Johne’s disease in two Korean black goats (Capra hircus coreanae) with clinical and subclinical infection: A case report

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ABSTRACT

Johne’s disease (JD) is a chronic enteric infection in ruminants caused by Mycobacterium avium subspecies paratuberculosis (MAP). JD infection is more difficult to diagnose in goats than cattle because MAP can insidiously affect small ruminants. Few reports have used pathological and molecular diagnosis for cases in Korean black goats. Here, we present our results from two MAP-infected goats. Case 1 was categorized as clinically significant (stage IV), with severe clinical signs and a high antibody titer (S/P ratio, 158.9%). Case 2 was in the subclinical stage (stage II); however, the goat suddenly died without any clinical signs (S/P ratio, 70.9%). DNA from the organ tissues and feces from Case 1 showed a strong positive PCR result for MAP, whereas Case 2 only exhibited a very weak reaction in the fecal sample. Moreover, fecal DNA from both cases was genotyped as C-type MAP using the PCR-REA method. Gastrointestinal organ tissues (jejunum, ileum, colon, and mesenteric lymph nodes) from Case 1 showed moderate-to-severe lesions, and acid-fast bacilli were observed. In contrast, Case 2 showed intact-to-mild pathological lesions, and acid-fast bacilli were detected in the colon, mesenteric lymph nodes, and liver. In addition, Case 2 was co-infected with Corynebacterium pseudotuberculosis, which caused caseous lymphadenitis. This case study provides valuable information regarding the pathological and molecular characteristics of JD-infected Korean black goats. The results highlighted the differences in pathological lesions between clinically and subclinically infected goats, which help veterinarians to develop better strategies to control MAP in goat farms.

Abbreviations
AFB, Acid-fast bacilli
H&E, Hematoxylin and eosin
JD, Johne’s disease
LN, Lymph nodes
MAP, Mycobacterium avium subspecies paratuberculosis
NIAS, National Institute of Animal Science

1. Introduction

Mycobacterium avium subspecies paratuberculosis (MAP) is the etiologic agent of paratuberculosis in ruminants, commonly known as Johne’s disease (JD). JD in cattle typically presents as watery diarrhea and weight loss, while the disease can affect small ruminants insidiously (Fecteau, 2018; Manning, 2001; Whittington et al., 2019; Windsor, 2015). Although MAP infection mainly occurs in neonatal ruminants, clinical signs of JD can be detected 2–5 years after infection (Waddell et al., 2016). Therefore, clinical examination and bacterial culture methods cannot consistently diagnose JD in small ruminants before they shed MAP or exhibit clinical signs (Fletcher et al., 2015; Windsor, 2015).

The prevalence of JD in goats is approximately 24.1%, 62.9%, and 7.1% in Greece, France, and Norway, respectively (Angelidou et al., 2014; Mercier et al., 2010; Nielsen & Toft, 2009). According to data from the Korean Statistical Information Service, the number of native Korean black goats (Capra hircus coreanae), the only breed indigenous to
Korea, increased annually from 271,000 to 551,000 goats between 2015 and 2020. A previous study estimated that the seroprevalence of MAP at the herd level in Korean black goats was 25.0% between 2005 and 2006, whereas the prevalence was only 8.2% at the individual level (Lee et al., 2006).

We speculated that this discrepancy in prevalence between herds and individuals was not only due to the low sensitivity of molecular diagnosis but also to the insidious clinical signs of JD in Korean black goats. Therefore, this study aimed to compare the histopathological and molecular diagnostic characteristics of two cases of JD in Korean black goats, which were at the clinical and subclinical stages of infection, with and without clinical signs of diarrhea, respectively.

2. Case presentation

2.1. Clinical history

In Case 1, a 18-month-old Korean black goat showing gradual weight loss was admitted to the animal hospital at the National Institute of Animal Science (NIAS) for disease diagnosis in July 2021. The MAP antibody titer was immediately evaluated using an IDEXX Paratuberculosis ELISA test (IDEXX Laboratories, Westbrook, USA). MAP antibody status was assigned according to the manufacturer’s instructions (i.e., S/P ratio ≥ 55%: positive; S/P < 55%: negative). The goat was positive, with an S/P of 57.3%, and was placed in an isolated room on the farm. The animal became progressively weaker and emaciated despite having a good appetite. Clinical signs emerged in January 2022, 10 d before the animal died. After the onset of clinical signs, the goat showed loss of appetite and died of severe emaciation and watery diarrhea. MAP antibody testing of the carcass yielded a strongly positive result (158.9%, S/P). In Case 2, a six-year-old Korean black goat presented with a suspected MAP antibody titer level (48.7%, S/P) in July 2021. The goat was also immediately housed in an isolation room, but it gradually lost weight. Finally, it suddenly died in January 2022 without any clinical signs (e.g., diarrhea and loss of appetite). The MAP antibody test was positive, with 70.9% S/P detected in the carcass.

2.2. Clinical findings and diagnostic assessment

Genomic DNA was extracted from all collected tissues (the rectum, cecum, mesenteric and gastrohepatic lymph nodes [LNs], ileum, jejunum, and lung) and fecal samples from both necropsied goats using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and QIAamp DNA Stool Mini Kit (Qiagen), respectively, according to the manufacturer’s instructions. The primers ISMan2-F (5′-GTTAGTGTGCGTACAGAT-3′) and ISMan2B2 (5′-GCATCAAGAGGACACCTGAC-3′) were used to amplify a 494-bp fragment to detect MAP, as in a previous study (Sung et al., 2004). All collected samples, except the lung, from Case 1 showed a positive reaction for MAP, while only fecal samples from Case 2 showed a weak positive reaction in the MAP PCR assay. PCR and restriction enzyme analysis (PCR-REA) was used for genotyping the fecal genomic DNA from each case. Briefly, fecal genomic DNA was amplified with IS1311 primers, as described previously (Kim et al., 2015). Subsequently, restriction enzyme analysis was performed by HinfI digestion of the PCR product, and a 2% agarose gel was used to visualize the band pattern. Genotyping of fecal DNA by PCR-REA showed that both MAP amplicons were “cattle type” (known as C-type), which had fragments of 67, 218, 285, and 323 bp (Fig. 1B).

Grossly, both cases showed severe congestive swelling lesions of the mesenteric LNs and presented mild hyperemic lesions in the ileum, jejunum, and cecum. In Case 1, a thickened and corrugated mucosal surface was observed in the ileum, as well as enlarged gastrohepatic LNs. In Case 2, a medium-sized abscess filled with yellow pus was observed in the bronchial LN.
3. Discussion

JD is one of the most critical chronic wasting diseases affecting small ruminants, including Korean black goats. MAP commonly resides in macrophages in the small intestinal lamina propria and associated LNs and triggers granulomatous inflammation and enteropathy (Whittington et al., 2019). Owing to these infectious characteristics, numerous diagnostic methods are inadequate for detecting MAP in infected animals. Notably, confirming JD in MAP-infected goats was more challenging than in infected cattle because the clinical signs are less evident in goats. This study provides the pathological findings and molecular diagnosis results of two MAP-infected Korean black goats at different clinical infection stages.

JD infection can be classified into four stages (stages I–IV) based on pathogen detection and clinical signs in infected animals (Whitlock & Buergelt, 1996). Accordingly, Case 1 was assessed as being a stage IV infection (158.9% S/P), and the animal was emaciated with fluid diarrhea. However, Case 2 was categorized as a subclinical stage (stage II) with no visible clinical signs (70.9% S/P). The PCR results also supported the classification of the JD infection stage of the two goats, which presented strong and very weak agarose gel bands in Cases 1 and 2, respectively.

Differences in pathological lesions were also observed between Cases 1 and 2. In Case 1, the goat showed enlarged gastrohepatic LNs and moderate congestive edema in the mesenteric LNs, indicating that the Case 1 goat was severely infected with MAP. Meanwhile, mild gross lesions were observed in the LNs in case 2. Histopathological examination of the gastrointestinal tissues also showed moderate-to-severe lesions in case 1, but mild or no lesions were observed in Case 2. Case 1 showed typical histopathological lesions in the jejunum, ileum, and colon of MAP-infected goats, as previously reported (Idris et al., 2021; Kim et al., 2015; Rhyoo et al., 2013), whereas we could not detect any specific lesions in the ileum and colon tissue from Case 2 goats. Although the ileum is known as the primary point of MAP invasion and localization within the host animal (Arsenault et al., 2014), AFBs were observed only in the jejunum tissue, not in the ileum and colon, in Case 2. These findings suggest that MAP infection did not progress within the ileum and colon. Given that the Case 2 goat was in the subclinical stage of JD infection, AFB may not be detected in the ileum tissue due to the difference in MAP localization and the location of the sampled tissue. Therefore, the diagnosis of MAP in subclinically infected goats should be performed in various locations in the gastrointestinal organs. In contrast, histopathological lesions of pulmonary edema (Case 1) and intestinal pneumonia (Case 2) were observed by H&E staining, but the lung tissue was negative for acid-fast staining. In the liver, both cases showed mild-to-moderate lesions by H&E staining, and Case 2 were weakly positive by acid-fast staining. Although gastrointestinal organs are known to be more susceptible to MAP than non-intestinal organs (Khol et al., 2010), our findings suggest that the liver of goats could be affected by MAP infection. The results also suggested that non-gastrointestinal tissue sampling could be helpful in the diagnosis of JD in goats. Furthermore, the Case 2 goat had an abscess in the bronchial LN related to the caseous lymphadenitis (CLA), which was caused by C. pseudotuberculosis. The results suggest that the sudden death in this subclinical MAP-infected case might be due to the co-infection of MAP and C. pseudotuberculosis. Given the high prevalence of CLA in Korean native black goats (7.3%) (Jung et al., 2015), sudden death could occur in subclinical MAP-infected goats (no clinical signs) when the animal was co-infected with C. pseudotuberculosis.

In this study, a discrepancy between the PCR results (negative) and histopathological lesions (AFB-positive) was observed in the subclinical JD infection case (Case 2). Previous studies have suggested that the methods or amount of tissue samples used for DNA extraction could significantly affect the yield of MAP DNA following PCR sensitivity...
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4. Conclusion

In conclusion, this study provides information about the clinical signs, pathological lesions, and molecular characterization of two cases of MAP infections in Korean black goats. The clinical JD-infected case (Case 1) presented a typical clinical presentation with a high MAP antibody value. DNA from case 1 tissue and feces showed a strong positive band in the PCR method and moderate-to-severe histopathological lesions in gastrointestinal organs. However, the subclinical JD-infected case (Case 2) showed no clinical signs of JD, and the DNA was very weakly positive only in the fecal sample. Notably, the ileum of Case 2 goat showed no specific histological lesions, and AFB was absent.

Fecal DNA sequences from both cases were revealed as belonging to the “C-type.” The overall results highlight the difficulties in diagnosing JD in subclinically infected goats, and this case study could be helpful for veterinarians in managing MAP in goat farms.

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Fig. 3. Microscopic lesions and acid-fast stain results of the case 2 goat. In the liver, inflammatory cell infiltration in the liver parenchyma (asterisk) and an acid-fast positive signal (arrows) are visible; inset: acid-fast bailli (arrow heads). C: Central vein. In the jejunum, inflammatory cell infiltration, villus fusion, and an acid-fast positive signal (arrows) are visible; inset: acid-fast bailli (arrow heads). In mesenteric lymph nodes (LNs), mild lymphoid depletion, and an acid-fast positive signal (arrows) can be seen; inset: acid-fast bailli (arrow heads).

(Foek-Chow-Tho et al., 2017; Gilardoni et al., 2012; Sting et al., 2014). These possibilities might have caused a difference in detection limits between PCR and acid-fast staining. Further studies are essential to increase the sensitivity of PCR for diagnosis in subclinical JD-infected animals.

MAP could be categorized into two strains infecting animal populations: the sheep type (S-type or type I), which is common in sheep, and the cattle type (C-type or type II), which is common in cattle and goats (Collins et al., 1990; Whittington et al., 1998). In this study, we genotyped fecal DNA from both cases using the PCR-REA method and revealed that all DNA samples belonged to the cattle type (known as C-type). This result is in agreement with that of a previous study on JD-infected Korean black goats (Kim et al., 2015). However, S-type infections in goats are not rare and have been reported in recent studies in Spain and Switzerland (De Juan et al., 2006; Scherrer et al., 2019). Therefore, continuous monitoring is required to detect emerging S-type MAP infections in Korean black goats, helping to deepen our understanding of the molecular epidemiology of MAP and providing better control strategies for MAP in the future.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data statement

Upon reasonable request, the datasets of this study can be obtained from the corresponding authors.

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