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Seronegative Findings on the Investigations of Equine Infectious Anemia in the Marmara Region of Turkey*

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Abstract: A total of 404 horses was examined from the selected cities (Istanbul, Bursa, Balıkesir) of the Marmara region of Turkey. Blood was collected from all horses and the sera were analyzed for the presence of antibodies to equine infectious anemia virus (EIAV) using an agar gel immunodiffusion (AGID) test. The results revealed that none of the horses were positive for antibodies to EIAV.

Key Words: EIAV, horse, AGID, Turkey

Marmara Bölgesinde İnfeksiyöz Anemi Yönünden İncelenen Atlarda Seronegatif Bulgular

Özet: Marmara bölgesinde seçilen kentlere (Istanbul, Bursa, Balıkesir) gidilerek toplam 404 at incelendi. Atlardan kan alınarak serum toplandı ve bu serumlarda atların infeksiyöz anemi virusuna (equine infectious anemia virus=EIAV) karşı oluşan antikorların varlığı agar jel immunodifüzyon (agar gel immunodiffusion=AGID) testi ile araştırıldı. İncelenen atlarda EIAV'una karşı antikor saptanmadı.

Anahtar Sözcükler: EIAV, At, AGID, Türkiye

Introduction

Equine infectious anemia (EIA) is caused by the equine infectious anemia virus (EIAV), which infects the animals in the family *Equidae* and is classified in the subfamily *Lentivirinae* of the family *Retroviridae*. This family also includes the immunodeficiency viruses of primates, bovine, feline and maedi-visna of sheep and arteritis-encephalitis virus of goats (1,2). EIAV is closely related to some of the aforementioned lentiviruses, caprine arthritis and encephalitis virus (CAEV), maedi-visna virus (MVV), feline and human immunodeficiency virus (FIV and HIV) as it shares some common nucleotide sequences (3,4). The cross-reactivity between EIAV, HIV and FIV has also been reported (5,6).

The AGID test, formerly named the Coggins test is commonly used for the diagnosis of EIA (7). The test is a

reference test and is used in many countries (7-9) during importation and exportation since EIA is on List-B as indicated by the Office International des Epizooties (OIE). Other tests such as enzyme linked immunosorbent assay (ELISA) and competitive ELISA (C-ELISA) have also been introduced for the diagnosis of EIA (8, 10-13). Recently, molecular biological techniques have been applied to detect EIAV in tissues and blood (14).

Turkey has a large horse population and these horses are used for working, racing and breeding. The aim of this study was first to determine whether or not EIA is present in the Marmara region of Turkey. This region borders Greece (European Union) and Bulgaria and is separated Asiatic and European parts by the Bosphorus. There is no study on EIA in this region at present. Secondly, to compare sideroleukocyte formation, blood

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biochemistry, PCV, erythrocyte count, leukocyte count and leukocyte percentages in seropositive and seronegative horses if the disease is present in serologically tested animals. Thirdly, to help the government to establish a compulsory monitoring scheme for all horses including horses held on location, being moved, imported or exported, in order to control the disease.

Materials and Methods

Study population and collection of sera

Horses included in the study were from the Marmara region of Turkey. This region is a predominantly agricultural region and has racing horses, army horses, private horses and the working horses of gypsies. The racing horses are mainly in the Turkish Jockey Club and Horse Sport Club located in Istanbul. The army horses are mainly in Bursa. The gypsies have one or two horses for carrying their belongings. Private horses belong to people who breed horses in a small stud either as a hobby or for commercial purposes.

The horses used in this study belonged to private units, the army and gypsies. Owners in selected cities were asked to participate in the study on the basis of willingness. All horses were bled if the owner wanted to participate in the study. Clinical examinations were performed by checking the mouth, nares, eyes, lymph nodes (axillar, mandibular and prescapular) and skin. The breed, sex and age of the animals were recorded. Blood was taken from the jugular vein using vacutainers without anticoagulant. A total of 404 horses was examined and sampled between June 1997 and June 1998. The expected proportion of positives was 1% with a 95% confidence limit and 5% accuracy.

Serological, biochemical and hematological studies

Blood samples were allowed to stand at room temperature for 1 hour. The sera were collected and stored at -20°C until required. The sera were analyzed for the presence of antibodies to EIAV using an AGID test using a commercial antibody test kit (IDEXX Laboratories, Milton Court, Churchfield Road, Buckinghamsire SL9 9EW, UK). The validation of this test was established by the manufacturer in terms of specificity and sensitivity.

The AGID test was performed as described by the manufacturer. Briefly, 1% Noble agar was prepared

using Sodium Hydroxide-Boric acid buffer and 15 ml of mixture was poured into plates (10X10 cm). The plates were cooled and a seven-well pattern of a center well surrounded by six wells were cut.

The center well was filled with the EIAV antigen supplied by the manufacturer. Three of the outer six wells were filled with the positive control sera and the other three with the test sera. The plates were then incubated in a humid chamber for 48-72 hours at room temperature. The presence of precipitin lines was checked every day and the partial identities were recorded.

Hematological and biochemical tests were not performed since no antibodies to EIAV were detected in any of the horses using the AGID test.

Results

Nine owners from the main cities (Istanbul, Balıkesir and Bursa) of the Marmara region agreed to cooperate in the study. A total of 404 horses was tested. These included, 24 stallions, 49 castrated horses and 331 mares. The age of the animals varied from 1 to 17 years old. The age, sex and breed of horses are shown in the Table. Clinical examination of the horses revealed that only the indigenous horses belonging to gypsies had gross lesions on the skin. Abrasions and wounds on the head, chest and legs were observed in these horses. Conjunctivitis and rhinitis were also observed.

All horses were negative for antibodies to EIAV (Table).

Discussion

This is the first study on the frequency of EIA in the Marmara region of Turkey. The study was carried out in selected cities (Istanbul, Bursa and Balıkesir) in which the horse population is high. A total of 9 owners joined the study and 404 horses were sampled. All horses were negative for antibodies to EIAV. In this study, the expected proportion of positives was taken to be 1%. The number of samples designed in this study gives a 95% confidence limit and 5% accuracy.

The AGID test used in this study has also been used in many other countries to detect antibodies to EIAV (1,2, 7,8). In this study, the validation of the test was not established since the specificity and sensitivity were

Table. Individual data of the horses analyzed for the presence of antibodies to EIAV using an AGID test.

Name of the Place	Breed	Sex	Age															Sex			Number of Sera	Number of Owners	Total Tested								
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Stallion				Castrated	Mare						
Kaynarca	Indigenous	Mare					2	2	6	5	3	8	1	2	1	1	1								32	32	32	32			
Aydınevler	Indigenous	Mare					2		4	4		16													26	26	26	26			
Yeni Sahara	Indigenous	Mare						1	3	2	3		1	1											11	11	11	11			
Örnek mh.	Indigenous	Mare					1	3	7	12	8	17	1	3											52	52	46	52			
	French	Castrated		1		2					2	1		2											8		8				
Yeniköy	Russian	Castrated			1	1	1			4	4		1												12		12				
		Stallion					1				1		1												3		3		36		
	English	Mare									1															1		1			
		Castrated			2	2		1				2	1	2	1											11		11			
Terkos	English	Mare												1												1		1			
		Castrated						1			1	1			1											4		4			
	Pony	Stallion				1				1	1			1													4		4		
		Mare						2			4	2		1			1										10	10	7	22	
Veterinary Faculty	Arabian	Castrated											1	1												3		3			
		Mare						1																			1		1		
	English	Stallion			1		1	1																		3		3	15	15	
		Mare					1			1			1														3		3		
	Indigenous	Castrated			1		1						1														3		3		
		Mare							1	1																	2		2		
BALIKESIR	Edremit	France Castrated								1			1														2		2		
		Stallion				2	2																				4		4		
	German	Mare			1										1	3											6		6		
		Castrated																								1		1			
	English	Mare				1								2				1									12		12		
		Castrated				1																				1		1			
	Indigenous	Stallion																								2		2			
		Mare				2		1				1			1												7		7		
	Belgian	Castrated				1	2																			3		3			
		Stallion			2	4						1															7		7		
BURSA	Indigenous	Mare			7	7	4	1				1															20		20		
		Castrated																								1		1			
	English	Mare													1	1	2	1									5		5		
		Castrated																													
	English	Mare			2	2	8		4	8	4	5	4	5	9	6	8	9	14								88		88		
		Castrated			1	3	2	2	6	7	4	4		9	3	2	4		4									51		51	1
TOTAL TESTED					10	17	20	26	14	21	41	40	35	55	22	26	12	17	15	20	13				24	49	331	404	140	404	
TOTAL POSITIVE																															0.0

determined by the manufacturer. Other tests with better sensitivity such as ELISA, C-ELISA and immunoblotting are also used to detect antibodies and antigens of EIAV in horses (10,11,13,15). The AGID test is cheap and easy to perform while ELISA has the advantage of testing large number of sera. False positives due to albumin and transferrin have been reported in ELISA (13). However, false positives can be reduced by using a competitive ELISA with monoclonal antibodies (10,13,16). The advantage of polymerase chain reaction (PCR) over other

tests has been discussed and PCR is not always recommended for routine diagnosis of EIA (14).

There is only one report on the frequency of EIAV in Turkey at present (17); 294 horses from 4 horse breeding studs were analyzed and no antibodies to EIAV were found. The results of this study are similar to those found in the previous study and indicate the absence of the disease in a large number of horses (17). However, several authors in other countries have reported the existence of EIA. In a study in Slovenia, the

seroprevalence was found to be 3.38% (18). The prevalence was 72.8% in Guyana (19). In the USA the prevalence was found to be between 0.27% and 12.8% depending on the region and year (2,20). In Japan, the incidence decreased after the control program introduced in 1978 and has not been reported since 1984 (1).

This study has shown that the EIA is not prevalent in the Marmara region of Turkey. This region borders the EU and there is horse movement because of exportation and importation. This region is important when considering the control of the disease. A control program in this region based on serological testing would promote

the prevention of the disease. Therefore the preventive policy would require a well-coordinated effort by the government and horse owners. These serological testing and slaughtering measures should be compulsory by law as they have proved successful in other countries such as France, England, Japan and the USA (1).

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