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Original Contribution

SEROEPIDEMIOLOGICAL STUDIES OF DONKEYS' BLOOD FOR DETECTION OF SOME VIRUS INFECTIONS ON UNGULATES

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ABSTRACT

The prevalence of antibodies against equine arteritis virus (EAV), equine influenza virus (EIV), equine herpes virus (EHV), equine infectious anemia virus (EIA) and African horse sickness virus (AHS) among donkeys in three regions of Bulgaria was studied. Serum samples of donkeys were tested by ELISA (enzyme-linked immunosorbent assay) for EAV, EHV and AHS, by inhibition hemagglutination test (HI) for EIV and by agar gel immunodiffusion test (test Coggins) for EIA. From 192 samples, 152 (79.1%) had antibodies to EAV (titres ranging from 1:20 to 1:20480), 126 (65.6%) were positive to EIV (1:80 to 1:1280) and 134 (69.7%) to EHV (1:40 to 1:10240). Antibodies against two viruses predominated (43.8% of seroreagents), followed by three (36.5%) and one (17.8%) virus. All sera were negative for EIA and AHS.

Key Words: donkeys, antibodies, prevalence, ELISA, HI test, Coggins test

INTRODUCTION

The donkey is one of the most important work animals, and plays a key role in agriculture and the economy, especially in developing countries. In some industrialized countries, donkeys are kept specifically for recreation, breeding, showing, or companionship. According to data by FAO, the number of these animals worldwide is more than 40 million. Donkeys make up a significant share of all ungulate animals in our country. In 2005, their number was 200000, while the number of horses was 150000, and of mules and hinnies – 15000. Donkeys are specifically useful in the mountain regions of our country, where they are applied to a wide range of activities related to agriculture and forestry.

Even though they are more resistant to contagious diseases than horses (1), donkeys are vulnerable to a number of infections with viral etiology. The main among them are: arteritis, influenza, herpes virus infections, infectious anemia, African horse sickness (1, 2). Equine arteritis virus (EAV), equine influenza virus (EIV) and equine herpes virus (EHV) infections are widespread in the equine populations worldwide (3, 4).

Until now, serological studies in our country have been performed to detect the presence of antibodies against the abovementioned diseases for horses only. A high percentage of seroreagents against the viruses of arthritis, influenza, and herpes virus infections was established. No positive reaction in animals against the viruses of equine infectious anemia (EIA) and African horse sickness (AHS) were found. In the beginning of 2003, influenza was discovered in some regions of the country, caused by the A2 Miami 1/63 strain.

From all presented information, it is apparent that no specific serological studies on donkeys have been ever performed in Bulgaria, to prove the presence of antibodies against the most widely spread viral infections in ungulates.

The aim of the current study was to establish the presence of antibodies against the viruses of arteritis, influenza, rhinopneumonitis,

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infectious anemia, and African horse sickness in donkeys from three regions of Bulgaria.

MATERIALS AND METHODS

Samples

A total number of 192 blood serum samples were examined, obtained from 76 male and 116 female donkeys, aged between 5 and 26 years, from three country regions: northwest (Montana, n=146), central (Stara Zagora, n=20), and northeast (Varna, n=26). The samples collected in March and April from the three regions were uneven in numbers because of the difficulties related to the organization of their obtaining. After separation of sera, we stored them at the temperature of -20˚C until analysis. Samples were studied to detect the presence of antibodies against the EAV, EIV, rhinopneumonitis (EHV), EIA, and AHS in ungulates.

Serological tests

ELISA (*enzyme-linked immunosorbent assay*), was used to determine the presence of antibodies (indirect ELISA) against the causative agents of EAV, AHS, and EHV. The reaction was carried out according to all instructions by the producer of the ELISA kit - Ingenasa-Madrid (5). The reaction was read on an ELISA reader (Dynatex) at wavelength of 450 nm.

The cut-off value was calculated by adding 0.2 to the value of the negative control result. Sera exhibiting absorption values lower than the cut-off value were accepted as negative. Sera exhibiting absorption values higher than the cut-off value + 0.15 were accepted as positive. Serum samples with medium values were considered to be doubtful and were tested again for presence of antibodies.

Hemagglutination inhibition test (HI). To determined the presence of antibodies against the equine influenza virus, we worked with reagents prepared in the laboratory as followed way: U-bottom microplates were used, with the sera being pretreated with 1.90M KJO_4 at room temperature for 60 minutes with subsequent addition of 1% glycerol, dissolved in physiological saline. Additionally, we performed inactivation at 62˚C for 30 minutes to remove nonspecific inhibitors. Tested sera were diluted in ratios from 1:10 to 1:1280 with saline solution. As an antigen, allantoic fluid, containing the virus strains A2 Miami 1/63(H3N8) and A1 Prague 1/56(H7N7), after inoculation in 10-day chicken embryos was used (6). We used the antigens in 4 HA units (4x minimum agglutinating dose, i.e. titre/4 per well) and these are kept at room temperature (23°C ±2°C). After gentle mixing, the chicken RBCs (1% [v/v]) were added in the reaction later. The results of the reaction were read as the highest dilution of serum giving complete inhibition of agglutination in U-bottomed plates.

Agar gel immunodiffusion test (test Coggins). To detect the presence of antibodies against the EIA we used the commercial kit of IDEEX (USA). The conduction of the reaction, including the preparation of the agar, the preparation of the wells, and the used amounts of sera and reagents were done according to the producer's instructions. In brief, immunodiffusion reaction was carried out in a layer of 1% Noble agar (15-17 ml; pH 8.6 ± 0.2) in Petri dishes with 100 mm in diameter. Six wells with 5.3 mm in diameter and 2.4 mm apart were punched out of the agar surrounding a centre well of the same diameter. The dishes were maintained at room temperature in a humid environment. After 24–48 hours the precipitation lines were examined over a narrow beam of intense, oblique light and against a black background.

RESULTS

The summarized results of the serological study of blood sera from donkeys **(Table 1)** showed that 79.1% (from 78% to 84.6% for certain regions) of samples contained antibodies against the EAV, 65.6% (61.5%-70.0%) – against the EIV, and 69.7% (63.0%-100%) – against the EHV. The presence of antibodies against serotype EHV-4 of the herpes virus in ungulates, which is related to EHV-1 was found in donkeys. All sera were negative for the viruses of EIA and AHS **(Table 1)**.

From **Table 2**, it is obvious that only 2% of the samples did not contain antibodies against the EAV, EIV and EHV. In some samples, we found antibodies only against a single virus - 17.8%. Most of them were against EAV – 11.5%, followed by EHV – 4.7% and EIV – 1.6%. In the region of Stara Zagora, seroreagents against a single virus were not discovered. The predominant antibodies were against two viruses - 43.8% (EAV and EHV - 16.7%, EAV and EIV - 15.1%, EIV and EHV - 12.0%). Antibodies against the three viruses were detected in 36.5% of the samples (34.2% in the Montana region, 38.5% in the Varna region, and 50% in the Stara Zagora region).

Table 1. *Summarized results from the serological study of blood sera from donkeys, to detect the presence of antibodies against the EVA, EIV, EHV, EIA and AHS viruses*

Region	Number of sera	Positive			Negative	
		EAV %	EIV %	EHV %	EIA %	AHS %
Montana	146	114 78.0	96 65.7	92 63.0	146 100.0	146 100.0
St. Zagora	20	16 80.0	14 70.0	20 100.0	20 100.0	20 100.0
Varna	26	22 84.6	16 61.5	22 84.6	26 100.0	26 100.0
Total	192	152 79.1	126 65.6	134 69.7	192 100.0	192 100.0

Table 2. *Results from the serological study of blood sera from donkeys for one, two, or three viruses.*

Region	Number of sera	Number of sera without antibodies % %	No. of sera with antibodies						
			Against one virus			Against two viruses			Three viruses
			EAV %	EIV %	EHV %	EAV EIV %	EAV EHV %	EIV EHV %	EAV EIV EHV %
Montana	146	4 2.0	20 13.6	3 2.0	7 4.7	26 17.8	19 13.0	17 11.6	50 34.2
St. Zagora	20	- 0	- 0	- 0	- 0	- 0	6 30.0	4 20.0	10 50.0
Varna	26	- 0	2 7.7	- 0	2 7.7	3 11.5	7 26.9	2 7.7	10 38.5
Total	192	4 2.0	22 11.5	3 1.6	9 4.7	29 15.1	32 16.7	23 12.0	70 36.5

The titres of the antibodies varied for the specific viruses **(Figure 1)**. The highest titres were established for antibodies against EAV (1:20 to 1:20480) with samples with titre of 1:1280 predominating. Titres of positive samples against EHV were 1:40 to 1:10240 (mostly 1:80) and against EIV – 1:80 to 1:1280 (1:80).

DISCUSSION

Our results showed the presence of antibodies in blood sera of donkeys, against the viruses of arteritis, influenza, and equine rhinopneumonitis, for all three studied regions of Bulgaria. No antibodies against the viruses of equine infectious anemia and African horse sickness were found. The negative results on the presence of antibodies against both equine viruses correlate with epidemiological studies and surveys in the country for a period of 10 years. With regard to infectious anemia, the country is free, with the last case dating back to 1993. Until now, no antibodies against the virus of African horse sickness were found in blood sera of ungulates, even though the basic vectors of spreading – the insects of the *Culicoides* genus are available (7).

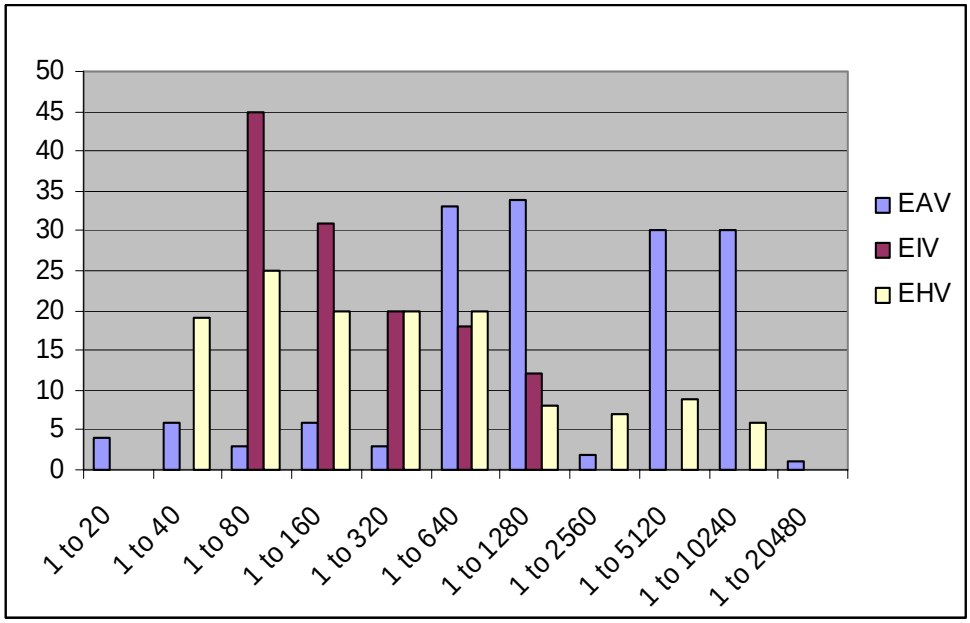

Figure 1. Titres of the antibodies in positive blood sera from donkeys, against EAV, EIV, and EHV.

Our results showed that the highest percentage of seroreagents could be found for the viral agent of arteritis – n=152. The observed titres suggested that there probably was an active circulation of a virulent viral strain, due to the lack of active prophylaxis against it. A study on horses for antibodies against the arteritis virus in five stables in Bulgaria found out 69.16% positively reacted animals (16). The values we got for donkeys: 79.1%, are close to the values for horses. Seroepidemiological studies showed that the horse arteritis virus is predominant in most European countries (8, 9). Our results are close to those reported from Hungary – 65% (10), South Africa – 99.2% (11), and Australia – 73% (12), and are very different from those for Austria-10.9% (13), North-East Tunisia – 8.75% (14), Brazil – 2.2% (15) and the USA – 1.9% of all local horses (16). There was no evidence of the presence of equine viral arteritis among equine population in Thailand (17).

The wide spread of the causative agent of arteritis in the three studied regions of Bulgaria, and probably in the other regions as well, is possibly due to the fact that an active specific immunoprophylaxis is not applied, as well as to the similar clinical features with herpes virus infections on ungulates. Last but not least, is the usage of unchecked animals for mating and breeding purposes, which are a primary factor in the epidemiology and the spreading of diseases among the population of susceptible animals (18). Established high titres of antibodies againt the EAV are possibly due to recent infection, while low levels to past infection (19).

Regarding the situation with the agents of influenza (n=126), and rhinopneumonitis infections (n=134), the overall image is somewhat obscured because of the performed active immunoprophylaxis against these two diseases. Unfortunately, it is not well planned and regularly performed, and that makes it difficult to perform proper prevention and control against these two dangerous virus diseases on ungulates.

In conclusion, in a serological study of blood sera from donkeys, the presence of antibodies against the viruses EAV, EIV, and EHV was detected for all three studied regions of Bulgaria. The antibodies against two viruses (43.8%) were predominant, followed by three (36.5%), and a single virus (17.8%). The primary problem with both medical and economical effect is the equine viral arteritis. Further research on the subject is needed to clarify the epidemiological conditions regarding the three diseases on donkeys, mules and hinnies in the whole country, and to

undertake adequate measures to limit and prevent their incidence.

REFERENCES

1. Gupta, A.K., Rattan, B., Malik, P., Kaur, D., Singh, B.K., Yadav, M.P., Experimental infection of donkeys with EHV-1 of horse origin – a study. *J Equine Sci*, 11:29-33, 2000..
2. Turnbull, A.,Wernery, U., Wernery, R., Anandh, J.P., Kinne, J., Survey of six infectious diseases of feral donkeys in the United Arab Emirates. *Equine Vet Educ*, 14:33-38, 2002
3. Livesay, G.J., O'Neil, T., Hannant, D., Yaday, M.P, Mumford, J.A., The outbreak of equine influenza (H3N8) in the United Kingdom in 1989: diagnostic use of an antigen capture ELISA. *Vet Rec*, 133:515-519, 1993.
4. Paweska, J.T., Gerdes, T., van Heerden, J., Serological relationship between a donkey alphaherpesvirus (isolateM7/91) and equid herpesvirus type 1 and 4. J. S. *Afr Vet Assoc*, 65: 64-66, 1994.
5. Timoney, P.J., The increasing significance of international trade in equines and its influence on the spread of infectious diseases. *Ann N Y Acad Sci*, 916:55-60, 2000.
6. Yamagishi, H., Nagamine, T., Shimoda, K., Ide, S., Igarashi, Y., Yoshioka, I., Matumoto, M., Comparative measurement of equine influenza virus antibodies in horse sera by single radial hemolysis, neutralization, and hemagglutination inhibition tests. *J Clin Microbiol*, 15:660-662, 1982.
7. Chenchev, I., Georgiev, G., Nedelchev, N., Study of the possibilities of occurrence of the African horse sickness virus and Culicoides spp. In Bulgaria in the period 2000-2003. Veterinarna medicina, 8:7-15, 2004.
8. Francesco, C.E., Carluccio, A., Marsilio, F., Sebastiani, C., Scialandrone, M., Infections with herpesvirus and arterivirus and in Martina Franca donkeys. *Obiettivi Doc Vet*, 24:43-46, 2003.
9. Newton, J.R., Wood, J.L., Castillo-Olivares, F.J., Mumford, J.A., Serological surveillance of equine viral arthritis in the United Kingdom since the outbreak in 1993. *Vet Rec*, 145:511-516, 1999.
10. Szeredi, L., Hornyak, A., Palfi, V., Molnar, T., Glavits, R., Denes, B., Study on the epidemiology of equine arteritis virus infection with different diagnostic techniques by investigating 96 cases of equine abortion in Hungary. *Vet Microbiol*, 108:235-242, 2005.
11. Paweska, J.T., Binns, M.M., Woods, P.S. A., Chirnside, E.D., A survey for antibodies to equine arteritis virus in donkys, mules and zebra using virus neutralization (VN) and enzyme linked immunosorbent assay (ELISA). *Equine Vet J*, 29:40-43, 1997.
12. Huntington P.J., Forman, A.J., Ellis, P.M., The occurrence of equine arteritis virus in Australia. *Aust Vet J*, 67:432-435, 1990.
13. Kolbl, S., Schuller, W., Pabst, J., Serological studies of the recent infections of Austrian horses with the equine arteritis. *Dtsch Tierarztl Wochenschr*, 98:43-45, 1991.
14. Ghram, A., Chabchoub, A., Turki, I., Boussetta, M., Ibn Amor, H., Ghorbel, A., Rhinopneumonia and equine viral arteritis: seroepidemiological study in the northeast of Tunisia. *Arch Inst Pasteur Tunis*,71:5-12, 1994.
15. Diel, D.G., de Almeida, S.R., Weiblen, R., Frandoloso, R., Anziliero, D., Kreutz, L.C., Groff, F.H.S., Flores, E.F., Prevalence of antibodies to influenza virus, viral arteritis and herpesvirus in horses of the Rio Grande do Sul state, Brazil. *Cienc Rural*, 36:1467-1473, 2006.
16. Hullinger, P.J., Gardner, I.A., Hietala, S.K., Ferraro, G.L., MacLachlan, N.J., Seroprevalence of antibodies against equine arteritis virus in horses residing in the United States and imported horses. *J Am Vet Med Assoc*, 219:946-949, 2001.
17. Tantaswasdi, U., Punyahotra, R., Wongkasemjit, S., Indrakamhang, P., A serological survey of equine infectious diseases in Thailand. *T J V M*, 28:51-58, 1998.
18. Glaser, A.L., de Vries, A.A., Rottier, P.J., Horzinek, M.C., Colenbrander, B., Equine arteritis virus: a review of clinical features and management aspects. *Vet Q*, 18:95-99, 1996.
19. MacLachlan, N.J., Balasuriya, U.B., Equine viral arteritis. *Adv Exp Med Biol*, 581:429-433, 2006.