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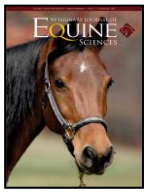

Original Article

A survey of equine anti-*Listeria monocytogenes* antibodies using Latex Agglutination Test in southeast of Iran

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ABSTRACT

Listeriosis is a zoonotic disease in humans and a wide range of domestic and wild animals and also some birds. The main purpose of the current study is to determination of anti-*Listeria monocytogenes* sero-prevalence in horses by latex agglutination test in southeast of Iran. A total of 163 serum samples were obtained from apparently healthy horses of equestrian clubs in Kerman and Yazd provinces - Iran. *Listeria monocytogenes* antibodies were found in 34 out of the 163 sera (20.85%). The latex agglutination test can be considered as an appropriate screening test in the early stages of diagnosis.

ردیابی پادتن ضد لیستریا مونوسیتوژنز با روش لاتکس اگلوتیناسیون در اسبان جنوب شرق ایران

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چکیده

لیستریوز یک بیماری مشترک میان انسان و طیف وسیعی از دامهای اهلی، وحشی و پرندگان می باشد. هدف از انجام مطالعه ی حاضر ردیابی پادتن ضد لیستریا مونوسیتوژنز با روش لاتکس اگلوتیناسیون در اسبان جنوب شرق ایران می باشد. برای این منظور تعداد ۱۶۳ نمونه سرم از اسب های به ظاهر سالم باشگاه های سوارکاری استان های یزد و کرمان تهیه شد. نتایج مطالعه حاکی از حضور پادتن در ۳۴ نمونه از مجموع ۱۶۳ سرم مورد بررسی (۲۰/۸۵ درصد) می باشد. بر این اساس به نظر می رسد که لاتکس اگلوتیناسیون آزمایش مناسبی برای غربالگری اولیه به منظور تشخیص بیماری می باشد.

واژه های کلیدی: لیستریا مونوسیتوژنز، آزمایش لاتکس اگلوتیناسیون، اسب، ایران

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Introduction

Listeriosis is one of the food-borne zoonosis diseases around the world caused by Listeria species. This causes considerable morbidity and mortality in humans and animals and the bacterium is considered an important food borne pathogen [1]. *Listeria monocytogenes* is ubiquitous, aerobic, gram positive and rod-shape bacterium, which is capable of causing severe disease in many species as sheep, cattle, goats, horse, humans and chickens [7,19]. There is six species of listeria in this genus that categorized into three groups as genome analyze, that first group contains *L. monocytogenes, L. innocua* and *L. welshimeri*, the second one *L. ivanovii*, and *L. seeligeri*, and the third include *L. grayi* [8]. Listeriosis occurs as a sporadic disease in horses with highly pathogenic intracellular, non-acid resistant bacteria and is clinically characterized by meningo-encephalitis. Signs associated with the nervous syndrome include paralysis of the mandibular and pharyngeal muscles, difficulty walking, inappetence, polydipsia, loss of body weight, and collapse. Following the first observation of signs, animals usually die within 3-10 days [9,11]

Materials and Methods

Sample collection and processing

A total of 163 serum samples were collected from 93 male and 70 female (clinically healthy) at 9 equestrian clubs in Kerman and Yazd provinces, Iran. The breed composition comprised of Arab and Arab cross horses (50 %) and non-Arab horses (50%) including Darreh–Shouri (5%), Turkmen (12 %), Thoroughbred (3 %) and mixed breeds (30 %). After collection, the samples were submitted to Microbiology laboratory of School of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran. Sera were stored at − 20°C until analysis.

Latex Agglutination Test (LAT)

Latex Agglutination Test Kit (Zist Faravard Pars Co, Rasht, Iran) was used according to the manufacture's protocol. Briefly, 10 µl of serum and 10 µl of antigen coated latex particles were added to on an agglutination card and mixed with a plastic stirrer. The card was rocked from side to side for up to 5 min to provoke the agglutination reaction. Specimens that showed agglutination during this period were recorded as positive, and otherwise negative. Incomplete agglutination recorded as suspected. Specificity and sensitivity assurance was carried out by testing positive and negative controls on daily basis.

Results

According to the Table 1 and 2, antibodies were detected in 34 sera (20.85%) out of 163 samples (21 cases ≤ 7 years old and 13 cases > 7 years old). Based on the results presented on Table 1 and 2, 21 (15 cases ≤ 7 years old and 6 cases > 7 years old) male (12.88%) and 13 (6 cases ≤ 7 years old and 7 cases > 7 years old) mares (7.98%) were sero-positive against listeriosis.

Table 1. Number and frequency (%) of positive, suspected and negative samples among 163 equine serum samples

Gender	Positive		Suspect		Negative		Total
	No*	F (%) **	No	F (%)	No	F (%)	No
Stallion	21	12.88	22	13.50	50	30.67	93
Mare	13	7.98	22	13.50	35	21.47	70
Total	34	20.86	44	27	85	52.14	163

*: Number of cases **: Frequency (percent)

Table2. The number and frequency of positive, negative and suspected samples (based on the presence of antibodies against *Listeria monocytogenes*) out of 163 horses in Kerman and Yazd provinces, Iran

Province	Gender	Age												Total					
		≤ 7 years old						> 7 years old											
		P		N		S	T	P		N		S	T	P		N		S	T
		No	F	No	F	No	F	No	F	No	F	No	F	No	F	No	F	No	F	No
Yazd	Stallion	7	14.58	31	64.58	10	20.83	48	1	14.28	1	14.28	5	71.42	7	8	14.54	32	58.19	15	27.27	55
	Mare	3	8.57	20	57.14	12	34.28	35	1	8.33	4	33.33	7	58.33	12	4	8.52	34	51.06	19	40.42	47
Kerman	Stallion	8	36.36	11	50	3	13.63	22	5	31.25	7	43.75	4	25	16	13	34.21	18	47.36	7	18.42	38
	Mare	3	27.27	6	54.54	2	18.18	11	6	50	5	41.66	1	8.33	12	9	39.13	11	47.82	3	13.04	23
Total		21	18.10	68	58.62	27	23.27	116	13	27.65	17	36.17	17	36.17	47	34	20.85	85	52.14	44	26.99	163

Discussion

Our results showed the total seropositivity for all 163 horses examined in this study was 20.85%. *Listeria monocytogenes* widely exists in the water, soil, plants, feces and feedstuff such as silage, vegetables and moldy forage. This bacteria is considered as one of the most important sources of infection in both domestic and wild animals and also some birds [16]. The prevalence of listeriosis has been mostly reported during the winter which can be due the fact that silage is frequently fed to animals in this season and also the pregnancy of animals predisposes them to this

infectious disease [12]. Listeriosis in farm animals often occurs in three main forms: encephalitis, septicemia and abortion [5]. However, the encephalitis form has a lower occurrence rate in horses [5,7]. Teruya *et al.*, (1977) conducted a study on equine listeriosis in Brazil using tube agglutination method and reported that 22.7% of 838 examined horses were seropositive. Solmaz *et al.*, (2002) carried out a similar study in Turkey using the same method and found 176 positive (86.7%) out of 203 horses [15,17]. Guclu *et al.,* (2007) showed 62 cases among 100 tested horses were sero-positive against *L. monocytogenes* using the Osebold absorption test. Anti *L. monocytogenes* antibodies were detected at 1:100, 1:200 and 1:400 titers in 29 (46.7%), 31 (50%) and 2 (3.2%) animals, respectively [20]. The results of the present study are similar to the study conducted by *Teruya et al.,* (1977) and Guclu *et al.,* (2007). However, higher prevalence in the study conducted by Solmaz *et al.,* can be due to factors such as climate variation, feedstuff and health management of the animals.

The results of the current study showed more sero-positivity among older males in contrast with previous study showed there is no relationship between age and sex and incidence of listeriosis. Saqib *et al.*, (2015) reported that 23.5% out of 183 equine serum samples were seropositive and number of seropositive males was higher than females [13]. Results of the present study in Yazd and Kerman provinces showed that the higher anti *L. monocytogenes* antibody titer found in horses in Kerman is possibly due to moderate climate and also the lower temperature and different management and any other factors or any combination(s). The obtained results of the separate studies in each province showed that the sero-positivity in the old males of Kerman follows the general rule which was mentioned earlier. The results of serological studies have shown that the presence of anti *L. monocytogenes* antibody may be widespread worldwide, but the absence of definitive clinical diagnosis and data makes it impossible to estimate the true incidence of listeriosis in animals and humans. These results have also revealed that the sero-prevalence of positive antibody titer against *Listeria monocytogenes* in animals varies widely with species tested, geographic location, season, assay type and the criteria used to define positive results [2,3,4,9,18]

It has been reported that the antigenic relationship between various serotypes of *L. monocytogenes* and a number of gram-positive and gram-negative bacteria (such as *Staphylococcus aureus*, *Streptococcus faecalis*, *Arcanobacter pyogenes*, *Bacillus subtilis* and *Escherichia coli*) may cause false-positive results in serological tests [10,14]. The latex agglutination test can be considered as an appropriate screening test in the early stages of diagnosis, because mentioned method has a favorable sensitivity and specificity. It is also a fast and cheap method which can be performed in all laboratories.

Conclusion

The current study detected anti *L. monocytogenes* antibody in the horses' population of Kerman and Yazd provinces, Iran. Mentioned results showed that the higher antibody titer found in Kerman province is possibly due to moderate climate and also the

lower temperature and different management and any other factors or any combination(s).

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Conflict of Interest

There is no conflict of intrest.

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