable for large-scale testing and therefore currently unlikely to replace serological tests in epidemiological studies. Blood samples in EDTA tubes are the preferred sample. Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. (See Reference Ranges – Section 3) Specimens are stored and transported at 4°C to the laboratory.  
*Note*: it is advisable to contact the laboratory prior to submitting samples for PCR to establish whether this technique is suited to the investigation being performed.

**Culture**

*Ehrlichia ruminantium* may be isolated from the blood of an infected host by cultivation on ruminant endothelial cells and the presence of characteristic morulae may be confirmed by immunofluorescence or immunoperoxidase techniques using a specific antiserum. Blood samples in EDTA tubes are required for this assay. Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. (See Reference Ranges – Section 3). Specimens are stored and transported at 4°C to the laboratory.
Immunohistochemistry (IHC) and Histopathology

IHC in conjunction with histopathology enables identification of *Ehrlichia ruminantium* antigen within histological lesions thereby confirming a diagnosis. A full set organ samples (brain, lung, heart, liver, spleen, kidneys, lymph nodes, gastrointestinal sections and muscle) are collected in 10% buffered formalin. The brain is the organ of choice for IHC staining.

Further Reading

Coxiella burnetti (Q-Fever)

Introduction

Q-fever is caused by *Coxiella burnetti*, a Gram-negative organism, currently classified in the genus *Coxiella*, family *Rickettsiaceae* and order *Rickettsiales*. *Coxiella burnetti* is widespread in domestic and sylvatic mammals, birds, fish and arthropods as well as causing Q-fever in man. In ruminants (sheep, cattle and goats) most infections are asymptomatic, although sporadic or outbreaks of abortions, may be seen occasionally in these species. Abortion in cattle is related to placental oedema, placental necrosis and foetal bronchopneumonia. In cattle retained placentas, metritis and infertility may also be seen. Ruminants appear to be the major reservoir of the organism. It is found in most species of ticks where it can be passed transovarially or trans-stadially. Transmission routes include direct contact with contaminated body tissues and fluids, aerosolisation and vector borne transmission. Small particle aerosol is probably the most important route. Transmission to man is through inhalation or the handling of contaminated tissues, especially placentas, but not tick bites. Humans usually develop a self-limiting febrile response but severe life threatening infection (pneumonia, hepatitis and chronic endocarditis) occurs in some individuals.

Sample Guide

Coxiella Antibody Detection – Serology

Enzyme-linked immunosorbent assay (ELISA) / immunofluoresence assays (IFA) / complement fixation tests (CFT)

Blood samples are collected into serum tubes with / without clot activator. Following collection samples are allowed to stand for 20 minutes at room temperature to enable good clot formation. Blood specimens are then preferably centrifuged and the serum poured off. Centrifuged serum or clotted whole blood is then transported at 4°C to the laboratory.

- ELISA has a high sensitivity, good specificity, is easy to perform and currently the method of choice.
- The CFT is specific but less sensitive, detects seroconversion later than ELISA or IFA and antibodies may persist longer.
Coxiella Antigen Detection

Polymerase chain reaction

- Milk, vaginal swabs, placental tissues, faecal samples and bulk milk tank samples are suitable for PCR assays.
- This assay has also been applied to environmental samples (dust samples) to try assess risk of aerosol exposure.
- Take all the necessary precautions when handling clinical samples as the samples are potentially highly infectious with a zoonotic risk.
- Wear protective clothing (overalls and gum boots), protective eye-ware, surgical mask, surgical gloves and post mortem gloves.
- Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996 and transported at 4°C to the laboratory.

Coxiella Isolation

- From live animals - milk, colostrum, genital tissues / swabs, placental tissues and faecal samples are preferred.
- In aborted foetuses - liver, lung and the stomach content are required.
- Take all the necessary precautions when handling clinical samples as these samples are potentially highly infectious with a zoonotic risk.
- Wear protective clothing (overalls and gum boots), protective eye-ware, surgical mask, surgical gloves and post mortem gloves.
• Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996 and transported at 4°C to the laboratory.

**Histopathology and Immunohistochemistry (IHC)**

• Tissue samples are collected into 10% buffered formalin.
• In aborted foetuses, placentitis is an important and consistent finding and therefore, placenta must always be included.
• Using IHC staining in conjunction with histopathology enables demonstration of *Coxiella burnetti* antigen within histological lesions.
• *Coxiella burnetti* are also acid-fast and therefore readily demonstrated in placental smears and histological sections with acid-fast stains (Ziehl-Nielson, Stamps).

**Further reading**

Leptospirosis

Introduction

Leptospirosis is an important spirochaetal infection of animals and humans. It is particularly important as a cause of abortion and infertility in livestock (cattle, pigs), but also causes losses through acute disease (septicaemia, hepatitis, interstitial nephritis, meningitis, haemolytic crisis, mastitis, periodic ophthalmitis in horses) in various animal species. *Leptospira interrogans* contains 19 sero-groups with 180 serovars. There are 2 broad categories namely host-adapted (infected animals become maintenance or reservoir hosts) and non-host adapted (causes accidental or incidental disease in susceptible animals). Infection in incidental / accidental hosts is severe followed by little urinary shedding, while infection in maintenance hosts is subtle with development of chronic renal carriers and urine shedding. Each serovar is adapted to and causes disease in its maintenance host, while some also induce disease in other incidental hosts. The natural reservoir of pathogenic leptospira is the proximal convoluted tubules of the kidney of maintenance hosts. Far all the non *hardjo-bovis* leptospiras cattle are incidental hosts and the maintenance hosts are wildlife (*L. grippotyphosa*, *L. icterohaemorrhagiae*), dogs (*L. canicola*) or swine (*L. pomona*).

Sample Guide

**Leptospira Serum Antibody Detection**

**Microscopic Agglutination Test (MAT)**

These assays demonstrate the presence of antibodies against various *Leptospira* serovars. Serum samples collected in a red (without clot activator) or yellow (with clot activator) stopper tube are required. Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should be centrifuged and the serum poured off.

- The current "gold standard" diagnostic test for leptospirosis is the *Leptospira* Microscopic Agglutination Test (MAT) performed during the acute stage of disease; a second (convalescent) serum sample should be obtained within 3 to 4 weeks.
- This assay is serovar specific and is run against a panel of different serovars important in South Africa.
- Antibodies are first detected within 7 to 10 days post-infection. In unvaccinated animals titres may initially be low, 1:100 to 1:200, but may rise in the convalescent sample to 1:800 to 1:1600 or higher if the homologous *Leptospira* serovar is used as antigen in the panel.
• In vaccinated animals, low-level acute titres (>1:400) are often found, but they depend on when the animal was last vaccinated.
• Responses to infection in previously vaccinated dogs generally result in anamnestic responses only to the homologus serovars.
• Generally, a four-fold rise in antibody titre to a *Leptospira* serovar is considered significant.
• When titres to a particular serovar reach high levels, e.g. 1:3200 to 1:6400, it is not unusual to see elevated titres to other serovars, which is likely due to cross-reactions.
• For accurate comparisons, all serum samples within the specimen group should be tested at the same time.
• Antimicrobial treatment adversely affects the development of antibody titres. Therefore, the first serum samples should be obtained before antibiotic treatment has begun.

**Leptospira Antigen Detection**

**Polymerase chain reaction (PCR)**

• PCR tests are rapid and allow for genus and serovar specific detection.
• Various clinical specimens including body fluids (urine, milk, cerebrospinal, thoracic and peritoneal fluids, semen, vaginal discharges) blood (EDTA) plus post mortal tissues are suitable.
• Another advantage is that these PCR assays can detect bacteria at very low levels, which has application for identification of carrier animals.
• Can be prone to a low specificity (false positive results).
• Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996 and transported at 4°C to the laboratory.
Leptospira culture

From **live animals**: urine, blood (EDTA), milk, cerebrospinal, thoracic and peritoneal fluids, semen, vaginal discharges are collected into sterile containers.
From **dead animals**: kidney, liver, lung, brain, adrenal gland and aqueous humor are the tissues of choice.
From **aborted foetuses**: kidney, liver, thoracic fluid, abomasal fluid, aqueous humor and placenta are preferred.

- Isolation of these bacteria is demanding and time-consuming (may be 10 days to 26 weeks depending on serovar).
- Transit time between sampling and setting up culture is crucial and limited to 3 hours. The use of specific transport media, liquid culture media or direct inoculation of samples on to culture media may increase the chances of success.
- Kidney is the sample of choice for identification of chronic carriers.
- The use of diuretics (furesomide) may also aid in a positive urine culture in live animals.
- A single negative culture does not guarantee a true negative status and three negative cultures over a period of three weeks are advised before considering an animal as negative.
- Determining the serovar of any isolate is important in disease control.
- Serovar determination is usually achieved by running MAT serovar specific serology in conjunction with isolation.
- Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996 and transported at 4°C to the laboratory.
Dark-field microscopy of urine and blood samples may be attempted but is generally of little practical use. Often the dark-field examination of urine is inconclusive. It is difficult to read, and requires fresh urine in order to observe intact leptospiral cells. **Fluorescent antibody (FA) testing** of centrifuged urine sediments is a more definitive test and leptospires do not need to be viable.

- Urine should be submitted to the laboratory on ice by overnight courier to ensure that the specimen is of good quality.
- It is essential to correlate FA results with the clinical and vaccine history since leptospires are commonly seen in the urine of seronegative carrier animals and in animals with clinical disease as early as 1 week post-infection.
- FA examination - FA should be done on all tissues submitted for postmortem examination, especially important are kidney and liver specimens.

**Histopathology and Immunohistochemistry (IHC)**

- Organisms can be demonstrated in formalin-fixed tissue sections by immunohistochemical stains utilising monoclonal antibodies.
Livestock Diseases

- Demonstrating the bacteria within histological lesions gives the pathologist confidence in confirming the diagnosis. Efficacy of IHC staining is dependent on the number of organisms present within the tissue.
- Do not identify the infecting serovar and results must be interpreted in conjunction with serological MAT results.

Further reading


NOTES
Mycoplasma bovis

Introduction

Mycoplasmas are often associated with respiratory diseases in cattle and are thought to exacerbate diseases associated with bacteria and viruses. *Mycoplasma mycoides* subsp. *mycoides* (small colony type - MmmSC) and *Mycoplasma bovis* are considered the most important mycoplasmas of cattle. MmmSC is the cause of contagious bovine pleuropneumonia (see Disease Diagnostics – Controlled and Notifiable Diseases). *Mycoplasma bovis* has been associated with subacute or chronic suppurative bronchopneumonia plus fibrinosuppurative and necrotizing arthritis and tenosynovitis. Other reported disease manifestations in cattle include fibrinopurulent or caseous otitis media, subcutaneous decubital abscesses, mastitis, meningitis; infectious keratoconjunctivitis and genital tract infection. *Mycoplasma bovis* is believed to frequently occur together with other viral and bacterial agents as part of the bovine respiratory disease complex and pneumonia arthritis syndromes.

Sample Guide

**Mycoplasma Isolation**

- Pathology is characterised by a cranioventral bronchopneumonia with multiple foci of caseous necrosis and concurrent fibrinosuppurative bronchopneumonia. All animals with arthritis have concurrent pulmonary lesions. Image above-left demonstrates the classic pulmonary lesions with multiple small foci of caseous necrosis in the cranial lobes. Image above-right is of a fibrinous arthritis.
- The distinctive features are visible on cut surface of these nodules which appear macroscopically as bulging, white-yellow, dry and friable and containing caseous material surrounded by fibrous tissue. In contrast *Mannheimia haemolytica* lesions are described as irregularly shaped, red-tan, not friable, sunken and delineated by a white rim from the adjacent tissues.
- Fresh lung, joint and genital tissues collected into sterile containers or swabs with transport medium, may be submitted for isolation of the organism.
- Isolation procedures are complicated and time consuming and therefore, failure to isolate any mycoplasmas is in no way exclusive of their potential role.
• Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. (See Reference Ranges – Section 3). Specimens are stored and transported at 4°C to the laboratory.

**Molecular DNA screening**

![Image of molecular DNA screening](image)

• Fresh lung, joint and genital tissues, nasal swabs / flushes, joint aspirates or genital discharges, may be submitted for the detection of *Mycoplasma bovis* nucleic acids.
• Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. (See Reference Ranges – Section 3). Specimens are stored and transported at 4°C to the laboratory.

**Histopathology and Immunohistochemistry (IHC)**

![Image of histopathology and IHC](image)

• Target tissues for histopathology include lung, genital tract and synovial membranes.
• Use of IHC stains in conjunction with histopathology enables demonstration of intralesional mycoplasmas.

**Further reading:**

Porcine Circovirus-2 (PCV-2)

Introduction

Porcine circovirus 2 (PCV 2) has been conclusively linked with PCV-2 associated systemic infection, previously termed post-weaning multisystemic wasting syndrome (PMWS). PCV-2 is also associated with various other disease syndromes in pigs (Porcine circovirus associated diseases - PCVAD) including porcine dermatitis and nephropathy syndrome (PDNS), PCV-2 associated reproductive failure, PCV-2 associated respiratory disease, PCV-2 associated enteritis, congenital tremor, necrotising lymphadenitis and renal tubular necrosis and interstitial haemorrhage (turkey-egg kidney). A previously un-described neurovascular disorder characterised by acute haemorrhage and oedema of cerebella meninges and parenchyma has now been described.

These Porcine circovirus associated diseases (PCVAD) may manifest as a severe herd problem accelerated by complicating bacterial or viral infections, or it may only be a sporadic individual animal problem. Transmission is thought to occur through direct contact via oronasal, faecal and urinary routes. However, vertical transmission has been demonstrated in individual sows in the field and PCV-2 has been isolated from semen. Lymphoid depletion and lymphopaenia in peripheral blood is a consistent feature of animals developing PCVAD. Although PCV-2 is essential for the development of PCVAD, co-factors of the virus namely host (breed of pig), co-infection with other pathogens [porcine parvovirus (PPV), porcine resproductive respiratory syndrome virus (PRRSV), Mycoplasma hyopneumoniae] and immune modulation (suppression of cell-mediated immunity) all influence the outcome of PCV-2 infection. Two different genotypes namely porcine circovirus-2a and PCV-2b are now distinguished.

Sample Guide

PCV-2 Antigen Detection

Histopathology and Immunohistochemistry (IHC)

IHC stains for PCV-2 viral antigen on formalin fixed, paraffin-embedded tissue sections, remains the gold standard for the diagnosis of porcine circovirus associated diseases.

- Demonstration of PCV-2 viral antigen within the typical lymphohistiocytic infiltrates of various organs enables confirmation of the diagnosis.
• Tissues stored in 10% buffered formalin for 6 months showed no loss of staining intensity.
• Target tissues in piglets and adults include tonsils, lymph nodes, spleen, intestine with Peyer’s patches, lung, liver and brain.
• Target tissue in aborted foetuses is the myocardium, with necrotising or fibrosing myocarditis being a hallmark lesion of in utero PCV-2 infection.

Polymerase Chain Reaction (PCR)

• Variants of the regular PCR including multiplex, nested and real-time PCR’s are available for detection of PCV-2 genetic material in clinical specimens.
• The primary application of the PCV-2 PCR is for the detection of PCV-2 nucleic acids in semen from breeding boars or semen destined for AI, to maintain negative herd status.
• The vast majority of the pig population (healthy and diseased) are infected with PCV-2 at some stage of their life and therefore many healthy pigs are positive for PCV-2.
• These PCR assays cannot replace clinical assessment of pigs or histological evaluation of tissues combined with IHC staining.

Virus isolation (VI)
Tissue homogenates and body fluids are the preferred samples, although VI is rarely employed for PCV-2 diagnostics. The primary applications of VI today include determining if PCV-2 shed in semen is infectious and to recover PCV-2 for vaccine production.

Electron Microscopy (EM)
A high level of virus (>10^5 virus particles) is required in tissue to be detectable by EM and therefore this procedure is rarely employed due to this low sensitivity.
PCV-2 Antibody Detection – Serology

Indirect Fluorescent Antibody (IFA) / ELISA / Serum Virus Neutralisation (VN)

Serological studies in pigs indicate that PCV-2 infection of swine is widespread worldwide. Therefore, the prime application of PCV-2 serology is to identify PCV-2 naïve populations which allow for the implementation of management strategies to reduce the risk of subsequent PCV-2 exposure in these populations.

Serum samples are collected into red (without clot activator) or yellow (with clot activator) stopper tubes. Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should be centrifuged and the serum poured off.

Further Reading

Porcine Reproductive and Respiratory Syndrome Virus
(PRRSV)

Introduction

Porcine Reproductive Respiratory Syndrome (PRRS) is a highly contagious disease of swine and an important economic problem for swine producers worldwide. The causative agent is an Arterivirus, with considerable genetic variation amongst isolates, which manifests as reproductive failure and respiratory disease in young pigs.

Reproductive failure is characterized by outbreaks of late gestation abortions, stillbirths, mummified foetuses, weak neonates and high rate of return to oestrus. Problems tend to persist and recur for many months.

Respiratory disease is most commonly observed in younger pigs with fever, anorexia, dyspnoea and coughing and weight loss with high mortality rate in young pigs. Mortality is low in older pigs and breeding stock.

Pigs usually become infected in the nursery following introduction of infected pigs into the herd. Use of infected semen is also an important route for virus introduction into previously un-infected herds. Once introduced into a herd the virus circulates through aerosol exposure, faeces, body discharges, direct contact and vertically. Persistent infection for several months is common and these PI pigs drive the ongoing endemic infection.

Sample Guide

PRRSV Antibody Detection – Serology

ELISA / Indirect Fluorescent Antibody (IFA) / Virus Neutralisation (VN)

These assays demonstrate the presence of antibodies against PRRSV. Serum samples collected in a red (without clot activator) or yellow (with clot activator) stopper tubes are required. Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should be centrifuged and the serum poured off. An acute serum sample collected as early as possible during the outbreak and a convalescent sample collected two to three weeks after the outbreak are the preferred samples. Acute centrifuged and separated sera are stored at -20°C until the convalescent sera arrive. Both acute and convalescent sera are tested concurrently. All serum samples are transported at 4°C to the laboratory.
Livestock Diseases

- ELISA is considered the “gold standard” serological procedure for the identification of PRRSV antibodies and is a procedure with high sensitivity and specificity.
- Demonstration of seroconversion (>4 fold titre increase) on acute and convalescent sera is the most definitive way of identifying infection serologically.
- Serology can not be used for the diagnosis of PRRSV in vaccinated or previously infected herds, as serology cannot distinguish between the antibodies arising from initial infection, re-infection or vaccination.
- Single serum samples are of little diagnostic use.
- Positive antibody titres in suckling and nursery pigs may be due to maternal immunity, which can persist until 3-5 weeks of age.
- Persistently infected pigs can become seronegative complicating interpretation of results.
- False positive ELISA titres are reported in 0.5-2% of samples.

**PRRSV Antigen Detection**

**Polymerase Chain Reaction (PCR)**

- PCR analyses for PRRSV are procedures with high sensitivity and specificity.
- Target tissues in live animals for sampling include tonsil and oropharyngeal scrapings plus lung lavages.
- The PCR has had limited success with thoracic fluid and tissues from stillborn foetuses.
- PCR is capable of detecting PRRSV nucleic acids for up to 250 days post infection.
- The PCR is used as both a herd screening test as well as a confirmatory test for suspicious serological results.
- All samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996 and transported at 4°C to the laboratory. (See Reference Ranges – Section 3).

**Immunohistochemistry (IHC)**
Livestock Diseases

- IHC enables detection of viral antigen within tissues fixed in 10% buffered formalin.
- Utilizing un-buffered formalin can severely compromise the accuracy of IHC stain interpretation.
- Target tissues include lung, heart, kidney, lymph nodes, spleen, thymus and tonsil.
- Tissue sections are stained with PRRSV monoclonal antibodies and counter stained with haematoxylin, to detect viral antigen in the cytoplasm of infected cells.

Virus Isolation

**Foetuses:** Target tissues include lung, spleen, heart, kidney, thymus, umbilical cord and umbilical cord blood.

**Piglets and Adults:** Live - serum, lung lavages, tonsil and oropharyngeal scrapings.

- Dead - lung, spleen, lymph nodes, heart, kidney, tonsil and thymus.

- All samples for virus isolation are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996 and transported at 4°C to the laboratory. (See Reference Ranges – Section 3).
- Ensure fresh tissues are packed in separate containers to the formalin-fixed tissues.

Further Reading

Porcine Parvovirus (PPV)

Introduction

Porcine parvovirus (PPV) still remains an important cause of embryonic and foetal deaths in swine worldwide, usually without any visible symptoms in sows. Infection usually follows oronasal exposure of naïve sows to PPV during the first half of gestation (60-70 days). Transplacental infection at this stage results in embryo/foetal infection with embryonic / foetal death. Foetuses older than 70 days are usually immunocompetent and able to withstand PPV infection. In addition to its reproductive effects PPV potentiates the effects of porcine circovirus-2 (PCV-2) in the clinical effects of post weaning multisystemic wasting syndrome.

Sample Guide

PPV Antigen Detection

Direct Fluorescent Antibody Assay (FAT)

- The identification of PPV antigen in tissues by immunofluorescence is a reliable and sensitive diagnostic procedure.
- Mummified foetuses, with crown-rump length < 16cm, or lung tissue from such foetuses are considered the samples of choice for PPV demonstration.
- Larger foetuses (>16cm), are usually older than 70 days and these foetuses usually contain antibody which interferes with the FAT test. Therefore, these larger foetuses, stillborn pigs and neonatal pigs are not considered suitable for PPV FAT assays.
- All samples for FAT testing are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996 and transported at 4°C to the laboratory. (See Reference Ranges – Section 3).
Polymerase Chain Reaction (PCR)

- The PCR is a sensitive and specific assay for PPV nucleic acids and is not affected by the presence of antibody. Therefore, the assay can be run on foetal, stillborn and neonatal tissues.
- Target tissues include lung, liver and kidney.
- Individually packed tissues, tissue homogenates and PCR swabs are suitable.
- All samples for FAT testing are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996 and transported at 4°C to the laboratory. (See Reference Ranges – Section 3).

PPV Antibody Detection – Serology

Haemagglutination Inhibition (HI) / Virus Neutralisation / Immunodiffusion / ELISA

These assays demonstrate the presence of antibodies against PPV. Serum samples are collected in a red (without clot activator) or yellow (with clot activator) stopper tubes. Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should be centrifuged and the serum poured off. An acute serum sample collected as early as possible during the outbreak and a convalescent sample collected two to three weeks after the outbreak are the preferred samples. Acute centrifuged and separated sera are stored at -20°C until the convalescent sera arrive. Both acute and convalescent sera are tested concurrently. All serum samples are transported at 4°C to the laboratory.

- Serological procedures are usually only employed as diagnostic procedures if mummified foetal tissue is unavailable.
Livestock Diseases

- The most valuable serological result is a seronegative response in sows or gilts suffering reproductive failure, as this would confidently exclude PPV infection.
- Demonstration of PPV antibodies in foetal thoracic fluid or heart blood, foetal tissue suspensions or in neonatal pigs that have not ingested colostrums, indicate *in utero* infection.
- Demonstration of seroconversion in acute and convalescent sera of sows and gilts suggest infection. However, collection of acute phase sera is practically impossible as the acute phase of infection has usually passed before infection is suspected.

**Virus isolation** is rarely employed as a diagnostic procedure due to poor sensitivity and specificity, especially on autolysed foetal tissues. The procedure is time consuming, labour intensive and cell cultures vary in their ability to detect virus.

**Further Reading**