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Survey on the Infection Source of *Listeria monocytogenes* for Korean Native Goats

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Introduction

Listeriosis is an acute infectious disease of various mammalian and avian species and abortion, encephalitis and septicemia are the main clinical manifestations. It has a worldwide distribution and is of growing importance in sheep of some countries in recent years.[11,17,18]

The causative organism, *Listeria monocytogenes(L. monocytogenes)*, is widespread in soil, vegetation, silage, animal and human feces and often listeriosis is associated with the feeding of poor quality or mouldy silage in animals.[8-10,12] Outbreak of listeriosis in the Korean native goat was first reported by Yeo *et al.*[21] and the authors stated the necessity of survey on the distribution of *L. monocytogenes* in nature in order to confirm the reservoirs of the organism.

The purpose of this investigation was to assess the infection source of *L. monocytogenes*, and the colonizing organs and antibiotic susceptibilities of *L. monocytogenes* isolates were determined in the present paper.

Materials and Methods

Samples tested: Ninety seven feces and 103 nasal exudates were sampled aseptically by rectal or nasal swabbing from 103 korean native goats, and 5 samples of both feeds and ground soil were randomly collected into sterile polyethylene bottles from 9 farms in suburb of Chinju city, Korea from March to October, 1987. Also included were tissue samples such as brain, spinal cord, lung, heart, liver, spleen, and lymph node from 25 Korean native goats. They are consisted of 7 heads submitted to University Veterinary Clinic for autopsy and 18 heads in slaughter house. Samples were brought back to the laboratory for immediate bacteriological examination for *L. monocytogenes*

Isolation of L. monocytogenes: Isolation of *L. monocytogenes* from the samples was carried out using modification of the methods described by Low and Renton[18], Davies[6] and Fenlon[9].

Feces, feed, soil and tissue samples were ground with the sterilized mortar and pestle to make 10% suspension with listeria enrichment broth (LEB: Difco tryptose broth base, 500ml; 5% nalidixic acid, 0.4ml; thallium acetate, 0.1g) and nasal exudate samples were washed into 5ml of LEB.

The 0.1ml of each suspension was plated directly onto two 5% sheep blood agar plates containing the above doses of nalidixic acid and

thallium acetate(BA) and a listeria agar plate(LA: LEB added agar to 1.5%), and 1ml of the suspension was inoculated into two separate tubes containing 9ml of LEB.

Sample preparations directly plated on BA were incubated in duplicate for 24 to 48 hours at 37℃ under aerobic and 5% CO condition and those on LA were incubated for 48 hours aerobically at 37℃.

Two tubes of LEB inoculated with sample preparations, were incubated aerobically at 37℃, and at 4℃, respectively The tubes incubated at 37℃ and 4℃ were monitored daily for 7 days and weekly for 3 months, respectively, by plating o.1ml onto LA and incubating aerobically at 37℃ for 24 to 49 hours.

Identification of L. monocytogenes: Preliminary screening of Listeria isolates was done by the colonial appearances such as smooth, round, convex and gummy texture, hemolysis, and catalase positive and gram positive bacillus. Further identification of the isolates as *L. monocytogenes* was based on cultural and biochemical characteristics described by Carter[4], Fenlon[8] and Sneath *et al*.

Susceptibility of L. monocytogenes isolates to antibiotics: Susceptibility testing of L. monocytogenes isolates to 16 antibiotics was carried out by the disk diffusion method of Bauer *et al*.

Experimental inoculation of L. monocytogenes to goats: To determine the colonizing organs of *L. monocytogenes* after infection, the strain isolated previously from the brain of a Korean native goat died from encephalitic listeriosis[21] was inoculated into each 2 healthy korean native goats of both sexes aged 4 to 5 months by the methods of Barlow and McGorum[1], Gitter *et al.*[13] and Smith *et al.*[19]

The organism was passaged through mice to restore virulence before inoculation. Then overnight cultures on BA at 37℃ were washed and suspended in sterile phosphate buffered saline to the concentration of 1.0×10^9/ml. Out of 4 goats, 2 were intravenously injected once and the others were orally inoculated daily for 3 successive goats with 5ml of the inoculum. Organs from goats died or sacrificed afterwards were as explained for the recovery of *L. monocytogenes*.

Results and Discussion

Eight strains of *L. monocytogenes* were isolated. Their cultural characteristics (Table 10) and properties of the carbohydrate fermentation(Table 2) were agreed with those of *L. monocytogenes* described by Carter[4], Fenlon[8] and Sneath *et al.*[20].

The results in isolation of *L. monocytogenes* from the total of 459 samples from Korean native goats and their breeding environment were shown in Table 3. Eight(8.2%) of 97 fecal samples were positive for the *L. monocytogenes* and all of the nasal exudate, tissue, feed and soil samples

Table 1. Cultural Characteristics of 8 Isolates of *L. monocytogenes*

Characteristics	Isolate
Gram-positive bacillus	+
Beta-hemolysis	+
Motility at 25℃	+
Growth at 4℃	+
6% NaCl tolerance	+
Catalase	+
Urease	−
Indole	−
Methye red	+
Voges-Proskauer	+
H_2S	−
KNO_3 reduction	−
Citrate utilization	−
Oxidation-Fermentation	ermentation
Esculin hydrolysis	+
Hippurate hydrolysis	+
Oxidase	−

+ : Positive.
− : Negative.

Table 2. Carbohydrates Fermentation of 8 Isolates of *L. monocytogenes*

Carbohydrates	Acid Production
Glucose	+
Maltose	+
Lactose	+
Sucrose	+
Fractose	+
Mannose	+
Rhamnose	+
Salicin	+
Trehalose	+
Galactose	−
Adonitol	−
Arabinose	−
Dulcitol	−
Inositol	−
Inulin	−
Mannitol	−
Raffinose	−
Sorbitol	−
Xylose	−

+ : Positive.
− : Negative.

Table 3. Isolation Rate of *L. monocytogenes from* Korean Native Goats and their Environment

Kinds of samples	No. of samples cultured	No. of samples organism isolated
Feces	97	8 (8.2)
Nasal exudate	103	0
Feed	45	0
Soil	45	0
Tissues		
Brain	25	0
Spinal cord	22	0
Lung	25	0
Heart	22	0
Liver	25	0
Spleen	25	0
Lymph node	25	0
Total	459	8 (1.7)

Figures in parentheses are the percentages.

were negative. The authors, therefore, thought the feces may be the important source of infection in Korea. In many countries silage was known as another important source of *L. monocytogenes* infection. The relationship between listeriosis in ruminants and the feeding of silage inferior in quality had been well documented.[14,16,18]

Though we did not assay for the silage since

Table 4 Comparison of Isolation Methods of *L. monocytogenes* from Feces of Korean Native Goats

Strain No.	Methods of isolation				
	Direct culture[a]			Enrichment culture[b]	
	BA-Aerobic	BA-5% CO_2	LA-Aerobi	37℃-1Week	4℃-3 Months
F-17	+	−	−	−	−
F-33	−	−	−	+	−
F-46	+	−	−	−	−
F-64	+	−	−	−	−
F-75	+	−	−	−	−
F-77	+	−	−	−	−
F-79	+	−	−	−	−
F-91	+	−	−	−	−

[a] Samples were cultured for 24-48 hours at 37℃.
[b] Organisms were monitored on listeria agar daily from 37℃ and weekly from 4℃ enrichment in listeria broth tubes.
BA : Blood agar.
LA : Listeria agar.
+ : Organism isolated.
− : Organism not isolated.

this is not fed to Korean native goats in general, good quality silage sealed against aerobic deterioration and fermented below pH 5.6 may be needed for feeding. because most *Listerias* spp. died out at pH 5.6 and below.[15)]

Selective isolation procedures have been required for the detection of *L. monocytogenes* from suspected materials. Comparison of 5 different isolation methods was done in this study(Table 4). Seven of 8 strains were originated from fecal samples which were cultured directly on BA for 24 to 48 hours aerobically at 37℃, and this is considered to be a simple and rapid method of isolation. Doyle and Schoeni[7] and Bearns and Girard[3)], however, reported that isolation of *L. monocytogenes* from the specimens in cold enrichment is generally successful when attempts to isolate from direct plating of suspected specimens onto conventional media occasionally fail. For the rapid and precise diagnosis of listeriosis in clinical specimens, therefore, prompt isolation of *L. monocytogenes* by direct culture of the specimens on BA is recommended and confirmation of the organism by lengthy procedure of enrichment culture must be done for the negative specimens in direct culture.

As revealed in Table 5, all strains of 8 *L. monocytogenes* isolates were resistant to amikacin, colistin, kanamycin and neomycin but were sensitive to nitrofurantoin. The majority of the isolates were also resistant in order of prevalence to clindamycin, gentamicin, lincomycin, methicillin and refampin(87.5%), ampicillin and oleandomycin(75.0%), chloramphenicol(62.5%), tetracycline(50.0%) and cephalothin (37.5%). In contrast to this result, Yeo *et al.*[21)] reported a strain isolated from the brain of the Korean native goat died from listeric encephalitis was sensitive to amikacin, kanamycin, clindamycin, gentamicin, chloramphenicol and tetracycline but was resistant to colistin and lincomycin. Also Chong *et al.*[5)] showed two

Table 5. Prevalence of Antibiotic Resistance in 8 Strains of *L. moccytogenes* Isolates

Antibiotics (μg)[a]	No. of strains resistant	% of strains resistant
Amikacin (30)	8	100.0
Ampicillin (10)	6	75.0
Cephalothin (30)	3	37.5
Chloramphenicol (10)	5	62.5
Clindamycin (2)	7	87.5
Colistin (10)	8	100.0
Gentamicin (10)	7	87.5
Kanamycin (30)	8	100.0
Lincomycin (2)	7	87.5
Methicillin (5)	7	87.5
Neomycin (30)	8	100.0
Nitrofurantoin (300)	0	0
Oleandomycin (15)	6	75.0
Penicillin (10)[b]	7	87.5
Rifampin (5)	7	87.5
Tetracycline (30)	4	50.0

[a] Disk content.
[b] Unit

strains of *L. monocytogenes* isolated from human meningitis were both sensitive to cephalothin, chloramphenicol, tetracycline and gentamicin, and further surveys on the antibiotic susceptibilities of *L. monocytogenes* are necessary in order to assess the effective therapeutics and prophylactics.

Among 4 goats experimentally infected with *L. monocytogenes* 2 goats inoculated intravenously were died 5 days post infection and each one of 2 goats inoculated orally was died and killed respectively 8 days post infection. The pathological findings at post mortem examination will be discussed in other paper and results in cultural attempts were arranged in Table 6.

Recovery of the organism from most parenchymal organs and blood revealed that septicemia had developed in goats infected by both routes and these organs were the predilection sites for colonization of *L. monocytogenes* after infection. A small fetus was found in a goat (No. 2) which

Table 6. Recovery of *L. monocytogenes* from Organs of the Korean Native Goats after Experimental fa Infection

Organs	Intravenous infection			Oral infection	
	Goat[a] 1	Goat 2	Fetus[b]	Goat 3	Goat 4
Brain	+	+	NT	+	+
Spinal cord	+	+	NT	+	+
Heart	+	+	NT	+	+
Lung	+	+	+	+	−
Liver	+	+	+	+	+
Spleen	+	+	NT	+	+
Kidney	+	+	N+	+	−
Stomach	+	+	NT	−	+
Intestines	+	+	+	+	+
Mesenteric lymph Node	−	−	NT	+	−
Lymph Node	+	+	NT	+	+
Tonsil	−	−	NT	+	+
Endometrium	NT	+	NT	NT	NT
Placenta	NT	+	NT	NT	NT
Amniotic fluid	NT	NT	+	NT	NT
Blood	+	+	NT	+	+

[a] Goat 1 and 3, male; 2 and 4, female.
[b] A fetus gained from goat 2.
+ : Organism recovered.
− : Organism not recovered.
NT : Not tested.

was in pregnancy even though it was 5 months of pregnancy and also *L. monocytogenes* was recovered from fetal lung, liver, kidney, intestines and amniotic fluid. Some investigators[13,18,19] also reported the recovery of *L. monocytogenes* from lung, liver, kidney, spleen, brain of ewes and/or their fetuses naturally or experimentally infected by oral route. These results give support to the fact that oral infection of *L. monocytogenes* leads the hematogenous invasion and causes the septicemic listeriosis.

Summary

Attempts were made to isolate *L. monocytogenes* from a total of 459 samples including feces, nasal exudates, tissues from Korean native goats, feeds and soils by direct cultivation and enrichment procedures. Also trials to determine the antibiotic susceptibilities of isolates and colonizing organs of *L. monocytogenes* after experimental infection were done, and the results were summarized as follows.

L. monocytogenes was isolated only from feces and the isolation rate was 8.2% of 97 fecal samples. The feces seemed to be an important source of infection for Korean native goats.

Direct culture of the sample suspension on blood agar for 24 to 48 hours aerobically at 37℃ was one of the proper isolation methods of *L. Monocytogenes*.

The majority(37.5 to 100%) of 8 *L. monocytogenes* isolates were resistant with different prevalence to a number of antibiotics tested but all strains were sensitive to nitrofurantion.

Septicemia was noticed in Korean native goats

inoculated either *per os* or intravenously with *L. monocytogenes* and most parenchymal organs were the sites of organism's colonization after infection.

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한국 재래산양에 대한 *Listeria monocytogenes*균의 감염원 조사

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초 록

한국 재래산양에 대한 *L. monocytogenes*균의 감염원을 조사하기 위하여 총 459개의 재래산양 분변, 비즙, 장기, 사료 및 토양재료로부터 균분리를 시도하였으며, 분리균의 항균성 물질에 대한 감수성 및 인공감염시의 *L. monocytogenes*의 침입장기를 조사하였던 결과는 다음과 같다.

*L. monocytogenes*는 분변에서만 분리되었으며 (분리율 8.2%) 분변이 본균의 주요 감염원으로 인정되었다.

가검재료로부터 *L. monocytogenec*의 분리방법으로는 유제화 재료를 혈액배지로써 37℃에서 24~48시간 동안 호기배양함이 우수하였다.

분리된 *L. monocytogenes* 8주 중 100%의 균주가 amikacin, colistin, kanamycin, neomycin에 내성을 나타내었으며, clindamycin, gentamicin, lincomycin, methicillin 및 refampin에 87.5%, ampicillin 및 oleandomycin에 75.0%, Chloramphenicol에 62.5%, tetracycline에 50.0% 및 cephalot hin에 37.5%의 균주가 내성이었다. Nitrofurantoin에는 전 균주가 감수성을 나타내었다.

*L. monocytogenes*를 재래산양에 경구 및 정맥내로 접종하였을때 패혈증이 인정되었으며 대부분의 실질장기가 이 균의 침입부위인 것으로 관찰되었다.