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Occurrence of selected parasites and viral infections in horses and donkeys in Turkey

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Summary

The present study aimed to investigate the prevalence of certain protozoa (*B. caballi*, *T. equi*, *T. gondii*, *Neospora* sp.) and viral agents (equine influenza, equine viral arteritis, equine herpesviruses) of equids in Balikesir and its surroundings, in Turkey. Plasma and serum samples were collected from 66 horses and 96 donkeys. *Babesia caballi*, *T. equi* and *Neospora* sp. antibodies were detected with c-ELISA, whereas *T. gondii* antibodies were revealed by the Sabin Feldman Dye test. Viral agents were detected by the PCR technique. The prevalence rates of the protozoa in horses were 12.12% for *B. caballi*, 34.84% for *T. equi*, 9.09% for *T. gondii*, and 10.6% for *Neospora* sp. The molecular prevalence of the viral agents amounted to 3.03% for equine influenza virus and 6.06% for equine herpesvirus 5. Equine viral arteritis virus and other herpesviruses (1, 2 and 4) were not detected in any of the samples. The rates of seropositivity in donkeys were 1.69% for *B. caballi*, 71.87% for *T. equi*, 90.62% for *T. gondii*, 23.95% for *Neospora* sp., 1.04% for equine influenza virus, 0% for equine viral arteritis virus, 3.12% for equine herpesvirus 5, and 0% for other herpesviruses. This study is the first to report the existence of anti-*Neospora* sp. antibodies in donkeys and the seroprevalence of *T. gondii* in horses and donkeys in Western Anatolia, Turkey.

Keywords: equine, equine influenza, equine herpesvirus, infectious disease, *Neospora* sp., *Toxoplasma gondii*, virological agent

Infectious diseases, both parasitic and viral, are a serious problem in equine breeding. Parasitic diseases include *Babesia caballi*, *Theileria equi*, Toxoplasma and Neospora infections. The obligatory intraerythrocytic protozoa *B. caballi* and *T. equi* cause equine piroplasmosis, a tick-borne disease (33). Clinical signs of equine piroplasmosis include icterus, fever, anemia, hemoglobinuria, and bilirubinuria. *Toxoplasma gondii* is a zoonotic and obligate intracellular apicomplexan parasite that can infect nearly all warm-blooded animals. Domestic and wild felids are the definitive hosts of the parasite, and they shed its oocysts with their feces (17). In addition, clinical toxoplasmosis was determined at necropsy in a horse that had shown colic, fever, soft feces, and increased levels of liver enzymes, which is the first case of clinic toxoplasmosis reported in this species (17). In many different species, *Neospora* sp. is one of the most prominent causes of neurological disease and abortion (16). In recent years, there has been an increased interest in using donkeys for onotherapy and as a milk source for pets and especially for children with allergy to cow's milk (8). Additionally, in some countries, donkeys are grown for their meat to be used in traditional Chinese medicine (9).

Many viral diseases have a strong impact on horse breeding (e.g. racehorses, show horses, riding horses) and certain industries. Among these diseases, there are many that settle in the respiratory system (equine influenza virus, etc.) (47), digestive system (equine rotavirus, etc.) (3) and genital system, as well as mortal diseases with a multisystemic character (equine herpesviruses, etc.) (13).

Dagalp et al. (13) reported that EHV-1 is responsible for most of herpesvirus abortions, whereas some abortions are also caused by EHV-4. While EHV-1 causes abortion in mares, as well as neurological disease and respiratory disease in young animals, EHV-4 is commonly responsible for pulmonary disorders. EHV-2 causes immunosuppression, keratoconjunctivitis, pharyngitis, ulcerative lesions of the oral mucosa, upper respiratory tract signs, and poor race performance (19), whereas EHV-5 may cause upper respiratory tract signs and equine multinodular pulmonary fibrosis (38).

Equine influenza virus (EIV), member of the *Orthomyxoviridae* family, causes severe respiratory disease, and infected horses show characteristic clinical signs of the disease, such as pyrexia, dyspnoea, anorexia, and coughing (47).

Equine viral arteritis (EVA) is a contagious acute disease of equids caused by the equine arteritis virus. Its clinical signs include anorexia, depression, leucopenia, edema, urticaria, abortion, and severe pneumonia in foals (14).

The present study was carried out on horses and donkeys in the Balikesir province of Turkey to detect selected protozoa (*T. equi*, *B. caballi*, *T. gondii*, and *Neospora* sp.), and viruses (EHV-1, EHV-2, EHV-4, EHV-5, EIV, and EVA). Furthermore, the objective of this study was to investigate differences in the presence of diseases between regions.

Material and methods

Study design and sample collection. A cross-sectional study was designed to analyze the presence of *T. equi*, *B. caballi*, *Neospora* sp., and *T. gondii*, as well as agents of equine viral diseases, namely, EIV, EVA, EHV-1, 2, 4, and 5, in horses and donkeys in the Balikesir province in the Southern Marmara region of Turkey. Blood samples were collected from horses and donkeys living at five different locations whose approximate coordinates are 39°34'N 26°59'E, 39°12'N 28°18'E, 38°37'N 27°23'E, 39°44'N 27°28'E, and 39°40'N 27°55'E. The animals included in this study had to be apparently healthy and come from the Balikesir province and its surroundings. The horses included in the study were used for sports and were housed individually or in herds of 5-10 animals. The donkeys selected for the study were used for the production of milk for human consumption. All blood samples were collected only for serologic surveillance, and not for therapeutic purposes. The study was conducted with the permission of the Balikesir University Animal Experiments Ethics Committee (confirmation number: 2020/5-12, Date: Aug. 20, 2020). None of the horses and donkeys included in the study had been vaccinated.

The horses were of various breeds. Their ages ranged from 6 months to 20 years, and their mean age was 7.3 years. Out of the 66 horses, 23 (35%) were female and 43 (65%) were male. The donkeys were also of various breeds, aged from 3 months to 17 years, and their mean age was 6.4 years. Out of the 96 donkeys, 94 (98%) were female and 2 (2%) were male.

All blood samples were collected from the jugular vein into Vacutainer tubes and delivered to the laboratory under cold chain conditions. The whole blood samples were centrifuged at 2000 rpm for 5 minutes on the same day, and after separation of the sera, the samples were stored at –80°C until analysis. To assess the health status of the animals, hemogram analyses were performed with an Abacus Junior Vet 5 - Diatron immediately after the blood samples were taken. All animals were given a clinical examination. Animals that were found to be sick according to the results of hematological analysis were not included in the study. Animals that had a fever (above 38.6°C) or any clinical signs, such as diarrhea, coughing, or lameness, that could increase the WBC count atypically (above 20.000 per microliter) were excluded. In the parasitological part of the study, our test algorithm was to investigate the presence of antibodies. In this study, primarily since antibodies could not be determined in the acute period, healthy animals were selected for the study by looking at their hemogram results (neutrophil, lymphocyte, and monocyte count at normal levels) to unbiased exampling. Hemogram analyses (Tab. 5) were performed for both horses (N = 66) and donkeys (N = 96).

Serological examination

***Theileria equi* and *Babesia caballi* analysis method.** A competitive enzyme-linked immunosorbent assay (cELISA) method was used for the detection of *B. caballi* and *T. equi* agents (Veterinary Medical Research & Development cELISA, catalog no: 273-2, VMRD®, Pullman, USA). Similarly, for *T. equi*, Veterinary Medical Research & Development cELISA (catalog no: 274-2, VMRD®, Pullman, USA) was used. The analyses were made according to the manufacturer's instructions (53). The average optical density (OD) at 630 nm wavelength was determined for each microplate using an ELISA reader (ELx 800 UV, Universal Microplate Reader, Bio-Tec Instruments, Inc.). The percent inhibition value for each sample was calculated by the formula % I = 100 – [(sample O.D. value × 100) ÷ (O.D. value of the mean of negative controls)]. Samples with a percent inhibition value of at least 40 were considered positive, whereas samples with a value of less than 40 were considered negative.

***Toxoplasma gondii* analysis method.** For *T. gondii* analysis, the Sabin Feldman Dye test (SFDT) was used (41). Sabin Feldman Dye was performed in the Parasitology Laboratory of Refik Saydam Epidemic Diseases Research Directorate. In this analysis, the *T. gondii* Tr-01 strain was investigated, and an antibody titer of 1/16 or higher was accepted as positive.

***Neospora* sp. analysis method.** A c-ELISA test kit (VMRD®, Pullman, USA) was used to detect anti-*Neospora* sp. antibodies (38). An average optical density (OD) at 630 nm wavelength was determined for each microplate in an ELISA reader (ELx 800 UV, Universal Microplate Reader, Bio-Tec Instruments, Inc.). The percent inhibition value for each sample was calculated by the formula % I = 100 – [(sample O.D. value × 100) ÷ (O.D. value of the mean of negative controls)]. Samples with a percent inhibition value of at least 30 were considered positive, whereas samples with a value lower than 30 were considered negative.

Virological analysis method

Preparation of blood samples. After the blood samples were centrifuged in a temperature of + 4°C for 20 minutes

Tab. 1. PCR primers, genome localizations, and product sizes used in the study

Disease	Primary name	Primary index	Product (bp)	Annealing degree (°C)	Reference
Equine viral arteritis	M1	CTGAGGTATGGGAGCCATAG	411	60	(18)
	M10	GGCCTGCGGACGTGATCG			
Equine influenza virus	INFV-M52C	CTT CTA ACC GAG GTC GAA ACG	244	55	(39)
	INFV-M253R	AGGGCATTTTGGACAAAGCGTCTA			
Equine herpes virus 1	FC2	CTTGTGAGATCTAACCGCAC	1150	60	(49)
	RC	GGGTATAGAGCTTTCATGGG			
	FC3	ATACGATCACATCCAATCCC	188		
	R1	GCGTTATAGCTATCACGTCC			
Equine herpes virus 4	FC2	CTTGTGAGATCTAACCGCAC	1150		
	RC	GGGTATAGAGCTTTCATGGG			
	FC3	ATACGATCACATCCAATCCC	677		
	R4	CCTGCATAATGACAGCAGTG			
Equine herpes virus 2	EHV2L1	GATGGTCTCACCTCTAGCAT	1111		
	EHV2R1	CTGGTGTAACACAGGTCTTC			
	EHV2L2	GGTCTCACCTCTAGCATAAC	817		
	EHV2R2	GCCACACTCTCTTCCTTAGT			
Equine herpes virus 5	EHV5L1	CCAACACAGAAGACAAGGAG	1339		
	EHV5R1	CACGGTGATACAGTCAGAGA			
	EHV5L2	CCAACACAGAAGACAAGGAG	410		
	EHV5R2	AGTTGACCGTCGTTCTAGTG			

at 2000 rpm, 200 µl was taken from the upper liquid and the buffy coat and transferred to a 1.5 ml sterile anRNAase-free tube. It was stored in sterile tubes at –80°C.

Viral nucleic acid extraction. The RNA and DNA of the viruses to be investigated were obtained using the GF-1 Viral Nucleic Acid Extraction Kit (Vivantis, Malaysia). With this kit, total nucleic acid was isolated from the samples using a silica gel spin column.

Reverse transcription of viral RNA. RevertAid ™ First Strand cDNA Synthesis Kit (First Strand cDNA Synthesis Kit Thermo Scientific, Germany) was used for reverse transcription of RNA viruses from nucleic acids obtained after extraction.

Polymerase chain reaction (PCR) technique. Some of the viral infections of horses were examined in our study. For this purpose, 6 different viruses were screened: EVA, EIV, EHV-1, EHV-4, EHV-2, and EHV-5. To obtain relevant products for the gene regions of the viruses, a PCR reaction was performed using the primers and method reported in Table 1. PCR mix components and PCR time/cycle information for the gene regions used in the study are given in Table 2.

Tab. 2. PCR mix components, concentration and program conditions

Component	Concentration	Program
Taq DNA polymerase (5 u/µl)	0.5 µl	
10 X Taq buffer (tampon)	3 µl	
$MgCl_2$ (25 mM)	2.4 µl	95°C – 5 minutes
10 mM dNTP mix	0.5 µl	94°C – 1 minute
Primary – forward (10 pmol/µl)	0.5 µl	(*) C – 1 minute 35 cycles
Primary – reverse (10 pmol/µl)	0.5 µl	72°C – 2 minutes
Deionized water	19.6 µl	72°C – 10 minutes
cDNA	3 µl	
Total	30 µl	

Agarose gel electrophoresis of PCR products. To visualize the amplification products, 1% agarose (Prona, Germany) containing ethidium bromide (SafeView classic, ABM, Canada) was prepared. The PCR products were carefully placed in wells formed by removing the combs by mixing with the loading dye (6 × Loading Dye, Thermo Scientific, Germany). 1 µl of a 100 bp marker (Thermo Scientific, Germany) solution was loaded to determine the approximate product size. Then, the products were subjected to electric current, and DNA bands formed as a result of PCR were visualized in a gel imaging system approximately 25 minutes later.

Statistical analysis. Descriptive statistics were used to analyze data for the whole blood and age. All statistical analyses were performed using SPSS 25.0 (SPSS Inc., Chicago, Illinois).

Results and discussion

A total of 8 horses (12.12%) were seropositive and 58 (87.88%) were seronegative for *B. caballi*, whereas 23 horses (34.84%) were seropositive and 43 (65.16%) were seronegative for *T. equi*. Only one donkey (1.69%) was seropositive and 95 (99%) were seronegative for *B. caballi*, whereas 69 donkeys (71.87%) were seropositive and 27 (28.13%) were seronegative for *T. equi*.

Six (9.09%) of the 66 horses were seropositive for *T. gondii*, and the rest (91%) were seronegative. Among donkeys, 87 animals (90.62%) were seropositive and only 9 (9.38%) were seronegative. With regard to *Neospora* sp. antibodies, 7 (10.6%) of the 66 horses were positive and 59 (89.4%) were negative, whereas 23 (23.95%) of the 96 donkeys were positive and 73 (76.05%) were negative.

Out of the 66 horses, 2 (3.03%) were positive and 64 (96.97%) were negative for the equine influenza virus, while among the 96 donkeys, only one (1.04%) was positive and 95 (98.96%) were negative. No sample from either species was found to be positive for the equine viral arteritis virus. None of the samples were found to be positive for any herpesvirus strain, except EHV-5. A total of 4 horses (6.06%) were found to be positive and 62 (93.94%) were negative for EHV-5,

Tab. 3. Disease detection rates in horses

Region		*B. caballi* (Ab)	*T. equi* (Ab)	*T. gondii* (Ab)	*N. caninum* (Ab)	EVA (Ag)	EHV-1 (Ag)	EHV-2 (Ag)	EHV-4 (Ag)	EHV-5 (Ag)	EIV (Ag)
City Center	Positive (N)	8	9	6	3	0	0	0	0	2	1
	Negative (N)	29	27	29	31	35	35	35	35	34	35
	Detection Rate (%)	21.62%	25.00%	17.14%	8.82%	0%	0%	0%	0%	5.88%	2.77%
Sindirgi province	Positive (N)	0	6	0	2	0	0	0	0	1	1
	Negative (N)	17	12	18	17	18	18	18	18	16	17
	Detection Rate (%)	0%	33.33%	0%	10.50%	0%	0%	0%	0%	5.88%	5.55%
Balya province	Positive (N)	0	8	0	2	0	0	0	0	1	0
	Negative (N)	12	4	13	11	13	13	13	13	12	12
	Detection Rate (%)	0%	66.66%	0%	15.30%	0%	0%	0%	0%	7.69%	0%
Total	Detection Rate (%)	12.12%	34.84%	9.09%	10.60%	0%	0%	0%	0%	6.06%	3.03%

Explanations: Ab – antibody; Ag – antigen (virus nucleic acid detection)

Tab. 4. Disease detection rates in donkeys

Region		*B. caballi* (Ab)	*T. equi* (Ab)	*T. gondii* (Ab)	*N. caninum* (Ab)	EVA (Ag)	EHV-1 (Ag)	EHV-2 (Ag)	EHV-4 (Ag)	EHV-5 (Ag)	EIV (Ag)
Manisa	Positive (N)	1	47	59	5	0	0	0	0	2	1
	Negative (N)	58	12	1	54	59	59	59	59	57	58
	Seropositivity Rate (%)	1.69%	79.66%	98.3%	8.4%	0%	0%	0%	0%	3.38%	1.69%
Sindirgi	Positive (N)	0	7	9	7	0	0	0	0	0	0
	Negative (N)	11	4	2	4	11	11	11	11	11	11
	Seropositivity Rate (%)	0%	63.63%	81.81%	63.63%	0%	0%	0%	0%	0%	0%
Edremit	Positive (N)	0	15	19	11	0	0	0	0	1	0
	Negative (N)	26	11	6	15	26	26	26	26	25	26
	Seropositivity Rate (%)	0%	57.69%	76,00%	42.30%	0%	0%	0%	0%	3.84%	0%
Total	Seropositivity Rate (%)	1.04%	71.87%	90.62%	23.95%	0%	0%	0%	0%	3.12%	1.04%

Explanations: Ab – antibody; Ag – antigen (virus nucleic acid detection)

Tab. 5. Whole blood analysis of horses and donkeys

Species		White Blood Cell 10*9/l	Lymphocyte 10*9/l	Monocyte 10*9/l	Neutrophile 10*9/l	Eosinophile 10*9/l	Basophile 10*9/l	Red Blood Cell 10*12/l	Haemoglobine g/dl	Pocket Cell Volume %	Platelet 10*9/l
Horse	N	66	66	66	66	66	66	66	66	66	66
	Mean	10.80	3.72	0.29	6.14	0.54	0.15	9.34	16.9	48.1	133
	Std Deviation	3.52	1.84	0.24	2.72	0.57	0.78	2.11	14.25	39.1	60.7
Donkey	N	96	96	96	96	96	96	96	96	96	96
	Mean	14.51	6.73	0.50	6.19	0.91	0.22	6.65	12.6	38.4	162
	Std Deviation	4.21	2.74	0.41	2.83	0.70	1.04	1.43	2.69	7.8	69.3

whereas among the donkeys, 3 animals (3.12%) were positive, and 93 (96.88%) were negative for that virus. Data on the occurrence of parasitic diseases and virologic detection are shown in Table 3 for the horses and in Table 4 for the donkeys. The results of the whole blood analysis are given in Table 5.

In the present study, the occurrence of some important parasitological and virological agents was investigated in horses and donkeys in Balikesir and its surroundings, in Turkey. The results suggest that piroplasmosis agents are generally common in both species. The exception is *B. caballi*, which is not common among donkeys. In contrast, *T. equi* was very common in donkeys. Whereas *B. caballi* was detected in both species at only one sampling location, *T. equi* was detected in both species at all sampling locations. In contrast to the present study, Acici et al. (1) found that *B. caballi* was more prevalent than *T. equi* in both horses and donkeys. Likewise, Teodorowski et al. (46) reported a *T. equi* seroprevalence of only 7.2% in horses in Poland. They used the PCR method, which may explain their low seroprevalence result. Furthermore, Machado et al. (31) reported seroprevalence rates of 93% for *B. caballi* and 73% for *T. equi* in donkeys. In contrast to these studies, Garcia-Bocanegra et al. (20) reported seroprevalences of *B. caballi* lower than those of *T. equi* in both horses and donkeys. According to these authors (20), the seroprevalence of *B. caballi* was 7.9% in horses and 17% in donkeys, whereas the seroprevalence of *T. equi* was 17% in horses and 47.2% in donkeys. Considering that the chronic form of *T. equi* is more prevalent in donkeys than in horses, and the acute form of the disease is rarely observed in donkeys, the results of that study are highly compatible with ours (52). *Theileria equi* was detected significantly more often than *B. caballi* in both species, and our results appear to be in agreement with previous reports from endemic countries (39, 45).

In this study, a prominent difference was found between the occurrence of *T. gondii* in both species. In humans and horses, an association has been described between low income and increased exposure to *T. gondii*. High exposure to the parasite may be due to poor sanitary conditions in low-income populations, which may increase the probability of contamination in water sources or elsewhere (30, 41). Similar factors apply to both horses and donkeys, but the danger of donkeys being infected with toxoplasma oocysts is increased by the extensive rearing of donkeys, their uncontrolled wandering in the environment, being housed in barns devoid of biosecurity measures (e.g. domestic cats can easily enter barns and mangers), and drinking from unhygienic stagnant water. In the farms and barns where the samples were collected, it was observed that domestic cats could easily enter the barns, conditions in the donkeys' barns were extremely bad, and their diet consisted of only pasture and hay. The donkeys whose blood samples we collected were kept in poor sanitary conditions, compared with those of the horses, which can explain the difference in the presence of specific antibodies to the parasite between the two species. The seroprevalence of the parasite in horses is highly variable from one country to another, ranging from 1% in Sweden (27) to 71% in Iran (24). The difference in seroprevalence may be related to differences in hygienic conditions, managing, and feeding practices (30). According to seroprevalence results from different countries of the world, the parasite is more prevalent in donkeys than in horses, e.g. its seroprevalence in Brazil (35) was reported as 72% in donkeys and 27% in horses. However, because there are fewer studies evaluating both species in the same cohorts, it is difficult to determine whether these results are caused by the naturally higher susceptibility of donkeys or their poor sanitary conditions. Nevertheless, there are several suggestions that horses are naturally resistant to *T. gondii* infection (17) or that they develop very low antibody titers, which cannot be detected by serological tests (15). Likewise, Garcia-Bocanegra et al. (21) reported that the seroprevalence of *T. gondii* was higher in animals that were kept outdoors than in those kept indoors. Moreover, Alvarado-Esquivel et al. (4) observed that horses generally received better care than donkeys in terms of the quality of fodder and drinking water. Our results are compatible with those of previous studies from different countries (20, 35), which show higher seroprevalence in donkeys. Studies on the seroprevalence of *T. gondii* in horses in Turkey were performed by Gazyagci et al. (22) and Karatepe et al. (28), who reported it as 36% and 7%, respectively. The result of this study (9.09%) is in agreement with the above reports, especially that by Karatepe et al. (28). Unfortunately, there are not many reports on the seroprevalence of *T. gondii* in donkeys in Turkey. Balkaya et al. (7) found that the prevalence of the parasite was 62%, which is also compatible with our result. However, *T. gondii* is common not only in equids, but also in other mammalian species, such as sheep, cattle, cats, dogs, and humans, in Turkey and worldwide (21, 25, 48). It can be suggested that *T. gondii* is highly prevalent in Turkey in both humans and other mammalians, which is a very prominent problem for public health (7, 15).

Neosporosis is not considered to be zoonotic, and its most important effect are economic losses caused by the disturbance of reproduction of cattle and small ruminants (16). However, because of the consumption of donkey and horse meat in some countries and increased interest in the general use of these animals (e.g. for onotherapy or as wood/baggage carriers), several studies have been performed. Waap et al. (50) reported that the seroprevalence of *Neospora* sp. in horses and donkeys was 9%. Bartova et al. (8) found that the seroprevalence of the parasite in horses was 8%, but all donkeys were negative. Kligler et al. (29) found the seroprevalence of *Neospora* sp. in horses to be 12%, which was significantly less than the seroprevalence for donkeys living in similar locations in Israel. In contrast to studies reporting low or no seropositivity (8), Gharekhani et al. (23) found the seroprevalences of *Neospora* sp. to be 40% in horses and 52% in donkeys. According to Tirosh-Levy et al. (48), it was as high as 70% in donkeys. Sevgili et al. (44) reported the seroprevalence of *Neospora* sp. in Thoroughbred mares in Sanliurfa, Turkey, to be 8%. In the present study, the seroprevalence of *Neospora* sp. was 10.6% in horses and 23.95% in donkeys, which is compatible with earlier reports (8, 49), especially those from the rural regions of Israel (29). Furthermore, to the best of our knowledge, this is the first report on the presence of anti-*Neospora* sp. antibodies in donkeys in Turkey.

Reports on the seroprevalence of EVA are globally inconsistent, as it ranges from 0% to 20% between countries (12, 32, 49). It is believed that this variation depends on horse population and surveillance in a given country (49). Nejat et al. (37) found the seroprevalence of EVA to be 4% in Iran, and Cruzet et al. (12) reported it as 16% in Spain. Similarly, Marenzoni et al. (32) and Turan et al. (49) reported the seroprevalence of EVA in horses in Turkey as 16% and 14%, respectively. In contrast to previous studies that detected antibodies, our study aimed at the viral detection of EVA. In this study, there was no sample positive for the EVA virus in either of the species living in the same region of Turkey. The rates of detection of EVA are highly changeable not only in Turkey, even within the same region.

Many seroprevalence studies were performed regarding EIV in horses and donkeys, and its seropositivity rate is highly changeable. The seropositivity rate of EIV in horses was 38% in Mexico according to Blitvich et al. (10), 11% in Pakistan according to Sajid et al. (43), and 93% in Australia according to Happold and Rubira (26). Ataseven and Daly (5) performed a large-scale EIV seroprevalence study on more than 600 equids from five different regions of Turkey. The overall seroprevalence of the virus was found to be 31%. The highest overall rate for EIV was found in the Marmara region (60%), where the present study was also performed, and the seropositivity for horses (41%) was more than four times as high as it was for donkeys (9%). According to Timurkan and Aydin (47), the seropositivity for antibodies to EIV in jereed horses in the province of Erzurum in eastern Turkey amounted to 26.3%, but they could not detect virological positivity. In the present study, the virological presence of EIV was revealed in 3% of horses and 1% of donkeys. Virus detection studies in the world are very limited. Therefore, in order to discuss the situation in the world and in Turkey, seroprevalence studies are examined in this section. It can be said that our detection rate was comparatively low because previous studies were aimed at detecting the presence of antibodies. The present study aimed to detect viruses by the PCR method, whereas in other studies the ELISA method was used to detect antibodies. Therefore, a complete comparison could not be made, but it was determined that the virus was circulating in horses and donkeys in the region where the study was conducted.

Among viral diseases with the strongest impact on the horse industry are those caused by herpesviruses. That is because herpesviruses have latent characteristics and are a group of diseases that spread in the heard from time to time and threaten the herd (13). Therefore, the presence of herpesviruses was investigated in this study. Many studies of EHV-1, 2, 4, and 5 in both healthy and unhealthy horses have been performed, producing different and contradictory results. The presence of antibodies to the virus, has been examined in many countries, usually by serological studies. Cruz et al. (11) reported that the seroprevalence of EHV in purebred horses in Spain was 26% for EHV-1 and 2% for EHV-4. However, neither EHV-1 nor EHV-4 was detected in the present study. In Turkey, Dagalp et al. (13) reported seroprevalence rates of 3.4% for EHV-1, 58.6% for EHV-4, 58.6% for EHV-2, and 75.9% for EHV-5. It is important to note that in all studies seroprevalence was detected by either PCR or ELISA. It is possible that the failure to detect EHV-1 and EHV-4 in our study was due to the inclusion of random but healthy horses, the use of PCR, and the fact that both viral agents caused subclinical infections. Negussie et al. (36) investigated the presence of EHV-1, -2, -4, and -5 in equids with and without clinical symptoms. In horses with clinical symptoms, detection rates for both EHV-1 and EHV-4 were found to be lower (8.1% and 7.5%, respectively) than those for EHV-2 (25%) and EHV-5 (28%). Furthermore, in horses without clinical symptoms, the detection rates for EHV-2 and EHV-5 were 7% and 17%, respectively, but no horses were positive for EHV-1 and EHV-4. Similarly, Akkutay et al. (2) investigated horses with and without clinical signs and found seroprevalence rates of 59% for EHV-2 and 62% for EHV-5. According to our literature search, there are fewer studies on the seroprevalence of herpesviruses in donkeys. Negussie et al. (36) found several types of EHV in donkeys with clinical signs, and their seroprevalence rates were 19% for EHV-1, 4% for EHV-2, 9% for EHV-4, and 7% for EHV-5. However, asymptomatic donkeys were all seronegative. Mekonnen et al. (34) found that the positivity for antibodies to EHV-1/EHV-4 in donkeys in Ethiopia amounted to 74%. EHV-2 antibodies were found more often in horses (54%) than in donkeys (4%). In contrast, EHV-5 antibodies were found more often in donkeys (56%) than in horses (18%). Ataseven et al. (6) reported a 24% detection rate for EHV-1 in donkeys in Turkey. The proportion of equids showing clinical signs was significantly higher among EHV-2-positive animals than among EHV-2-negative ones. Since there have been almost no virological studies in our country, we chose to search for the virus directly, rather than for the antibodies. Research in the present study was also carried out at the level of herpesviruses. For this latent infection, only positivity for EHV-5 (6.06%) was detected. Antigenic determinations are very difficult in periods when latent infections are not reactivated by nature. As mentioned earlier, we managed to detect EHV-5 in our study population, but no other herpesviruses were detected, because of the random collection of samples and the inclusion of animals without clinical signs in the study. In future studies, it is recommended to determine the presence of the virus by selecting target-oriented populations with clinical findings.

Most of the samples came from unregistered animals with general use such as hobby and carrying. In Turkey's rural areas, such equids are used as transport and pack animals or in traditional gaming. Piroplasmosis can be considered prevalent in the research-exampling field. *Theileria equi* was found to be very common in donkeys, which can be an infection source for racehorses. Surprisingly, *Toxoplasma gondii* was also highly prevalent in donkeys. The management and health conditions of donkeys have been observed to be poor. They had close contact with domestic felids, were allowed to range freely in the environment, and received insufficient anti-parasitological drug therapy. Considering the increase in donkey's milk consumption and in the use of donkeys in recent years, it is important to monitor the seroprevalence of *T. gondii* in donkeys. Furthermore, the fact that their meat or carcasses are consumed by wild and domestic felids, can be a serious problem for public health because these carnivores can shed oocysts into the environment and water sources with their feces. Similarly, *N. caninum* can create serious problems, especially in ruminant breeding, because it causes abortions and other economic losses. The prevalence of viral agents examined in this study was not critically high. However, their presence was determined only by PCR. The reason for the higher detection rates of viral agents in horses than donkeys may be that horses are raised in close contact in poorly ventilated and cramped barns.

This study was comprehensive in the sense of investigating both protozoal and virological diseases in horses and donkeys. Similar studies in different regions of Turkey will provide valuable information about the prevalence of the aforementioned infectious agents, which pose a serious threat for human and animal health. Moreover, this research is important because it is the first to confirm the presence of anti-*Neospora* sp. antibodies in donkeys in Turkey and to detect antibodies to *T. gondii* in horses and donkeys in western Turkey.

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