

1-1-2006

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Recommended Citation

BÖRKÜ, M. KAZIM; URAL, KEREM; GAZYAĞCI, SERKAL; ÖZKANLAR, YUNUSEMRE; BABÜR, CAHİT; and KILIÇ, SELÇUK (2006) "Serological Detection of Listeriosis at a Farm," *Turkish Journal of Veterinary & Animal Sciences*: Vol. 30: No. 2, Article 20. Available at: <https://journals.tubitak.gov.tr/veterinary/vol30/iss2/20>

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Serological Detection of Listeriosis at a Farm

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Received: 28.03.2005

Abstract: Sera of 50 goats, 3 horses, 1 dog, and 6 herdsmen were analyzed for the presence of antibodies against *Listeria monocytogenes* by the Osebold agglutination assay on a commercial farm in Akyurt, Ankara. Some of the goats exhibited clinical signs such as apathy, unilateral facial paralysis, incoordination, and abortion.

Of the goat samples, 23 (46%) were seropositive and 10 (20%) were at the level of the suspected titer and 17 (34%) were below the cut-off titer in this assay. All horses, the dog and 5 herdsmen were seropositive. The analysis of cerebro-spinal fluid, taken from an agonic goat whose antibody titer was 1/400, revealed a total leukocyte count of 1000 white blood cells per decaliter and total protein of 228.4 mg/dl.

The results of this study suggest that listeriosis is prevalent in different animal species and humans, and this infection should be considered by both veterinarians and public health officials.

Key Words: Listeriosis, goat, Osebold method

Bir Çiftlikte Listeriosis'in Serolojik Belirlenmesi

Özet: Ankara Akyurt yakınlarında bir ticari işletmede bulunan 50 keçi, 3 at, 1 köpek ve 6 hayvan bakıcısının kan örnekleri *Listeria monocytogenes* antikorlarının varlığı yönünden Osebold methodu ile kontrol edildi. Keçilerden bazıları apati, unilateral fasiyal paraliz, inkoordinasyon ve abort gibi semptomlar gösteriyordu.

Bu çalışmada keçi serumlarından 23'ünde (% 46) seropozitivite ve 10 serum (% 20) şüpheli ve 17 serum (% 34) sınır değerinde tespit edildi. Atların tamamı, köpek ve 5 hayvan bakıcısı seropozitif olarak bulundu. Antikor titresi 1/400 olan agoni halindeki bir keçiden alınan serebro-spinal sıvı analizinde total lökosit 1000 WBC/dekalitre ve total protein 228,4 mg/dl belirlendi.

Bu çalışmada elde edilen bulgular listeriosis'in farklı hayvan türleri ve insanlarda bulunabileceğini ve bu enfeksiyonun veteriner hekimler ve halk sağlığı yetkilileri tarafından birlikte ele alınması gerektiği fikrini vermektedir.

Anahtar Sözcükler: Listeriosis, keçi, Osebold metodu

Introduction

Listeriosis, caused by a facultative intracellular microorganism called *Listeria monocytogenes*, has a worldwide sporadic distribution especially in temperate climates (1-4). Listeriosis occurs commonly in ruminants, but it can affect fowl and humans as well (2). Although silage and heavy browse consumption have been proposed as the source of the infection, listeriosis was

reported in ruminants that were not fed silage (3,5). *L. monocytogenes* may be transmitted not only venereally in ruminants, but also by feces of latent carriers or by fomites (5). The disease has 3 distinct clinical syndromes: abortion following the infection of the pregnant uterus, septicemia in fetuses or neonates, and encephalitis in adult animals (3,6). Among ruminants, encephalitic listeriosis has been reported more commonly in sheep than in goats, although it affects all goat breeds (3).

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The purpose of this study was to demonstrate the presence of antibodies to *L. monocytogenes* on a farm including goats with clinical signs (i.e. encephalitis, apathy, unilateral facial paralysis, incoordination, and abortion) and asymptomatic goats, horses, a dog and herdsmen.

Case History

The present study was carried out in a commercial farm, with a total of 50 goats, which included 1- to 6-year-old goats. Age distribution of the goats was as follows: 1 year old, n = 14; 2 years old, n = 12; 3 years old, n = 6; 4 years old, n = 5; 5 years old, n = 7; and 6 years old, n = 6. Of the 50 goats, 23 were showing clinical signs such as encephalitis, apathy, unilateral facial paralysis, incoordination and abortion. Three horses, 1 dog, and 6 herdsmen at the same farm were also included in this study. Respiratory rate and rectal temperature were recorded shortly after the animals were clinically examined.

In an attempt to identify possible unapparent carriers among the animals, rectal and vaginal swab specimens were collected for bacteriologic examination from 7 goats known to have aborted. All the swab samples were put into tubes containing Listeria Enrichment Broth (Leb, Merck, USA) (7).

Cerebro-spinal fluid from an agonic goat was collected into an EDTA-treated tube and submitted to the laboratory immediately. Cheeses produced from unprocessed milk consumed by herdsmen on the same farm were cultured to investigate the presence of Listeria agents (7).

Blood samples were taken from all the goats exhibiting clinical signs (n = 23) and also from apparently healthy goats (n = 27), 3 horses, 1 dog and 6 herdsmen. The obtained sera were stored at -20 °C until analysis.

An antibody titration test to detect antibodies for *L. monocytogenes* was carried out according to the method described by Osebold et al. (8). The test antigen used in the present study was prepared in the Laboratories of Refik Saydam National Hygiene Center, Department of Communicable Diseases Research, and the assay was carried out in 3 steps. For the first step, the whole cell antigens were prepared from *Staphylococcus aureus* (ATCC 29213) strains by the Osebold method (8). In the second step, the antigens were prepared from *L. monocytogenes* 1/2a, 1/2b, 4b, 4c and 4d strains and

were combined in the same suspension. In the last step an agglutination test was performed after the absorption of sera samples with *S. aureus* antigen (8).

A 1-1.5 g cube consisting of surface and interior material was cut from each cheese. The cube was macerated and blended with peptone water by a stomacher. In addition, 10-fold serial dilutions for 1 ml of macerate were performed in peptone water. For each dilution step, a 0.1-ml sample was surface-inoculated onto Listeria Selective Agar (LSA, Merck) (7). The cheeses were stored at +4 °C in the laboratory until required.

In order to evaluate serum samples for seropositivity, titers of <1/50, 1/50-1/100, and ≥1/100 were considered negative, suspected, and positive, respectively.

Results and Discussion

Of the goat sera tested against *L. monocytogenes* "O" antibodies, 17 (34%) were seronegative, 10 (20%) were suspected, and 23 (46%) were seropositive. Titrations of seropositive samples were 1/100 (20%), 1/200 (24%) and 1/400 (2%) (Table). Five herdsmen (1/100), all horses (1/200) and the dog (1/100) were also seropositive, while 1 herdsman was suspected (1/50) (Table).

Total leukocyte counts and total protein in CSF sample were 1000 white blood cells per decaliter (reference range, 0-7 WBC/dl) and 228.4 mg/dl (reference range, 24-40 mg/dl) (2), respectively.

No *L. monocytogenes* was isolated from the rectal and vaginal swab samples or from on-farm manufactured cheeses.

Encephalitic listeriosis has been suggested as a cause of considerable morbidity and mortality. Morbidity and mortality may reach 10% or more, during winter in Angora breed goats and other goats that browse heavily (3,9,10). Young animals have been reported to be more susceptible to listeriosis (11).

In a previous study in 5 goats with experimentally induced listeriosis, severe infections occurred in the youngest animals (4). Generally, young animals are more prone to Listeria infection than old animals (11). Similarly, the present study, based on humoral immune response, most of the affected goats were younger, as described previously (11). In the 12 goats, consisting of 2 adults and 10 young animals, titers were determined as

Table. Anti-*L. monocytogenes* "O" antibody titers.

	Titers of serum samples					Total
	0 Seronegative	1/50 Suspected	1/100 Seropositive	1/200 Seropositive	1/400 Seropositive	
Goats	17	10	10	12	1	50
Dogs	-	-	1	-	-	1
Horses	-	-		3	-	3
Herdsmen	-	1	5	-	-	6

1/200 for all (Table). The formation of high level of specific antibodies in the present study might be related with the age of animals and habitat of food intake.

Cerebrospinal fluid (CSF) analysis has been used as a tool in the diagnosis of listeriosis although the cell and protein concentrations in the CSF samples do not correlate with the symptoms or prognosis (4). Total leukocyte counts were 1000 white blood cells per decaliter and total protein was 228.4 mg/dl. This may help in the diagnosis of listeriosis along with clinical signs and serology.

During an outbreak of listeriosis, *L. monocytogenes* can be isolated from the feces of apparently normal animals (12). In addition, *L. monocytogenes* is shed in feces of symptomatic carriers during the final period of pregnancy and during the lambing season (2). In a previous study involving experimentally infected goats, pre-existing antibodies in serum were found in association with rapid clearance of *L. monocytogenes* from the gastrointestinal tract and the absence of clinical signs, suggesting resistance against the organism. Moreover, in the latter study, a similar association was reported between the constitution of high levels of specific IgG antibodies and the disappearance of *L. monocytogenes* from the feces, suggesting the role of humoral defense involved in eliminating listeriosis (4). In the present study, in order to identify possible unapparent carrier animals, rectal and vaginal swab specimens were collected for microbiological culture from 7 goats known to have aborted. However, no *L. monocytogenes* was isolated from the swab samples. This may be related to the formation of high levels of specific antibodies in the goats of the present study that might contribute to humoral defense involved in the elimination

and disappearance of *L. monocytogenes* from the feces, as reported previously (4).

Results of earlier investigations support the determination of antibody levels during the infection period by means of agglutination, complement fixation, immune precipitation and passive immunohemolysis tests with killed bacterial antigens, although detection of antibodies against *L. monocytogenes* does not prove the diagnosis of the active status of the infection. A large percentage of the sera from humans and animals contain antibodies against *L. monocytogenes* in the absence of the infection (13-15). 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) can cause false-positive results (3,15,16). In the method described by Osebold et al. (8) to detect *L. monocytogenes* 'O' antibodies, sera samples were treated with the whole cell antigens of *S. aureus* in order to eliminate antibodies against *S. aureus*. Additionally, the chemical and immunological composition of *L. monocytogenes*' surface structures showed that treatment of the antigen with trypsin increased its sensitivity and eliminated cross reactions (8).

Cut-off values in agglutination tests have been controversial. Some previous authors concluded that titers exceeding 1/100, 1/160, 1/200 and 1/320 should be considered positive (3,16-18). However, using the Osebold method, as cross reactions are eliminated and test sensitivity increased, a cut-off value equal or higher than 1/100 should be regarded as positive (14-16), as was the case in this study.

Human listeriosis, as a foodborne infection, has received attention, and 10 cases reported in the United Kingdom and Republic of Ireland consisted of a driver, wholesale butcher, veterinarians and farmers (19). In the present study, the high seropositivity results against *L. monocytogenes* in herdsmen were found in association with a previously reported study in Ankara, Turkey (20).

In conclusion, higher rates of seropositivity against *L. monocytogenes* in goats, horses, a dog and herdsmen on a commercial farm suggest that *L. monocytogenes* infection should be taken into consideration by veterinarians and public health officers.

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