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STUDIES ON ARTERIVIRAL INFECTION IN HORSES IN BULGARIA

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Summary

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A total of 227 equine blood sera were tested. One hundred and fifty eight (69.16%) were positive for equine viral arteritis (EVA) infection, including samples from all age groups. The positive samples in all five tested stables were from horses of different categories and breeds. The sera, which were investigated in dilution, showed titers from 1:5 to 1:20 (for screening and minimum 1:40 in individual test).

EVA has to be considered as a major problem of both veterinary and economical importance. Our results showed that the disease was spread widely among horses in Bulgaria. The EVA infection was probably imported by semen.

Our findings were the first positive cases for EVA in Bulgaria.

Key words: equine arteritis virus, equine viral arteritis, EVA, horses

INTRODUCTION

Equine viral arteritis (EVA) has a considerable global significance, having been identified in many countries of the EU and in America. Serological evidence indicate that the infection occurs in other areas of the world as well. The major consequences of EVA are that the infection may be abortigenic and that a significant percentage of stallions which become infected recover clinically but become long-term venereal "carriers" of the virus (Mumford, 1985; Clayton, 1986; Glaser *et al.*, 1997a, 1997b; Timoney, 1999, 2000a, 2000b).

Direct economic consequences attributable to equine arteritis virus (EAV) infection include those due to: 1) outbreaks of abortion or death in very young foals; 2) denied export markets for carrier stallions, virus infected semen and other categories of naturally seropositive horses; and 3) reduced commercial value of carrier stallions and reduced need to breed these animals. To this moment the disease is not established in Bulgaria (Chenchev *et al.*, 2002).

The equine arteritis virus belongs to the *Arterivirus* genus of the Arteriviridae family (Doll *et al.*, 1957, Cavanagh, 1997, Glaser *et al.*, 1997a). The viral particles are 60 nm in diameter and consist of an isometric nucleocapsid surrounded by a lipid envelope.

Despite its worldwide distribution, EAV causes disease outbreaks only exceptionally. The most severe and first recognized outbreak occurred in 1953 at stud farms in Bucyrus, Ohio. Since then

epizootics have been reported from Austria, Canada, Italy, Poland, Switzerland and the United States of America. The symptoms observed during these outbreaks were milder than those during the 1953 epizootic and affected horses of various breeds. The most recent outbreaks occurred at a riding school in the Netherlands, a stud farm in Great Britain, in racing clubs in France (Timoney, 2000a).

EVA is unusual in that that both respiratory and venereal routes spread it. If stallions become infected their internal genital organs may become persistently infected and they may shed the virus in their semen for the rest of their lives i.e. they become permanent carriers of infection and thereafter permanently unsuitable for breeding purposes (Timoney and McCollum, 1993). This may render them commercially valueless. Mares mated with, or inseminated by, infected stallions may become infected and may then spread the infection to others in contact with them on the same premises, by the respiratory route. In contrast to stallions, mares, geldings and immature colts, eliminate virus within a few weeks of infection and they do not become long-term carriers (Glaser *et al.*, 1997a).

The aim of these studies is to estimate the existence of EVA infection in equine population in Bulgaria.

MATERIALS AND METHODS

This study was carried out in 5 stables where cases of reproductive failures and heavy respiratory syndrome in equines were observed in February, spring and summer of 2002 (Chenchev *et al.*, 2002). Four of them were private horse riding clubs and one was a state stable. The stables were situated on the territory of four regions in the different parts of country. The number of animals in these units was from 5 to 25 equines from different categories and breeds. The trial period included the time from February 2002 to December 2002.

Clinical and pathological inspection was performed using descriptions by Timoney (1999). Two hundred twenty seven blood samples were taken from different categories of equines at the 14th day after the first clinical sings. The blood sera were tested by ELISA (Ingenasa-Madrid), according to Timoney (2000a). The samples were determined as EAV positive when their optical density values were lower than the cut off value (in our studies, a values between 0.152 and 0.176). All samples were preliminary diuted to 1:40 accordicng the instructions of the commercial screening kit.

RESULTS

The epizootological investigation revealed that the horses from all categories in affected horse-riding clubs were vaccinated against equine herpes virus and equine influenza and the results from the serological investigation for herpes viral infections and influenza were negative. The stallions and mares did not attended competitions in the last year. In 2000 and 2001 there were some cases of import of semen from stallions and the stallions were mated with mares from different part of neighbors regions.

The clinical sings of disease in the different categories were: fever, anorexia, depression, oedema of the limbs, conjunctivitis with lacrimation, rhinitis and nasal discharge, urticaria of the head, neck and trunk, and leucopenia. Occasionally, the infection of pregnant mares resulted in abortion, that occured 10-30 days after the viral infection even if mares showed no

Table 1. Results from tests of sera for equine viral arteritis by ELISA

Stable code	Horses < 2 years		Horses from 2 to 5 years		Adult stallions and mares		All horses	
	All	+	All	+	All	+	All	+
Kn	8	6	6	6	16	16	30	28
Sm	3	3	2	2	6	5	11	10
Ha	20	10	45	25	55	50	120	85
Ba	5	2	5	1	40	25	50	28
Rz	4	-	4	1	8	5	16	6
All stables	40	21	62	35	125	101	227	157

+ = number of positive samples

clinical signs during the first four months. Initially, they manifested depression and anorexia and then, conjunctivitis with discharges from the medial ocular angle was visible. The body temperature in some animals increased to 40-40.2^{o} C. Some disturbances of the oestrus have been registered. The percentage of abortions increased to 18%. The abortions were mostly during the first part of gestation and the animals recovered without treatment. In some animals dermatitis was observed.

The growth of sick animals was retarded and frequently they suffered from secondary bacterial infections, such as colienteritis, salmonellosis, and pneumonia.

The outcome of infection with EAV was determined by the strain and infectious dose of the virus, the age and physiological condition of the infected horse, the environmental conditions and the route of infection.

The results from ELISA for antibodies detection are shown in Table 1. It shows that 158 out of 227 tested blood sera (69.16%) were positive, including samples from all age groups. The positive samples were from all five tested stables. Tested sera, analysed in the specified dilution of 1:40, were determined as positive vs the control sera used in the reaction.

DISCUSSION

Stallions may become persistent EAV carriers and transmit the infection venereally to mares. The duration of the carrier state can be several years, even for the life of an individual stallion. The clinical signs in the different horse categories that we estimated in all 5 stables corresponded to those described by other authors (Mumford, 1985; Paweska and Barnard, 1993; Timoney and McCollum, 1993; Glaser *et al.*, 1997a; Timoney, 1999; 2000a) and were characteristic for EVA infection. Laboratory tests are required to diagnose outbreaks of clinical disease, to screen for subclinical infection and to identify carrier stallions.

Horses are often moved over long distances both nationally and internationally, especially to attend races and for breeding. Other members of the Equidae, including mules, donkeys and zebras are also being transported around the world in ever increasing numbers. Of particular importance in the control of EVA is the detection of carrier stallions, in order to ensure that they are not used for breeding

purposes. A proportion of stallions that recover from infection will become long-lasting shedders of virus in semen. Currently there is no simple way of identifying these animals, e.g. by a blood test, and instead test mating of susceptible mares must be performed, or else semen must be collected and tested for the presence of virus.

The diagnosis is to be confirmed by ELISA positive results for antibodies against EAV virus and by the negative results for the other viral and bacterial infections. (Clayton, 1986; Paweska and Barnard, 1993; Cho *et al.*, 2000; Timoney, 2000b; Chenchev *et al.*, 2002).

In conclusion, EVA has to be considered as a major problem of both medical and economical importance. Our results showed that the disease is spread widely among horses in Bulgaria. The EVA infection was probably imported by semen.

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