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Equine Infectious Anemia
Diagnosis and Prevention

by James E. Pearson,* D.V.M.

Equine infectious anemia (EIA) was first reported in 1843 and Valle and Carre demonstrated in 1904 that the causative agent was a filterable virus. It is an acute, subacute or chronic disease of Equidae characterized by fever, anemia, depression, edema and emaciation.

Progress in elucidating the pathogenesis, developing diagnostic tests and characterizing the etiologic agent has been limited by the apparent lack of a susceptible laboratory animal or cell culture system. Positive diagnosis has depended on horse inoculation tests. In the past 10 years two developments have helped characterize the disease: 1) Kobayashi and Kono propagated the virus in bone marrow and horse leukocyte cultures, and the work has been confirmed by other workers; 2) Coggins and Norcross in 1970 and Nakajima in 1971 described an immunodiffusion test for serological confirmation of the disease. The test has proven reliable and has become widely used in the United States and Canada.

This paper will deal primarily with diagnosis and prevention and is based on a review of applicable literature and personal experience in this laboratory. A complete review of the literature was not attempted and the reader is referred to the publication by Dreguss and Lombard and other reviews for more detailed information.

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Etiology

Although it has been well established that EIA is caused by a virus, the characteristics of the agent have been poorly understood. Research in this area has been limited until recently by the lack of a cell culture system or laboratory animal for viral assay. Nakajima, et al. described the agent as being a RNA virus dependent on DNA for replication, 80-120 nm in diameter and similar in structure to the RNA tumor viruses. He found it to be sensitive to ether and resistant to trypsin. Kono verified some of the same physiochemical characteristics of the virus. Other workers described the agent as being 30-70 nm in size. The virus was found to be inactivated at 60°C for 60 min., resistant to phenol, and inactivated by formalin.

Distribution

Even before the advent of a diagnostic test the disease had been reported on all continents from the subpolar areas to the tropics. In the United States the disease has been reported in most states. The current availability of a diagnostic test, other than horse inoculation, will provide a more practical method for determining the incidence and distribution. It has been observed that the incidence was higher in low lying swampy areas which led to the name "swamp fever."

Transmission

The virus has been found in all body secretions including semen and milk,
however, contact and vertical transmission appeared to be rare. Insects, particularly blood sucking flies, have been incriminated as the main source of transmission. It has been demonstrated that as little as 1.0 ml. of a $10^{-4}$ dilution of serum from some carrier animals was infective; therefore, contaminated surgical instruments, needles and syringes could play a role in transmission. Information on insect and vertical transmission has been limited by the lack of a virus isolation procedure other than horse inoculation.

Clinical Signs

The clinical signs, pathology and prognosis vary greatly among individuals. The disease can be acute, subacute or chronic with possible conversion from one form to the other. The incubation period is usually 8–21 days but periods of up to 61 days have been observed. The length of the incubation period does not appear to be correlated with the severity of the disease. In the acute form the animal may have febrile attacks (104–108°F) at short intervals or the temperature may remain high until death (Fig. 1). The acutely infected animal rarely dies within 14 days of the initial temperature rise unless the disease is complicated by secondary bacterial infection or heavy parasitism.

Shortly after the first exacerbation the horse becomes depressed. If the acute form continues there is an increasing debilitation, anemia, submucosal hemorrhage, profuse bleeding from wounds, and edema of dependent parts. The febrile reaction may continue with the animal showing incoordination, becoming comatose and dying. The temperature may return to normal and the disease revert to the subacute or chronic form at any time. Subacute cases exhibit febrile attacks similar to those of the acute cases but the and occurs less frequently (Fig. 2). This form can go unnoticed with the only clinical sign being slight depression and ano-

Figure 1. Temperature, packed cell volume and hemoglobin graphs of a pony which exhibited acute clinical signs of equine infectious anemia.

Clinical Pathology

In the acute form anemia is observed. Serial packed cell volume (PCV) and temperature determinations are accurate indications of the stage of the disease (Fig. 1–3). Anemia does not usually occur until

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several days after the temperature rise and PCV will slowly increase after the temperature returns to normal. Equine infectious anemia infection causes hemolytic anemia. Bilirubinuria, hyperbilirubinemia and icterus may occur but hemoglobinuria does not occur. Circulating immature erythrocytes are extremely rare in the equine but have been observed in cases of prolonged acute EIA. An increase in Heinz bodies and subtle changes in RBC are observed if blood smears are examined serially during the infection.

Circulating leukocyte changes are variable. An initial leukopenia is often observed with a relative lymphocytosis and monocytosis developing later. Sideroleukocytes (leukocytes containing non-hemoglobin iron) are often detected in the circulating blood of infected animals. Siderocytes can be used to aid in the diagnosis of EIA but they also occur in other hemolytic anemias.

The serum lipoprotein increases markedly during and following febrile reactions. The lipid in the serum and clot can be detected by gross examination of the clotted blood. At the same time there
is an increase in serum glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH) and gamma globulin. A. Baetz, unpublished data.

Immunity

It has been conclusively demonstrated that antibody against EIA virus is produced by the infected horse.\textsuperscript{11,22,24} As reviewed elsewhere, a variety of serologic tests have been tried with doubtful specificity or sensitivity.\textsuperscript{4,8,9,13,26} However, several tests have been used to successfully detect antibody against EIA virus and will be described here.

An agar gel immunodiffusion (ID) test described by Coggins \textit{et al.}\textsuperscript{1,3} has been evaluated and shown to be sensitive and specific.\textsuperscript{1,2,3,5} The test detects a specific EIA antibody and due to the chronicity of the disease the animal is assumed to be a virus carrier. A variety of experimentally infected horses, including asymptomatic animals infected as long as four and five years have been positive on the test. Two types of false reactions have been observed: 1) the antibody cannot be detected for 14 to 40 days postinfection\textsuperscript{35} so a negative reacting animal may in reality be in the incubation period; 2) foals nursing infected mares will have a positive test for up to 6 months after birth due to colostral antibody.\textsuperscript{2}

The ID test is set up with a pattern of seven wells cut in agar. Antigen is placed in the center well and serum in the six outer wells. A known positive serum is placed on each side of the serum to be tested. A total of three serums can be tested on each pattern. A specific precipitin line forms between each positive serum and the antigen while none is observed between the negative serum and

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{immunodiffusion_test}
\caption{Immunodiffusion test which has antigen in center well; control positive serum in wells A, C and E; negative serum in well B; strong positive serum in well D; and weak positive serum in well F.}
\end{figure}
antigen (Fig. 4). The antigen used is splenic extract from a horse or pony with acute EIA. Characteristics of the antigen have been established by Norcross and Coggins.35

Nakajima et al. described a similar immunodiffusion test using purified cell culture propagated virus as the antigen.34 This test also appeared to be highly specific.

A complement fixation test has been described by Kono and Kobayashi:18,19 Virus propagated in horse leukocyte cultures was used as the antigen. The test has been found to be very specific and has been utilized by Henson et al.7 and in this laboratory. It was generally not capable of detecting the chronic carrier (greater than 90 DPI). The test does serve as a method for detecting the virus in leukocyte cultures. Modifications are being attempted to make the test more sensitive.

A virus neutralization test in horse leukocyte cultures has been described.6,22 This test appeared to be specific and sensitive; however, the technical difficulties in horse leukocyte culture production will probably limit it to the research laboratory. It is a method of detecting neutralizing antibody and is useful in differentiating between strains for future vaccine evaluations.

A fluorescent antibody technique using infected horse leukocyte cultures has been described by Ushimi.40 Nonspecific fluorescence of the cultures has posed a problem in some cases. Localization of the viral antigen in tissues using fluorescent antibody tissue section techniques has been described by McGuire et al.25

Necropsy Findings

As with the clinical signs the gross lesions are quite variable. The only gross lesions seen repeatedly is hemorrhagic hyperplasia of the splenic and renal lymph node. In some animals petechial or ecchymotic subserosal hemorrhage is observed along with splenomegaly. In the acute case of long duration, evidence of anemia, icterus, and edema may be observed.

Histopathological examination can be helpful. Common lesions are lymphocytic proliferation in the spleen and lymph node and a lymphocytic infiltration of the liver, bone marrow, heart and kidney. Hemosiderin deposits may be observed in the liver, especially early in the disease. The lymphoproliferative lesion has been described as being similar to a neoplasm.37

Treatment

No specific treatment is available. Supportive treatment for the anemia, such as blood transfusions, may reduce the severity of the clinical signs. When treating an animal it should be remembered that it is and will remain a virus carrier.

Prevention

An understanding of the etiology, clinical picture, necropsy findings, and diagnostic techniques available are useful for planning the prevention of the disease. Control on a farm should be based on positive diagnosis of suspected cases, isolation or destruction of infected animals, and prevention of transmission. It should be assumed that any horse may be a potential virus carrier and all potentially contaminated instruments and equipment should be heat sterilized. Fly and mosquito control programs should be practiced particularly for the tabanid (horse fly).

Immunization has been attempted using a variety of different preparations but no immunity has been produced.4,10 The only exception was reported recently by Kono et al.2,4 who successfully immunized 11 animals with modified live virus propagated in horse leukocytes. The animals resisted challenge with homologous virus material but were susceptible to virus from a heterologous source. These results suggest that there may be several strains of EIA virus which will make the production of a reliable vaccine more difficult.

Summary

During the past decade and particularly in the last three years, techniques have been developed that have enhanced the study of EIA. When the only method of diagnosis was inoculation of a susceptible
horse, progress was very slow. Now with a diagnostic test and a susceptible cell culture available the characteristics of the disease should be elucidated. There are many areas that are not understood such as the pathogenesis, distribution, incidence, role of insects in transmission, methods of prevention, and treatment. The development of additional cell culture systems and improved fluorescent antibody techniques will assist in obtaining virus isolations. Work now in progress will help solve these problems.

REFERENCES

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