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RESEARCH ARTICLE

Detection of The Presence of Leptospirosis in Horses by ELISA Method

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ABSTRACT

Leptospirosis is one of the common zoonotic diseases worldwide. It significantly affects humans and animals, causing socio-economic losses. Equine leptospirosis often manifests itself with recurrent genital tract infections, reproductive disorders, abortion, embryonic death, and birth of weak foals. Studies on Leptospirosis, especially in horses, are quite limited. For this reason, our study was planned to investigate the transmission cycle in terms of farm animals and public health, and also to determine the presence of Leptospirosis in horses. For our research, 187 horse blood serum samples were collected from different settlements in Turkey. In the collected samples, *Leptospira* spp. ELISA test was used to investigate the presence of antibodies. A total of 8 samples (4.27%) were detected as seropositive in horse serum samples. The agent, a significant pathogen for both human and animal health, was identified as seropositive. In addition, the high rate of transmission of leptospirosis through contact with infected horses and the potential for spread through different animals should be taken into consideration. Leptospirosis was determined as seropositivity by ELISA in the horses sampled. Therefore, it is important to diagnose and control the infection rapidly.

Keywords: ELISA, Horse, Leptosipirosis, Serologic Test, Zoonoses

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Atlarda Leptospirosis Varlığının ELISA Yöntemi ile Tespiti

ÖZ

Leptospirosis dünya çapında yaygın zoonotik hastalıklardan biridir. İnsanları ve hayvanları önemli ölçüde etkileyerek sosyo-ekonomik kayıplara neden olur. At leptospirozu sıklıkla tekrarlayan genital sistem enfeksiyonları, üreme bozuklukları, abort, embriyonik ölüm ve zayıf tayların doğumu ile kendini gösterir. Özellikle atlarda Leptospirozis üzerine yapılan çalışmalar oldukça kısıtlıdır. Bu sebeple, çiftlik hayvanları ve halk sağlığı açısından bulaşma döngüsünü araştırmak, aynı zamanda atlarda Leptospirozis'in varlığını belirlemek amacıyla çalışmamız planlanmıştır. Araştırmamız için Türkiye'nin farklı yerleşim yerlerinden 187 adet at kan serumu örneği toplandı. Toplanan örneklerde, *Leptospira* spp. antikorlarının varlığını araştırmak için ELISA testi kullanıldı. At serum örneklerinde toplam 8 örnek (%4,27) seropozitif olarak tespit edildi. İnsan ve hayvan sağlığı için önemli bir patojen olan etken seropozitif olarak teşhis edilmiştir. Ayrıca, leptospirozis hastalığının enfekte atlarla temas yoluyla bulaşma oranının yüksek olması ve farklı hayvanlar ile yayılma potansiyeli göz önüne alınmalıdır. Örnek alınan atlarda Leptospirosis ELISA ile seropoziti olarak belirlenmiştir. Bu nedenle enfeksiyonun hızla teşhis edilerek kontrol altına alınması önem taşımaktadır.

Anahtar Kelimeler: At, ELISA, Leptosipirosis, Serolojik Test, Zoonozlar

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INTRODUCTION

The epidemiology of leptospirosis is complex as it occurs in humans, domestic and wild animals. Leptospirosis exhibits a complex epidemiological cycle involving humans, domestic animals, and wildlife populations. There are a wide variety of animal species that can be seen as potential reservoirs for Leptospirosis disease, which is a common zoonosis, as in Brucellosis infections. (Andersen-Ranberg et al., 2016; Kaya et al., 2017; Kepenek et al., 2021). Leptospiral infection can have fatal consequences and the disease can cause abort and other reproductive problems in horse, cattle, goats and pigs. In humans, symptoms range from subclinical infection to acute febrile illness, pulmonary hemorrhage, and renal failure (Yamaguchi et al., 2018).

Most animals are either reservoir hosts that chronically retain leptospira in their kidneys or are incidental hosts. (Levett et al., 2001). According to epidemiological information to date, rodents are identified as the primary reservoir host for Leptospirosis and other different zoonotic infections (Levett et al., 2001; Ayçiçek, 2004; Temel et al., 2020). Rodents are considered the primary reservoirs of Leptospira and play a key role in the transmission of leptospirosis to other hosts such as horses, domestic animals, dogs, and humans (Levett et al., 2001). Climate changes also contribute to the transmission cycle. Especially floods, disproportionately increasing temperatures, poor hygiene conditions, housing problems due to inadequate economic conditions, large numbers of mice and other reservoir animals cause Leptospirosis. Many of the areas known to have high incidence of leptospirosis have been affected by these factors (Lau et al., 2010). Leptospirosis can spread to the environment, especially through the urine of animals. Seropositive animals in the population are also thought to contribute to the transmission cycle through genital and urinary secretions. (Yamaguchi et al., 2018).

In the diagnosis of Leptospirosis, ELISA tests and microagglutination tests (MAT) are used to test for the presence of Leptospirosis in individual animals and herds. It can be used to confirm the diagnosis, especially in samples. (Mulla et al., 2006). In particular, screening herds and detecting antibodies with these serological tests is a very useful method for animal and human health. Leptospirosis serovars are classified into 28 serogroups based on serological similarity. These serovars were identified using cross agglutination absorption testing (CAAT). According to previous reports, different methods are used for serological diagnosis of Leptospirosis. It has been reported that specificity issues between these serological tests can be overcome by treating an antigen produced by acetic acid extraction with various enzymes or by using Mab-based competitive ELISA (Costa et al., 2012; Saito et al., 2013; Bourhy et al., 2014).

The objective of this study is to ascertain the presence of leptospirosis in equine blood sera by employing the ELISA method, a serological test.

MATERIAL and METHODS

Blood Sera Sampling

Horse blood samples were collected from Konya (n=35), Mersin/Tarsus (n=50), Adana (n=23), Mersin/Mezitli (n=17)Niğde/Kemerhisar(n=62). A total of 187 horse blood samples were collected in February 2024. The horses included in the study were 187 horses aged 24 months and over between. Horses were kept on different farms. According to the information received from farm owners from time to time, cases of fever, genital system infections were seen (Figure 1).

Figure 1: Locations of farms where horse blood serum is collected

Method

Serological analysis

Blood serum samples were collected from farms in different settlements across Türkiye. The samples were then subjected to a centrifugal process at 1000g for a duration of 20 minutes. Thereafter, the samples were stored at a temperature of -20°C until utilisation. Blood samples brought to the laboratory were analyzed after separating their serums. For the qualitative detection of IgG class antibodies against Leptospira spp., the Horse Leptospira spp. Antibody ELISA Kit (Abbexa, United Kingdom) was used. The test was performed according to the manufacturer's recommendations.

All samples were run in 96-well microtiter plates for ELISA testing. Optimal working concentrations/dilutions of antigens/antibodies or reagents were determined by titration. Standards/controls and diluted samples (diluted 5-fold) were dispensed into microplate wells in a volume of 50 µl and plates were covered with plate sealer. Incubated for 30 minutes at 37°C. Cover were removed and washed the plate 5 times with 1X Wash Buffer. After washing, 50 µl of Detection Reagent to each well were added. Plate were covered with a plate

sealer and incubated for 30 minutes at 37°C. Then cover were removed, liquid were discarded and repeated the wash process as described, 5 times. 50 µl of TMB Substrate A and 50 µl of TMB Substrate B were added into each well. The plate were covered with the plate sealer and gently tapped the plate to mix thoroughly. Incubated at 37°C for 10-15 minutes, avoided exposure to light. 50 µl of Stop Solution were added for ending process. The results were read at 450 nm on the ELISA reader. ELISA optical density (OD) reading were transformed to serum / positive percentage (S / P) according to a specific equation cited by manufacturer (Abbexa, 2024).

Interpretation of ELISA Results

Mean OD of the Positive Control should be ≥ 1.0.
Mean OD of the Negative Control should be ≤ 0.2.
CUT OFF value = Negative Control + 0.15

Ethics statement

This research was performed with the permission of SÜVDAMEK dated 01.02.2024 and numbered 2024/006.

RESULTS

Eight (4.27%) of 187 horse blood sera samples were seropositive for Leptospirosis. These findings suggest that leptospirosis is present in horses in these provinces (Table 1).

Table 1. Seropositivity results of horse blood sera tested by ELISA

Location	Number of Horses	Number of Seropositive Horses	Seropositive Percentages
Adana	23	1	0.53%
Tarsus/Mersin	50	3	1.60%
Mezitli/Mersin	17	-	-
Ereğli/Konya	35	1	0.53%
Kemerhisar/Niğde	62	3	1.60%
Totaly	187	8	4.27%

DISCUSSION

Leptospirosis can be observed in many domestic and wild mammals, primarily cattle, sheep, dogs, and horses. The disease may appear in various clinical forms in animals. In general, it manifests with clinical signs such as fever, anorexia, jaundice, abortion, and organ involvement. In horses, leptospiral infections are particularly characterized by abortion, infertility, genital tract infections, renal disorders, and uveitis in the eyes. It can cause significant economic losses and serious animal health problems. Therefore, leptospirosis in horses is an infection that should be carefully considered in terms of both herd health and its zoonotic potential (Ellis, 2023; Šimpraga et al., 2024)

Leptospirosis often causes a mild and self-limiting febrile disease in humans, but it can reach lifethreatening severity in rare cases. Due to its systemic course, various organs may be affected during the disease. Jaundice and renal failure, called Weil's disease, are the most clinically significant manifestations of leptospirosis.

Leptospirosis poses a worldwide problem for both animal and public health, affecting humans, domestic animals, wildlife and even reptiles, spanning many species and serovars. Seropositivity rates for leptospirosis vary widely depending on the species, geographic region, and the population studied. Generally, seropositivity rates in humans range between 5% and 20%, while these rates tend to be higher in animals, reaching up to 30% in some species. (Levett et al., 2001; Ellis 2014; Pissawong et al., 2020).

Cases of leptospirosis are also commonly reported in coastal countries of the Mediterranean and in Africa. Leptospirosis Seropositivity was observed in 1.3% of human samples in Morocco. Epidemiological assessments have highlighted that there is a significant increase in seropositivity rates in areas with high rodent numbers and poor hygiene conditions. (Bourhy et al., 2014).

The clinical presentation of Leptospira infection in horses varies, often making definitive diagnosis difficult. The majority of equine cases are asymptomatic. Clinical signs are mostly related to the mare's reproductive system and kidneys. Studies on this have been reported (Ellis et al., 2014; Divers et al., 2019). In addition, Leptospira infection contributes to the development of recurrent uveitis in horses, which appears to be the most important clinical outcome (Lowe, 2010; Spiess, 2010).

In 2021, Wasiński et al. demonstrated the serological presence of leptospirosis in Arabian horses. They

found a very high rate of 33.2% seropositivity. In this study conducted on randomly selected horse populations, despite high Leptospira seropositivity, it was clinically reported that the number of cases in the herd was low. For this reason, they thought that the disease was exposed to different ways. Additionally, when this study is compared to ours, there are large differences in climate in selected regions. Although seropositivity has been detected in horses on many farms, especially in a tropical region such as the Mediterranean region, seropositivity values do not show parallelism with this study. The areas we took as a sample have a much milder climate than Poland. We know that leptospira is actually a tropical climate bacteria. Here the different result emerges. The authors supported idea that very high seropositivity has been found in the region due to the increase in Leptospira carrier populations. Moreover, many horses may have only been exposed to this infectious disease for a short period of time. Therefore, it has been reported that horses may have merely seroconverted without any clinical consequences. Another point awaiting clarification is the occurrence and duration of infections in horses that do not show clinical signs. In our study, Eight (4.27%) of 187 horse blood sera samples were seropositive for Leptospira. Although regionally low seropositivity was shown, the presence of the disease was revealed.

In our study, the samples were taken on February in a cold climate also affected the results. In addition, previously reported genital tract infections and fever were not observed at the time the sample was taken. These symptoms have been seen from time to time on the mentioned farms. Therefore, even if horses do not have any clinical symptoms, previous exposure to the infection is evident. In parallel with this study, seropositivity has serologically demonstrated the presence of the disease, even though no clinical symptoms are observed. It should not be ignored that horses with leptospirosis seropositivity may pose a risk, especially for people involved in care and nutrition.

Microscopic Agglutination Test (MAT) is considered as the reference test for the diagnosis of leptospirosis as it allows the detection of serovar-specific antibodies with high sensitivity and specificity. However, it has some difficulties due to its reliance on live Leptospira cultures and technical complexity.

In 2014, Ye et al. compared the Microagglutination test and ELISA test in horse blood serum. They emphasized that the use of some antigens in the ELISA test is more sensitive and specific. They reported that the ELISA test was easier to apply, especially among serological tests, and the results were compatible with the standard. Our aim for our study was to quickly detect the disease. Although there is no comparison between serological tests such as this study in terms of specificity, we can say that the ELISA test gives very easy and fast results.

ELISA tests offer many advantages over MAT (Microagglutination Test). It uses non-hazardous reagents, relatively sensitive and specific. In addition, the stringent quality control criteria established for this test ensure the reproducibility of objectively interpreted results. The use of frozen antigen-coated plates is relatively long-lasting. Because of these advantages described, ELISA could potentially be used as a screening test for leptospirosis and other spesific diseases. Serum that scores positive in this screening ELISA can then be tested with serovar-specific ELISAs or MAT to identify the infecting serovar. Today, this method has been applied to both leptospira and other reported infections (Niloofa et al., 2015 ;Behera et al ., 2022).

Complementary serological tests are very important, especially when considering cases where MAT gives negative results when clinical symptoms indicate the disease (Haake et al., 2015; Day, 2021). ELISA tests are reported as complementary serological tests, especially with MAT (Levett et al., 2001, Haake et al., 2015; Day, 2021). For this reason, ELISA tests are important for the early diagnosis of acute Leptospirosis infection and detect immunoglobulin M antibodies, which can be detected before MAT produces a positive result (Goris et al., 2012; Tan et al., 2014, Kaya, et al., 2017). Previous studies have reported that MAT is less reliable than ELISA tests in chronic leptospiral infections (Who, 2003; Day, 2021).

The ELISA test offers numerous advantages in the serological diagnosis of leptospirosis. Its rapid turnaround time, high sample throughput, and compatibility with automation provide practicality in field studies and large-scale population screenings. ELISA tests, based on antigen-antibody interactions, enable reliable detection of the specific immune response elicited by the infection. Additionally, ELISA requires less technical expertise and does not depend on live Leptospira cultures, thereby enhancing laboratory safety and ease of implementation. The ELISA test offers several advantages in the serological diagnosis of leptospirosis in animals. A study conducted in Egypt analyzed 600 bovine serum samples using an ELISA kit. The results demonstrated that ELISA is a reliable method for detecting leptospiral antibodies and provides a practical alternative to traditional diagnostic techniques (İbrahim et al., 2022). Furthermore, research carried out in South Sudan highlighted the importance of serological methods such as ELISA for epidemiological surveillance of Leptospira spp. in cattle (Rosa et al., 2017). Collectively, these studies underscore the role of ELISA in enhancing the accuracy and efficiency of leptospirosis diagnosis in veterinary practice. While planning our research, we took these studies as a reference, with the primary aim of investigating the presence of infection in the region using the ELISA test. Due to its practicality, safety, ability to rapidly

detect specific antibodies, and compatibility with automation, ELISA proved to be a particularly suitable method, especially under field conditions. In this respect, our findings are consistent with the aforementioned studies and confirm that ELISA is an advantageous test for the serological diagnosis of leptospirosis in animals.

Leptospirosis, which is endemic in many tropical regions, can cause major epidemics, especially after heavy rains and floods, and is more common in individuals who have contact with contaminated water or soil (Saito et al., 2013). Professional groups such as veterinarians, slaughterhouse workers, farmers, hunters and laboratory workers who have direct contact with infected animals in daily life are at risk for leptospirosis found leptospirosis positivity in only two of 102 slaughterhouse workers in Ankara (Türkiye) (Babür et al., 2003). These results emphasize the need to protect people, especially those in the risk group, from Leptospirosis disease.

CONCLUSION

In our study, the presence of Leptospirosis seropositivity in horses were revealed by ELISA method. Especially for leptospirosis, environment, transmission routes and climatic factors are very effective. To prevent leptospirosis in horses, good hygiene practices, minimizing contact with rodents, and vaccinating other species and pets are important.

The incidence and significance of leptospirosis in horses remains unclear. The incidence reported in almost all epidemiological studies varies greatly depending on the geographical region examined. Large-scale studies are needed to evaluate the disease from a general perspective and offer solution suggestions.

Conflict of interest: The authors declared that there is no conflict of interest.

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Ethical approval: This research was performed with the permission of SÜVDAMEK dated 01.02.2024 and numbered 2024/006.

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