CHAPTER 2.5.6.

EQUINE INFECTIOUS ANAEMIA

SUMMARY

Equine infectious anaemia (EIA) is a persistent viral infection of equids. The causative agent, EIA virus (EIAV) is a lentivirus in the family Retroviridae, sSubfamily Orthoretrovirinae. Other members of the lentivirus genus include: bovine immunodeficiency virus; caprine arthritis encephalitis virus; feline immunodeficiency virus; human immunodeficiency virus 1; human immunodeficiency virus 2; and maedi/visna virus. EIA can be diagnosed on the basis of clinical signs, pathological lesions, serology and molecular methods. Infected horses remain viraemic carriers for life and, with very rare exceptions, yield a positive serological test result. Antibody response usually persists and antibody-positive animals, older than 6–8 months, are identified as virus carriers (below 6–8 months of age, serological reactions can be due to maternal antibodies; status can be confirmed by molecular techniques). Infected equids are potential virus reservoirs. Biting flies are mechanical vectors for the virus in nature.

Identification of the agent: *Virus from a horse can be isolated by inoculating suspect blood into a susceptible horse or on to leukocyte cultures prepared from susceptible horses. Recognition of infection in horses that have been inoculated experimentally may be made on the basis of clinical signs, haematological changes and a positive antibody response determined by an immunodiffusion test or enzyme-linked immunosorbent assay (ELISA) or by molecular techniques. Successful virus isolation in horse leukocyte cultures is confirmed by the detection of specific EIA antigen, by immunofluorescence assay, polymerase chain reaction, reverse-transcriptase assay, or by the inoculation of culture fluids into susceptible horses. Virus isolation is rarely attempted because of the time, difficulty and expense involved.*

Serological tests: *Agar gel immunodiffusion (AGID) tests and ELISAs are simple, reliable tests for the demonstration of EIAV infection. When ELISAs are positive they should be confirmed using the AGID test. EIA antigens can be prepared from infected tissue cultures or by using recombinant DNA technology.*

Requirements for vaccines and diagnostic biologicals: *There are no biological products currently available.*

A. INTRODUCTION

Equine infectious anaemia (EIA) occurs world-wide. The infection, formerly known as swamp fever, is limited to equids. The disease is characterised by recurrent febrile episodes, thromboctopenia, anaemia, rapid loss of weight and oedema of the lower parts of the body. If death does not result from one of the acute clinical attacks, a chronic stage develops and the infection tends to become inapparent. The incubation period is normally 1– 3 weeks, but may be as long as 3 months. In acute cases, lymph nodes, spleen and liver are hyperaemic and enlarged. Histologically these organs are infiltrated with nests of immature lymphocytes and plasma cells. Kupffer cells in the liver often contain haemosiderin or erythrocytes. The enlarged spleen may be felt on rectal examination. Differential diagnoses include equine viral arteritis (Chapter 2.5.10), and other causes of oedema, fever, or anemia.

Once a horse is infected with EIA virus (EIAV), its blood remains infectious for the remainder of its life. This means that the horse is a viraemic carrier and can potentially transmit the infection to other horses (4). Transmission occurs by transfer of blood from an infected horse. In nature, spread of the virus is most likely via interrupted feeding of bloodsucking horseflies on a clinically ill horse and then on susceptible horses. Transmission can also occur by the iatrogenic transfer of blood through the use of contaminated needles. *In utero* infection of the fetus may occur (9). The virus titre is higher in horses with clinical signs and the risk of transmission is higher from these animals than the carrier animals with a lower virus titre.

B. DIAGNOSTIC TECHNIQUES

Agar gel immunodiffusion (AGID) tests (7) and enzyme-linked immunosorbent assays (ELISAs) (17) are accurate, reliable tests for the detection of EIA in horses, except for animals in the early stages of infection and foals of infected dams. In other rare circumstances, misleading results may occur when the level of virus circulating in the blood during an acute episode of the disease is sufficient to bind available antibody, and if initial antibody levels never rise high enough to be detectable (18). Although the ELISA will detect antibodies somewhat earlier and at lower concentrations than the AGID test, positive ELISAs are confirmed using the AGID test. This is because false-positive results have been noted with ELISAs. The AGID test also has the advantage of distinguishing between EIA and non-EIA antigen–antibody reactions by lines of identity.

The EIAV is a lentivirus in the family Retroviridae, subfamily Orthoretrovirinae. Other members of the lentivirus genus include: bovine immunodeficiency virus; caprine arthritis encephalitis virus; feline immunodeficiency virus; human immunodeficiency virus 1; human immunodeficiency virus 2; and maedi/visna virus. Nucleic acid sequence comparisons have demonstrated a marked relatedness among these viruses.

1. Identification of the agent

a) Virus isolation and identification

Virus isolation is usually not necessary to make a diagnosis.

Isolation of the virus from suspect horses may be made by inoculating their blood on to leukocyte cultures prepared from horses free of infection. Virus production in cultures can be confirmed by detection of specific EIA antigen by ELISA (16), by imunofluorescence assay (20), by molecular tests or by subinoculation into susceptible horses. Virus isolation is rarely attempted because of the difficulty of growing horse leukocyte cultures.

When the exact status of infection of a horse cannot be ascertained, the inoculation of a susceptible horse with suspect blood should be employed. In this case a horse that has previously been tested for antibody and shown to be negative is given an immediate blood transfusion from the suspect horse, and its antibody status and clinical condition are monitored for at least 45 days. Usually, 1–25 ml of whole blood given intravenously is sufficient to demonstrate infection, but in rare cases it may be necessary to use a larger volume of blood (250 ml) or washed leukocytes from such a volume (5).

b) Polymerase chain reaction

A nested polymerase chain reaction (PCR) assay to detect EIA proviral DNA from the peripheral blood of horses has been described (13). The nested PCR method is based on primer sequences from the *gag* region of the proviral genome. It has proven to be a sensitive technique to detect field strains of EAV in white blood cells of EIA infected horses; the lower limit of detection is typically around 10 genomic copies of the target DNA (13, 14). A real-time reverse-transcriptase PCR assay has also been described (8). To confirm the results of these very sensitive assays, it is recommended that duplicate samples of each diagnostic specimen be processed. Because of the risk of cross contamination, it is also important that proper procedures are followed. Methods to insure the validity of PCR testing are discussed in detail in Chapter 1.1.5 Validation and quality control of polymerase chain reaction methods used for the diagnosis of infectious diseases.

The following are some of the circumstances where the PCR assay maybe used for the detection of EIAV infection in horses:

- Conflicting results on serologic tests;

- Suspected infection but negative or questionable serologic results;

- Complementary test to serology for the confirmation of positive results;

- Confirmation of early infection, before serum antibodies to EIAV develop;

- Ensuring that horses that are to be used for antiserum or vaccine production or as blood donors are free of EIAV;

- Confirmation of the status of a foal from an infected mare.

2. Serological tests

Due to the persistence of EIAV in infected equids, detection of serum antibody to EIAV confirms the diagnosis of EIAV infection.

a) Agar gel immunodiffusion test (the prescribed test for international trade)

Precipitating antibody is rapidly produced as a result of EIA infection, and can be detected by the AGID test. Specific reactions are indicated by precipitin lines between the EIA antigen and the test serum and confirmed by their identity with the reaction between the antigen and the positive standard serum. Horses in the first 2–3 weeks after infection will usually give negative serological reactions. In rare cases the post-infection time prior to the appearance of detectable antibody may extend up to 60 days.

Reagents for AGID are available commercially from several companies. Alternately, AGID antigen and reference serum may be prepared as described below.

o   Preparation of antigen

Specific EIA antigen may be prepared from the spleen of acutely infected horses (6), from infected equine tissue culture (11), from a persistently infected canine thymus cell line (3), or from proteins expressed in bacteria or baculovirus using the recombinant DNA technique (2, 10). Preparation from infected cultures or from recombinant DNA techniques gives a more uniform result than the use of spleen cells and allows for better standardisation of reagents.

To obtain a satisfactory antigen from spleen, a horse must be infected with a highly virulent strain of EIAV. The resulting incubation period should be 5–7 days, and the spleen should be collected 9 days after inoculation, when the virus titre is at its peak and before any detectable amount of precipitating antibody is produced. Undiluted spleen pulp is used in the immunodiffusion test as antigen (6). Extraction of antigen from the spleen with a saline solution and concentration with ammonium sulphate does not give as satisfactory an antigen as selection of a spleen with a very high titre of EIA antigen.

Alternatively, equine fetal kidney or dermal cells or canine thymus cells are infected with a strain of EIAV adapted to grow in tissue culture (American Type Culture Collection). Virus is collected from cultures by precipitation with 8% polyethylene glycol or by pelleting by ultracentrifugation. The diagnostic antigen, p26, is released from the virus by treatment with detergent or ether (11). EIAV core proteins, expressed in bacteria or baculovirus, are commercially available and find practical use as high quality antigens for serological diagnosis.

The p26 is an internal structural protein of the virus that is coded for by the *gag* gene. The p26 is more antigenically stable among EIAV strains than the virion glycoproteins gp45 and gp90 (12). There is evidence of strain variation in the p26 amino acid sequence; however there is no evidence to indicate that this variation influences any of the serological diagnostic tests (21).

o   Preparation of standard antiserum

A known positive antiserum may be collected from a horse previously infected with EIAV. This serum should yield a single dense precipitation line that is specific for EIA, as demonstrated by a reaction of identity with a known standard serum. It is essential to balance the antigen and antibody concentrations in order to ensure the optimal sensitivity of the test. Reagent concentrations should be adjusted to form a narrow precipitation line approximately equidistant between the two wells containing antigen and serum.

o   Test procedure (1, 6, 15)

i)   Immunodiffusion reactions are carried out in a layer of agar in Petri dishes. For Petri dishes that are 100 mm in diameter, 15–17 ml of 1% Noble agar in 0.145 M borate buffer (9 g H_3BO_3 plus 2 g NaOH per litre), pH 8.6 (± 0.2) is used. Six wells are punched out of the agar surrounding a centre well of the same diameter. The wells are 5.3 mm in diameter and 2.4 mm apart. Each well must contain the same volume of reagent.

ii)   The antigen is placed in the central well and the standard antiserum is placed in alternate exterior wells. Serum samples for testing are placed in the remaining three wells.

iii)   The dishes are maintained at room temperature in a humid environment.

iv)   After 24–48 hours the precipitation reactions are examined over a narrow beam of intense, oblique light and against a black background. The reference lines should be clearly visible at 24 hours, and at that time any test sera that are strongly positive may also have formed lines of identity with those between the standard reagents. A weakly positive reaction may take 48 hours to form and is indicated by a slight

bending of the standard serum precipitation line between the antigen well and the test serum well. Sera with high precipitating antibody titres may form broader precipitin bands that tend to be diffuse. Such reactions can be confirmed as specific for EIA by dilution at 1/2 or 1/4 prior to retesting; these then give a more distinct line of identity. Sera devoid of EIA antibody will not form precipitation lines and will have no effect on the reaction lines of the standard reagents.

v)   *Interpretation of the results:* Horses that are in the early stages of an infection may not give a positive serological reaction in an AGID test. Such animals should be bled again after 3–4 weeks. In order to make a diagnosis in a young foal, it may be necessary to determine the antibody status of the dam. If the mare passes EIA antibody to the foal through colostrum, then a period of 6 months or longer after birth must be allowed for the maternal antibody to wane. Sequential testing of the foal at monthly intervals may be useful to observe the decline in maternal antibody. To conclude that the foal is not infected, a negative result must be obtained (following an initial positive result) at least 2 months after separating the foal from contact with the EIA positive mare or any other positive horse. Alternatively PCR could be performed on the blood of the foal to determine the presence/absence of EIA proivirus.

b)   Enzyme-linked immunosorbent assay

There are four ELISAs that are approved by the United States Department of Agriculture for the diagnosis of equine infectious anaemia and are available internationally; a competitive ELISA and three non-competitive ELISAs. The competitive ELISA and two non-competitive ELISAs detect antibody produced against the p26 core protein antigen. The third non-competitive ELISA incorporates both p26 core protein and gp45 (viral transmembrane protein) antigens. Typical ELISA protocols are used in all tests. If commercial ELISA materials are not available, a non-competitive ELISA using p26 antigen purified from cell culture material may be employed (16).

A positive test result by ELISA should be retested using the AGID test to confirm the diagnosis because some false-positive results have been noted with the ELISA. The results can also be confirmed by the immunoblot technique. A standard antiserum for immunodiffusion, which contains the minimum amount of antibody that should be detected by laboratories, is available from the OIE Reference Laboratories (see Table given in Part 3 of this *Terrestrial Manual*). Uniform methods for EIA control have been published (19).

C.   REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

No biological products are available currently.

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NB: There are OIE Reference Laboratories for Equine infectious anaemia (see Table in Part 3 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list: www.oie.int).