

Equine Leptospirosis in Egypt: Seroprevalence and Risk Factors

Key words

Leptospirosis;
seroprevalence;
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Abstract: Most leptospiral infections in horses are asymptomatic; however, acute disease manifestations as well as reproductive failure and recurrent uveitis have been reported. Horses are considered accidental hosts. The data about equine leptospirosis in Egypt are scarce. Hence, the present study aimed to investigate presence of antibodies against *Leptospira* sp. in horse in four Egyptian governorates and determine the associated risk factors for the infection. To determine the seroprevalence in 305 serum samples, the microscopic agglutination test (MAT) was carried using eight *Leptospira* serovars antigens. The results revealed 104 animals were positive for at least one of the serovars (34.1%; 95%CI: 29.01-39.59). The most common reaction was reported to Icterohaemorrhagiae serovar (15.14%), followed by Canicola (14.75%), Bratislava (11.47%), Copenhageni (8.19%), Pomona (7.86%), and Hardjo (6.88%). The most prevalent was observed among females, older horses raising in pasture or in contact with ruminants or dogs and lack of rodents control. The significant seroprevalence suggests that Egyptian horses living in the studied area are at high risk of infection or exposure by *Leptospira* sp. Thus, the establishment of emergency surveillance and control program is very crucial for this zoonotic pathogen.

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Introduction

Leptospirosis is one of the most common zoonoses in the world and is caused by gram-negative spirochetes from *Leptospira* genus (*Leptospiraceae* family, order *Spirochaetales*) (1, 2). It affects both human and animals, mainly in tropical regions where the organism thrives in optimal temperature and humidity circumstances (3). Infections in horses are most occurred through direct contact with urine or placenta secretions of diseased animals, or indirectly through a contaminated surroundings (4).

Leptospira sp. infection is predominantly subclinical in equines and is thought to be a significant cause for abortion, stillbirth, delivery weak foals, and neonatal mortality (5, 6). However, the main symptoms in horses are anorexia, moderate fever, jaundice and recurrent uveitis (7).

Moreover, pulmonary hemorrhage and deaths due to interstitial nephritis may be occurred (8).

Leptospirosis infection in horses is one of the leading causes of significant economic loss in the equine agribusiness sector due to the numerous consequences of the disease, including the cost of treating diseases animals, the interruption of training, a decline in performance, and the exclusion of affected animals from auctions and competitions (9, 10). Moreover, the infected animals in either acute or chronic phases are thought of as reservoirs and play a significant part in the transmission of disease that poses a risk to public health (11).

Leptospirosis has traditionally been diagnosed in a laboratory using serological tests. The standard serological test

is the microscopic agglutination test (MAT), where a single detection of a high antibody titer associated with clinical symptoms and a four-fold or more shift in antibody titers in paired acute and convalescent samples are considered diagnostic. It is preferable to isolate the spirochete, however this is a challenging and time-consuming (12). Moreover, the polymerase chain reaction (PCR) have been used as a specific test for detection of *Leptospira* DNA in tissue of premature born foals (13, 14) and in the vitreous fluid of horses with recurrent uveitis. (15).

Leptospiral infection in horses is frequently detected using serology. The most common reported serovars are *L. interrogans* sv. Bratislava, *L. interrogans* sv. Pomona, *L. kirschneri* sv. Grippotyphosa and *L. interrogans* sv. Icterohaemorrhagiae (16).

In Egypt, most of previous studies on leptospirosis were focused on human or pets like dogs, cats (17) and few studies in cattle (18). Nonetheless, there is no epidemiological data about the *Leptospira* infection in horse particularly in Nile Delta of Egypt.

Therefore, the objective of the current study was to assess the prevalence of leptospirosis among horses in the four governorates of northern Egypt as well as potential risk factors for leptospiral infection.

Materials and methods

Ethical statement

The study protocol was approved by ethical committee of the Faculty of Veterinary Medicine, Benha University, Egypt, which complies with all applicable Egyptian regulations on research and publication. The Committee's Animal Ethical Rules and Guidelines were followed in the collection and handling of serum samples.

Study location

A serological survey was conducted from January 2020 to April 2021 in the northern Egyptian governorates of Cairo, Giza, Kafr ElSheikh, and Qalyubia, which situated geographically at 30°2'40"N 31°14'9"E, 29.9870°N 31.2118°E, 31.3°N 30.93°E and 30.41°N 31.21°E, Figure 1.

According to Köppen, the climate of Giza is similar to an arid, hot desert. Because of its proximity to Cairo, it has a similar climate to Cairo. The average temperature is 25 °C and highest temperature reported in August, while the rain is rare in this area with average rainfall of 100-200 mm annually. In addition, the weather in Kafr ElSheikh and Qalyubia governorates is hot, muggy, arid summers and chilly, dry, windy, mainly clear winters. The temperature falls between 15 to 30 °C throughout the year.

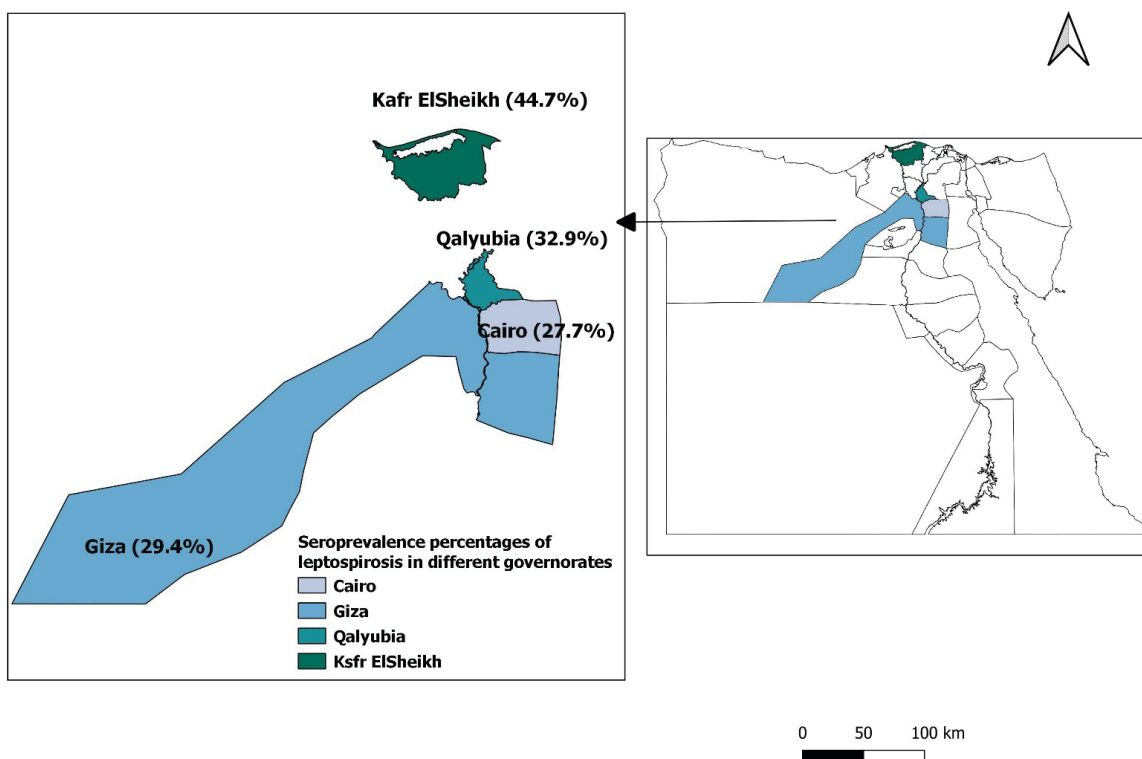


Figure 1: MAP demonstrate studied governorates and seropositivity percentages to *Leptospira* sp.

Sampling and epidemiological data

Win Epi 2.0 was used to estimate the sample size, which was 305 horses, with a predicted prevalence of 50%, a 95% confidence range, and an accepted error of 5%. Because there haven't been any epidemiological study that describe the state of horse leptospirosis in the examined region, the predicted prevalence of 50% was chosen. The examined horses had not specific signs for the disease and have not been vaccinated against leptospirosis. In the present study, no exclusion criteria or repeat sampling of the same horses were applied. The blood samples were taken after obtaining the farmer's informed consent from horse by puncturing the jugular vein with vacuum tubes without an anticoagulant. The samples were centrifuged at 3000 xg for 10 min to separate the serum, which kept at -20 °C until serological analysis.

In order to determine risk variables related with seropositive horses, a questionnaire was made accessible to the owners of the study's horses. Questionnaire was set to collect the data associated with the potential risk factors for leptospira infection. The questionnaire included the data about sex (male or female), age (<6 years, 6-10 years, and >10 years), season (winter, autumn, summer, and spring), the presence of ruminants (yes/no), the presence of dogs (yes/no), the type of housing (Stable, pasture, mixed), and control of rodents (yes/no).

Serological testing

The microscopic agglutination test (MAT) was used to screen serum samples for *Leptospira* antibodies. A variety of *Leptospira* serovars were used as antigens in the microscopic agglutination reaction, including serogroup Icterohaemorrhagiae, serovar Copenhageni and Icterohaemorrhagiae; serogroup Canicola, serovar

Canicola; serogroup Grippotyphosa, serovar Grippotyphosa; serogroup Pomona, serovar Pomona; serogroup Sejroe, serovar Hardjo; serogroup Tarassovi, serovar Tarassovi and serogroup interrogans serovar Bratislava. The serum samples were tested at 1:100 dilution by mixing of 25 µL of diluted serum 1:14 in sterile saline with 150 µL of the diluted *Leptospira* suspensions. Sera that produced a positive reaction were titrated further in a series of two-fold dilutions, commencing at 1:125 and continuing until the titer endpoint. Antibody titers were calculated as the reciprocal of the highest serum dilution that resulted in a 50% or more reduction in the amount of free leptospores in the suspension when compared to a sterile saline-based negative control. A titer of ≤ 100 was considered positive, meaning it indicated exposure to or infection with *Leptospira*.

Statistical analysis

Data were organized and statistically analyzed using SPSS software version 24 (IBM, Chicago, USA). Chi-square test was used to evaluate the relationship between the results of the serology (positive and negative) and the factors. Findings with *P*-value less than 0.05 were regarded as statistically significant. First, the univariate binary logistic regression analysis was performed to identify important risk factors linked to *Leptospira* seropositivity. Then, all variables with *P*<0.05 in univariate regression model were passed to multivariate logistic regression model. The odds ratios (ORs) and 95% confidence intervals were calculated to evaluate the degree of risk. The model's fit was evaluated using the Hosmer and Lemeshow goodness test.

Results

At a serum dilution of 1:100, 104 horses (34.1%, 95%CI: 29.01-39.59) from four governorates showed positive MAT titers to one or more *Leptospira* serovars. The majority of

Table 1: MAT antibody titer distribution for different *Leptospira* serovars

Serovar	Number of positive horses in each titer						No of positive animals (%)
	125	250	500	1000	2000	4000	
Icterohaemorrhagiae	15	12	7	8	6	5	53/305 (15.14%)
Canicola	14	11	8	4	3	5	45/305 (14.75%)
Bratislava	13	10	6	3	1	2	35/305 (11.47%)
Copenhageni	11	9	5	0	0	0	25/305 (8.19%)
Pomona	11	8	3	1	1	0	24/305 (7.86%)
Hardjo	9	6	5	0	1	0	21/305 (6.88%)
Grippotyphosa	0	0	0	0	0	0	0/305 (0)
Tarassovi	0	0	0	0	0	0	0/305 (0)

Table 2: Number of seropositive animals to single and multiple serovars

No of serovar	Number of animals reacted positive (%)
1	59/104 (56.7%)
2	22/104 (21.1%)
3	18/104 (17.3%)
4	5/104 (4.8)
5	0/104 (0)

horses tested positive for *Icterohaemorrhagiae* serovar, followed by *Canicola*, Bratislava, Copenhageni, Pomona, and Hardjo, but no animals tested positive for *Grippotyphosa* or *Tarassovi* serovars, Table 1. The seropositive horses reacted with single serovar were 59/104 (56.7%), while 43.3% (45/104) of the seropositive horses tested positive to numerous serovars, Table 2.

In this study, it was discovered that 104 animals possessed *Leptospira* antibodies, representing a seroprevalence of 34.1%. Horses from Kafr ElSheikh had the greatest prevalence rate (44.7%), followed by those from Qalyubia (32.9%), while animals from the Giza governorate had the lowest prevalence (27.7%), as indicated in Table 3.

Considering sex as a potential risk factor among the tested animals, gelding horses and male vs female horses showed a significant difference ($P < 0.05$). Significantly, the infection rate among females was the greatest (39%), while the prevalence rate among male animals was the lowest (24.3%). In addition, age was associated with a greater seroprevalence that was statistically significant ($P = 0.001$). In comparison to horses in the age groups < 6 and 6 to 10 years old, all horses over 10 years old had greater prevalence rates (49.1%), table 3. The highest prevalence rates were seen in the autumn (50.6%) and spring (33.3%), with a significant seasonal difference in *Leptospira* seropositivity ($P < 0.05$). According to the findings, horses raised in close proximity to ruminants or dogs had higher seropositivity rates, which were 39.9% and 38.5%, respectively. Also, the findings show that, in comparison to horses living in stables or mixing houses, horses roaming freely in pastures had the highest prevalence rate (42%). It's interesting to note that rodent control has a substantial effect on the prevalence of *Leptospira* in horses, where the prevalence rate significantly rose in the presence of rodent control, Table 3.

The result of multivariate logistic regression analysis summarized in table 4 and showed that sex, age, season, housing, contact with ruminants or dogs and control of rodents were identified as risk factor for *Leptospira* infection. Females (OR= 1.27, 95%CI: 0.49-5.70) within age group more than 10 years old (OR=3.25, 95%CI: 1.31-8.11) was found to be a greater risk than gelding and other age

groups. Moreover, horses raised in contact with ruminants (OR=2.43, 95%CI: 1.35-4.36) or dogs (OR=1.36, 95%CI: 0.66-2.78) and living freely in pasture (OR=2.30, 95%CI: 1.16-4.52) had high risk of infection than other animals. Also, autumn season (OR=2.66, 95%CI: 1.21-5.83) and presence of rodents control (OR=3.26, 95%CI: 1.55-6.85) increased risk of infection, Table 4.

Discussion

Horses are not frequently thought as a potential source of leptospirosis spread unlike other domestic and wild animals. Nevertheless, horses may have *Leptospira* in their kidneys, making them carriers and transmit the bacterium in the environment (5). In horses, the disease varies geographically in terms of prevalence and serovars involved, and its effects are still unknown (8, 19). The current study used the MAT to determine the prevalence of antibodies against eight different *Leptospira* serovars and related risk variables in horses living in the four Egyptian governorates.

The findings of present study revealed that 34.1% of horses had positive MAT titers for at least one *Leptospira* serovar, while 53.3% of seropositive horses had antibodies for several serovars. Several infections or cross-reactions may cause seropositivity to multiple serovars. Additionally, because none of the horses were immunized against leptospirosis, we concluded that the presence of antibodies indicated *Leptospira* exposure or illness.

The *Leptospira* sp. seroprevalence in Egyptian horses was lower than that reported in Europe. In Switzerland, Italy and the Netherlands, seroprevalences were determined to be 58.5% 67.2% and 79%, respectively (20-22). On the other hand, the seroprevalence rate in the current study was higher than those reported in Italy, which were 11.4% and 1.5%, respectively (23, 24). Additionally, in a Brazilian studies, the seropositivity rates were 71.4% (25), 28.75% (26), 45.9%, (27) and 8% (28). Furthermore, according to a recent study conducted in various States of the American Midwest, healthy horses have a 77% seroprevalence rate (29). The findings of present investigation were attributed to the type of environment (30-34). The studied horses were kept under good care conditions and in regions with little stagnant water (35).

The present findings revealed that the most prevalent serovar was *Icterohaemorrhagiae* (15.14%), followed by *Canicola* (14.75%). These findings are consistent with earlier serological studies conducted on Brazilian horses (21, 27, 36). It's interesting that no animals tested positive for the *Grippotyphosa* serovar. Although this serovar very seldom affects horses, it is thought to be the one most frequently linked to equine recurrent uveitis (ERU) in Europe (20). The obtained findings were consistent with those reported by several authors, where most of seropositive or infected horses have no symptoms (7, 8).

Table 3: The risk factors associated with *Leptospira* spp. seropositivity in horse

Variable	Total tested horses	No of positive	No of negative	% of positive	95% CI	Statistic
locality						
Giza	85	25	60	29.4	20.78-39.82	$\chi^2=6.322$ df=3 $P=0.097$
Cairo	65	18	47	27.7	18.29-39.58	
Kafr ElSheikh	85	38	47	44.7	34.6-55.28	
Qalyubia	70	23	47	32.9	23-44.5	
Sex						
Male	74	18	56	24.3	15.97-35.2	$\chi^2=7.552$ df=2 $P=0.023^*$
Female	210	82	128	39.0	32.7-45.79	
Gelding	21	4	17	19.0	7.67-40	
Age						
<6 years	64	11	53	17.2	9.88-28.22	$\chi^2=13.805$ df=2 $P=0.001^*$
6-10	186	66	120	35.5	28.96-42.59	
>10	55	27	28	49.1	36.38-61.92	
Season						
Winter	73	17	56	23.3	15.08-34.17	$\chi^2=15.067$ df=3 $P=0.002^*$
Spring	75	25	50	33.3	23.71-44.58	
Summer	76	21	55	27.6	18.84-38.57	
Autumn	81	41	40	50.6	39.95-61.23	
Contact with ruminants						
Yes	198	79	119	39.9	33.33-46.85	$\chi^2=8.451$ df=1 $P=0.004^*$
No	107	25	82	23.4	16.35-32.21	
Contact with dogs						
Yes	231	89	142	38.5	32.49-44.94	$\chi^2=7.968$ df=1 $P=0.005^*$
No	74	15	59	20.3	12.87-31.18	
Housing						
Stable	81	17	64	21.0	13.54-31.07	$\chi^2=12.599$ df=2 $P=0.002^*$
Pasture	181	76	105	42.0	35.04-49.27	
Mixed	43	11	32	25.6	14.93-40.24	
Control of rodents						
No	76	12	64	15.8	9.27-25.6	$\chi^2=15.100$ df=1 $P<0.0001^*$
Yes	229	92	137	40.2	34.03-46.63	
Total	305	104	201	34.1	29.01-39.59	

*The result is significant at $P < 0.05$

Table 4: Multivariate logistic regression analysis for variables associated with horse leptospirosis

Factors	B	S.E.	OR	95% CI for OR		P value
				Lower	Upper	
Sex						
Male	0.240	0.662	1.27	0.35	4.65	0.717
Female	0.518	0.624	1.68	0.49	5.70	0.407
Age						
6-10	0.749	0.401	2.11	0.96	4.64	0.062
>10	1.180	0.466	3.25	1.31	8.11	0.011
Season						
Spring	0.300	0.410	1.35	0.60	3.02	0.464
Summer	0.068	0.416	1.07	0.47	2.42	0.870
autumn	0.978	0.400	2.66	1.21	5.83	0.015
Contacts with ruminants						
Yes	0.886	0.299	2.43	1.35	4.36	0.003
Contacts with dogs						
Yes	0.306	0.366	1.36	0.66	2.78	0.403
Housing						
Pasture	0.831	0.346	2.30	1.16	4.52	0.016
Mixed	0.373	0.489	1.45	0.56	3.79	0.446
Control of rodents						
Yes	1.182	0.379	3.26	1.55	6.85	0.002

B: Logistic regression coefficient, SE: Standard error, OR: Odds ratio, CI: Confidence interval

According to our data, older horses are significantly more likely than young horses to be seropositive to *Leptospira* sp. This finding is consistent with results from previous studies (9, 37) and could be explained by the fact that exposure to *Leptospira* is more likely as horse get older and that seropositivity can last for a very long time. Nevertheless, other researchers did not observe significant relationship between age and seropositivity (27, 38).

The present findings are consistent with previous studies showing that females more susceptible to get *Leptospira* infection than males (27, 28). These findings confirm that females are more vulnerable than males since they are allowed to roam more freely on pasture on stud farms and are often included in batches of the most animals (39-47).

According to our findings, the seroprevalence rate of *Leptospira* was highest during autumn in comparison with other seasons, which come in accordance with findings of Trimble and colleagues (29). Since leptospire may survive and spread infection for a longer period of time in warm and humid climates. Thus, spring and fall have higher seroprevalences.

The study's findings confirmed the necessity of sanitary management when different species cohabit, with the presence of animals such as sheep, goats, or cows on nearby farms serving as risk factors for exposure to *Leptospira* sp. in study participants' animals (9). *Leptospira* infections in ovine and caprine are frequent, and these species, like bovines, can serve as significant reservoirs of the disease and

have epidemiological implications (39, 48-52). Moreover, according to findings reported by Chiareli and colleagues (53), equines that coexist with other species share communal drinking and pasture, have direct or indirect contact with contaminants of aborted materials, graze on pasture contaminated with infected urine, and have contact with animals on neighbouring properties are more likely to be exposed to *Leptospira* spp.

In the horses' indoor habitat, rats are probably the most common species of animals, and *Leptospira interrogans* prevalence was positively correlated with the density of the rat population (54). The possibility of the *Leptospira* spreading to horses was greatly decreased by the existence of pest control methods. Rodent control is seen as a key element in prevention (55) despite the fact that it can occasionally be challenging to assess the role that rodents play in the transmission of leptospiral serovars (56).

In agreement with findings of Pinna and colleagues (57), appropriate management, such as the availability of veterinary support on the property, control measures for presence of rodents, and quarantine of recently arriving animals, suggested probable circumstances of less exposure to leptospirosis. In contrast, Batista and colleagues (58), observed no significant association between presence of rodents and livestock farming and seropositivity for *Leptospira* sp. and they attributed this to inaccurate data, a non-conclusive epidemiological questionnaire, the presence of confounding variables, and unmeasured environmental variables.

Conclusion

The present study confirms presence of antibodies against *Leptospira* sp. among Egyptian horses and horses living in studied areas high risk exposure to pathogenic leptospires. Age, sex, season, the presence of ruminants or dogs, and the absence of rodent controls were the risk factors targeted in this investigation for the occurrence of horse leptospirosis. Thus, control of rodent is important factor to reduce the spreading of infection among horses and consequently for human.

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authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate. The study protocol was approved by ethical committee of the Faculty of Veterinary Medicine, Benha University, Egypt, which complies with all applicable Egyptian regulations on research and publication. The Committee's Animal Ethical Rules and Guidelines were followed in the collection and handling of serum samples.

Consent for publication not applicable.

Availability of data and materials. All data generated or analysed during this study are included in this published article

Competing interests. There are no conflicts of interest declared by the authors.

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Leptospiroza konj v Egiptu: seroprevalenca in dejavniki tveganja

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Izveček: Večina leptospiroz pri konjih je asimptomatskih, vendar obstajajo poročanja o akutnih bolezenskih znakih, reprodukcijskih motnjah in ponavljajočih se uveitisih. Konji veljajo za naključne gostitelje. Podatki o leptospirozi konj v Egiptu so pomanjkljivi. Zato je bil namen te študije raziskati prisotnost protiteles proti bakteriji *Leptospira* sp. pri konjih v štirih egiptovskih pokrajinah in določiti z njimi povezane dejavnike tveganja za okužbo. Za določitev seroprevalence v 305 vzorcih seruma je bil opravljen mikroskopski aglutinacijski test (MAT) z osmimi antigeni serovara *Leptospira*. Rezultati so pokazali, da so bile 104 živali pozitivne na vsaj enega od serovarov (34,1 %; 95-odstotni indeks: 29,01–39,59). Najpogostejša reakcija je bila na serovar Icterohaemorrhagiae (15,14 %), sledili so Canicola (14,75 %), Bratislava (11,47 %), Copenhageni (8,19 %), Pomona (7,86 %) in Hardjo (6,88 %). Najpogostejše so bile pri kobilah, starejših konjih, konjih, ki se redijo na pašnikih ali so v stiku s prežvekovalci ali psi in pri pomanjkanju nadzora nad glodavci. Velika seroprevalenca kaže, da so egiptovski konji, ki živijo na proučevanem območju, izpostavljeni velikemu tveganju za okužbo z bakterijo *Leptospira* sp. Zato je za ta zoonotski patogen zelo pomembna vzpostavitev programa nujnega nadzora in obvladovanja.

Ključne besede: Leptospirosis; seroprevalence; risk factors; horse; Egypt