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REGULAR ARTICLES

Serology and clinical relevance of *Corynebacterium pseudotuberculosis* in native Korean goats (*Capra hircus coreanae*)

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Abstract This study was conducted to assess the seroprevalence and clinical relevance of *Corynebacterium pseudotuberculosis*, which is the causative agent of caseous lymphadenitis (CLA), in native Korean goats (*Capra hircus coreanae*). A total of 466 native Korean goats from 40 herds (11 to 12 samples per herd) were randomly selected throughout the nation and evaluated by direct palpation, bacterial isolation, ELISA, and PCR. In serological examinations, 267 (57.3 %) of the goats tested were positive against *C. pseudotuberculosis*. When seroprevalence was analyzed according to age, region, and season, statistically significant differences were observed in relation to all three parameters ($P<0.05$). For clinical examination, the superficial lymph nodes of all goats were palpated to diagnose CLA. Pus samples taken from superficial abscesses were used for bacterial isolation. Among the 466 goats tested, 34 (7.3 %) were presumptively diagnosed with CLA, and *C. pseudotuberculosis* was isolated from 24 goats (70.6 % of goats with CLA lesions) whose infections were confirmed by PCR. Considering the high seroprevalence and bacterial isolation rate from most of the superficial CLA lesions, it is suspected that many internal CLA lesions exist in this goat population. These results suggest that *C. pseudotuberculosis* infection is widespread in native Korean goats, and appropriate control programs need to be established.

Keywords Bacterial isolation · Caseous lymphadenitis · ELISA · Native Korean goats · PCR

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Introduction

Corynebacterium pseudotuberculosis is known worldwide to cause pseudotuberculosis or caseous lymphadenitis (CLA) in sheep and goats (Brown and Olander 1987; Dorella et al. 2006). The superficial form of the disease causes lesions, primarily on the head and neck, which are noticeable upon inspection (Gezon et al. 1991). Because of poor responses to drug therapy, long-term survival of the organism in the environment, and difficulties in detecting infected animals, it is challenging to eradicate the disease, which may continue to cause a serious problem in goats.

While multiple studies on CLA in goats have been reported in many different countries (Skalka et al. 1998; Seyffert et al. 2010), limited information is available from Korea, except in one case where *C. pseudotuberculosis* was isolated from a Saanen dairy goat (Shin et al. 2010). In Korea, goats are a popular livestock, and the population is approximately 250,000 (KOSIS 2010). Thus, this study was aimed at determining the serology and clinical relevance of *C. pseudotuberculosis* in native Korean goats (*Capra hircus coreanae*).

Materials and methods

Sample collection

Between November 2009 and August 2010, a total of 466 native Korean goats from 40 herds (11 to 12 samples per herd) were randomly selected for serum collection throughout the nation, and information about age, region, and season were recorded for statistical analysis. All the tested goats were reared in free stalls and had no contact with other domestic animals. None of the sampled goats had been vaccinated against CLA. The serum samples were divided into three different groups based on age: young goats (<1 year, 148

samples), adult goats (≥1 year, 284 samples), and unknown (34 samples). Sample collection regions were divided into three, i.e., the northern, central, and southern regions, according to administrative boundaries (Fig. 1). During the cold season, between October and March, 123 serum samples were obtained. During the warm season, between April and September, 343 samples were collected. The sera obtained were kept at −20 °C until testing.

Serology and clinical examination

All serum samples were tested using a commercial enzyme-linked immunosorbent assay (ELISA) kit (ELITEST CLA, HYPHEN BioMed, France) according to the instructions of the manufacturer.

During blood sample collection, individual goats were also examined by direct inspection and superficial lymph node palpation for CLA. Pus samples were taken from superficial abscesses of these goats using sterile syringes (Fig. 2). Part of the sampled contents was inoculated onto a 5 % sheep blood agar plate. The plate was aerobically and anaerobically incubated for 24 to 48 h at 37 °C. Isolates were identified using PCR and API Coryne kit (bioMérieux, France).

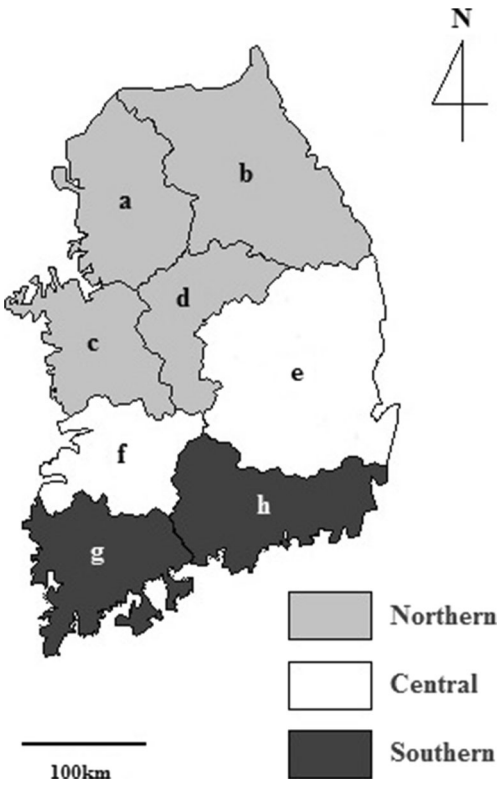

Fig. 1 To evaluate the seroprevalence of *Corynebacterium pseudotuberculosis*, sample collection regions were divided into three areas, i.e., northern [Gyeonggi-do (**a**), Gangwon-do (**b**), Chungcheongnam-do (**c**), and Chungcheongbuk-do (**d**)], central [Gyeongsangbuk-do (**e**) and Jeollabuk-do (**f**)], and southern [Jeollanam-do (**g**) and Gyeongsangnam-do (**h**)] regions, according to administrative boundaries

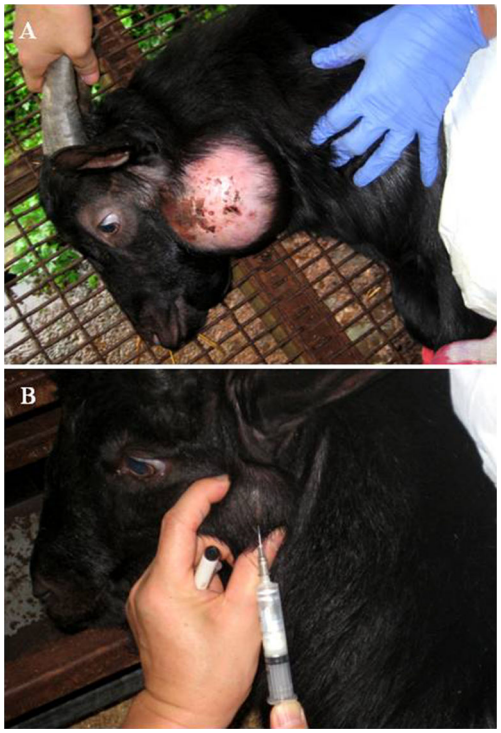

Fig. 2 Gross appearance of native Korean goats with caseous lymphadenitis. **a** Superficial form of caseous lymphadenitis at the parotid lymph node. **b** Creamish pus taken from caseous lymphadenitis at the parotid lymph node using a sterile syringe

DNA extraction and PCR

For PCR detection of *C. pseudotuberculosis*, DNA was extracted from pus samples and isolates using a DNeasy Blood and Tissue kit (Qiagen, USA) according to the manufacturer's instructions. DNA from each pus sample and isolate was tested by a duplex PCR according to the methods described by Pacheco et al. (2007). The target genes for the duplex PCR were *C. pseudotuberculosis* 16S rRNA and *pld*, which encodes the exotoxin phospholipase D. The estimated amplicon sizes for *C. pseudotuberculosis* 16S rRNA and *pld* genes were 816 and 203 bp, respectively.

The PCR reactions were carried out in a Mastercycler® pro PCR system (Eppendorf, Germany), and the PCR amplicons were separated by agarose gel (1.5 %) electrophoresis and viewed using an UV transilluminator.

Statistical analysis

A chi-square test was performed to analyze the differences between various groups using the SPSS software (ver. 17.0; SPSS Inc., Chicago, IL). P values <0.05 were considered statistically significant. Confidence intervals (CI, 95 %) were also calculated.

Results

Among the 466 goats tested, 267 (57.3 %) were seropositive for *C. pseudotuberculosis* (Table 1). When prevalence was analyzed by age, 73 (49.3 %, CI 41.3–57.4) of the 148 young (<1 year) goats, 176 (62.0 %, CI 56.3–67.6) of 284 adult (≥1 year) goats, and 18 (52.9 %, CI 36.2–69.7) of 34 goats in the unknown age group were seropositive for *C. pseudotuberculosis*. In the analyses according to region, 51 (46.4 %, CI 37.0–55.7) of 110, 51 (65.4 %, CI 54.8–75.9) of 78, and 165 (59.4 %, CI 53.5–65.1) of 278 goat samples were seropositive in the northern, central, and southern regions, respectively. In the analyses based on season, the seroprevalence was higher in the cold season (65.0 %, 80/123; CI 56.6–73.5) than that in the warm season (54.5 %, 187/343; CI 49.3–59.8). A significant difference was observed according to age, region, and season ($P<0.05$).

Among the 466 goats tested, 34 (7.3 %) were presumptively diagnosed with CLA by superficial abscesses. In most cases, parotid, mandibular, and cervical lymph nodes were affected, and the affected lymph nodes were characterized by enlargement and hairless skin around them (Fig. 2). All cases yielded a creamish pus (Fig. 2b). Of these 34 goats, *C. pseudotuberculosis* was isolated from 24 (70.6 % of goats with CLA lesions; Table 2). All isolates were identified as *C. pseudotuberculosis* with biochemical tests and PCR. In total, 95.8 % (23/24) of the goats in which *C. pseudotuberculosis* was isolated were adults (data not shown). Of the 10 goats with superficial abscesses from which *C. pseudotuberculosis* was not isolated, two goats were positive for *Staphylococcus aureus* (Table 2).

When the pus samples from the 24 goats in which *C. pseudotuberculosis* was isolated were assessed by duplex PCR for the detection of *C. pseudotuberculosis* 16S rRNA and *pld* genes, all 24 samples showed estimated amplicon sizes of 816 and 203 bp for *C. pseudotuberculosis* 16S rRNA and *pld* genes, respectively (Fig. 3).

Table 1 Seroprevalence of *Corynebacterium pseudotuberculosis* in 466 native Korean goats according to age, region, and season

Group		No. tested	No. positive (%)	95 % CI
Age[a]	Young (<1 year)	148	73 (49.3)	41.3–57.4
	Adult (≥1 year)	284	176 (62.0)	56.3–67.6
	Unknown	34	18 (52.9)	36.2–69.7
Region[a]	Northern	110	51 (46.4)	37.0–55.7
	Central	78	51 (65.4)	54.8–75.9
	Southern	278	165 (59.4)	53.6–65.1
Season[a]	Cold (October–March)	123	80 (65.0)	56.6–73.5
	Warm (April–September)	343	187 (54.5)	49.3–59.8
Total		466	267 (57.3)	52.8–61.8

CI confidence interval

[a] Significant difference was observed ($P<0.05$)

Table 2 Comparison between *Corynebacterium pseudotuberculosis* isolation and ELISA results in 34 native Korean goats with superficial abscesses

		C. pseudotuberculosis isolation		Total
		Positive (%)	Negative	
ELISA	Positive	24 (70.6)	10[a]	34
	Negative	0	0	0
Total		24 (70.6)	10	34

[a] *Staphylococcus aureus* was identified from 2 out of 10 goats in which *C. pseudotuberculosis* was not isolated

Discussion

In the present study, CLA prevalence was higher in adult goats (≥1 year, 62.0 %) than in young goats (<1 year, 49.3 %), which was statistically significant ($P<0.05$). Consistent with the data obtained from another study, seroprevalence tended to increase with age (Seyffert et al. 2010). One possible explanation for the higher seroprevalence in adult goats is that the frequency of abscess discharge increases with age.

When analyzed by region, a statistically significant difference was observed ($P<0.05$). The authors cautiously suspect that the difference was attributed to the degree of sanitation at each farm. However, because of a lack of information, this study has the limitation of being unable to deduce the exact reason for the significant difference observed according to region.

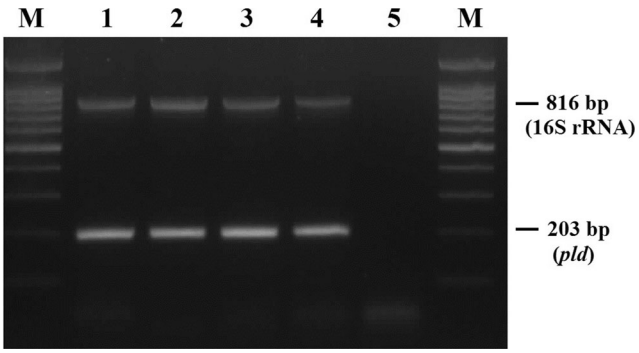

Fig. 3 Duplex PCR detection of *Corynebacterium pseudotuberculosis* 16S rRNA and *pld* genes. *Lane M*, 100-bp DNA ladder; *Lanes 1–3*, field isolates of *C. pseudotuberculosis*; *Lane 4*, a positive control (ATCC43926) of *C. pseudotuberculosis* biovar *ovis*; and *Lane 5*, a negative control. The DNA fragments produced were analyzed on agarose gel (1.5 %) and visualized by ethidium bromide staining and UV transillumination. Estimated sizes of amplicons are indicated on the right for *C. pseudotuberculosis* 16S rRNA and *pld* genes at 816 and 203 bp, respectively

According to season, the seroprevalence was significantly higher in the cold season than that in the warm season ($P<0.05$). Physical transfer of purulent discharges from superficial CLA lesions has been suggested as an important mode of infection (Ashfaq and Campbell 1980). In the cold season, native Korean goats are typically raised in dense populations within barns for warmth. The authors suggest that this type of handling makes transmission easier due to close contact with infected goats. Furthermore, low temperatures could extend the survival period of *C. pseudotuberculosis* in the environment (Augustine and Renshaw 1986).

Among 34 goats that were presumptively diagnosed with CLA by direct inspection and superficial lymph node palpation, *C. pseudotuberculosis* was isolated from 24 (70.6 %) goats. Of the 10 goats in which *C. pseudotuberculosis* was not isolated, *S. aureus* was identified in two. These results are partially consistent with those from another study showing that *Arcanobacterium pyogenes* and *S. aureus* infections were characterized by superficial abscesses in goats (Gezon et al. 1991). The failure to isolate *C. pseudotuberculosis* from pus samples may be due to specimens being collected from exudates from the center of the abscess, or from inspissated pus, which may not yield bacterial growth.

Because *C. pseudotuberculosis* was isolated from goats that showed positive ELISA results, the authors suggest that the ELISA kit used was sensitive in detecting *C. pseudotuberculosis* infections. These findings are consistent with a previous study in which ELISA plates coated with recombinant phospholipase D were highly specific and sensitive for detecting *C. pseudotuberculosis* infection (Menzies et al. 1994). Moreover, Ellis et al. (1990) suggested that the recombinant antigen could be useful for overcoming problems with nonspecific cross-reaction in serological assays. Despite reports of cross-reaction with *Mycobacterium paratuberculosis* (Pepin et al. 1987) and of no significant relationship between the extent of CLA lesions and antibody titer (Ellis et al. 1990), ELISA has been shown effective in assessing the eradication of CLA in sheep and goats (Dercksen et al. 1996; Baird and Malone 2010). While only 6.3 % (29/461) of the tested goats presented CLA lesions, a high seroprevalence of 57.3 % (264/461) for *C. pseudotuberculosis* was observed. This could be explained by a high frequency of internal CLA lesions, which could not be evaluated in this study, because all the animals were alive at the time of testing.

Typical goat production in Korea involves an intensive handling system with little management practice. Furthermore, there is no licensed vaccine against CLA currently available. To the best of our knowledge, this study describes for the first time the prevalence of CLA in native Korean goats. Considering the high seroprevalence and bacterial isolation from most of the superficial CLA lesions, it is suspected that many internal CLA lesions also exist. This study indicates that *C. pseudotuberculosis* infection is widespread among goats in Korea and that bacterial infection was associated with superficial abscess. Thus, appropriate control programs need to be established to prevent transmission of *C. pseudotuberculosis*.

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Conflict of interest The authors have no financial, personal, or organizational conflict with respect to the work reported in this manuscript.

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