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First molecular evidence of bovine hemoplasmas in houseflies (*Musca domestica*)

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Abstract

Background Hemoplasma infections in cattle are caused by *Mycoplasma wenyonii* and *Candidatus Mycoplasma haemobos* and induce asymptomatic or chronic infections but occasionally lead to life-threatening hemolytic anemia. Despite the global distribution of bovine hemoplasmas, information regarding their transmission vectors and prevalence is still lacking in the Republic of Korea. Therefore, this study aims to investigate the infection rate of bovine hemoplasma in cattle and houseflies and to assess the risk factors associated with hemoplasma infection in cattle.

Methods Overall, 376 blood samples were collected from Korean indigenous cattle (male, 10–13 months old), along with 2,690 houseflies (*Musca domestica*) from the same farm where the cattle were raised. PCR assays targeting the 16S rRNA gene were performed to detect hemoplasmas, and positive samples were sequenced.

Results The infection rate of bovine hemoplasmas was 50.8% (191/376) in cattle and 7.4% in pooled houseflies. Among cattle, 18.6% (70/376) and 20.0% (75/376) tested positive for *M. wenyonii* and *Candidatus M. haemobos*, respectively. Conversely, in houseflies, *Candidatus M. haemobos* was more frequently detected (5.9%) than *M. wenyonii* (0.7%). Co-infection was 12.2% (46/376) in cattle and 0.7% in flies. Furthermore, hemoplasma infection was significantly associated with the grazing experience of their dams. Cattle born to cows with grazing experience exhibited a higher risk for *M. wenyonii* infection (odds ratio [OR] = 1.62; 95% confidence interval [CI]: 1.03–2.55; $P=0.045$), whereas these cattle had a lower risk for *Candidatus M. haemobos* infection (OR = 0.32, 95% CI: 0.19–0.74; $P=0.000$) than animals born to cows without grazing experience. The sequences obtained from houseflies were confirmed as *Candidatus M. haemobos*, which displayed high similarity (98.2–100%) to those from cattle obtained in this study.

Conclusions To our knowledge, this study represents the first report of bovine hemoplasmas identified in houseflies. This molecular evidence suggests that houseflies may be possible vectors for *Candidatus M. haemobos*.

Keywords Bovine hemoplasma, *Mycoplasma wenyonii*, *Candidatus mycoplasma haemobos*, Houseflies, Transmission route

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Background

Hemotropic mycoplasma (hemoplasmas) are small, pleomorphic, and wall-less bacteria that parasitize the surface of red blood cells, causing infections in various animals, including humans [1, 2]. Hemoplasmas are emerging pathogens in a wide variety of animals [2]. To date, two distinct hemoplasmas have been identified in cattle: *Mycoplasma wenyonii* and *Candidatus Mycoplasma haemobos* [3, 4]. The clinical signs of these bovine hemoplasmas include anemia, pyrexia, lymphadenopathy, anorexia, decreased milk production, and reproductive problems; however, most infections in animals remain subclinical [1, 4–8]. Acute infections are rare in cattle, but occasionally they can lead to overt life-threatening hemolytic anemia [9, 10].

Bovine hemoplasmas are widely distributed worldwide [4, 8, 11–16], but their transmission routes are poorly understood. Several studies have reported that transmission can occur through direct contact with infected blood, contaminated surgical instruments, blood-sucking arthropods (such as ticks, flies, lice, and mosquitoes), or via transplacental infection [3, 14, 17–20]. Although *M. wenyonii* and *Candidatus M. haemobos* DNA has been detected in several vectors [17, 20–23], the evidence that arthropod vectors were involved has not been sufficiently proven in the Republic of Korea (ROK).

The housefly (*Musca domestica*) is the most abundant and widely distributed fly species [24] and can carry more than 100 pathogens responsible for diseases in animals and humans [25] and consequently poses a substantial health risk [26]. However, despite their role in disease transmission, little is known about the specific pathogens that houseflies carry, and farmers often overlook their influence. To date, houseflies were not considered to be potential candidate vectors for transmitting bovine hemoplasmas. However, previous results have detected bovine hemoplasmas in blood-sucking flies [17]. Moreover, a recent study showed that houseflies could be potential carriers of *Mycoplasma* spp. [27]. Flies are predominantly found at cattle farms during the summer and autumn and readily contaminate food and eating utensils. Considering their adaptability, mobility, and affinity for various environments, houseflies may be involved in disseminating bovine mycoplasmas with other pathogens between animals or on farms. Since the exact vector of this disease has not yet been identified, the role of houseflies in transmitting bovine mycoplasmas needs to be determined.

Compared to other countries, a minimal number of studies on hemoplasmas have been conducted in the ROK. So far, only one study on bovine hemoplasmas is available [12]. A limitation of previous study was focused on elucidating the presence of hemoplasmas and the association between hemoplasma infection and anemia

[12]. Therefore, considering that houseflies are found abundantly on farms, we aimed to investigate the occurrence of bovine hemoplasmas in houseflies, evaluate the possible transmission routes of bovine hemoplasma using molecular analysis, and determine the risk factors associated with hemoplasma infection in cattle.

Materials and methods

Ethical statement

All animal-related experimental procedures were conducted in strict adherence to the relevant guidelines and regulations for the use of animal samples as approved by the Institutional Animal Care and Use Committee (IACUC) at Kyungpook National University (No. KNU-2023-0280). Written informed consent was obtained from the cattle owners for the collection of blood samples.

Sample collection

During the summer of 2023, 376 blood samples were collected from the jugular vein of each bull reared on a single farm in the ROK. The cattle used for blood collection were 10–13 months old, male, clinically healthy, and had never grazed on pasture. To assess potential variation in infection rates based on the grazing experience of their dams, the animals were classified into two groups: those with grazing experience ($n=127$) and those without ($n=249$). Because some of the dams were pregnant, the owner did not want to collect blood from them. Moreover, some had already been sold to other farms and blood collection from cows cannot be conducted. For these reasons, this study focused on how the grazing experience of their dams affects bovine hemoplasma infection in their offspring. The difference in infection rate was also evaluated according to age: <1 year old ($n=346$) and over 1 year old ($n=30$). Heparin–anticoagulated blood was kept on ice and transferred to the laboratory at Kyungpook National University and then used for DNA extraction. A total of 2,690 houseflies were trapped on the same farm where the cattle were raised during the summer. This sample size was deemed sufficient balancing logistical feasibility and process. The collections were performed near the bulls using an entomological net. All fly specimens were promptly frozen (-80°C) upon collection and transferred to the laboratory for species identification and hemoplasma detection. Other fly species were excluded.

DNA extraction and polymerase chain reaction

DNA was extracted from blood and fly samples using the AccuPrep® Genomic DNA Extraction Kit (Bioneer, Daejeon, ROK) according to the manufacturer's instructions. For the blood samples, 200 μL was used. Regarding fly samples, 12 specimens were pooled to obtain

approximately 25 mg, resulting in the extraction of 269 DNA. Subsequently, all DNA were stored at -80°C until further analysis. Extracted DNA were screened for *M. wenyonii* and *Candidatus M. haemobos* detection using a PCR assay targeting the 16S rRNA gene. The PCR methods for detecting hemoplasmas and identifying fly species were performed as previously described [8, 28]. Positive and negative controls were included in each PCR run. Two samples confirmed as positive in our previous study were used as positive controls [12] and distilled water was used for negative control. All PCR products were separated via electrophoresis on 1–1.5% agarose gels and then visualized by staining with ethidium bromide.

Sequencing and phylogenetic analyses

All PCR products were purified using the AccuPrep® PCR Purification Kit (Bioneer) according to the manufacturer's instructions and then directly sequenced (Bioneer). The nucleotide sequences obtained from this study were aligned using the BioEdit software (version 7.2) and compared among themselves and then with other sequences from the GenBank database using the BLAST in National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST>). The percentage of nucleotide sequences similarities between our sample sequences and the reference sequences was assessed in Geneious Prime software. Phylogenetic analysis of hemoplasmas identified in cattle and houseflies was conducted using the maximum-likelihood method implemented in MEGA11. The best substitution model used in this study was Hasegawa-Kishino-Yano (HKY)+G. The reliabilities of clusters were assessed using bootstrap values with 1,000 replicates. The nucleotide sequences obtained in this study were assigned the following accession numbers: PP544180–PP544184 and PP544185–PP544194 for *M. wenyonii* and *Candidatus M. haemobos*, respectively.

Statistical analysis

Statistical analysis was performed using the SPSS Statistics 29 software package for Windows (SPSS Inc, Chicago, IL, USA). Associations between bovine hemoplasma-positive samples and the grazing experience of dams were analyzed using the Chi-square test. Binary logistic regression analysis was used to determine the relationship between the prevalence of hemoplasma infections and the grazing experience of the dams. Odds ratio (OR) with 95% confidence intervals (CIs) were calculated to assess the likelihood of association. A *P*-value of <0.05 (two-tailed) was considered statistically significant.

Results

The overall infection rate of hemoplasmas in cattle was 50.8% (191/376; 95% CI: 45.7–55.9). Among them, 70 (18.6%; 95% CI: 14.7–22.5) and 75 cattle (19.7%; 95% CI:

Table 1 The infection rate of hemoplasmas detected in cattle ($n=376$) and pooled houseflies ($n=269$)

Pathogens	No. of positive (%) in cattle	No. of positive (%) in flies*
<i>M. wenyonii</i>	70 (18.6%)	2 (0.7%)
<i>Candidatus M. haemobos</i>	75 (20.0%)	16 (5.9%)
Co-infection	46 (12.2%)	2 (0.7%)
Total	191 (50.8%)	20 (7.4%)

*To extract DNA from housefly samples, 12 pooled specimens were used

Table 2 Risk factors associated with hemoplasma infection

Variables	<i>M. wenyonii</i>		<i>Candidatus M. haemobos</i>	
	No (%). of positive	χ^2 (<i>P</i> -value)	No (%). of positive	χ^2 (<i>P</i> -value)
Age				
<1 year ($n=346$)	30.6% (106/346)	0.094 (0.759)	32.4% (112/346)	0.071 (0.790)
≥ 1 year ($n=30$)	33.3% (10/30)		30.3% (9/30)	
Grazing				
No ($n=249$)	27.3% (68/249)	3.86 (0.049)*	39.8% (99/249)	18.38 (0.000)***
Yes ($n=127$)	37.8% (48/127)		17.3% (22/127)	

Grazing refers to the mother cows that were grazing before delivery

* $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$

15.9–23.9) tested positive for *M. wenyonii* and *Candidatus M. haemobos*, respectively. Furthermore, 46 cattle (12.1%; 95% CI: 8.9–15.5) were co-infected with both species (Table 1). The flies used in this study were identified as houseflies (*Musca domestica*) using species identification primers (Supplementary Fig. 1). The positive infection rate of bovine hemoplasmas in pooled houseflies was 7.4% (95% CI: 4.3–10.5). *Candidatus M. haemobos* DNA was most frequently detected (5.9%; 95% CI: 3.1–8.7), while the positivity rate of *M. wenyonii* was 0.7% (95% CI: -0.3–1.7). Co-infection with both species was 0.7% (95% CI: -0.3–1.7). Overall, *Candidatus M. haemobos* was more frequently identified in houseflies than *M. wenyonii* (Table 1).

The associations between hemoplasma-positive samples and the age of cattle and the grazing experience of their dams were evaluated. As shown in Table 2, no significant difference in hemoplasma infections was noted according to the age of cattle. However, the grazing experience of the dams was statistically significant for both types of hemoplasma infections. Compared with cattle born from housed cows, the infection rates of *M. wenyonii* were significantly greater in cattle born to cows with grazing experience ($\chi^2=3.86$, $P=0.0495$), whereas *Candidatus M. haemobos* infection rates were significantly lower in these cattle ($\chi^2=18.38$, $P=0.000$). Additionally,

the risk of being positive for *M. wenyonii* was 1.62-fold higher in cattle born to cows with grazing experience (95% CI: 1.03–2.55; $P=0.045$), while the likelihood of being positive to *Candidatus M. haemobos* was 0.32-fold lower in these cattle (95% CI: 0.19–0.74; $P=0.000$; Table 3).

To investigate the genetic relationships of bovine hemoplasmas detected in cattle and houseflies, ten sequences (five for *M. wenyonii* and five for *Candidatus M. haemobos*) from cattle blood samples were selected and successfully obtained, whereas only five sequences for *Candidatus M. haemobos* were obtained from houseflies. Therefore, five *M. wenyonii* and ten *Candidatus M. haemobos* sequences were included in our analysis. According to the phylogenetic analysis based on the 16S rRNA gene, our sequences were classified into *M. wenyonii* and *Candidatus M. haemobos* (Fig. 1). The five *M. wenyonii* sequences identified from cattle exhibited 98.0–100% identity to each other and demonstrated 93.9–99.7% identity to those previously reported in the ROK [12], whereas these sequences showed 97.5–100% identity to cattle sequences reported from Cuba, Germany, Japan, Kyrgyzstan, Mexico, Nigeria, Turkey, and USA. Five sequences of *Candidatus M. haemobos* from houseflies showed 98.0–100% identity, whereas five from cattle exhibited 98.7–99.8% identity. Five sequences obtained from houseflies displayed 98.2–100% identity to cattle detected in this study, 99.5–100% identity to cattle in Brazil, 98.2–99.3% identity to cattle in China and Japan, 98.4–99.6% identity to cattle in India, and 98.8–100% identity to cattle in Kyrgyzstan, Nigeria, and Turkey.

Discussion

In this study, *M. wenyonii* and *Candidatus M. haemobos* were detected in both cattle and houseflies. While bovine hemoplasmas are not typically considered pathogenic in the ROK, they may have persisted for a long time under various environmental conditions. Our findings revealed that the sequences of *Candidatus M. haemobos* from houseflies closely resembled those found in cattle. To our knowledge, this is the first study to identify the presence of bovine hemoplasmas in houseflies (*Musca domestica*).

Hemoplasmas infection in cattle has been frequently reported in several countries [4, 11–16]. However, there is little information on hemoplasmas in the ROK. Nevertheless, the occurrence of hemoplasmas in this study

was relatively high compared to other countries [8, 10, 15, 21, 29]. Several studies have shown that hemoplasma infections are associated with agroclimatic zones, sex, age, and rearing system [10, 15, 30]. However, the cattle used in this study were all of one sex (male) with a single indoor raising system and differed only in age. Nevertheless, our findings revealed that bovine hemoplasma infections were not associated with the age of cattle, contrary to previous studies that indicated adult cattle (≥ 1 year) have a higher risk of hemoplasma infection than young animals [13, 31–33]. It is believed that other factors, such as farm management, hygiene, and environment, may influence hemoplasma infection. One limitation of this study is that all animals were of the same sex (male), and the number of samples was inconsistent between the two groups, preventing a comparison based on sex and age from being performed. The clinical importance and economic impact of hemoplasmas on the livestock industry have not been fully explored in the ROK; however, given their high infection rate, it should not be overlooked.

According to a recent study performed by our group, *M. wenyonii* was found to be more prevalent in grazing cattle than *Candidatus M. haemobos* [12]. However, this study showed a slightly higher prevalence of *Candidatus M. haemobos* (20%) than *M. wenyonii* (18.6%), which differs from previous findings [12]. The most significant difference between the two studies is that the cattle in this study had not experienced grazing and were raised solely in the barn. The grazing experience of their dams may have influenced *M. wenyonii* and *Candidatus M. haemobos* infections. Our results showed that in cattle born from cows with grazing experience, *M. wenyonii* infection was high, whereas *Candidatus M. haemobos* infection was low. Given that the dams had grazed prior to delivery, the possibility that they were infected by ticks cannot be excluded. Thus, *M. wenyonii* could have been transmitted to the dams by ticks and passed the infection to their offspring via the placenta. In contrast, cattle born from cows with no grazing experience demonstrated a high positive rate of *Candidatus M. haemobos* infection, suggesting that vectors other than ticks may be involved in its transmission. Previous studies suggest that vertical transmission could be a possible route for bovine hemoplasmas [14, 18, 19]. However, another limitation of the current study is that we could not examine hemoplasma infection from their dams; hence, we cannot confirm whether *M. wenyonii* and *Candidatus M. haemobos* are

Table 3 Univariate logistic regression analysis for each hemoplasma infection according to grazing experience of the mother cows

Grazing	<i>M. wenyonii</i>		<i>Candidatus M. haemobos</i>	
	P-value	OR (95% CI)	P-value	OR (95% CI)
No (Ref.)	–	1.00	–	1.00
Yes	0.045*	1.62 (1.03–2.55)	0.000**	0.32 (0.19–0.74)

* $P < 0.05$, ** $P < 0.005$ vs. reference (Ref.)

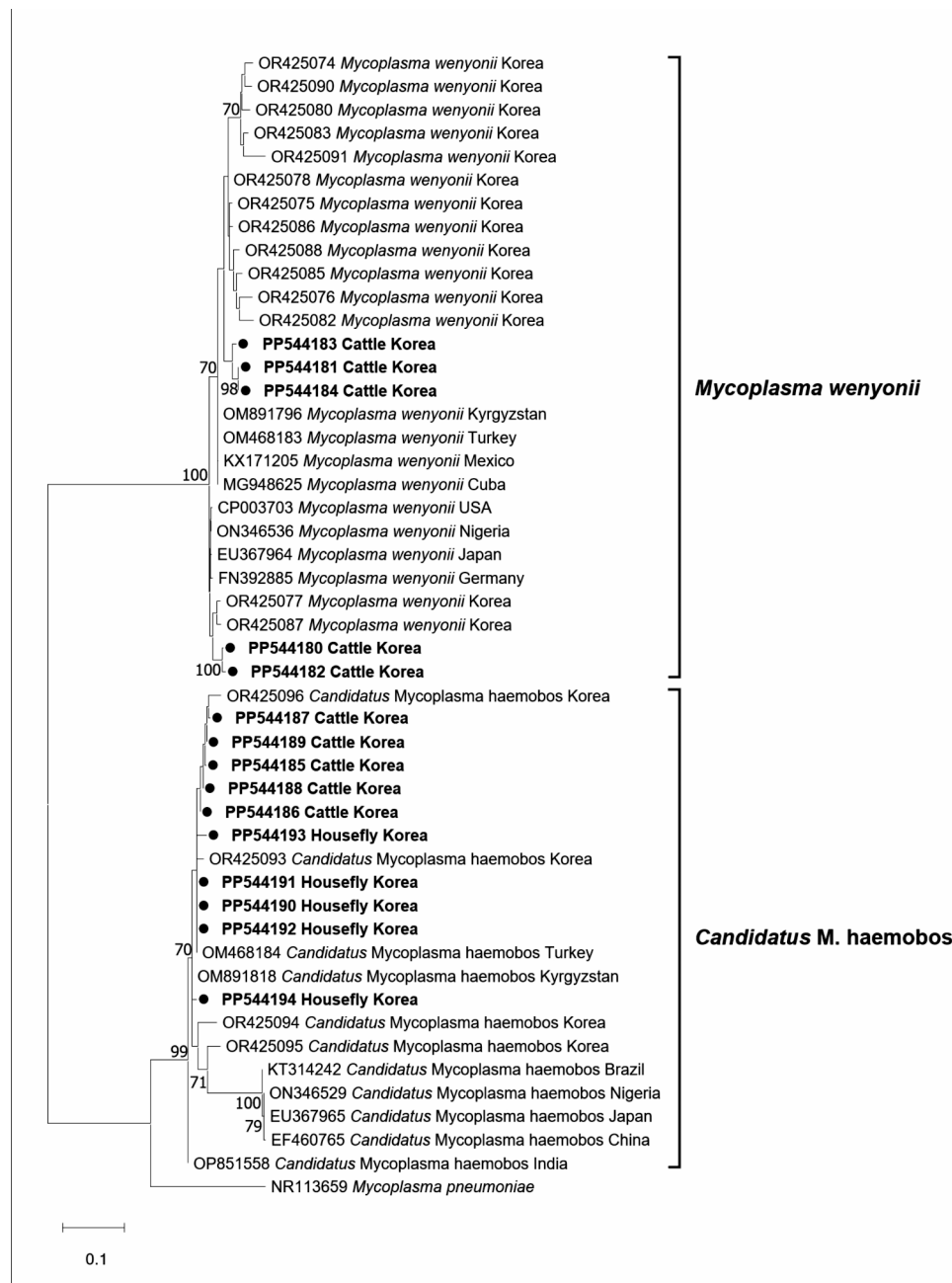


Fig. 1 Phylogenetic tree inferred using maximum-likelihood analysis, employing the HKY + G model. This tree represents the 16S rRNA gene sequence of *Mycoplasma wenyonii* and *Candidatus M. haemobos*. Bootstrap values, expressed as a percentage of 1,000 replicates, are presented at the nodes. Values less than 70% are not shown. The scale bar represents the number of nucleotide substitutions per site. Sequences analyzed in this study are indicated by bold circle symbol

transmitted by transplacental infection. Therefore, further studies are warranted to examine offspring and their dams to elucidate vertical transmission.

Bovine hemoplasma infection is a vector-borne disease; however, the primary vectors responsible for transmitting bovine hemoplasmas remain unclear in the ROK. Several studies have reported the identification of *M. wenyonii* DNA in ticks, lice, flies, and mosquitoes [9, 11, 17,

34–36], whereas the presence of *Candidatus M. haemobos* was confirmed in flies and *Rhipicephalus microplus* and *Haemaphysalis longicornis* ticks [20, 23]. However, experimental proof of bovine hemoplasma transmission by arthropods is rare. Compared to findings from previous literature, our findings demonstrated that *Candidatus M. haemobos* DNA was predominantly detected in houseflies, with identical sequence homology between

cattle and fly DNA. Additionally, *M. wenyonii* DNA was also identified in houseflies, although its sequence could not be obtained, possibly owing to the small amount of *M. wenyonii* DNA present in houseflies. To our knowledge, this is the first time *Candidatus M. haemobos* DNA has been identified in houseflies. The infection rate of *Candidatus M. haemobos* was significantly decreased in cattle born to cows with grazing experience, whereas its infection rate was 2.3-fold higher in cattle born to cows with no grazing experience. Furthermore, considering that *Candidatus M. haemobos* DNA was detected in houseflies and that the bulls used in this experiment were raised in the barn, it is possible that *Candidatus M. haemobos* infection may be transmitted to cattle by flies rather than ticks. Collectively, these findings indicate that the vectors responsible for transmitting each hemoplasma may differ.

Phylogenetic analysis revealed that the sequences of *Candidatus M. haemobos* from cattle and houseflies shared high similarity with those reported in other countries. The infection rate of *Candidatus M. haemobos* was low in houseflies; however, the genomic relatedness of *Candidatus M. haemobos* DNA sequences from cattle detected at the same farm suggests that houseflies can serve as a vector for *Candidatus M. haemobos*. According to a previous study, *M. arginini* was identified in houseflies, and their role as carriers of *Mycoplasma* spp. was also demonstrated [27]. Although *M. arginini* and *Candidatus M. haemobos* cause different clinical symptoms in cattle, our results support that houseflies may act as possible vectors for *Mycoplasma* spp. At this point, how houseflies transmit disease to cattle remains unclear, as they are not blood-sucking vectors. However, houseflies may use mucous membranes, such as the nasal area, to transmit the bacteria [37]. Given that flies can travel 7–32 km daily, keeping them away from cattle is crucial [38, 39]. Most importantly, houseflies comprised 100% of all Diptera collected and identified on this farm. In the cattle farm environment, feces and manure attract houseflies, making them carriers of many pathogens [40]. These findings suggest that houseflies may be potential vectors of *Candidatus M. haemobos* and may influence livestock health. Therefore, our findings underscore the importance of managing and controlling houseflies through environmental improvement practices to protect animal health.

Conclusion

The present study has established the first molecular evidence of *Candidatus M. haemobos* in houseflies. Our findings suggest that bovine hemoplasma infection is high in the ROK. The sequences of *Candidatus M. haemobos* identified from houseflies showed high genomic relatedness to cattle detected at the same farm.

Additionally, this study provides new insights into the vector transmission of bovine hemoplasmas in the ROK. Further studies are warranted to identify the transmission routes of hemoplasma infection through ocular or nasal discharges in cattle to evaluate the role of houseflies as vectors.

Abbreviations

16S rRNA	16S ribosomal RNA
<i>Candidatus M. haemobos</i>	<i>Candidatus Mycoplasma haemobos</i>
CO1	cytochrome c oxidase subunit 1
<i>M. wenyonii</i>	<i>Mycoplasma wenyonii</i>
PCR	polymerase chain reaction
ROK	Republic of Korea

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12917-024-04343-x>.

Supplementary Material 1

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Author contributions

KSC designed the study. MHP and SJC performed laboratory analysis. YK performed sampling. HCC, YJP, MJJ, and JS analyzed data. MHP, SJC, and KSC wrote the manuscript. All authors read and approved the final manuscript.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. The nucleotide sequences obtained in this study were assigned the following accession numbers: PP544180-PP544184 for *M. wenyonii* and PP544185-PP544194 for *Candidatus M. haemobos*, and are available in the repository, WEB LINK (<https://www.ncbi.nlm.nih.gov/nuccore/PP544180.1-PP544184.1>).

Declarations

Ethical approval and consent to participate

This study was reviewed and approved by the ethics committee of Institutional Animal Care and Use Committee (IACUC) at Kyungpook National University (approval number: KNU-2023-0280). Written informed consent was obtained from the cattle owners for the collection of blood samples.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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