

Evaluation of Antibody Against *Leptospira* in Horses

Farzaneh Fallah Taherpazir¹, Soheila Moradi Bidhendi^{2*}, Pezhvak Khaki²

¹Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, Iran

²Department of Microbiology, Reference Laboratory *Leptospira*, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

ABSTRACT

Objective: The incidence of leptospirosis in temperate regions is high among farm workers and animals. The disease in animal's causes fever, hemoglobinuria, icterus, abortion, mastitis and reduces milk production and impaired reproduction and death. Therefore, the condition of this disease and the study of common serotypes are essential for the development of a vaccine, as well as the high prevalence of infection in horses in regions of Iran.

Material and Methods: From 2015 to 2017, 317 serum samples were collected from the horses of Kerman, Zarand, Shahrehabak, Kish, Hashemabad, Soltanabad, Najafabadsemirom, Golpayegan. The serum samples were analyzed by microagglutination test (MAT).

Result: Results showed that in Kerman 80% of serums were positive followed by Kish (70.8%), Zarand (65%), Shahrehabak (58.3%), Hashemabad (57.1%), Soltanabad (57.5%), Najafabad semirom (43.2%) and Golpayegan (13.9%). The predominant serovars observed were *Leptospira icterohaemorrhagiae* (70.3%) and *Leptospira sejroe hardjo* (24.1%) followed by *Leptospira autumnalis* (13.1%), *Leptospira grippotyphosa* (10.9%), *Leptospira canicola* (13.1%) and *Leptospira Pomona* (6.5%).

Conclusion: The result showed that leptospiral antibody is present in a number of horses in these areas, and that responsible authorities should pursue the prevention and control of this multi-faceted disease in a more serious way than the spread of the disease in these areas.

Key words: *Leptospira*, Microscopic agglutination test, Horse

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Corresponding author: Soheila Moradi Bidhendi

e-mail: s.bidhendi@rvsri.ac.ir

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INTRODUCTION

Leptospirosis is an important disease between animal and human worldwide. *Leptospira* belong to family *Leptospiraceae*, it's spiral and thin. Their major antigenic component is lipopolysaccharide (LPS). This bacterium can see in urban and rural places especially in tropical and subtropical region) Khurana et al. [1] and there are also reports of sporadic symptoms because it can survive in a temperate climate for a longer time Tilahun et al. [2]; Fentahun et al. [3]. It is one of the six diseases that marked by Office International des Epizooties (OIEs) that discuss in the section of climate changes and diseases related to animal that is the main source of rodents and wildlife [4]. Horses can also be infected with the disease. The route of transmission is direct or indirect with urine, placenta fluid and environment [5]. Different carrier play an important roles for contaminate the environment such as rodents, marsupial and mammals [6,7]. Because of complexity in the taxonomy of *Leptospira* spp. more

than 300 serovars, grouped in 20 serogroups [8] that circulate between rats, other rodents, livestock and domestic pets [9]. Other animals which are not natural carriers of infection have mild to severe infection and even death. The leptospires may cause reproductive problems mainly abortions Hamond et al. [10]. Mother to fetus transmission is also common [11,12]. This disease is an occupational and has manifestations of different symptoms from asymptomatic infections to death in human [13]. Although the importance of zoonosis in the transmission of the disease in horse is not relevant but it should be cautious when abortion or the birth of a baby occurs [14]. The symptoms of the disease are different in the horse, which can include asymptomatic, mild and acute varying from fever, anorexia, and listlessness to renal failure, pulmonary haemorrhages, intracerebral haemorrhage, multisystem organ failure, anemia, conjunctival suffusions, petechial hemorrhages and hematuria. [11,15,16]. These symptoms cause a high economic loss in the horse breeding industry [17]. Between these disease uveitis is common in equine leptospirosis [18]. In laboratory for detecting *leptospira* microagglutination test (MAT) is widely used, [19] and it's qualitative and quantitative test which is hazardous

and time-consuming process [20]. The purpose of this research was to determine the serological evidence and the prevalence of *leptospira* in horses in areas of Iran by MAT.

MATERIAL AND METHODS

Serum samples

The experiments were carried out on horses from different regions of Iran during the years 2014 to 2016. From this, 317 serum samples were collected from different parts of Iran including 76 samples from Kerman, 43 samples from Zarand, 12 serum samples from Shahrehabak, 24 serum samples from Kish, 37 serum samples from Najafabadsemirom, 43 serum samples from Golpayegan, 49 serum samples from Hashemabad and 33 serum samples from Sultanabad.

Culture collection

The experiments were done at the Leptospirosis reference laboratory of the Razi Vaccine and Serum Research Institute of Karaj, Iran. This serum sample was stored at -20°C. Twenty live *L. interrogans* reference strains was used as the antigen that include *autumnalis*, *canicola*, *grippotyphosa*, *serjoe hardjo*, *icterohaemorrhagiae*, *pomona*, *serjoe serjoe*, *serjoe wolfii*, *pamona*, *malaysia*, *celledoni*, *lyme*, *djasiman*, *pyrogenes*, *ballum*, *javanica*, *australis*, *bataviae*, *serjo hardjobovis*, *semaranga*. Different serovars were cultivated separately in selective medium with supplement and kept in at 28°C.

Leptospirosis antibody detection and diagnosis

Detection of the antibody was done by MAT and using 20 live *L. interrogans* reference strains [21]. Briefly, in a 96-well microtiter plate serum samples were diluted and mixed with an equal volume of viable *Leptospira* strains. After incubation at 30°C for 2 h, the samples were examined for agglutination by dark-field microscopy. Titers that showed 50% agglutination, it is considered as a positive titer. A positive titre was considered at $\geq 1/100$.

RESULTS

Antibodies were found in 182 (57.4%) of 317 serum samples of horses in different areas of Iran (Figure 1).

The results from 317 serum samples of horses showed that 70.3% had titer against *L. icterohaemorrhagiae*, followed by 24.1% with *L. serjoe hardjo*, 10.9% with *L. grippotyphosa*, 13.1% with *L. autumnalis*, 13.1% with *L. canicola* and 6.5% with *L. pomona* (Figure 2).

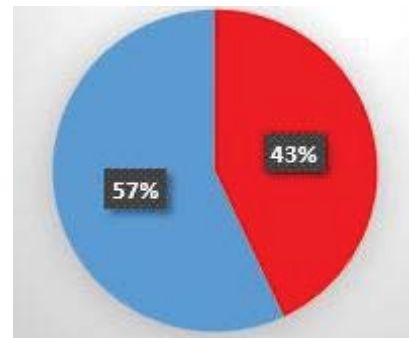


Figure 1: Distribution of relative frequency of positive and negative serological cases in all studied horses. Blue: Positive, Red: Negative.

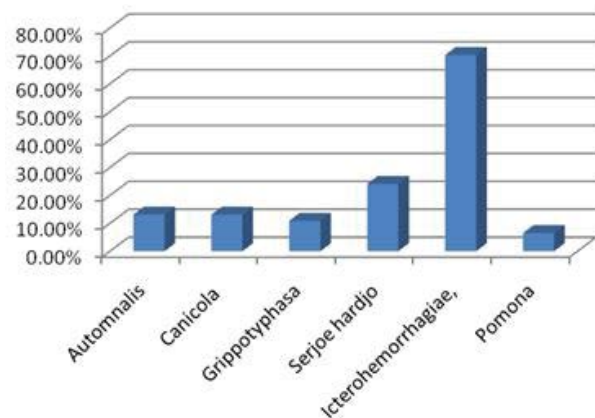


Figure 2: Frequency (%) of dominant serovars of leptospira.

According to Table 1, the highest prevalence of positive sera by MAT was found in Kerman 61 (80%), followed by Zarand 28 (65.1%), Shahrehabak 7 (58.3%), Kish 17 (70.8%), Najafabad semiram 16 (43.2%), Golpayegan 6 (13.9%), Hashemabad 28 (57.1%) and Sultanabad 19 (57.5%).

Table 1: Number and frequency (%) of positive sera in different areas

Cities	No.	Positive	Negative
kerman	76	61 (80%)	15 (19.7%)
zarand	43	28 (65.1%)	15 (34.8%)
Shahr babak	12	7 (58.3%)	5 (41.6%)
kish	24	17 (70.8%)	7 (29.1%)
Hashem abad	49	28 (57.1%)	21 (42.8%)
Sultan abad	33	19 (57.5%)	14 (42.4%)
Najaf abad semirom	37	16 (43.2%)	21 (56.7%)
Golpaygan	43	6 (13.9%)	37 (86.0%)

Table 2 shows relative frequency distribution of different serotypes of *leptospira* in serum samples of horses in different cities. The predominant serovars for Kerman were *L. icterohaemorrhagiae* and *L. serjoe hardjo*, Zarand were *L. icterohaemorrhagiae* and *L. canicola*, Shahr babak were *L. icterohaemorrhagiae* and *L. autumnalis* and *L. pomona*, kish were *L. icterohaemorrhagiae* and *L. autumnalis*, Hashem abad were *L. icterohaemorrhagiae* and

Table 2: Relative frequency distribution of different serotypes of leptospira in serum samples of horses

Cities	Count	Positive	Negative	Positive with <i>Automnalis</i>	Positive with <i>Canicola</i>	Positive with <i>Grippotyphasa</i>	Positive with <i>Serjoe hardjo</i>	Positive with <i>Icterohemorrhagiae</i>	Positive with <i>Pomona</i>
kerman	76	61	15	11	11	7	22	37	6
zarand	43	28	15	2	10	2	6	17	1
Shahr babak	12	7	5	-	-	-	7	1	6
kish	24	17	7	2	-	-	1	17	-
Hashem abad	49	28	21	6	1	5	10	18	3
Sultan abad	33	19	14	1	1	4	4	14	1
Najaf abad semirom	37	16	21	2	1	2	-	13	-
Golpaygan	43	6	37	-	-	-	1	5	-
Total	317	182	135	24	24	20	44	128	12

Table 3: Number of serum positive samples against different serotypes of leptospira in different dilutions

Serovars \titer	1/200	1/400	1/800	1/1600	1/3200	Total
<i>Automnalis</i>	7	11	7	-	-	25
<i>Canicola</i>	3	8	8	6	3	28
<i>Grippotyphasa</i>	3	11	5	4	-	23
<i>Serjoe hardjo</i>	19	16	9	3	1	48
<i>Icterohemorrhagiae</i>	28	45	36	22	7	138
<i>Pomona</i>	6	3	8	-	-	17
Total	66	94	73	35	11	279

Table 4: The number of serum samples with which number of antigens are positive

	No. of positive samples with 1 antigen	No. of positive samples with 2 antigen	No. of positive samples with 3 antigen	No. of positive samples with 4 antigen	No. of positive samples with 5 antigen
kerman	19	18	6	2	1
zarand	15	10	-	-	-
Shahrebabak	6	1	-	-	-
kish	14	3	-	-	-
Najafabad semirom	14	2	-	-	-
Golpaygan	6	-	-	-	-
Hashem abad	16	9	3	-	-
Soltan abad	13	5	1	-	-

L. serjoe hardjo, Sultan abad were *L. icterohemorrhagiae*, *L. serjoe hardjo* and *L. grippotyphasa*, Najaf abad semirom were *L. icterohemorrhagiae*, *L. grippotyphasa* and *L. automnalis* and Golpaygan were *L. icterohemorrhagiae* and *L. serjoe hardjo*.

Also in this study, antibody titer included 1/200 for 66 (23.6%), 1/400 for 94 (33.6%), 1/800 for 73 (26.1%), 1/1600 for 35 (12.5%), 1/3200 for 11 (3.9%) samples (Table 3).

In this study 48 (15.1%) out of 317 serum samples had titer against 2 serovars followed by 10 (3.1%) out of 317 serum samples had titer against 3 serovars, 2 (0.6%) out of 317 serum samples had titer against 4 serovars and 1 (0.3%) out of 317 serum samples had titer against 5 serovars (Table 4).

DISCUSSION

Leptospirosis is a zoonotic disease and considered as emerging disease that caused by *leptospira* [22]. Very

little is known about the seroepidemiology of *leptospira* infection in horses in Iran.

In this research 317 horse serum samples collected from different area in iran conclude Kerman, Zarand, Shahrebabak, Kish, Najafabad, Golpayegan, Hashemabad and Sultanabad. The seroprevalence of *Leptospira* in the studied population was 70.3%, and the type of serovars was dominated by *L. icterohemorrhagiae* because *Leptospira* is a common disease between humans and animals and is a work-related illness that is directly or indirectly caused by direct or indirect contact with the disease, the disease should be detected in the community.

In the research was done by Hajikolahi et al. [23] the result showed that serovar *grippotyphosa* is present in 33.33% of horses in Ahavaz area in Iran. The highest titer among the samples were 1/100 and 1/200 (71.43 and 28.57% of positive horses, respectively) [11].

Many research in different country in horse showed that different serovars were predominant such as serovar Bratislava (25%) in Ireland, Pomona (30.5%)

in Queensland, Pomona (12.47%) in California, and Pomona (48.7%) in India [8] Khoushah et al. collected 90 horse serum samples in Ardebil. They worked with 7 live antigens, *Pomona*, *Canicola*, *Hardjo*, *Ballum*, *Icterohaemorrhagiae*, *Australis* and *Grippotyphosa*. The prevalence of leptospiral infection was 7.77% in horses. The dominant serovar was *Hardjo*, followed by *L. icterohaemorrhagiae* (29%), *L. pomona* (14%) and *L. grippotyphosa* (14%). Our result was in agreement with their results with slight difference in the species identification. *L. icterohaemorrhagiae* was predominant serovar in our study.

This indicates that over the past several years, serovars have been involved and their percentage has changed. The geographic region and the type of weather and carriers are involved in the spread of these serotypes. The infection is caused by more than one serovar, which means that according to the common strain in the region, the vaccine should be made and used. Although *Leptospiral* disease is not common in horses but studies have shown that the disease is widespread and the predominant serovars varying from region to region. Since antibody production appear within a few days after infection and persist for weeks or months but in chronic disease antibody titers may fall to undetectable levels. So its need sensitive methods to detect the bacteria in urine or the genital tract of chronic carriers. These results confirmed that *leptospiral* infection [24] may exist in the horse population in Iran and the presence of antibodies in the absence of infection indicates exposure to the organism and must be acknowledged. In addition, these results confirmed that the majority of *leptospiral* infections are asymptomatic.

CONFLICT OF INTEREST

There is no of conflict of interest.

FUNDING SOURCES

Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

ETHICAL CONSIDERATION

This work was approved by the ethical committees of Department of Microbiology, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

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