Uludag Univ. J. Fac. Vet. Med.
24 (2005), 1-2-3-4: 95-98

Determination of Seroprevalence of *Listeria Monocytogenes* Antibodies in Cattle in Bursa Province of Turkey

Engin KENNERMAN* Cahit BABUR** Selçuk KILIÇ**

Geliş Tarihi: 01.02.2006
Kabul Tarihi: 07.03.2006

Summary: To determine the seroprevalance of antibodies to *L. monocytogenes* in cattle in Bursa province of Turkey was aimed in this study. Two hundred nine, aged 1-5 years, healthy Holstein cattle were randomly selected from 6 different districts and after clinical examination blood samples were collected. The herd size (number of cattle in each herd) and the animals fed silage were recorded. Antibodies to *L. monocytogenes* were determined by agglutination test according to the method described by of Osebold et al. Hematological parameters (white blood cell count, circulating red blood cell count, hematocrit and differenciated cell counts) were analyzed.

Antibodies to *L. monocytogenes* were found in 101 (48.3 %) of the 209 cattle tested. Of 209 cattle tested 54 (25.8%) had 1/50, 74 (35.4%) had 1/100, 23 (11.0%) had 1/200 and 4 had (1.9%) 1/400 agglutination titer whereas no agglutination was detected in 54 (24.88%) cattle. 53.7% (58/108) of female and 42.5% (43/101) of male cattle have been found to be seropositive. The seropositivity in silage fed cattle (57.5%, 92/160) was found statistically higher than cattle that were not feed silage (18.3%, 9/49) ($p<0.001$). The higher seropositivity (55.8%) was observed in cattle from large herd size (50 – 100 cattle).

As a result, in this study seroprevalence of antibodies to *Listeria monocytogenes* in cattle in Bursa province of Turkey was found as 48.3 % and it was concluded that silage feeding is an important factor in the epidemiology of listeriosis. Larger and detailed prophylactic studies must be planned to control this zoonosis infection for animal and human health.

Key Words: Listeriosis, cattle, Turkey.

Bursa Bölgesindeki Sığırlarda *Listeria monocytogenes* Antikor Seroprevalansının Belirlenmesi

Özet: Bu çalışmada Bursa bölgesindeki sığırlarda L. *monocytogenes* antikor seroprevalansının belirlenmesi amaçlandı. Çalışmada Bursa bölgesinde 6 farklı ilçeden rasgele seçilen Holstein ırkı, 1-5 yaşlı, sağlıklı 209 sığırın klinik muayeneleri yapıldı ve kan örnekleri alındı. Sürüdeki hayvan sayısı (sürü boyutu) ve silajla beslenen hayvanlar kaydedildi. L. *monocytogenes* antikor düzeyleri Oesborn ve ark. tarafından belirtilen aglutinasyon testi kullanılarak belirlendi. Tüm sığırların hematolojik muayeneleri (total lökosit, eritrosit, hematokrit ve formül lökosit) yapıldı. L. *monocytogenes* antikor düzeyi 1/100 ve üzeri olanlar seropozitif olarak kabul edildi.

İki yüz dokuz sığırın 101'inde (% 48.32) *L. monocytogenes* antikorları belirlendi. Çalışmadaki sığırların 54'ünde (% 25.8) 1/50, 74'ünde (% 35.4) 1/100, 23'ünde (% 11.0) 1/200 ve 4'ünde (% 1.9) 1/400 düzeyinde antikor titresi belirlenirken, 54 sığırda (% 24.8) antikor saptanmadı. Dişilerin % 53.7'sinin (58/108) erkeklerin % 42.5'inin (43/101) seropozitif olduğu belirlendi. Silajla beslenen sığırlarda seropozitiflik oranının (% 57.5, 92/160), silajla beslenmeyenlerden (18.3%, 9/49) istatistiki olarak ($p<0.001$) daha yüksek olduğu saptandı. Büyük sürülerde (50-100 sığır) seropozitiflik oranının daha yüksek (% 55.8) olduğu gözlendi.

* University of Uludag, Faculty of Veterinary Medicine, Department of Internal Medicine, Bursa, Turkey
** Laboratories of Refik Saydam Hifzissiha National Hygine Center, Department of Infectious Disease, Ankara, Turkey.

Sonuç olarak, bu çalışmada Bursa bölgesinde *Listeria monocytogenes* antikor seroprevalansının % 48.3 olduğu belirlendi ve listeriosisin epidemiyolojisinde silajla beslenmenin önemli bir faktör olduğu ortaya konuldu. İnsan ve hayvan sağlığı açısından önemli bir zoonoz enfeksiyon olan listeriosisle mücadele için daha geniş kapsamlı profilaktif çalışmaların yapılmasının gerekliliğine karar verildi.

Anahtar Kelimeler: Listeriosis, sığır, Türkiye.

Introduction

Listeriosis is an infectious disease of man and animals occurring worldwide caused by pleiomorphic bacterium *Listeria monocytogenes*.[1] The organism can be isolated from surface soil, pasture, surface water, food, and feces and milk of animals. Although the organism is widespread in the environment *L. monocytogenes* infections have frequently been associated with the feeding of silage.[2, 3]

Listeriosis is manifested by meningoencephalitis, abortion and septicemia. Only one clinical form of the disease usually occurs in a group of animals or an individual animal. In addition of these three major signs of disease; mastitis, myelitis, iritis and keratoconjunctivitis have also been associated with *L. monocytogenes*[1, 4].

Listeriosis can be diagnosed in the laboratory by cultivation of the organism, demonstration of the infectious agent or its products in tissues or body fluids and detection of a specific immune response.[5] Serodiagnostic techniques used to detect *L. monocytogenes* infections are agglutination, florescent antibody, complement fixation and ELISA.[1, 5]

The objective of this study was to determine the seroprevalance of antibodies to *L. monocytogenesis* in cattle in Bursa province of Turkey.

Materials and Methods

Two hundred nine healthy cattle, aged 1 - 5 years, Holstein bred, were randomly selected from six different districts of Bursa province of Turkey. After clinical examination of each animal, blood samples were collected by jugular puncture. The herd size (number of cattle in each herd) and the animals fed silage were recorded.

Venous blood samples were collected into vacutainer tubes with EDTA and no anticoagulant. Hematological parameters (white blood cell count, circulating red blood cell count, hematocrit and differenciated cell counts) were analyzed using automatic analyzer (System 1999; Sereno Baker Diagnostic Inc, Pennsylvania-USA).

The obtained sera were stored at –20^{o}C until tested. Diagnostic procedures were carried out at Refik Saydam Hygiene Center (RSHC), Department of Communicable Diseases Research in Ankara, Turkey.

The presence of anti-*L.monocytogenes* antibodies was determined by agglutination tests according to the method described by of Osebold et al.[6]. The antigen used in the present study was prepared in RSHC and the assay was carried out in 3 steps. Briefly, at the first step, the whole cell antigens were prepared from *Staphylococcus aureus* (ATCC 29213) strains with Osebold method. At the second step, the antigens were prepared from *L. monocytogenes* 1/2a, 1/2b, 4b, 4c and 4d strains and combined to prepare *L. monocytogenes* antigen pool. As a last step, agglutination test was performed after the absorption of sera samples with *S. aureus* antigen. Samples with a titer ≥1:100 were considered positive.

Chi square test (Statgraphics plus 5.1) was used to assess the variation in seroprevalence between age and herd size. Results of hematological examinations were analyzed by student t test (SPSS 10.01).

Results

In this study, antibodies to *L. monocytogenes* were found in 101 (48.3 %) of the 209 cattle tested. Of 209 cattle tested 54 (25.8%) had 1/50, 74 (35.4%) had 1/100, 23 (11.0%) had 1/200 and 4 had (1.9%) 1/400 agglutination titer whereas no agglutination was detected in 54 (24.8%) cattle (Table I).

In the present study 53.7% (58/108) of female and 42.5% (43/101) of male cattle have been found to be seropositive ($p<0.05$). The seropositivity in silage fed cattle (57.5%, 92/160) was found statistically higher than cattle that were not feed silage (18.3%, 9/49) ($p<0.001$). Although not statistically important ($p>0.05$), commonly affected age group was 2-3 years. The highest seropositivity (55.8%) was observed in cattle from herd size 50 – 100 ($p<0.01$) (Table II).

Table I. Distribution of antibodies to *Listeria monocytogenes* and agglutination titers on different locations in Bursa region

					Seropositive titers				
Location	Total	Negative	Pozitive	%	0	1/50	1/100	1/200	1/400
Merkez	50	18	32	68.1	7	11	25	7	-
İnegöl	30	25	5	16.6	19	6	5	-	-
Yenişehir	34	14	20	58.8	6	8	13	6	1
Keles	29	20	9	31.0	13	7	8	1	-
Karacabey	27	16	11	40.7	6	10	10	1	-
M.Kemalpaşa	39	15	24	61.5	3	12	13	8	3
Total	209	108	101	48.32	54	54	74	23	4

Table II. Prevalence of *Listeria monocytogenes* antibodies among cattle of different herd size

Herd size	Seronegative (%)	Seropositive (%)	Total
1-5	15 (60.0%)	10 (40.0%)	25
5-49	55 (56.2%)	43 (43.8%)	98
50-100	38 (44.2%)	48 (55.8%)	86
Total	108	101 (48.3%)	209

Results of hematological examination between seropositive and seropositive cattle showed no significant difference (Table III).

Table III. Haematological results of seropositive and seronegative cattle

Parameter	Seronegative X±Sx	Seropositive X±Sx
WBC (x10^3/µl)	8.24 ± 1.18	8.92 ± 1.46
RBC (x10^6/µl)	8.34 ± 0.96	8.82 ± 1.15
HCT (%)	33.15 ± 3.42	33.47 ± 4.28
Neutrophyl (%)	39.64 ± 1.86	42.26 ± 2.25
Lymphocyt (%)	57.91 ± 5.18	55.09 ± 4.51
Eosinophile (%)	1.89 ± 0.67	2.21 ± 0.73

Discussion

Seroepidemiological surveys have indicated the presence of listeriosis in several countries. In Turkey Erdogan et al.[7] tested anti-Listeriolisin O antibodies (LLO) to L. monocytogenes infection in cattle by ELISA in Kars district and 92.6% of cattle in Ardahan, 88.7% of cattle in the vicinity of Kars, and 75.3 % of cattle in central Kars and totally 81% of cattle were reported to have antibodies to *L. monocytogenes*. Tütüncü et al.[8] reported that 28.4% of cattle had anti-Listeriolisin O antibodies to *L. monocytogenes* by ELISA in Van district. In a previous study Sahal et al.[9] studied the seroprevalence of listeriosis in cattle by L. monocytogenes absorpsion test with Osebold modified method in Ankara, and reported that 44.9% of cattle (43/411) had antibodies to L. monocytogenes. In another study[10] in Bursa province, anti LLO antibodies were reported in sheep with 25.80 % seropositivity. Observed seroprevalence (48.32 %) in this study was lower than those of reported by Erdogan et al.[7] in the vicinity of Kars and higher than those of reported by Tütüncü et al.[8] in the vicinity of Van. On the other hand, our finding is similar with the earlier reported 44.9 % prevalence in Ankara.[9] The reasons for the difference in seropositivity among the different geographic areas might be due to lack of prevention and control of the disease as well as differences in serological test and antigens used.[11]

Listeriosis has been particularly associated with the feeding of poor quality silage and the disease is called silage sickness. Improperly fermented silage (pH > 5.0 to 5.5) that was contaminated initially by soil and crops can allow subsequent amplification of *L. monocytogenes* to high numbers; therefore, silage feeding appears to represent a common route of infection for farm animals.[5] In this study the seropositivity in silage fed cattle (57.5%, 92/160) was found statistically higher than cattle that were not feed silage (18.3%, 9/49) was in well agreement with the researchers reported that silage feeding is an important factor in the epidemiology of listeriosis.[2, 3]

Animals of both sexes and all ages may be affected by listeriosis.[12] In the present study 53.7% (58/108) of female and 42.5% (43/101) of male cattle have been found to be seropositive ($p<0.05$). These findings are well agreement with Sahal et al[9] who reported higher seropositiviy in female cattle. Higher seropositivity in females may be associated with more stress factors such as parturition and lactation in female cattle. It is reported that listeriosis is more common in young animals.[1] On the other hand, Gray and Killinger [12] stated that listeriosis is more common during the first 3 years of life. In this study observed higher seroprevalance of listeriosis in cattle 2-3 years old was parallel to that of reported by Gray and Killenger.[11]

Although listeriosis is manifested by meningoencephalitis, abortion and septicemia, it is reported that healthy animals can also be latent *L.*

monocytogenes carriers without clinical signs.[1] In the present study all sampled cattle were healthy and the seroprevalence of listeriosis was found as 48.32 %. Meng and Doyle [13] have shown that up to 50% of fecal samples collected from animals with no clinical signs of listeriosis may contain *L. monocytogenes.* It is concluded that there was a saprophytic existence of the organism in the plant-soil environment wherein this environment served as a reservoir.[11] In the present study the highest seropositivity (26.4%) was observed in cattle from large herd size (51 - 100 cattle). This result showed that farms may function as a natural reservoir for *L. monocytogenes* and, ultimately, as a primary source of food processing plant environment contamination.[5]

Although the prevalence of listeriosis was found as 48.3 % the results of hematological examination between seropositive and seronegative cattle showed no significant difference. That is because healthy animals can be latent carriers without clinical signs.[1]

As a result, in this study seroprevalence of antibodies to *Listeria monocytogenes* in cattle in Bursa province of Turkey was found as 48.3 % and it was concluded that silage feeding is an important factor in the epidemiology of listeriosis. Larger and detailed prophylactic studies must be planned to control this zoonosis infection for animal and human health.

References

1. LOW, J.C., DONACHIE, W. A review of *Listeria monocytogenes* and listeriosis. *The Veterinary Journal*, 153:9-29, 1997.
2. GARAYZABAL, J.B., BLANCO, M., BOLOND, J.A., BRIONES, V., GARCIA, J.A, DELGADO, C., DOMINGO, M., MARCO, J. A., DOMINGUEZ, L.A. A direct plating method for monitoring the contamination of *Listeria monocytogenes* in silage. *Journal of Veterinary Medicine B*, 39:513-518, 1992.
3. VAZQUEZ, B.J.A., DOMINGUEZ, L., BLANCO, M., ROCOURT, J., FERNANDEZ GARAYZABAL J.F., GUTIERREZ, C.B., TASCON, R.I., RODRIGUEZ FERRI, E.I. Epidemiologic investigation of a silage-associated epizootic of ovine listeric encephalitis, using a new listeria-selective enumeration medium and phage typing. *American Journal of Veterinary Research*, 53:368-371, 1992.
4. SMITH, B.P., MADGAN, J.E., DYBAL, N.O. *Large Animal Internal Medicine*, 1st Ed., WB Saunders Company, Philadelphia, 747-749, 1990.
5. NIGHTINGALE, K.K., SCHUKKEN, Y.H., NIGHTINGALE, C.R., FORTES, E.D., HO, A.J., HER, Z., GROHN, Y.T., MCDONOUGH, P.L., WIEDMANN1, M. Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. *Applied and Environmental Microbiology*. 70:4458-4467, 2004.
6. OSEBOLD, J.W., AALUND, A., CHRISP, C.E. Chemical and immunological composition of surface structures of *Listeria monocytogenes*. *Journal of Bacteriology*, 89: 84-88, 1965.
7. ERDOGAN, H.M., GOKCE, G., GOKCE, H.I., KIRMIZIGUL, A.H., GUNES, V., SURAL, E., YILMAZ, K. Use of ELISA technique in the investigation of *Listeria monocytogenes* infection in cattle in Kars District. *The Journal of Faculty of Veterinary Medicine University of Kafkas,* 5:43-46, 1999.
8. TÜTÜNCÜ, M., SOLMAZ, H., AKKAN, H.A., KARACA, M., AĞAOĞLU, Z. Seroprevalence of *Listeria monocytogenes* infection in cattle in Van, Turkey. İndian Vet. Journal, 82, 926-928, 2005.
9. SAHAL, M., GAZYAGCI, S., URAL, K., BABUR, C., KILIC, S., HANEDAN, B. Seroprevalence of antibodies to *Listeria monocytogenes* in cattle with and without clinical suspicious for listeriosis in Ankara in Turkey. XXII. World Buiatrics Congress 18-23 August, Hannover, Germany, 59, 2002.
10. KENNERMAN, E., ERDOGAN, H.M., ŞENTURK, S., GOLCU, E. Serological diagnosis of listeriosis by ELISA in sheep in Bursa region. *Turkish Journal of Veterinary Surgery,* 6:15-19, 2000.
11. ERDOGAN, H.M., CETINKAYA, B., GREEN, L.E., CRIPPS, P.J., MORGAN K.L. Prevalence, incidence, signs and treatment of clinical listeriosis in dairy cattle in England.. *Veterinary Record*. 149: 289-293, 2001.
12. GRAY, M.L., KILLINGER, A.H. *Listeria monocytogenes* and listeric infections. *Bacteriological Reviews*, 30: 309-382.
13. MENG, J., DOYLE, M.P. Emerging issues in microbiological food safety. Annual Review of Nutrition 17:255-275, 1997.