Orbivirus Diagnosis in an Endemic Country

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This presentation highlights the complexities of making a diagnosis in a situation where vaccination is taking place, and where there are multiple virus serotypes and related viruses in circulation. The main orbiviruses of interest are those of African horse sickness (AHS) and bluetongue (BT) and further complicated by the viruses of equine encephalosis (EE) and epizootic haemorrhagic disease of deer (EHD). South Africa is an endemic country with all 9 AHS serotypes and 22 of 24 BT serotypes present. Annual vaccination is carried out but a large pool of virus is available in unvaccinated zebra and donkeys for AHS, and indigenous sheep, cattle and ruminant wildlife for BT. Diagnoses of the two diseases are discussed separately.

AHS had its origin in zebras which, however, do not exhibit the disease, while up to 95% of susceptible horses die. Diagnosis requires recognition of recent infection, and a history of recent vaccination is essential. Diagnostic tests are based on detection of virus and/or antibodies. Use of antibodies is fast and inexpensive and based on immunoglobulin M or G (IgM or IgG) responses. All horses are seropositive, and paired samples taken 14 days apart are required to demonstrate recent exposure and a rise in titre. IgM enzyme-linked immunosorbent assays (ELISAs) for multiple serotype viruses are not available. The IgG ELISA result is based on a single dilution thus, in paired samples, a rise in titre is a qualitative estimate of exposure. The complement fixation test (CFT) is largely an IgM test which uses a dilution series, and paired samples can show a rise in titre. It is slow, old-fashioned and labour intensive, but it is the only useful serological option where there are pre-existing antibodies and a rising titre is required. Demonstration of virus or antigen or ribonucleic acid (RNA) presents advantages and disadvantages for each. Virus isolation on cells is slow for AHSV but faster intracerebrally in mice. Viral titres are low with circulating antibodies, and serotyping or sequencing to differentiate vaccine and field strains may be needed, an isolate becomes then necessary. Antigen detection by immunohistochemistry is useful, if only formalin material is received, or if tissue tropism is of interest. Polymerase chain reaction (PCR) in its many formats gives a rapid diagnosis but interpretation can be difficult. RNA is present for some months, therefore the time of infection and its significance is difficult to gauge. The antigen-capture ELISA is useful, particularly on tissue culture isolates to distinguish AHS and EE, if two PCR tests have not been done initially. As a primary diagnostic tool it lacks sensitivity, and an isolate is often required for additional tests. The best diagnostic option is to get a good history and to do as many different tests as possible in order to make an educated guess. This is because EE mimics AHS clinically, has the same vectors and co-circulates with AHS in the population. Tests do not cross-react, but it is important to focus on both viruses in order to make the proper diagnosis.

Much that has already been mentioned for AHS also applies for BT. The pool of wild-type, infectious virus becomes proportionately much larger when BT vaccine coverage is lower, causing the disease to occur widely. Little diagnostic material is received as mortality in sheep is only a low 2–30%; each year, however, at least 2–3 dominant serotypes are present. Similar tests are available in an IgG format in use. All sheep are generally seropositive because of multiple exposures. Negative sheep need to be sourced from high-altitude cold areas. The very helpful CFT cannot be used as this group-specific test cross-reacts significantly with EHDV. This problem is addressed in the competitive ELISA with a monoclonal antibody specific for BTV. This test is essential to obtain export certification. In South Africa mortality caused by BT is low and sporadic, and virus spread is slow because of homologous and heterologous background antibodies in sheep flocks. Diagnoses become important in the export sector for testing ruminant wildlife going to zoological collections or ovine embryos where donor sheep are tested. BT-free certification is obtained by PCR testing. As mentioned previously, RNA is present for a prolonged period of time. A negative PCR means no exposure, whereas a positive result requires virus isolation to certify that no live whole virus is present, as it could infect vectors. Field, not vaccine strains, require amplification in embryonated eggs first, then isolation from dead embryos on tissue culture. The cross-reactivity between BTV and EHDV in diagnostic tests is not an important consideration except when an isolate cannot be typed. If EHD viruses were as widely prevalent as those of EE, there would be a diagnostic problem, but EHDV isolates are not found in sheep. From time to time EHDV is found in alpaca and bovine cases. Finally, orbivirus diagnoses are challenging in an endemic country but always interesting and sometimes surprising in the final results obtained.

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