Acta Scientiae Veterinariae, 2024. 52: 1968.

RESEARCH ARTICLE
Pub. 1968

ISSN 1679-9216

SARS-CoV-2 in Horses in the Thrace Region of Turkey

Ali Uslu[1], Asli Balevi[1], Zafer Sayin[1], Cumhur Adiay[2], Mustafa Agah Tekindal[3] & Osman Erganis[1]

ABSTRACT

***Background*:** People spent more time at home as part of the precaution taken after the COVID-19 pandemic. In this stage, human beings began to own more pets and spend more time with them. One of the most significant questions during this period was whether COVID-19 could be transmitted to pets. Beta coronavirus was already known in the veterinary field to cause diseases with different pathogenicity in many animal species. Over time, research has shown that the disease can be transmitted to pets and wild animals. Many studies have shown that equines could be infected with this disease. This study serologically investigated whether COVID-19 was transmitted to horses from humans.

***Materials, Methods & Results*:** In this cohort study, 510 horse serums were collected from Canakkale (n = 80), Istanbul (n = 200), Tekirdag (n = 75), and Kirklareli (n = 155), provinces in the Thrace region of Turkey. The sampling was conducted in October and December 2021, when the SARS-CoV-2 pandemic. The samples were grouped as 0-1, 1-5, 6-10, and 11-15 ages. Enzyme-linked immunosorbent assay (ELISA) plates were coated with the whole inactive SARS-CoV-2 virus at five µg/ml. Hyperimmunized horse sera was used as a positive control, and newborn foal serums that did not drink colostrum were used as a negative control. Positive and negative control sera were also tested by virus neutralization assay (VNA). Primary and secondary serum dilution was determined using the checkerboard method. Accordingly, the test serum dilution was 1/12,800, and the secondary antibody dilution was 1/2,500. The classical indirect ELISA steps were followed. Horses, 192 (37.6%) were bred in riding clubs, and 318 (62.4%) were bred in individual barns. OD values ranged between 0.05-1.39. The cut-off value prepared by ROC analysis with 100% sensitivity, 99.78% specificity, and 95% confidence interval was defined as the OD 0.78 limit value in determining positivity. In this study, 60 (11.76%) horses tested serologically positive for SARS-CoV-2. The highest infection rates were in Istanbul (13%) and Kirklareli (13.3%), and the lowest were in Canakkale (8.75%). According to the chi-square test, there was no statistically significant difference in infection rates between cities ($P > 0.05$). It was determined that horses in the 6-10 age group and in riding schools had a statistically higher rate of contracting the disease than horses in other age groups and barn ($P < 0.05$). The infection rate was found to be very low in horses younger than 1-year-old and close to 15-year-old.

***Discussion*:** The infection was more intense in the 1-10 age group and riding clubs. The lower incidence of disease was seen in old and young horses because of their lack of use in equestrian activities. According to the results of this study, 11.76% of 510 horses were found to be SARS-CoV-2 positive. As a result of another study conducted on the presence of SARS-CoV-2 in 587 racehorses with a single or several fixed riders, it was reported that 5.9% was positive. In this respect, the results were found to be compatible. The SARS-CoV-2 seropositivity rate in cats and dogs has been reported as 4-43.8% and 3.4-23.5%, respectively. It was thought that the lower infection rate in horses as pets compared to dogs was related to the fact that horse riding was done outdoors, and COVID-19-positive individuals were prohibited from going out to do these activities in the acute period and rested at home during the shedding period. Compared to this study and other studies, it has been determined that the infection in horses is silent and has a short viremia period, and this related seropositivity can be detected. Sick humans should be more careful about zooanthropozoonosis diseases that can be transmitted from humans to horses.

Keywords: COVID-19, cohort study, horse, SARS-CoV-2, seropositivity, zooanthropozoonosis.

DOI: 10.22456/1679-9216.141369

Received: 20 July 2024 Accepted: 12 October 2024 Published: 29 October 2024

[1]Department of Microbiology, Faculty of Veterinary Medicine, Selcuk University. Konya, Turkey. [2]Pendik Veterinary Control Institute, İstanbul, Turkey. [3]Department of Biostatistics and Medical Informatics, Faculty of Medicine, Izmir Katip Celebi University. İzmir, Turkey. CORRESPONDENCE: A. Uslu [aliuslu@selcuk.edu.tr]. Faculty of Veterinary Sciences - Selcuk Üniversitesi. 42003 Konya, Turkey.

INTRODUCTION

The Coronavirinae virus family is divided into 4 subgroups: Alphacoronavirus (αCoV), Betacoronavirus (βCoV), gammavirus (γCoV) and Deltacoronavirus (δCoV) [1]. In the veterinary field, coronavirus infections are frequently seen in animals like: Bovine coronaviruses (BCoVs) (βCoV) in calves, Equine Coronavirus (ECoV) (βCoV) in foals, Canine coronavirus (CCoV) (αCoV) in dogs and Feline Coronavirus (FCoV) (αCoV) in cats [2,17]. Humanity encountered the coronavirus for the 1st time as an epidemic: SARS-CoV-1, which occurred in Foshan, Guangdong, China, in 2002 [8]. Then MERS-CoV infection originated in camels and appeared in the Arabian Peninsula in 2012 [16]. Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) emerged in Wuhan, China, in 2019 and caused the pandemic [31]. It is known that the source of SARS-CoV-2 emerged from a bat and then spread to the human population with ACE2 receptor [5]. Only interhuman infection was reported at the beginning of the pandemic, but it was unclear whether pets were infected. However, in some countries, symptoms similar to those observed in humans have been observed in pet animals [25]. Recent research has indicated that pets and even wild animals are infected [24]. It has been reported to be as pathogenic in the US white-tailed deer population as it is in humans [17]. In experimental infections of farm animals, contamination occurred, and antibodies were formed, but no virus shedding was observed [22,28]. Although not as close as pets, horses and humans have a close relationship. This study aimed to determine serologically, to what extent the SARS-CoV-2 virus was transmitted from humans to horses.

MATERIALS AND METHODS

Sample collection

The blood samples were collected from horses from individual horse breeding barns and riding clubs in the Thrace regions of Turkey between October and December 2021. Horses whose blood was taken from 4 different provinces: Canakkale (n = 80) [40°12′0″N, 26°24′0″E], Istanbul (n = 200) [41°0′49″N, 28°57′18″E], Kirklareli (n = 75) [41°40′48"N,27°26′ 1.4″E], and Tekirdag (n = 155) [41°1′19.2″N,27°25′21″E], a total of 510 healthy horses. During the study period, no samples could be taken from Edirne [41°17′16.8″N, 26°31′17.4″E] due to the pandemic measures in the Thrace region of Turkey. The samples were divided into 4 age groups: 0-1, 1-5, 6-10, and 11-15 years. Blood was drawn from the horses' jugular veins in a sterile condition. Samples were left for clotting, and serum was obtained by centrifugation (10 min at 3000 *g*) and stored at -20°C. The horses' serum samples were collected epidemiologically through a cohort study by random screening. The sample was carried out during the COVID-19 pandemic when the disease was still prevalent in people in Turkey, and the Ministry of Health was taking precautions.

Enzyme linked immunosorbent assay (ELISA) and virus neutralization assay (VNA)

The SARS-CoV-2 whole inactive virus hCoV-19/Turkey/Pen07/2020 strain (GISAID Access. ID: EPI_ISL_491476) (D614G mutant) used in coating the ELISA plate in this study was obtained from the project "TUBITAK 18AG020" named "Development of Inactive Covid19 Vaccine." CovidSera[1] obtained from hyperimmunized horses was used as positive control serum, and serum of newborn foals that did not drink colostrum was used as negative control. For the reliability of the ELISA test, positive and negative control sera were also subjected to virus neutralization assay (VNA) and assay procedures were performed as described in previous studies [10]. To determine the appropriate dilution rate with checkerboard test, positive and negative control serums were diluted up to 1/3,276,800, starting from 1/100 dilution. To determine the ideal sensitivity and specificity, the secondary antibody was diluted at a rate of 1/1,250 and 1/2,500, starting from the 1/5,000 dilution rate recommended by the commercial company. It was determined that the ideal sensitivity and specificity for samples and controls serum dilution were at 1/12,800 dilution, for secondary antibody dilution was at 1/2,500 dilution, and these dilutions were used for the test. SARS CoV-2 antigen (200 µg/mL) diluted with 0.01 M phosphate-buffered saline (PBS) pH 7.2 at 5 µg/mL in each flat bottom 96 well ELISA plates[2] and incubated overnight at +4°C. 0.01 M PBS 0.05% Tween 20, pH 7.2 (PBS/T) was used as the washing solution, and it was washed 4 times. Then, incubated with blocking solution (5% skim milk powder in PBS) for 2 h at room temperature, washed previously describe. Samples and positive negative control sera were diluted 1/12,800 with PBS/T and incubated overnight at +4°C. At the

end of incubation, after washing 5 times with washing solution, secondary antibody against horses produced in goat (product no:5220- 0370)[3] diluted with 1/2,500 PBS 5% milk powder was added and incubated for 2 h at room temperature. When the secondary antibody stage was completed, the washing step was completed as in the previous step, and o-phenylenediamine dihydrochloride[4] in phosphate-citrate buffer with sodium perborate was added and incubated for 30 min. A stop solution was added as a final step, and the results were evaluated after the plates were read with an ELISA reader at 450 nm [11,29]. For the test to be validated, the ELISA test was repeated 3 times for each sample, and the results were obtained by averaging these values.

Statistical analysis

The data were evaluated in the statistical package program IBM SPSS Statistics Standard Concurrent User V 26[5]. Descriptive statistics were given as number of units (n), percentage (%), mean ± standard deviation ($\bar{X}$ ± ss), median (M), minimum (min) and maximum (max) values. When the differences between 3 or more groups were wanted to be evaluated, the "Kruskal Wallis H test" was used because the data did not show a normal distribution. The "Roc Curve" analysis method was used to compare the diagnostic performances of 2 or more diagnostic or laboratory tests. Pearson and Fisher's exact tests were used to compare categorical variables with each other. A value of $P < 0.05$ was considered statistically significant.

RESULTS

According to the study results, 223 (43.7%) horses were male, and 287 (56.3%) were female. The horses included in the study were 65 (12.7%) in the 0-1 age group, 213 (41.8%) in the 1-5 age group, 128 (25.1%) in the 6-10 age group, and 104 (20.4%) in the 11-15 age group. In the study, there were 80 (15.7%) horses from Canakkale, 200 (39.2%) from Istanbul, 75 (14.7%) from Kirklareli, and 155 (30.4%) from Tekirdag among the provinces where the sampling was done (Figure 1). Of these horses, 192 (37.6%) were bred in riding clubs, and 318 (62.4%) were bred in individual barns. OD values ranged between 0.05-1.39 (Table 1).

According to the virus neutralization assay (VNA), neutralizing antibodies up to 1/102,400 were found in positive control serum samples, while no titers were detected in negative control sera. In order to precisely determine the true positivity and negativity limit, the cut-off value prepared by ROC analysis prepared from positive controls at a dilution rate of 1/12,800 was defined as the OD 0.78 limit value in determining positivity. Values higher than this value were considered positive (Table 2).

The OD under the curve was found to be 1,000 (100.0%) and was statistically significant ($P < 0.001$). This value showed that it was 100.0% strong in distinguishing the positive control value. The cut-off limit value was accepted as > 0.78.

The cut-off value was 0.78 according to the ROC curve analysis performed with 100% sensitivity, 99.78% specificity, and 95% confidence interval of the OD values (Figure 2). From 510 horse serum samples, 60 (11.76%) sera samples were found to be positive against coronavirus with an ELISA test. The highest SARS-CoV-2 sero-positivity rate in horses was in the Kirklareli province (13.3 %), then Istanbul (13%), Tekirdag (10.96%), and the lowest in Canakkale (8.75%) (Table 3).

According to result, horses are compared according to barn style and age (Table 4). There is a statistically significant difference in the horses housed in riding schools in the 6-10 age group compared to other age groups. The study results showed that infection rates in equestrian clubs did not differ by province [$P = 0.172$] (Table 5). No difference in the infection rate between provinces could be detected. Interestingly, considering the human mobility and infection rate in Istanbul, it is thought that the transmission to horses would be higher. However, a similar positivity was detected in horses in Kirklareli as in Istanbul. It was thought that this might occur due to a similar shedding rate among individuals who requested horse riding schools in these provinces. The positivity rate in Canakkale was the lowest in the province.

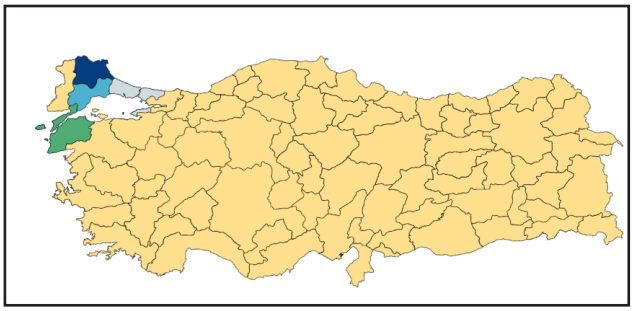

Figure 1. On the map of Turkey, the provinces where samples from the Thrace region were collected are Istanbul (n = 200) in gray, Kirklareli (n = 75) in dark blue, Tekirdag (n = 155) in cyan, and Canakkale (n = 80) in green.

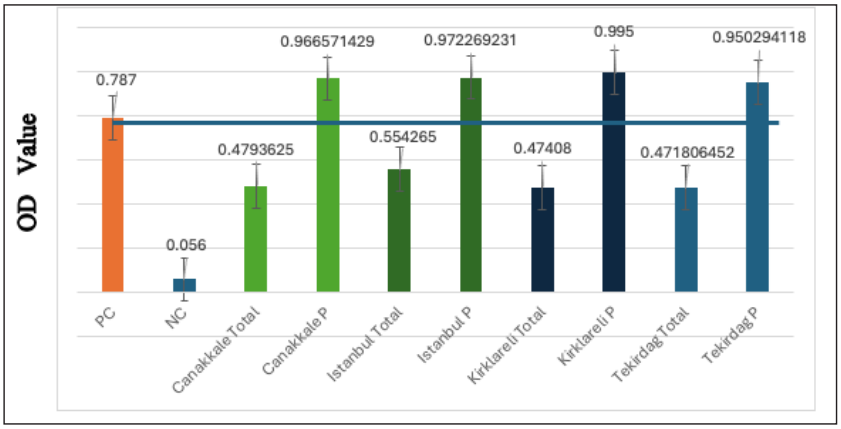

Figure 2. Mean(sd) compared results of groups ELISA Cut-off value-line (0.78) determined with 100% sensitivity and 99.78% specificity according to ROC analysis, [Total: Average OD values with standard deviation against SARS-CoV-2 of all horses in the city, P: Average OD value, including standard deviation ($P < 0.05$), of SARS-CoV-2 Positive horses with cut-off value OD above 0.78 in the city].

Table 1. Introductory features about the animals sampled in the study (mean ± SE).

Variables	Statistics
Gender, n (%)	
Male	223 (43.7)
Female	287 (56.3)
Age, n (%)	
0-1	65 (12.7)
1-5	213 (41.8)
6-10	128 (25.1)
11-15	104 (20.4)
City, n (%)	
Canakkale	80 (15.7)
Istanbul	200 (39.2)
Kirklareli	75 (14.7)
Tekirdag	155 (30.4)
Type of Stable, n (%)	
Horse Riding Club	192 (37.6)
Barn for individual Horse Breeding	318 (62.4)
OD	
$\bar{X}$ ± SD	0.5 ± 0.22
Cut-off ± SD	0.78 ± 0.07
M (min-max)	0.46 (0.05-1.39)

Average; SE: Standard Error; SD: Standard Deviation; M: Median; %: Row percentage.

Table 2. ROC analysis for positive control value (mean ± SE).

	The Area under the curve (AUC)	SE	*P*	Area under the curve (AUC) 95% Confidence limits		Sensitivity	Specifity	Cut-off Threshold
				Lower Limit	Upper limit			
OD	1.000	< 0.001	< 0.001	0.993	1.00	100.00	99.78	> 0.78

SE: Standard Error.

Table 3. Comparison of disease density by city (mean ± SE).

City	Number	Positive		Negative		Total
		%	N	%	N	
Canakkale	80	8.75	7	91.25	73	7/73 [a]
Istanbul	200	13%	26	87	174	26/174[a]
Kirklareli	75	13.30%	10	86.7	65	10/65[a]
Tekirdag	155	10.96%	17	89.04	160	17/160[a]
Total	510	11.76%	60	88.24	450	60/450

[a]No statistical difference could be detected between cities: $P > 0.05$, χ^2: (Chi-Square test); SE: Standard Error.

Table 4. Comparison of SARS-CoV-2 infection in horses according to type of barn and age (mean±SE).

City	Type of Stable	0-1		1-5		6-10		11-15		Total	
		%	N	%	N	%	N	%	N	%	N
Canakkale	Horse Riding Club	0	0	5%	3	6.66%	4	0	0	11.66	7
	Individual Barn	0	0	0	0	0	0	0	0	0	0
Istanbul	Horse Riding Club	1.66%	1	21.66%	13	15%	9	3.33	2	41.66	25
	Individual Barn	0	0	0	0	1.66%	1	0	0	1.66%	1
Kirklareli	Horse Riding Club	0	0	5%	3	6.66%	4	1.66%	1	13.33%	8
	Individual Barn	0	0	1.66%	1	1.66%	1	0	0	3.33	2
Tekirdag	Horse Riding Club	0	0	6.66%	4	18.33%	11	1.66%	1	26.66%	16
	Individual Barn	0	0	0	0	1.66%	1	0	0	1.66%	1
Total	Horse Riding Club	1.66%	1	38.33%	23	46.66%	28	6.66%	4	93.33%	56/136[x] %41.17
	Individual Barn	0	0	1.66%	1	5%	3	0	0	6.66%	4/314[y] %1.27
	Comparison of infected horses by total age groups		1/64[c] 65		24/189[b] 213		31/97[a] 128		4/100[c] 104	100%	60/510 11.76%

[a,b,c]In the same row. [x,y]In the same column, these different letters are statistically significant ($P < 0.05$; χ^2: (Chi-Square test). While the infection is most common in horses in the 6-10 age group, it is seen at the lowest rate in horses in the 1-5 age group and the 0-1 and 11-15 age groups. The infection rate was found to be higher in riding clubs. SE: Standard Error.

Table 5. Comparison of infection rate in equestrian clubs by provinces comparison of infection rate in equestrian clubs by provinces (mean ± SE).

	OD	Statistics	
	M (IQR)	H Value	*P* Value
Cities			
Canakkale	0.68 (0.34)		
Istanbul	0.57 (0.21)	4.996	0.172
Kirklareli	0.75 (0.51)		
Tekirdag	0.67 (0.26)		

M: Median; IQR: Inter Quartile Range; H: Kruskal Wallis test; SE: Standard Error.

DISCUSSION

As stated in the data of the Turkish Statistical Institute (TUIK), it has been reported that there are a total of 3,258 horses in the Thrace region, Canakkale (799), Istanbul (1086), Kirklareli (455) and Tekirdag (918) [26, 27].

During the period when the sample was taken, 9,482,051 SARS-CoV-2 human cases were reported in hospitals throughout Turkey until December 31, 2021. Since there was partial freedom, riding schools were operating during this period [21].

A genetic study of ACE-2 receptor from different animals, such as equids, livestock, primates, rodents, and marine mammals, found that animals with this receptor can bind the SARS-CoV-2 spike protein and promote virus entrance to host cell [15].

The reason for the low number of 1-year-old horses was interpreted as this age group not being used in equestrian activities yet and, therefore, having less contact with people. In this age group, positivity was detected only in a horse housed in a riding school in Istanbul. It was linked to human population density and the possibility of contact.

The total positivity rate in individual barns was 6.66%, and the rate in the entire sample was 0.78%. In horses of this type of breeding, the infection is seen in the age groups of 1-5 and 6-10, while it cannot be detected in the ages of 0-1 and 11-15, which is again attributed to the fact that these age groups have less contact with people. No positive horse was detected in the serological study conducted on 18 horses in China [9]. The study finished by Lawton *et al*. [14] 587 racehorses serum samples, they determined the Cut-off value as OD 0.507, which is 6 times the standard deviations above the average of the bloods taken from pre COVID-19 time 88 horse serums that were previously never chance to encounter with SARS-CoV-2. According to these results, 35/587 (5.9%) of the racehorses were infected with SARS-CoV-2 and could not find any PCR-positive horses in their study on SARS-CoV-2 [14]. This study used serum samples from vaccinated horses to produce hyper-immune serum against SARS-CoV-2 as a positive control. Blood serum samples taken from newly born foals without drinking colostrum were used as a negative control. Both positive and negative controls were validated by neutralizing antibody testing. To prevent false positivity at the cut-off value, according to the ROC analysis results with 100% sensitivity and 99.78% specificity, the OD was found to be 0.787, and 60/510 (11.76%) horses with a titer above this value were found to be positive. When 2 similar studies were examined in terms of age groups, Lawton *et al*. [14] stated that 587 racehorses were 2-7 years old. This study determined that 84.99% of SARS-CoV-2-positive horses were horses aged 1-10 years old. Both studies were found to be compatible with each other in terms of serological methods and results. The only difference between the 2 studies is that Lawton *et al*. [14] generally included single-owner or rider racehorses in the sample group. This study focused on horses from riding schools with many riders, and 93.33% of the positive samples were detected in the animals in this enterprise. In the study conducted by Pusterla *et al*. [20], 2 horses (8 and 21-year-old) whose owner was detected with COVID-19 (Delta B.1.617.2), were followed for 21 days. No signs of disease were seen in the horses. Although their feces and mucosa were found to be negative for coronavirus by RT-PCR, the younger one was determined COVID-19 seropositive that the antibody titer increased 1 week after the owner was detected with SARS-CoV-2 virus [20]. Some researchers focused on PCR shedding in horses. The study conducted on 667 horses found no positive SARS-CoV-2 horses with qPCR [13]. Again, in the study conducted on 34 Italian Trotters horses, all samples were found COVID-19 negative by qPCR [7]. The reason why shedding could not be detected with PCR in the studies is explained by the fact that it has a very short shedding period since horses and other animals like dogs, cats, and pigs are not the main hosts for SARS-CoV-2 [19,23]. Farm animals such as sheep, goats, cows, rabbits, and alpacas were experimentally infected with the 2019-nCoV/USA-WA1/2020 strain, and shedding was detected by RT-PCR only in cattle, goat, and rabbit nose swaps. The SARS-CoV-2 virus could not be detected with RT-PCR in horses. This study reported that since serological evaluation was not performed in horses, these human-adapted strains did not cause clinical symptoms due to their inability to replicate adequately outside the host [4].

The serologically detected infection rate in cats and dogs is 4-43.8% and 3.4-23.5%, respectively [3,6,12,18,30]. In addition, pandemic measures taken from country to country also vary, which is a factor that indirectly affects the number of individuals who come into contact with pets at home. Since horse people are

trained outdoors in close contact courses, they are not exposed to high doses of the virus, so it is thought that the transmitted virus causes seropositivity in horses without causing viremia. This may be why the infection rate in horses is below from pet animals like cats and dogs. On the other hand, in one serological cohort study with VNT conducted in Istanbul, Turkey, in November December 2020, virus-neutralizing antibodies were detected in none of 115 cats and only in 2 of 91 dogs [10]. When these 2 studies conducted on different animals in Turkey are compared, the difference can be explained by the fact that cats and dogs are adopted by one family; however, horses in riding clubs encounter more than 1 person. The serological study on cats and dogs was conducted in 2020, the beginning of the epidemic, when the total number of infected people was low.

Although less blood was taken from riding clubs in the sample (32.6%) than from individual barn samples (62.4%), the infection rate was 10.98% among all 56/510 animals from riding clubs, and just 4/510 (0.78%) horses were from individual horse barns. The reason for this is that these horses came into contact with many SARS-CoV-2 carrier riders during this period of the pandemic. The study was aimed at detecting the presence of infection serologically, and it did not focus on the presence of nasal and fecal shedding by qPCR. All studies conducted on horses have reported that horses might transmit the SARS-CoV-2 virus and cause subclinical disease but can show seropositivity without shedding the virus due to silent viremia. Our study determined that seropositivity occurred in horses that transmit from individuals carrying COVID-19. Horse owners should take the necessary biosecurity measures regarding diseases that can be transmitted from humans to animals.

CONCLUSIONS

According to the results of this study, it was determined that the infection could be transmitted from humans to horses, especially riding clubs, during the period when the infection was intense. Unlike another study result (5.9%), the SARS-CoV-2 positivity rate was found high (11.76%) because the horses in the riding club had more than 1 rider. This showed that more caution was needed in zooanthroponosis, which could be transmitted from humans to animals. Since shedding was not measured with PCR in the study, it is unknown whether horses can be transmitted from horses to humans or from horses to humans. Many research on SARS-CoV-2 contagion in horses have indicated that the infection is transmitted silently and causes seropositivity. It should not be forgotten that if the virus in horses, like white-tail deer, is passed from animal to animal, more pathogenic strains for humans may emerge.

MANUFACTURERS

[1]Vetal A.Ş. Adiyaman, Turkey.

[2]Kartell S.p.a. Noviglio, Italy.

[3]Sera Care. Milford, MA, USA.

[4]Sigma Aldrich Chemie GmbH. Steinheim, Germany.

[5]IBM Corp. Armonk, NY, USA.

***Acknowledgements*.** We would like to thank Vetal A.Ş. for the CovidSera, prepared from hyperimmunized horses with COVID-19 antigen, positive control serum samples the used in study optimization.

***Ethical approval*.** The Ethical Committee of Selcuk University, Faculty of Veterinary Medicine, Turkey (No. 2022/023) approved this study.

***Declaration of interest*.** The authors declare no conflicts of interest. The authors alone are responsible for the contents of this study.

REFERENCES

1 **Abdel-Moneim A.S. & Abdelwhab E.M. 2020.** Evidence for SARS-CoV-2 infection of animal hosts. *Pathogens*. 9(7): 529. DOI: 10.3390/pathogens9070529

2 **Alluwaimi A.M., Alshubaith I.H., Al-Ali A.M. & Abohelaika S. 2020.** The coronaviruses of animals and birds: their zoonosis, vaccines, and models for SARS-CoV and SARS-CoV2. *Frontiers in Veterinary Science*. 7(582287): 1-12. DOI: 10.3389/fvets.2020.582287

3 **Barrs V.R., Peiris M., Tam K.W., Law P.Y., Brackman C.J., To E.M., Veronica Y., Chu D.K., Perera R.A. & Sit T.H. 2020.** SARS-CoV-2 in quarantined domestic cats from COVID-19 households or close contacts, Hong Kong, China. *Emerging Infectious Diseases*. 26(12): 3071. DOI: 10.3201/eid2612.202786

4 **Bosco-Lauth A.M., Walker A., Guilbert L., Porter S., Hartwig A., Mcvicker E., Bielefeldt-Ohmann H. & Bowen R.A. 2021.** Susceptibility of livestock to SARS-CoV-2 infection. *Emerging Microbes & Infections*. 10(1): 2199-2201. DOI: 10.1080/22221751.2021.2003724

5 Burki T. 2020. The origin of SARS-CoV-2. *The Lancet Infectious Diseases*. 20(9): 1018-1019. DOI: 10.1016/S1473-3099(20)30641-1

6 Calvet G.A., Pereira S.A., Ogrzewalska M., Pauvolid-Corrêa A., Resende P.C., Souza Tassinari W., Pina Costa A., Keidel L.O., Rocha A.S.B. & Silva M.F.B. 2021. Investigation of SARS-CoV-2 infection in dogs and cats of humans diagnosed with COVID-19 in Rio de Janeiro, Brazil. *PLoS One*. 16(4): e0250853. DOI: 10.1371/journal.pone.0250853

7 Cerino P., Buonerba C., Brambilla G., Atripaldi L., Tafuro M., Concilio D.D., Vassallo L., Conte G.L., Cuomo M.C. & Maiello I. 2021. No detection of SARS-CoV-2 in animals exposed to infected keepers: results of a COVID-19 surveillance program. *Future Science OA*. 7(7): FSO711. DOI: 10.2144/fsoa-2021-0038

8 Cherry J.D. & Krogstad P. 2004. SARS: the first pandemic of the 21st century. *Pediatric Research*. 56(1): 1-5. DOI: 10.1203/01.PDR.0000129184.87042.FC

9 Deng J., Jin Y., Liu Y., Sun J., Hao L., Bai J., Huang T., Lin D., Jin Y. & Tian K. 2020. Serological survey of SARS-CoV-2 for experimental, domestic, companion and wild animals excludes intermediate hosts of 35 different species of animals. *Transboundary and Emerging Diseases*. 67(4): 1745-1749. DOI: 10.1111/tbed.13577

10 Enül H., Saraç F., Adıay C., Uzar S., Tuncer-Göktuna P., Arun S.S. & Hasöksüz M. 2022. Detection of neutralising antibodies against SARS-CoV-2 in companion animals in Istanbul. *Journal of Istanbul Veterinary Sciences*. 6(3): 128-133. DOI: 10.30704/http-www-jivs-net.1198979

11 Erganis O., Sayin Z., Hadimli H.H., Sakmanoglu A., Pinarkara Y., Ozdemir O. & Maden M. 2014. The effectiveness of anti-R. equi hyperimmune plasma against R. equi challenge in thoroughbred Arabian foals of mares vaccinated with R. equi vaccine. *The Scientific World Journal*. 480732: 1-10. DOI: 10.1155/2014/480732

12 Hamer S.A., Pauvolid-Corrêa A., Zecca I.B., Davila E., Auckland L.D., Roundy C.M., Tang W., Torchetti M.K., Killian M.L. & Jenkins-Moore M. 2021. SARS-CoV-2 infections and viral isolations among serially tested cats and dogs in households with infected owners in Texas, USA. *Viruses*. 13(5): 938. DOI: 10.3390/v13050938

13 IDEXX. 2020. SARS-CoV-2 (COVID-19) RealPCR Test Available at <https://www.idexx.com/en/veterinary/reference-laboratories/overview-idexx-sars-cov-2-covid-19-realpcr-test >

14 Lawton K.O., Arthur R.M., Moeller B.C., Barnum S. & Pusterla N. 2022. Investigation of the role of healthy and sick equids in the COVID-19 pandemic through serological and molecular testing. *Animals*. 12(5): 614. DOI: 10.3390/ani12050614

15 Liu Y., Hu G., Wang Y., Ren W., Zhao X., Ji F., Zhu Y., Feng F., Gong M. & Ju X. 2021. Functional and genetic analysis of viral receptor ACE2 orthologs reveals a broad potential host range of SARS-CoV-2. *Proceedings of the National Academy of Sciences*. 118(12): e2025373118. DOI: 10.1073/pnas.2025373118

16 Mackay I.M. & Arden K.E. 2015. MERS coronavirus: diagnostics, epidemiology and transmission. *Virology Journal*. 12: 1-21. DOI: 10.1186/s12985-015-0439-5

17 Palmer M.V., Martins M., Falkenberg S., Buckley A., Caserta L.C., Mitchell P.K., Cassmann E.D., Rollins A., Zylich N.C. & Renshaw R.W. 2021. Susceptibility of white-tailed deer (*Odocoileus virginianus*) to SARS-CoV-2. *Journal of Virology*. 95(11): 10.1128/jvi. 00083-00021. DOI: 10.1128/jvi.00083-21

18 Patterson E.I., Elia G., Grassi A., Giordano A., Desario C., Smith S., Anderson E., Prince T., Patterson G. & Lorusso E. 2020. Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. *Nature Communications*. 11(1): 6231. DOI: 10.1038/s41467-020-20097-0

19 Pickering B.S., Smith G., Pinette M.M., Embury-Hyatt C., Moffat E., Marszal P. & Lewis C.E. 2021. Susceptibility of domestic swine to experimental infection with severe acute respiratory syndrome coronavirus 2. *Emerging Infectious Diseases*. 27(1): 104. DOI: 10.3201/eid2701.203399

20 Pusterla N., Chaillon A., Ignacio C., Smith D.M., Barnum S., Lawton K.O., Smith G. & Pickering B. 2022. SARS-CoV-2 seroconversion in an adult horse with direct contact to a COVID-19 individual. *Viruses*. 14(5): 1047. DOI: 10.3390/v14051047

21 Ministry of Health Turkey. 2021. Coronavirus infection weekly and mothly list. Available at <https://covid19.saglik.gov.tr/TR-66935/genel-koronavirus-tablosu.html>

22 Schlottau K., Rissmann M., Graaf A., Schön J., Sehl J., Wylezich C., Höper D., Mettenleiter T.C., Balkema-Buschmann A. & Harder T. 2020. SARS-CoV-2 in fruit bats, ferrets, pigs, and chickens: an experimental transmission study. *The Lancet Microbe*. 1(5): e218-e225. DOI: 10.1016/S2666-5247(20)30089-6

23 Shi J., Wen Z., Zhong G., Yang H., Wang C., Huang B., Liu R., He X., Shuai L. & Sun Z. 2020. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS–coronavirus 2. *Science*. 368(6494): 1016-1020. DOI: 10.1126/science.abb7015

24 Singh D.K., Singh B., Ganatra S.R., Gazi M., Cole J., Thippeshappa R., Alfson K.J., Clemmons E., Gonzalez O. & Escobedo R. 2021. Responses to acute infection with SARS-CoV-2 in the lungs of rhesus macaques, baboons and marmosets. *Nature Microbiology*. 6(1): 73-86. DOI: 10.1038/s41564-020-00841-4

25 Sit T.H., Brackman C.J., Ip S.M., Tam K.W., Law P.Y., To E.M., Yu V.Y., Sims L.D., Tsang D.N. & Chu D.K. 2020. Infection of dogs with SARS-CoV-2. *Nature*. 586(7831): 776-778. DOI: 10.1038/s41586-020-2334-5

26 Ministry of Agriculture and Forestry Turkey. 2022. Horse number change in 2021. Available at <https://www.tarimorman.gov.tr/Konular/Bitkisel-Uretim/Iyi-Tarim-Uygulamalari/Istatistikler>

27 Turkish Statistical Institute. 2022. Number of horses bred in Turkey in 2021. Available at <https://www.tuik.gov.tr/media/announcements/istatistiklerle_Turkey_2021.pdf.>

28 Ulrich L., Wernike K., Hoffmann D., Mettenleiter T.C. & Beer M. 2020. Experimental infection of cattle with SARS-CoV-2. *Emerging infectious diseases*. 26(12): 2979. DOI: 10.3201/eid2612.203799

29 Yerken K., Uslu A., Bauyrzhan O., Sayin Z., Balevi A., Yerbol I., Ilban A. & Erganis O. 2023. Sero-epidemiology of the Rhodococcus equi in horses in Eastern Kazakhstan. *Eurasian Journal of Veterinary Sciences*. 39(2): DOI: 10.15312/EurasianJVetSci.2023.401

30 Zhang Q., Zhang H., Gao J., Huang K., Yang Y., Hui X., He X., Li C., Gong W. & Zhang Y. 2020. A serological survey of SARS-CoV-2 in cat in Wuhan. *Emerging Microbes & Infections*. 9(1): 2013-2019. DOI: 10.1080/22221751.2020.1817796

31 Zhu N., Zhang D., Wang W., Li X., Yang B., Song J., Zhao X., Huang B., Shi W. & Lu R. 2020. A novel coronavirus from patients with pneumonia in China, 2019. *New England Journal of Medicine*. 382(8): 727-733. DOI: 10.1056/NEJMoa2001017