Canine Parvovirus Enteritis (CPV-2)

Introduction

Canine parvovirus-2 (CPV-2) belongs to the feline parvovirus subgroup together with feline panleukopaenia virus (FPLV), mink enteritis virus, raccoon parvovirus and blue fox parvovirus. The exact origin of CPV-2 is still unclear but derivation from FPLV virus or FPLV-like virus of wild carnivores is suspected. CPV-2 is able to replicate in canine and feline cells and in dogs while FPLV replication only occurs in cats and feline or mink cell cultures.

Sample Guide

**CPV-2 - Serum antibody testing**

Haemagglutination inhibition / Virus neutralisation / 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.

Demonstrating rising antibodies in acute and convalescent serum samples (> 4 fold increase in antibody titre) are suggestive of CPV-2 infection. A high HI titre (> 1:320) in a single serum sample collected after an animal has been clinically sick for 3 or more days, is also considered indicative of CPV-2 infection. HI titres can persist for up to 3 weeks after the onset of diarrhoea.
CPV-2 - Viral antigen testing

Faecal antigen ELISA

This assay is rapid, sensitive and specific in identifying CPV-2 antigen and thereby infection in faecal material. The main drawback of this assay is that faecal shedding is short lived and within a few days virus excretion has often dropped to below detectable levels. In addition masking of the viral epitopes by secretory antibody and IgG leaking into the lumen in the later stages of infection, interfere with the test. Therefore, a negative faecal antigen ELISA assay can not exclude CPV-2 infection.

*Caution* - false positive results are possible 5-12 days following the use of MLV CPV-2 vaccines. In general vaccine responses are weak compared to natural infection.

Polymerase Chain Reaction (PCR)

Nucleic acid detection (PCR) - detects CPV-2 antigen in faeces and tongue epithelium as well as post mortal tissues (ileum, mesenteric lymph node, heart). Samples must be collected under strict aseptic conditions, in sterile containers and transported cooled (4°C) to the laboratory. This is a very specific assay with a positive result showing high correlation to a diagnosis of CPV-2 infection. In addition this assay distinguishes between field virus and vaccine virus.
Immunohistochemistry (IHC)

IHC detects CPV-2 antigen in formalin fixed tissues. This is a very sensitive procedure capable of demonstrating viral antigen within infected tissues (jejunum, ileum, mesenteric lymph node, spleen, heart). IHC utilizes a counter stain of haematoxylin enabling correlation of viral antigen distribution to the pathology. The tongue epithelium has proven a useful diagnostic sample for histopathology, IHC and PCR, especially in cases with severe necrotizing lesions in the intestine or in post mortal cases with autolytic change.

Virus isolation

CPV-2 virus is best isolated from the following tissues jejunum, ileum, mesenteric lymph nodes and other lymphoid organs, as well as faeces. Electron microscopic scan of faeces and tissues can also be used to demonstrate typical parvoviral particles. Samples must be collected under strict aseptic conditions, in sterile containers and transported cooled (4°C) to the laboratory.

*Caution* - post mortal decomposition reduces the chances of isolating virus.
Further Reading

Canine Diseases

Canine Coronavirus (CCV)

Introduction

Canine coronavirus (CCV) is a member of the virus family Coronaviridae and different members of the Coronaviridae infect various mammalian species. Infection appears to be widespread in dogs although clinical disease appears to be infrequent. Neonatal pups are more severely affected than pups of weaning age or adults. There are reports of puppy fatalities characterised by segmental necrotic enteritis of the small intestine.

Sample Guide

CCV - Serum Antibody Testing

Virus Neutralisation / ELISA

These serological assays detect IgM and IgG antibodies in serum. 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 4ºC to the laboratory.

Positive CCV titres and demonstration of serum conversion merely indicates exposure and many serologically positive animals do not show any clinical disease.

CCV – Antigen Detection

Polymerase Chain Reaction (PCR)

- The CCV PCR assay has high sensitivity for the detection of CCV genetic material in faecal samples.
- Faecal specimens should be collected into sterile containers / sampled with PCR swabs and transported at 4ºC to the laboratory.
Faecal Immunochromatographic Assay

- This is a rapid, qualitative immunochromatographic assay for canine coronavirus, which is performed on faecal material or faecal swabs.
- Fresh faecal material is required and therefore, specimens should be submitted to the laboratory immediately after collection.
- Samples must be transported cooled at 4°C to the laboratory.

Electron Microscopy (EM)

- CCV can be detected in faeces on EM examination.
- Faecal viral loads need to be fairly high for detection and therefore false-negative findings are a problem with this procedure.
- Samples must be transported cooled at 4°C to the laboratory.
- Fresh faecal material is required and therefore, specimens should be submitted to the laboratory immediately after collection.
Immunohistochemistry (IHC)

- IHC staining is performed in conjunction with histopathological examination and allows for the demonstration of viral antigen within histological lesions.
- These procedures have best applications on tissue specimens collected into 10% buffered formalin at post mortem.
- As tissues are formalin fixed samples can be transported at ambient temperatures.
- Formalin-fixation also allows for storage of samples for a period of time prior to submission to the laboratory.

Further Reading


NOTES
Canine Distemper (CDV)

Introduction

Canine distemper virus (CDV) is a highly contagious disease of dogs and other carnivores and is still common despite several decades of vaccination. CDV has a wide host range including multiple species in the families Canidae, Aluridae (pandas), Hyaenidae, Mustelidae, Procyonidae (raccoon), Ursidae (bears), Felidae (lions, tigers), Viverridae and Edentata (ant bears). Similar disease syndromes occur in marine mammals caused by distinct phocine, dolphin, porpoise, cetacean and pilot whale morbilliviruses. Outbreaks in seals have most commonly been associated with phocine morbillivirus, CDV and a newly characterised morbillivirus.

Sample Guide

CDV – Antibody testing

ELISA / Virus Neutralisation / Indirect Fluorescent Antibody

These serological assays detect IgM and IgG antibodies in serum and or cerebrospinal fluid. 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.

IgM titres in serum rise rapidly post infection (6-8 days) and rising titres can be measured in dogs surviving the acute phase of the disease. IgM titres usually disappear within 3 months post infection. High IgM titres reliably predict acute distemper but are less sensitive with chronic progressive inflammatory encephalitis. Transient IgM increases are also seen following first immunisation with CDV vaccine.

IgG titres in serum are very difficult to interpret on their own, with high titres being associated with past or present CDV infection or past CDV vaccination. When used in conjunction with IgM titres they enable more refined interpretation of the IgG titre. A high IgM titre (> 1:40) on a single serum sample and / or a four fold increase in IgG titres with acute and convalescent sera, are indicative of virus exposure.

*Caution* - CDV is an immunosuppressive virus and so may result in a low incidence of post infection seroconversion. Distinction between vaccine induced and field virus antibodies is not possible.
Anti-CDV IgG antibodies in cerebrospinal fluid are highly significant as they are locally produced and therefore their presence indicates evidence of distemper encephalitis.

*Caution* - blood contamination during collection or impairment of the blood-brain barrier can lead to false positive results.

**CDV - Viral antigen testing**

**Antigen capture ELISA**

Detects CDV antigen in serum, plasma (EDTA), ocular discharge, nasal discharge, saliva or urine. Samples should be collected in a sterile, sealed container and transported to the laboratory cooled (4°C).

*Caution* - testing dogs within 3 weeks of vaccination with a modified-live CDV vaccine may produce false positive results.

**Polymerase Chain Reaction (PCR)**

Nucleic acid detection (PCR) - detects CDV RNA inuffy coat (EDTA), whole blood (serum with polymer gel clot-activator) or CSF. Samples must be collected under strict aseptic conditions, in sterile containers and transported cooled (4°C) to the laboratory. This is a very specific assay with a positive result showing high correlation to a diagnosis of CDV infection. In addition this assay can distinguish between field virus and vaccine virus.

*Caution* - Sample handling is critical as viral RNA is very labile, therefore improper handling can result in false negative results. **Do not** pack together with any formalin-fixed tissues as formalin fumes damage viral RNA.
Direct Fluorescent Antibody Test

Immunofluorescence detects CDV antigen in cytological smears (conjunctival, corneal, tonsillar, genital and respiratory epithelium) by employing fluorescein-conjugated CDV antibodies. Cytobrush techniques improve recovery from conjunctival and corneal sites. Cytological slides should be pre-cleaned in alcohol before smears are made. Smears should then be thoroughly air-dried and fixed in acetone for 5 minutes and then transported to the laboratory (if acetone unavailable send air-dried smears).

*Caution* - smears must be transported in a sealed plastic sample envelope and **not** included in the same sample envelope with any formalin-fixed tissues as formalin fumes damage viral antigen integrity.

Immunohistochemistry (IHC)

IHC detects CDV antigen in formalin fixed tissues. This is a very sensitive procedure capable of demonstrating viral antigen and its distribution within infected tissues. IHC utilises a counter stain of haematoxylin enabling correlation of the site of viral antigen accumulation to the pathology.
Virus isolation enables detection of virulent CDV in buffy coat specimens or body tissues. Buffy coat specimens (EDTA blood samples) collected during the early phase of infection provide the best chance of isolating virus. Tissue samples (lymph node, thymus, spleen, lung, brain) collected at post mortem examination are the preferred tissue for isolation. In dogs with chronic disease or vaccine-induced encephalitis, virus isolation is usually unsuccessful.

*Caution* - buffy coat and tissue samples must be transported in a sealed plastic sample envelope and not included in the same package with any formalin-fixed tissues as formalin fumes may destroy any virus.

Further reading

Canine Adenovirus - 1 (CAV-1) - Infectious Canine Hepatitis

Introduction

Infectious canine hepatitis (ICH) is a systemic viral disease of young canines, bears and otters caused by canine adenovirus-1 (CAV-1) and characterised by unapparent to rapidly fatal disease. CAV-1 replication in endothelial cells and hepatocytes produces an acute, necrohaemorrhagic hepatitis typical of this condition. Pathology is most severe in young animals. Virus can be shed in the urine for up to 6-9 months following infection.

Sample Guide

ICH - Serum antibody testing

Indirect haemagglutination / ELISA / Complement fixation / Immunodiffusion

These assays detect IgM and IgG antibodies in serum. 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 4ºC to the laboratory.

Acute and convalescent samples (2 weeks apart) are required to best interpret the results as distinguishing a serological response following exposure to field virus from a response following vaccination with a MLV vaccine, can be problematical. A four-fold or greater increase in antibody titre levels in the convalescent sample suggest field virus exposure.

ICH - Viral antigen testing

Polymerase Chain Reaction (PCR)

Nucleic acid testing (PCR) is designed for rapid detection of CAV-1 viral antigen in clinical specimens (EDTA blood, urine, corneal scrapings), but can also be employed on necropsy tissue samples. Samples must be collected under strict aseptic conditions, in sterile containers and transported cooled (4ºC) to the laboratory.
Direct Fluorescent Antibody Test

Immunofluorescence detects CAV-1 viral antigen in various body tissues (liver, kidney, lymph nodes, cornea) i.e. mostly post mortal samples. Tissues should be fresh and transported on ice at 4°C.

*Caution* - smears / tissues must be transported in a sealed plastic sample envelope and not included in the same sample envelope with any formalin-fixed tissues as formalin fumes damage viral antigen integrity.

**Immunohistochemistry (IHC)**

IHC detects CAV-1 antigen in formalin fixed tissues from necropsies and employing the haematoxylin counter stain, enables visualisation of virus in cells and inclusion bodies, allowing correlation to the histopathology findings.

**Virus isolation**

CAV-1 virus can be readily isolated from any body tissue or secretion during the viraemic phase. The sample of choice that provides the greatest chance of isolation is the kidney, as this is the organ of persistent virus localisation with virus being isolated for up to 9 months.
following infection. Viral isolation from the liver can be difficult due the hepatic enzymes interfering with CAV-1 nucleic acid replication. Isolation from aqueous humour is usually only successful in the very early stages of infection before antibody infiltration and immune complex deposition. All body fluids for isolation should be collected into heparin tubes.

Samples for virus isolation should be packed on ice and transported at 4ºC to the laboratory.

**Further Reading**

Canine Adenovirus-2 (CAV-2)

Introduction

CAV-2 is implicated in respiratory disease and possibly enteritis, but is not associated with systemic infection. In infectious tracheobronchitis cases following oronasal CAV-2 infection, respiratory signs are consistent with damage to bronchial epithelial cells. However, the clinical infectious tracheobronchitis syndrome is usually only observed when CAV-2 infection is complicated by other viral or bacterial pathogens. These commonly include Canine Parainfluenza 2 (CPI2V), Canine Distemper Virus (CDV), Bordetella bronchiseptica, mycoplasmas and Streptococcus equi subsp. zooepidemicus.

Sample Guide

CAV-2 Antigen Detection

Polymerase Chain Reaction (PCR) / Virus isolation

As CAV-2 is restricted to the respiratory system and is not systemic, nasal swabs are the samples of choice. PCR nasal swabs with viral transport medium or swabs in PBS are the samples of choice. In cases of presumed adenovirus enteritis intestinal swabs or fresh faeces are submitted.

Following specimen collection 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.
In cases of **infectious tracheobronchitis** a battery of PCR assays are run including CAV-2, canine PIV, CDV, CHV, Mycoplasma spp. In addition nasal swabs / nasal flushes / tracheal washes are submitted for aerobic bacterial culture. Bacterial culture targets the primary bacterial agents *Bordetella bronchiseptica* (charcoal swabs preferred) and *Streptococcus equi* subsp. *zooepidemicus* (non-charcoal swabs preferred).

Serology has little clinical application in infectious tracheobronchitis.

**Further Reading**

Canine Herpesvirus (CHV)

Introduction

Canine herpesvirus (CHV) infection is restricted to Canines and infection has been documented in both domestic and wild dogs. The most common presentation is fatal disease in neonatal pups (1-4 weeks of age) due to low and poorly regulated body temperature. *In utero* infection can result in the death of the foetus (abortion) or pups shortly after birth. Infected dams develop a solid immunity and CHV related diseases are not observed in subsequent litters. Infection in adult dogs seems to be restricted to the upper respiratory tract, although CHV has been isolated from adult dogs with corneal ulceration and is also suspected in dogs with fertility disorders.

Sample Guide

**CHV - Serum antibody testing**

**Virus Neutralisation/ELISA/Haemagglutination Inhibition**

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.

Virus persistence and latent infections make interpretation of serological assays difficult. Acute and convalescent serum antibody testing enables for meaningful interpretation, but seropositive responses merely indicate exposure and not necessarily clinical disease.
CHV - Viral antigen testing

Virus isolation

Organ samples of choice for isolation from puppies dying of acute systemic infection are adrenal gland, lung, liver, kidney, spleen and lymph nodes. In older animals or animals that have recovered isolation is best achieved from the oral mucosa, upper respiratory tract and external genitalia. Two to 3 weeks following infection, isolation is usually unsuccessful. Electron microscopic scan of tissues can also be used to demonstrate typical herpesviral particles.

Polymerase Chain Reaction

Nucleic acid testing (PCR) is designed for rapid detection of CHV viral antigen in clinical specimens (EDTA blood, oral / tracheal swabs, swabs from external genitalia) or necropsy tissue samples. It is the assay of choice for the identification of latently infected animals. Samples should be transported on ice at 4°C. All tissues must be transported in a sealed plastic sample envelope and not included in the same sample envelope with any formalin-fixed tissues as formalin fumes damage viral antigen integrity.
Direct Fluorescent Antibody Test

Immunofluorescence detects CHV viral antigen in various body tissues, mostly post mortem samples. Tissues should be fresh and transported on ice.

*Caution* - smears / tissues must be transported in a sealed plastic sample envelope and not included in the same sample envelope with any formalin-fixed tissues as formalin fumes damage viral antigen integrity.

Immunohistochemistry (IHC)

Detects CHV antigen in formalin fixed tissues from necropsies and employing the haematoxylin counter stain, enables visualisation of virus in cells and inclusion bodies, allowing correlation of virus distribution to the histopathology findings.

Further Reading

Canine Babesiosis

Introduction

Canine babesiosis is a tick-transmitted, haemoprotezoal disease of domestic dogs (*Canis familiaris*) and African wild dogs (*Lycaon pictus*). Over 100 species of Babesia have been identified but only two, *Babesia canis* and *B. gibsoni*, are known to infect dogs. Genetic sequencing has confirmed 3 distinct subspecies of *B. canis* namely *B. canis canis*, *B. canis rossi* and *B. canis vogeli*. In addition at least 3 subtypes of small *Babesia* affecting dogs are thought to occur including *B. gibsoni* (Asian type), *B. conradae* (*B. gibsoni* - California type) and a *B. microti*-like organism.

South African canine babesiosis is a heterogeneous complex of disease presentations caused by *Babesia canis rossi*, transmitted by *Haemophysalis leachi* ticks. This strain of babesia is very widespread in South Africa and notoriously the most virulent. The small *Babesiae* are not known to occur in South Africa. *B. canis vogeli* is transmitted by the *Rhipicephalus sanguineus* tick and associated with mild, uncomplicated and possibly even asymptomatic disease. *B. canis canis* results in clinical symptoms which are generally of severity mid-way on the scale between the other two sub-species.

The uncomplicated clinical form of the disease is characterized by anaemia, fever and splenomegaly and can be further subdivided into mild or moderate (PCV > 15%) or severe (PCV < 15%). The former cases are usually successfully treated as outpatient cases. Complicated babesiosis on the other hand presents as one or more organ dysfunction including cerebral, acute renal failure, hepatic dysfunction, myocardial disease, rhabdomyolysis, adult respiratory distress syndrome, pancreatitis, dermal necrosis, haemorrhagic diathesis and immune mediated haemolytic anaemia; and this form of the disease has a high mortality rate.

Sample Guide

**Blood smear examination**

- Thin peripheral blood smears are the preferred specimens. Peripheral capillary blood is believed to yield higher parasitaemias compared to central venous blood smears.
- Clinical symptoms, history of possible tick exposure, haematology and biochemistry results can be highly suggestive of babesiosis, and if organisms can be identified in blood smears the disease is confirmed.
Canine Diseases

- Parasitaemia can be very low particularly in early *B. canis rossi* cases and blood smear evaluation may be negative.
- High parasitaemias have been associated with *E. canis* co-infection.
- Smears made from bone marrow aspirates or from the microhaematocrit, tube just below the buffy coat, may be more sensitive in detecting parasites.
- If clinical signs are highly suggestive but smears are negative further diagnostic tests (namely PCR) are recommended.

**Babesia Antigen Detection**

**Molecular DNA Assays**

Molecular assays using PCR and reverse-line blotting (RLB) are specific and sensitive confirmatory assays for canine babesiosis. A minimum of 2ml of EDTA blood is the preferred sample although Babesia DNA can also be effectively extracted from blood smears (stained / unstained).

- PCR allows for identification of *Babesia* down to species level.
- Positive serology in the face of negative PCR probably indicates previous exposure, with clearance of the organism.
- Samples should be transported on ice at 4°C to the laboratory.

**Babesia Antibody Detection – Serology**

**Indirect Immuno-Fluorescent Antibody (IFA) / ELISA**

Serology is the most commonly used screening method. Following collection of blood (minimum of 0.5ml) into serum tubes with / without clot activator, 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.

Seroconversion is confirmed by detecting rising antibody titres over a 2-3 week period. Titres greater than 1:80 are considered positive, but this varies between laboratories.

- Antibody titres are lower in sub-clinically and recently infected dogs.
- A negative result in a case with a high clinical suspicion, should have a PCR test run and the serology should be repeated in 2-3 weeks.
- Accurate titre measurement is best achieved with IFA. Most ELISA methods are only semi-quantitative.
- Sero-positivity should not be used as a diagnostic method when clinical signs are not present – this may only indicate previous exposure.

Further Reading

NOTES
Canine Ehrlichiosis

Introduction

Canine ehrlichiosis or canine monocytotropic ehrlichiosis (CME) caused by *Ehrlichia canis* is widely distributed in tropical and subtropical regions around the globe. Other species of “Ehrlichia” that have been documented in dogs include *Ehrlichia chaffeensis*, *Neorickettsia risticii*, *Ehrlichia ewingii*, *Anaplasma phagocytophilum* and *Anaplasma platys*. It has been shown that these other “Ehrlichia” organisms can cause disease in canines, but currently only *A. platys* (canine recurrent thrombocytopenia) has been reported in South Africa.

Disease caused by *E. canis* typically occurs in 3 phases
- Acute phase characterised by fever, malaise, lymphadenopathy, splenomegaly, thrombocytopenia, leukopenia and non-regenerative anaemia.
- Subclinical phase that can last for a few months to years, during which the carrier state exists.
- Chronic phase in which severe clinical ehrlichiosis (pancytopaenia) occurs

Differences in strain pathogenicity, concurrent or co-infections with other protozoa (*Babesia, Bartonella, Anaplasma*), host immune status, or other, as yet-undiscovered factors may affect the spectrum and severity of the clinical and pathological features of *E. canis* infection in dogs. Bone marrow suppression and hypoplasia with an associated peripheral blood pancytopenia contributes to the terminal stage of chronic *E. canis* infections. Dogs may die from bacterial septicemia, severe haemorrhagic diathesis or both.

Sample Guide

**Smear Examination**

*E. canis* morulae can be identified in peripheral blood smears, splenic aspirates, lymph node aspirates and bone marrow aspirates from live animals or those collected at post mortem.

- Detecting morulae in leukocytes within blood smears is diagnostic but lacks sensitivity, as estimated parasitaemias in early infection are only 1% of circulating leukocytes.
- Sensitivity can be improved by using buffy-coat, splenic, lymph node or bone marrow aspirate smears.
- A negative smear evaluation cannot exclude *E. canis* infection.
**E. canis Antigen Detection**

**Polymerase Chain Reaction**

- *Ehrlichia canis* nucleic acid detection (PCR) is considered a sensitive procedure to detect acutely infected animals before seroconversion has occurred.
- A minimum of 2ml of EDTA blood is required.
- This particular *E. canis* PCR is highly species specific with no cross-reaction with other Ehlichias or other rickettsial species.
- A negative *Ehrlichia canis* nucleic acid detection (PCR) result should be treated with some reserve if used for the initial diagnosis of canine mononuclear ehrlichiosis.
- The sensitivity of blood PCR in natural infections is variable, with false negative results caused by difficulties in extracting the organism, problems with the technique and inappropriate sample selection.
- Development of the nested PCR assay has gone some way to addressing the technique problem.
- Acute and convalescent serology should always be used in conjunction with the PCR Positive serology in the face of negative PCR probably indicates previous exposure, with clearance of the organism.
- The *E. canis* PCR is best suited to be used in conjunction with serology to detect acutely infected animals before seroconversion.
- PCR of splenic aspirates is considered a much more sensitive alternative to blood-PCR and is useful in distinguishing treated animals with persistent infection from those that retain high antibody titres after successful treatment.
- For localized infections PCR analysis of joint fluid, CSF or aqueous humour are likely to provide more meaningful diagnostic information. A minimum of 0.5 ml of fluid collected into a plane serum tube are required.
E. canis Antibody Detection – Serology

Indirect Immuno-Fluorescent Antibody (IFA) / ELISA

Serology is the most commonly used screening method. Following collection of blood into serum tubes with / without clot activator, 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 disease syndrome and a convalescent sample collected two to three weeks later are the preferred samples. A minimum of 0.5 ml clotted blood is required.

- Seroconversion in dogs can take up to 4-5 weeks post-infection.
- Positive serology is commonly accepted as titres greater than 1:64 – 1:80, but it varies between laboratories.
- Antibody titres are lower in sub-clinically infected dogs.
- A negative result in a case with high clinical suspicion should have a PCR run on spleen aspirates or EDTA blood and the serology repeated in 4 – 6 weeks.
- Accurate titre measurement is best achieved with IFA. Most ELISA methods are only semi-quantitative.
- ELISA assays accurately identified positive cases (by IFA) when titres were > 1:320 but were unreliable when titres were between 1:80 – 1:320.
- Antibodies can be detected in cerebrospinal fluid, but any positive result must be confirmed with PCR.
- *Positive titres in asymptomatic dogs merely indicate exposure.*
- Treated dogs should be retested by IFA at 2 months post-treatment. If the IFA titre does not drop significantly (>16-fold), additional treatments will be required.
- Antibodies to E. canis can cross-react with other Ehrlichia species in these assays, but this is probably of limited importance in South Africa.
Further Reading


NOTES
Canine Neosporosis

Introduction

*Neospora caninum*, is a strictly intra-cellular, protozoan parasite of the family *Apicomplexa* and is the causative agent of canine neosporosis. Neosporosis is an important cause of meningoencephalomyelitis, polymyositis and polyradiculoneuritis in dogs worldwide. The dog and coyote have been proven as definitive hosts with other wild canines being suspected. Canines also serve as intermediate hosts with tachyzoites and tissue cyst forming bradyzoites composing the intermediate host parasite stages. Tissue cysts are found principally in the CNS but have also been reported in the skeletal muscles of naturally infected dogs. Transplacental infection is common but post-natal infection is also believed to occur. Dogs may also acquire the infection through ingestion of infected material (aborted bovine foetuses, placenta, tissue cysts in meat of intermediate hosts).

Clinical neosporosis is most frequently seen in puppies less than 6 months, characterized by muscle rigidity leading to a hind-limb paresis or ataxia, progressing to affect forelimbs, swallowing and breathing. Rigid hyperextension develops in many cases. Other clinical signs that have been reported are head tremors, myocarditis, pneumonia and pyogranulomatous dermatitis. Disease is less commonly documented in adults.

Sample Guide

**Neospora Antigen Detection**

**Molecular DNA assays**

- Live animals: EDTA blood (minimum of 0.5ml) and / or CSF in a plain tube or sterile container (minimum 0.25ml) are the preferred samples for the Polymerase Chain Reaction (PCR).
- Dead animals: The PCR can also be applied to foetal and post mortal tissues.
- 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).
Neospora caninum IHC is widely employed, in conjunction with histopathology, on formalin-fixed tissues to identify protozoal tachyzoites and bradyzoite filled cysts in tissue sections. On HE-stained histological sections the tachyzoites and tissue cysts of Neospora caninum cannot be distinguished from those of Toxoplasma gondii. IHC stains however, enable accurate distinction of these 2 protozoa and allow for confirmation of neosporosis. The image above-right is of an HE-stained tissue section from a dog with a N.caninum tissue cyst (arrow) in a myofibre of the tongue.

Neospora Antibody Detection – Serology

Indirect Immunofluorescent Antibody (IFA) / ELISA / Immunoprecipitation

Serology is the most commonly used screening method. Following collection of blood into serum tubes with / without clot activator, 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 disease syndrome and a convalescent sample collected two to three weeks later are the preferred samples. A minimum of 0.5 ml clotted blood is required.

- Titres greater than 1:50 are generally considered positive.
- Antibody titres are lower in asymptomatic dogs.
- Titres > 1:800 with typical myo-neurological signs are highly suggestive of active infection.
- However, some symptomatic dogs can have titres between 1:50 – 1:800.
- Titres between 1:50 – 1:800 in asymptomatic dogs merely indicates exposure.
- Antibodies can be detected in cerebrospinal fluid but usually in lower concentrations – a titre > 1:50 being considered positive. Minimum of 0.25ml of CSF in a plane serum tube is required.
- Antibodies to *T. gondii* do not cross-react with *N. caninum*, at dilutions of 1:50 or less in serum or cerebrospinal fluid.

**Further Reading**


**NOTES**