

Characterization of *Trueperella pyogenes* isolated from caseous lymphadenitis lesions in Korean native goats

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The study characterized *Trueperella pyogenes* (*T. pyogenes*) from caseous lymphadenitis (CLA) in Korean native goats (KNG). CLA samples (n=191) were obtained from abattoir-slaughtered KNG. Pus were obtained and *Corynebacterium pseudotuberculosis* was detected by PCR using PLD gene. Three PLD-negative samples (1.57%) from supramammary lymph node were subjected to bacterial culture. Isolates were identified using 16S rRNA and biochemically characterized by APIZYM. Phylogenetic and biochemical analyses identified the isolates as *T. pyogenes*, and have shown separate clusters and varied enzyme reaction, suggesting genotypic and phenotypic diversity. This first report of *T. pyogenes*-induced CLA in KNG provided information for further investigations.

Key Words: Caseous lymphadenitis, Korean native black goat, *Trueperella pyogenes*

INTRODUCTION

Trueperella pyogenes (*T. pyogenes*) is a gram-positive, coryneform bacterium, ubiquitous in the environment, and commonly inhabits the skin and mucosa of the gastrointestinal, upper respiratory, and urogenital tracts of animals (Yassin et al, 2011). This Actinomycete has an opportunistic nature because it causes diverse clinical manifestations, particularly purulent or suppurative infections, ranging from abscesses, lymphadenitis, mastitis, pneumonia, and pyelonephritis, affecting a variety of livestock species including mainly cattle, pigs, goats and sheep (Winn Washington et al, 2006). The pathogenicity of this bacterium could be attributed to certain mechanisms such as the potent pyolysin which is the main virulence factor that promotes tissue damage; as well as mucosal adherence/colonization-associated proteins including neuraminidases, fimbriae, and collagen-binding proteins (Risseti et al, 2017).

In Korea, the Korean native black goat (KNG) is the only indigenous breed of goat, and its products has his-

torically served for alternative medicine, such as broths and extracts, rather than for meat consumption (Son 1999). This is due to the high level of polyunsaturated fatty acids (PUFA), and low levels of saturated fat and cholesterol, as well as the preference to other livestock meat (Son 1999). Recently, the trend on the demand for healthy food lead to the global increase in goat meat consumption due to its distinct nutritional components, compared to other red meats (Mazhangara et al, 2019).

Suppurative infections leading to lesions and lymphadenitis are among the main causes of reduced productivity and product quality in livestock causing significant economic losses in the industry (Rzewuska et al, 2019). These infections are mainly caused by pyogenic bacteria such as *Staphylococcus* spp., *Streptococcus* spp., *Corynebacterium* spp., *T. pyogenes*, and *Rhodococcus equi* (Rzewuska et al, 2019). One of these infections that causes damage to livestock production particularly in the goat industry is caseous lymphadenitis (CLA). CLA is a chronic, contagious disease characterized by a formation of abscesses in the lymph nodes. It is considered as

the main suppurative infection that affects small ruminants, specially goats, worldwide (Al-Traif et al, 2017). The known primary agent of CLA is *Corynebacterium pseudotuberculosis*, a non-spore forming, intracellular pathogen that can survive in the environment for long periods (Al-Gaabary et al, 2009). This persistent characteristic of *C. pseudotuberculosis* is accounted for its high transmission rate to animals within the flock. However, there are few studies claiming that other bacteria, like *T. pyogenes*, could also induce CLA in goats (Stefanska et al, 2007). This study describes the characterization of *T. pyogenes* isolated from CLA lesions in KNG.

MATERIALS AND METHODS

A total of 191 CLA lesion samples were taken from goat carcasses slaughtered at an abattoir located at Jeonnam province, Korea. All carcasses were examined by a veterinary inspector for observation of occurrence of CLA lesions. The lymph nodes with surrounding tissue or purulent lesions were excised and were immediately transported to the laboratory where the caseous or purulent material was collected. DNA was extracted using Qiamp DNA mini kit (Qiagen, Germany) from the sample, and molecular detection of a 203-bp region of *C. pseudotuberculosis* phospholipase-D (PLD) gene was amplified by PCR to identify which samples were *C. pseudotuberculosis* positive/negative following a published method (Sá et al, 2013). Then, bacterial isolation was carried out on all the samples by streaking a loopful of caseous material/pus on blood agar supplemented with 5% defibrinated sheep blood, then subsequently

incubated for 24~72 h at 37°C.

Single colonies were isolated and purified using the same medium. After cultivation, a loopful of each isolate was used for DNA extraction and PLD was detected. Afterwards, PLD-negative samples were subjected to biochemical test using APIZYM (BioMerieux, France) to evaluate the enzyme activity of isolates following the manufacturer's protocol. Lastly, molecular identification were performed by amplification of the 16S rRNA gene. PCR products were submitted to Macrogen (Macrogen, Inc., Korea) for sequencing. Sequences were assembled and identified using the Basic Local Alignment Search Tool (BLAST). Phylogenetic analysis of isolates was done using Molecular Evolutionary Genetics Analysis version 7 (MEGA7) by Maximum Likelihood method.

RESULTS AND DISCUSSION

Among the 191 CLA lesions collected and assessed, three (1.57%) samples were PLD-negative. The presentation of CLA lesions were all superficial in the supramammary lymph node, and these abscesses contains more fluid purulence (Fig. 1A), compared to PLD-positive samples which contains cheesy (more solid) caseous material. This corroborated with the bacterial isolates as PCR amplification of purified isolates from these CLA lesions were also PLD negative. The cultural characteristics: small, irregular smooth and whitish colonies (Fig. 1B); and microscopy of Gram's stained samples showed no difference among all isolates (Fig. 1C).

Caseous lymphadenitis could afflict significant economic damage in the goat industry. In Korea where intensive goat farming is increasing (Kim et al, 2019), very

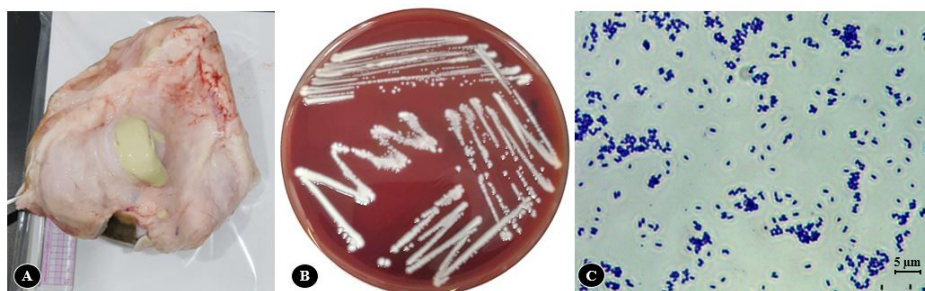


Fig. 1. CLA lesion excised from the supramammary lymph node with purulent material discharge (A). Plate purification of *T. pyogenes* showing the plate culture characteristics (B), and Gram's stained smear of *T. pyogenes* isolate showing Gram-positive pleomorphic or coccoid rods (C).

few studies are currently available with regards to CLA. Previously, the relevance of *C. pseudotuberculosis* in KNG revealed that CLA is prevalent in the country, and calls for a proper strategy to control the disease (Jung et al, 2015). However, in case of *T. pyogenes* in CLA, no study in KNG is available. Here, we report the first isolation and characterization of *T. pyogenes* causing CLA in the indigenous breed of goat in Korea.

On the basis of cultural and differential staining, it is difficult to pinpoint the difference between *T. pyogenes* culture to *C. pseudotuberculosis*, therefore, more specific identification such as PCR should be considered. The result of this study also showed that only a low percentage of CLA occurrence is caused by *T. pyogenes* infection compared to those induced by *C. pseudotuberculosis*. In a previous retrospective study, the occurrence of *T. pyogenes* causing CLA was higher in goats in Brazil (Ribeiro et al, 2015) and in Spain (de la Fuente et al, 2017). *T. pyogenes* isolates originated from CLA lesions which formed in the supramammary lymph node. It was previously reported that *T. pyogenes* in intra-

mammary gland is an important contributor in clinical mastitis in bovine (Alkasir et al, 2016). In goats, however, CLA in the supramammary lymph nodes was previously reported to also be mainly caused by *C. pseudotuberculosis* (C Schreuder et al, 2011). Even though KNGs are not bred for milk production, they can serve as reservoir of *T. pyogenes* which poses a potential threat in the biosecurity leading to future disease outbreaks, as well as zoonoses and antibiotic resistance. Furthermore, abscess disease which also proliferates in the lymph nodes is caused by *Staphylococcus aureus* subsp. *anaerobius* and mainly affects younger goats up to six months of age. A previous investigation in Spain reported that this bacteria, together with *C. pseudotuberculosis* and *T. pyogenes* could occur concomitantly causing CLA and abscess disease in goat and sheep herds (de la Fuente et al, 2017). Additionally, internal CLA caused by *T. pyogenes* also occurred in very small proportion in Polish sheep (2.00%) (Didkowska et al, 2020).

The enzyme activity showed positive result for the alkaline phosphatase, esterase (C4), leucine arylamidase,

Table 1. Biochemical test of *Trueperella pyogenes* isolates using APIZYM compared to *T. pyogenes* and *C. pseudotuberculosis* type strains

No.	Enzyme assayed for	<i>T. pyogenes</i>		<i>C. pseudotuberculosis</i>
		This study (n=3)	Type strain (Yassin et al, 2011)	DSM20689 (Wink 2012)
2	Alkaline phosphatase	+	+	+
3	Esterase (C4)	+	+	+
4	Esterase Lipase (C8)	-	+	+
5	Lipase (C 14)	-	+	-
6	Leucine arylamidase	+	-	-
7	Valine arylamidase	-	+	-
8	Cystine arylamidase	-	-	-
9	Trypsin	-	-	-
10	α -chymotrypsin	-	-	-
11	Acid phosphatase	+	-	+
12	Naphthol-AS-B1-Phosphoamidase	+	+	-
13	α -galactosidase	-	-	-
14	β -galactosidase	+	-	-
15	β -glucuronidase	+	+	-
16	α -glucosidase	+	+	+
17	β -glucosidase	-	+	-
18	N-acetyl- β -glucosaminidase	+	-	-
19	α -mannosidase	-	-	-
20	α -fucosidase	-	-	-

+, positive reaction; -, negative reaction.

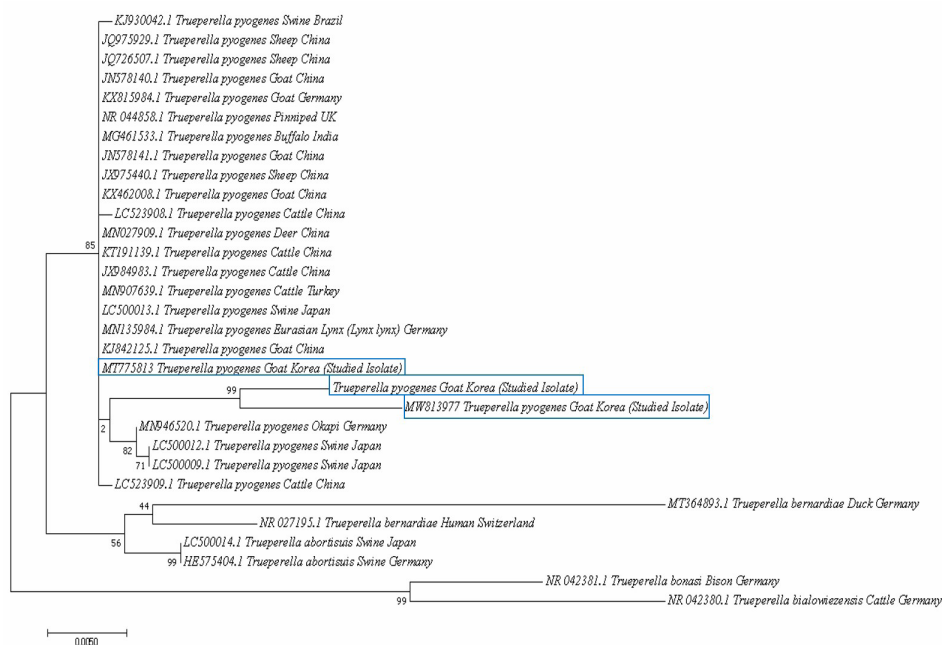


Fig. 2. Phylogenetic tree constructed using the maximum likelihood method based on 16S rRNA gene sequences *T. pyogenes*, and other *Trueperella* species. The tree was bootstrap resampled 1000 times. The bar represents 0.005 substitutions per nucleotide position. The analysis involved 25 nucleotide sequences *T. pyogenes* (3 from our study and 22 from the prior study), and six other *Trueperella* species retrieved from the GenBank® database. Evolutionary relationship was calculated using MEGA7.

acid phosphatase, naphthol-AS-B1-phosphoamidase, α -glucosidase, β -galactosidase, β -glucuronidase, and N-acetyl- β -glucosaminidase and negative for esterase lipase (C8), lipase (C14), cystine arylamidase, valine arylamidase, α -galactosidase, α -chymotrypsin, β -glucosidase, α -fucosidase and α -mannosidase. Compared to the type strain obtained from a previous characterization (Yassin et al, 2011), the enzyme activity of our isolates was different as presented in Table 1. The biochemical test result of *T. pyogenes* isolates showed variation in the enzyme reactivity compared to a previous study which suggests phenotypic diversity among strains (Yassin et al, 2011).

Molecular identification of the three PLD-negative isolates using the 16S rRNA gene revealed these samples as *T. pyogenes*, and the phylogenetic tree (Fig. 2) showed the evolutionary relationship to *T. pyogenes* from other country/host, and other members of genus *Trueperella*. One of the isolates closely grouped with the subclade containing most *T. pyogenes* isolates from diverse sources, but two of the isolates formed a distinct clade within the node containing all *T. pyogenes*. Additionally, the phylogenetic tree showed that two of our isolates may have further evolved from the other

isolates. The phenotypic and genotypic diversity of *T. pyogenes* revealed in this study, as also observed previously, could provide essential information in the characterization of this bacterium to better understand its pathogenesis (Rogovskyy et al, 2018).

In conclusion, with the involvement of several bacteria in CLA, proper identification of which agent is involved is essential to lessen the risk of development of antimicrobial resistance among the pathogens present. Since this is the first reported cases of *T. pyogenes*-induced CLA in KNG, this study provided information that could be used for establishing diagnosis, treatment and control measures for CLA in the country. Currently, limited information is available with regards to *T. pyogenes* as a causative agent of CLA in goats. Further investigations should be conducted in order to deeply understand the pathogenic mechanisms, risk factors, and impacts of this bacterium in CLA.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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