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SEROLOGICAL DIAGNOSIS OF EQUINE INFECTIOUS ANEMIA VIRUS INFECTION IN THE CENTRAL REGION OF THE RIO GRANDE DO SUL STATE

DIAGNÓSTICO SOROLÓGICO DA INFECÇÃO PELO VÍRUS DA ANEMIA INFECCIOSA EQÜINA NA REGIÃO CENTRAL DO ESTADO DO RIO GRANDE DO SUL

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RESUMO

This paper consists of a brief review on equine infectious anemia added of the results of serological diagnosis of this infection performed at the Federal University of Santa Maria, RS, Brazil, between 1979 to 1990. A total of 7,035 serum samples were tested by the agar gel immunodiffusion test (Coggins test), to which 40 (0,57%) reacted positively. This percentage of positivity is lower than in other regions of Brazil, probably due to the lack of predisposing factors for equine infectious anemia virus spread, such as, a low density of blood-sucking insects in the South of Brazil and the seldom use of massive therapies and vaccinations in the region.

Key words: equine infectious anemia, diagnosis, immunodiffusion.

RESUMO

Este trabalho consiste de uma breve revisão sobre a anemia infecciosa eqüina e de resultados dos diagnósticos sorológicos desta infecção realizados na Universidade Federal de Santa Maria, RS, Brasil, entre os anos de 1979 e 1990. Um total de 7.035 amostras de soro foram testadas pela prova de imunodifusão en gel de ágar (teste de Coggins), ao qual 40 (0,57%) apresentaram-se positivas. Este percentual de positividade é baixo, comparado com outras regiões do Brasil, provavelmente devida à falta de fatores predisponentes para a disseminação do vírus da anemia infecciosa eqüina, tais como, baixa densidade de insetos hematófagos no sul do país e pouca freqüência de terapêuticas e vacinações massivas na região.

Palavras-chave: anemia infecciosa eqüina, diagnóstico, imunodifusão.

INTRODUCTION

Equine infectious anemia (EIA) is a viral disease of the members of the equine family. The worldwide distribution of EIA has been reviewed by JOHNSON (1976) who reported outbreaks throughout the world and the existing literature from 1966 to 1975.

The disease usually is clinically diagnosed in a chronic form, with a high percentage of affected horses demonstrating weight loss, depression, and reduced hematocrit values, platelet counts and hemoglobin (ISSEL & COGGINS, 1979). Chronically affected horses show the most marked lesions, such as splenomegaly, lymphadenopathy, accentuated hepatic lobular architecture, anemia, emaciation, edema and hemorrhage (HENSON & McGUIRE, 1971). Microscopic lesions include early lymphoid necrosis followed by lymphoproliferative changes. Acute EIA is most often associated with the first exposure to the virus and is characterized by a sudden rise in body temperature and disseminated hemorrhages. Neurologic signs, such as ataxia, have been associated with EIA (McCLURE et al, 1982; HELD et al, 1983). Acute cases that survive tend to become chronic, and chronic cases to become inapparent (KEMEN, 1977). Assimptomatic carriers may exhibit clinical signs in stressful circumstances as shown

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by Kono et al (1976) using corticosteroids. The production and release, or infection with a novel antigenic strain of the virus may also cause the recrudescence of EIA (KONO et al, 1973).

Equine infectious anemia virus (EIAV) is now an established member of the genus **Lentivirus** in the family Retroviridae, since it has a high molecular weight RNA, an internal virion reverse transcriptase (CHARMAN et al, 1976), a RNA-dependent DNA polymerase (ARCHER et al, 1977) maturation process (McCONNELL et al, 1977) and morphologic properties (GONDA et al, 1978) that resemble visna and maedi viruses of sheep and other retroviruses. SHEN et al (1977) demonstrated that EIAV is readily inactivated by a variety of chemical disinfectants, such as sodium hydroxide, organic phenolic compounds, ethanol, formalin and chlorhexidine.

The transmission of EIA has long been known to occur by means of blood-sucking insects (HYSLOP, 1966; ISHI, 1963), and recently HALL et al (1988) described a propagating, epizootic EIA where biting flies were of major importance in the spread of the infection. Although an apparent propagation of the EIAV in a mosquito ovarian cell line has been reported (BREAUD et al, 1976), suggesting a biological transmission, the natural transmission of EIAV by insect vector is known to occur mechanically, that is, the insect merely transfers infectious material. This mechanism depends on a variety of factors, such as viremia titer, vector population levels and diversity, vector behavior, factors affecting the interruption of vector feeding and the distance separating infected and susceptible hosts (ISSEL & FOIL, 1984).

Stable fly (**Stomoxys calcitrans**), deer fly (**Chrysops flavidus**), horse flies (**Hybomitra lasiophtalma, Tabunus quinquevittatus** and **Tabanus sulcifrons**) have been shown to transmit the virus (FOIL et al, 1983; KEMEN et al, 1978). WILLIAMS et al (1981) could not demonstrate transmission by mosquitoes (**Aedes sollicitans, Psorophora columbiae**).

Utilizing acutely infected horse, with high viremia titers, experimental vector transmission trials have been successful, even with a single horse fly (HAWKINS et al, 1976). However, insect transmission from inapparent carriers have been demonstrated (KEMEN et al, 1978; ISSEL et al, 1982).

TASHJIAN (1984) has demonstrated EIAV in semen of a chronic EIA-infected stallion, as well as a stallion to mare transmission, where a contaminated semen probably had introduced EIAV through an injury in the female genitalia. Clinically and inapparently infected mares may transmit EIAV to their offspring, by intrauterine, insect or colostrum transmission (KEMEN & COGGINS; TASHJIAN, 1984).

Equine infectious anemia virus is readily transmitted by blood transfusion or by inoculation of small quantities of blood, since the inoculation of suspect blood into a susceptible horse has been used as the most sensitive and certain mean of detecting EIAV (ISSEL & COGGINS, 1979). WILLIAMS et al (1981) showed EIAV infective on hypodermic needles for up to 96 hours.

Although clinical, hematological and pathological aspects of the disease may direct a possible diagnosis, none of them are pathognomonic for EIA. Laboratory tests have been used to detect EIAV and EIAV antibody. EIAV has been demonstrated by the horse inoculation test and direct fluorescent antibody technique (McGUIRE et al, 1971a). Complement-fixation (CF) and CF inhibition tests (McGUIRE et al, 1971b), neutralization (HENSON et al, 1969), precipitation (COGGINS & NORCROSS, 1970; COGGINS et al, 1972) and ELISA (SUZUKI et al, 1982; SHANE et al, 1984; ARCHABAULT et al, 1989) have been used to detect EIA antibody.

The agar gel immunodiffusion (AGID) test has been the most useful method for the diagnosis of EIA. It can test a large number of horses at the same time, provides the results within 48 hours, and it is accurate and inexpensive (COGGINS & NORCROSS, 1970). Precipitating antibodies have been demonstrated to be present for as long as viremia exists (COGGINS et al, 1972) while CF antibodies usually become undetectable in a relatively short period of time (NAKAJIMA et al, 1971). Although neutralizing antibodies persist for long periods, they are detected later in the clinical infection.

As with any serological test, the AGID test does not detect the infection prior to antibody production, and may give positive results in the suckling foal that has received antibody in colostrum from its dam (ISSEL & COGGINS, 1979). If the foal is not infected, it will usually become test negative by the time it is 6-8 months of age, after the lost of maternal antibody (KEMEN & COGGINS, 1972; BURNS, 1974).

Hypericin and pseudohypericin (naturally occuring polycyclic quinones) have been recently shown to inhibt infectivity of several retroviruses **in vitro**, including EIAV (KRAUS et al, 1990). However, the antiviral activity of hypericin was completely dependent on the presence of light (CARPENTER & KRAUS, 1991), and there is still no certain treatment available against EIA.

Vaccines have been developed, but none has been succesful againsr antigenically heterologous virus. Only recently SHEN & WANG (1991) reported a protective rate around 80% using EIA donkey leucocyte attenuated virus, but there is no commercially available vaccine yet.

In the last decade, data from Argentina (GALASSI et al. 1980; TRIONI, 1981), Paraguay (SOLAIRES, 1981) and some states of Brazil (PAVEZ et al, 1981; NASCIMENTO & RIBEIRO, 1982) showed a mean percentage ranging from 1.6 to 48.0% of positive horses to the AGID test. Recently SOUZA et al (1991)

found 4% EIA-positive horses in 300 draft animals from Goiânia city, Brazil.

The objective of this paper is to present data related to EIA in the central region of the Rio Grande do Sul state, its control and risks to the horse population. This paper also recalls attention to EIA as a constant threat to equine herds.

MATERIAL AND METHODS

A total of 7,035 equine serum samples, without a definite breed or age, from counties of the central region of the Rio Grande do Sul state, Brazil, were submitted to the Federal University of Santa Maria (UFSM), during 1979 to 1990. Serum samples were tested by the AGID test (COGGINS et al, 1972), using 90mm diameter Petri dishes. A pattern of 6 wells arranged equidistantly from a central well was used, each one having 4mm in diameter, cut in the layer of agar (15ml of agar per dish). Fifty microliters of commercially available reagents[1] (antigen and serum controls) were used.

RESULTS

The results of the AGID test were separated by year as shown in table 1. The highest percentage of positive horses occured in 1979, the first year of diagnosis. The percentage of positivity was decrescent and no positive reaction was observed in the last two years, 1989 and 1990.

TABLE 1 - Results of the equine infectious anemia (EIA) agar gel immunodiffusion (AGID) test on equine serum samples from 1979 to 1990, UFSM, RS, Brazil.

YEAR	Nº SERUM SAMPLES	AGID TEST RESULTS	
		POSITIVE	% POSITIVES
1979	298	9	3.02
1980	500	6	1.20
1981	366	4	1.09
1982	515	3	0.58
1983	526	1	0.19
1984	550	1	0.18
1985	598	2	0.33
1986	1334	9	0.67
1987	675	2	0.30
1988	581	3	0.52
1989	617	0	0.00
1990	475	0	0.00
TOTAL	7035	40	0.57

DISCUSSION

Equine infectious anemia virus is characteristically a persistent virus which develops a carrier state in the host with continuing multiplication and discontinuing transmission, as reviewed by PASTORET et al (1987). This persistence must be due to constant glicoprotein antigens variations, to which neutralizing antibodies are produced. Another possibility could be the integration of viral genome into the cellular DNA.

It has been sugested that carrier animals, even though viremic, are not a threat to other horses, that a very high plasma virus titer (around 10^6 $TCID_{50}$/ml) is necessary for an efficient insect transmission which is only compatible with very severe, recent infections (CRAWFORD, 1979). On the other hand, inapparent carriers may exibit clinical signs in stressful circumstances (KONO et al, 1976), and insect transmission from animals without clinical signs has already been reported (KEMEN et al, 1978; ISSEL et al, 1982). Thus the evidence indicates that seropositive horses should be considered at least potentially dangerous when allowed to mingle at pasture with uninfected horses (KEMEN, 1977). Thus, when the statements above are taken into consideration, the equine population studied was under some threat, mainly during the first years of the study, when more animals reacted positively to the AGID test.

The percentage of positive reactions to the AGID test observed during 1979 to 1990 at the Federal University of Santa Maria was rather low (0.57%). The highest percentage was observed in 1979 (3.02%) and apparently decreased until 1989 and 1990, when none of the serum samples tested reacted positively. In 1986 a light increase of positivity was observed, but it should be due to the large amount of serum samples tested in this year. The low percentage of positivity should be an effect on the use of AGID tests in the control of EIA in racetracks, horse-shows and fairs, as well as of the euthanasia of positive horses, utilized in the central region. Another possibility should be the lack of predjsposing factors for EIAV dissemination, such as low density of blood-sucking insects in the South of Brazil,

and the seldom use of massive therapies and vaccinations in equine herds of the central region of the Rio Grande do Sul state.

Data from the **"BOLETIM DE DEFESA SANITÁRIA ANIMAL"** (1986) showed that EIAV prevalence in Brazil remained around 3.0% in the last ten years. The Middle West and North regions of Brazil have the highest indices of positivity, 12.70 and 11.77% respectively, perhaps due to climate factors and management system appropriate for viral dissemination. PAVEZ et al (1981) revealed a 12% prevalence of EIA by the AGID test and 20% of affected herds in Goias state (Middle West region) and that a high percentage of owners and breeders performs massive treatments using the same needle for all animals.

The AGID test has been considered the simplest and an efficient test to identify EIA-positive horses, and this is believed to be a key to the control of EIA. In the United States of America, the percentage of immunodiffusion-positive samples decreased significantly as the number of AGID tests increased (PEARSON & KNOWLES, 1984). Strategies of control should be directed concerning the epidemiological status of the population. EIA-free herds should only admit seronegative horses and quarantine recent arrivals before introducing them into the herd. Periodic retesting of all animals and euthanasia of possible positive horses should be indicated. Endemic or enzootic populations should choose a control or an eradiction program. If the target is control of EIA, this could be achieved by the identification and isolation of infected animals. If one chooses eradication, the reservoir (the infected equine) should be destroyed. General control measures, such as control of bloodsucking insects and disinfection of hypodermic needles or other instruments that could carry infective blood should be practiced (KEMEN, 1977).

Although EIA apparently is not a problem in the Rio Grande do Sul state, veterinarians should consider the potential threat of EIA. The disease is present, mainly in the Middle West and North regions of Brazil (BOLETIM DE DEFESA SANITÁRIA ANIMAL, 1986), where a differential diagnosis should be done, as well as in neighbour countries (GALASSI et al, 1908; SOLAIRES, 1981; TRIONI, 1981) and therefore the commercialization of horses could disseminate the infection. Seropositive animals may be viremic for life, and chronically affected animals or even inapparent carriers may show recurrent cycles of clinical disease (STEIN et al, 1955). Thus considering the possibility of occuring outbreaks, leading to deaths and decreased performance of infected equines, it is indispensable the accomplishment of strategies of control or eradication of EIA in the Rio Grande do Sul state.

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