Turk. J. Vet. Anim. Sci.
2007; 31(4): 271-273

Short Communication

The Seroprevalence of *Listeria monocytogenes* in Sport Horses Bred in Ankara Province

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Received: 05.01.2006

Abstract: The aim of this study was to determine the seroprevalence of *Listeria monocytogenes* in horses bred for sport in the province of Ankara. Sera collected from 100 clinically healthy horses were tested for the presence of *L. monocytogenes* antibodies using the Osebold absorption test. Agglutination titers ≥ 1:100 were accepted as positive results and a seropositivity rate was determined. Of the 100 serum samples, 62 were seropositive for *L. monocytogenes* antibodies detected at dilutions of 1:100 (n = 29, 46.7%), 1:200 (n = 31, 50%), and 1:400 (n = 2, 3.2%).

Key Words: *Listeria monocytogenes*, Osebold absorption test, horse

Ankara Yöresinde Sportif Amaçlı Yetiştirilen Atlarda *Listeria monocytogenes*'in Seroprevalansı

Özet: Bu çalışma, Ankara Yöresinde sportif amaçlı yetiştirilen atlarda *Listeria monocytogenes*'in seroprevalansının saptanması amacıyla yapılmıştır. Klinik olarak sağlıklı toplam 100 attan elde edilen serumlar Osebold absorbsiyon testi ile *L. monocytogenes* antikorları yönünden incelenmiştir. 1/100 ve üzerindeki titrasyon basamaklarındaki aglütinasyon varlığı pozitif olarak kabul edilmiştir. 100 serum örneğinden 62 (% 62) serum örneği çeşitli dilusyonlarda pozitif olarak değerlendirilmiştir. Seropozitif bulunan serumların antikor titreleri incelendiğinde, 29'u (% 46,7) 1/100, 31'i (% 50) 1/200, 2'si (% 3,2) 1/400 titrede *Listeria monocytogenes* antikorları saptanmıştır.

Anahtar Sözcükler: *Listeria monocytogenes*, Osebold absorbsiyon testi, at

Listeriosis is an infectious disease characterized by meningoencephalitis, abortion, septicemia, and mastitis in animals. Known to cause similar dysfunctions in humans, listeriosis is a zoonotic disease (1,2). The disease is caused by facultative, intracellular, gram-positive, non-acid resistant, regular round-ended *Listeria* species that are 0.5-2.0 × 0.4-0.5-µm-sized short rods that do not form spores and do not have capsules.

This microorganism is found in the normal microflora of the digestive system of healthy humans, mammals, and avian species (3). Taxonomically, it is divided into 6 species (*Listeria monocytogenes, Listeria ivanovii, Listeria seeligeri, Listeria innocua, Listeria welshimeri*, and *Listeria grayi*), of which only *L. monocytogenes* and *L. ivanovii* are potentially pathogenic. *L. monocytogenes* has been determined to cause listeriosis in horses (2-4).

The disease displays a sporadic course in horses and is generally characterized by meningoencephalitis. Other symptoms that may be observed include paralysis of the mandibular and pharyngeal muscles, difficulty walking, inappetence, polydipsia, loss of body weight, and collapse. Following the first observation of symptoms, animals die within 3-10 days (5,6).

In January and March 2004 we collected 10-ml blood samples, in accordance with the routine method, from 100 horses (25 female, 75 male; age range: 3-16 years) bred in Ankara province, Turkey, for sportive purposes. Sera were harvested from the blood samples by centrifugation (Labofuge 200, Hareus, Germany) at 4000 rpm for 10 min at room temperature. Serum samples were kept at –20 °C until tested.

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Diagnostic procedures were carried out at the Department of Communicable Diseases Research at Refik Saydam Hygiene Center (RSHC) in Ankara.

The presence of anti-*L. monocytogenes* antibodies was determined by agglutination tests according to the method described by Osebold et al. (7). *Listeria ivanovii* was excluded from this study. The antigen used in the present study was prepared in RSHC and the assay was performed in 3 steps. Briefly, first the whole cell antigens were prepared from *Staphylococcus aureus* (ATCC 29213) strains with the Osebold method. In the second step the antigens were prepared from *L. monocytogenes* 1/2a, 1/2b, 4b, 4c, and 4d strains, and then combined to prepare the *L. monocytogenes* antigen pool. The third step was performing the agglutination test after absorption of the sera samples with *S. aureus* antigen. Samples with a titer ≥ 1:100 were considered positive. Positive and negative controls were used in each test.

Among the 100 horses tested for the presence of *Listeria monocytogenes* antibodies using the Osebold absorption test, 62 (62%) were found to be seropositive. Among the 62 seropositive animals, *L. monocytogenes* antibodies were detected at dilutions of 1:100, 1:200 and 1:400 in 29 (46.7%), 31 (50%), and 2 (3.2%) animals, respectively.

Serological studies have suggested that infection may be widespread worldwide, and that the lack of clinical data and diagnosis renders it impossible to estimate the true incidence of the disease in animals and humans (2,3,6,8,9).

Studies in recent years have indicated that *L. monocytogenes* infection displays different clinical courses and that this aspect should be taken into consideration. Clark et al. (10) determined a combined immunodeficiency (B and T lymphocytes) syndrome in a 1-month-old Arabian foal with typical symptoms of the central nervous system. This animal died despite intense antibiotic treatment, and widespread lymphocytic hypoplasia, granulomatous inflammation of the brain, heart and adrenal glands, spinal meningitis, and focal hepatic necrosis were observed at necropsy. *L. monocytogenes* was reported to have been isolated from a primary culture of the brain. Wallace and Hathcock (11) reported diarrhea, lethargy, fever, and leucopenia in a foal, and isolated *L. monocytogenes* from the blood culture. Wilkins et al. (12) diagnosed *L. monocytogenes* septicemia in a 6-day-old foal. Sanchez et al. (13) reported pain and lacrimation in the right eye of a mare and isolated *L. monocytogenes* from the corneal lesions. Evans et al. (14) diagnosed an eye infection in a horse caused by *L. monocytogenes*.

The prevalence of *Listeria* spp. infection in animals varies widely with species tested, year, geographic location, assay type, and criteria used to define positive results. Seropositivity to *L. monocytogenes* in healthy horses has been reported by numerous researchers (10-16). The reasons for this discrepancy may be explained by spatial, temporal, strain, and many other factors determining the prevalence of listeriosis in horses, as well as possible differences among laboratories and testing procedures.

The disease is reported to be diagnosed by serological methods in many countries. In Brazil, Teruya et al. (15) tested 838 horse serum samples using the tube agglutination method and determined a seropositivity of rate 22.7%.

Listeriosis in animals was first recognized when a small outbreak was observed in 200 pregnant mares in Turkey in 1945. Among these animals, 12 displayed the encephalitic form of the disease and 5 died due to listeriosis within 5 days (16).

L. monocytogenes was first diagnosed in equine animals by means of serological methods in Turkey by İnci et al. (16). The researchers performed the Osebold absorption test on 120 serum samples pertaining to 67 horses, 20 mules, and 33 donkeys bred in the province of Kayseri, Turkey, and 27 (40.29%), 3 (15%), and 10 (30.3%), respectively, were seropositive. Among the 120 equines that were tested, 40 (33.3%) were seropositive for *L. monocytogenes*.

The seropositivity rate of 62% found in the present study is higher than the percentage previously determined in the province of Kayseri, which might be related to geographical variation and different breeding practices.

Results of previous studies point out that the identification of *L. monocytogenes* antibodies by serological techniques does not prove the diagnosis of active infection. A large percentage of sera from humans and animals contain antibodies against *L. monocytogenes* in the absence of the infection (17,18). Without longitudinal and repeated sampling, seroprevalence studies do not provide information on the incidence of

current infection in animals nor indicate whether an animal is infectious. Instead, the results can only be interpreted in animals as evidence of previous infection (past exposure) (19).

It has been reported that the antigenic relationship between different serotypes of *L. monocytogenes*, and some gram-positive and gram-negative bacteria (*Staphylococcus aureus, Streptococcus faecalis, Corynebacterium pyogenes*, *Bacillus subtilis*, and *Escherichia coli* K8) may cause false-positive results in serological tests (8,20). To determine *L. monocytogene* O antibodies, as described by Osebold et al. (7), by treating serum samples with the whole cell antigens of *S. aureus* and the Listeria antigen with trypsin in order to eliminate antibodies against *S. aureus*, increases test sensitivity and eliminates cross reactions.

In conclusion, the present study found a high rate of seropositivity in Ankara that is similar to the results of a previous study in Kayseri, which indicates that clinical disease might also be observed in Turkey and that the disease may have been overlooked by veterinarians until now. Therefore, horses displaying symptoms similar to those described herein should also be tested for *L. monocytogenes*.

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