

Molecular detection of *Babesia* sp. in sheep at farms in Sleman, Daerah Istimewa Yogyakarta

Tabitha Andrea Putri¹, Diah Adhenia Ernisa Putri Mustikaingtyas¹, and Sugi Winarsih², Trang Thuy Nguyen³, Aan Awaludin⁴, Wisnu Nurcahyo⁵, Yudhi Ratna Nugraheni^{5*}

¹Faculty of Veterinary Medicine, Universitas Gadjah Mada, Jl. Fauna No.2 Karangmalang, Catur Tunggal, Depok, Sleman, D.I.Yogyakarta, Indonesia

²Puskesmas Ngemplak (Animal Health Center Ngemplak), Department of Agriculture, Food, and Fisheries, Sleman Regency. Jl. Kragilan No.002/002, Balong, Bimomartani, Ngemplak, Sleman, Daerah Istimewa Yogyakarta, Indonesia

³School of Biotechnology, International University, Vietnam National University of Ho Chi Minh City, VNUHCM Township, Quarter 33, Linh Xuan Ward, Ho Chi Minh City, Vietnam

⁴Livestock Production Study Program, Department of Animal Science, Politeknik Negeri Jember. Jl. Mastrip PO BOX 164, Jember, Jawa Timur, Indonesia

⁵Department of Parasitology, Faculty of veterinary Medicine, Universitas Gadjah Mada Jl. Fauna No.2 Karangmalang, Catur Tunggal, Depok, Sleman, D.I.Yogyakarta, Indonesia

Abstract. Babesiosis is a tick-borne disease caused by protozoan parasites of the genus *Babesia* that can impair animal health, reduce livestock productivity, and result in economic losses. Sheep, an important livestock species in Indonesia, are particularly susceptible, especially under traditional farming systems. This study aimed to detect *Babesia* sp. infection in sheep from Sleman Regency using microscopic and molecular approaches and to characterize the detected isolate at the species level. A total of 35 blood samples were collected from smallholder sheep farms. Microscopic examination was performed using 10% Giemsa-stained blood smears, while molecular detection was conducted using Polymerase Chain Reaction (PCR) with panpiroF1 and panpiroR1 primers targeting the 18S rRNA gene. One sample tested positive for *Babesia* sp. by both microscopic and PCR examinations, with a low parasitemia level of 1.2%. The observed prevalence was 3.0% (95% CI: 0.0–15%). Sequencing and phylogenetic analysis demonstrated that the detected isolate was closely related to *Babesia motasi*. Although based on a single detection, these findings provide preliminary molecular evidence of *Babesia motasi* infection in local sheep and underscore the importance of early detection for improving animal health and supporting effective disease surveillance in small ruminant farming systems.

1 Introduction

Sheep (*Ovis* sp.) are among the most widely kept livestock species in Indonesia and have long been favored by local farmers for their adaptability, productivity, and economic value. In the Special Region of Yogyakarta (DIY), sheep farming is conducted at both smallholder

* Corresponding author: yudhi.ratna.n@mail.ugm.ac.id

and commercial scales, with a recorded population of 140,398 heads in 2022. Sheep are primarily raised for meat production, as a form of household savings or investment, and their manure is commonly utilized as organic fertilizer. Compared to cattle, sheep exhibit higher reproductive potential and require relatively lower maintenance costs, making them an efficient and accessible option for small-scale farmers [1].

Despite these advantages, the productivity and sustainability of sheep farming are frequently constrained by suboptimal management practices and the occurrence of infectious diseases. These diseases adversely affect animal welfare and result in significant economic losses due to reduced growth performance, decreased reproductive efficiency, abortion, and mortality. Among the most persistent health problems in livestock production systems in tropical and subtropical regions, including Indonesia, are parasitic infections. Parasites are organisms that depend on their hosts for nutrients during part or all stages of their life cycle [2-3]. Parasitic infestations interfere with normal physiological functions and suppress immune responses, ultimately leading to impaired productivity. The associated economic impacts include decreased body weight in cattle, reduced meat and milk quality, compromised fertility, and increased mortality rates.

One of the most important blood parasites affecting small ruminants is *Babesia* sp., an intracellular protozoan parasite that infects erythrocytes and causes babesiosis. Babesiosis has been reported in various livestock species, including cattle, buffaloes, goats, and sheep. The main *Babesia* species infecting sheep are *Babesia motasi* and *Babesia ovis* [4]. The disease is transmitted through tick vectors such as *Rhipicephalus bursa*, *Ixodes ricinus*, *Dermacentor reticulatus*, and *Haemaphysalis punctata* [4]. Following transmission, *Babesia* sp. multiplies within red blood cells, leading to erythrocyte destruction and clinical manifestations including anemia, fever, hemoglobinuria, and, in severe cases, death [5].

Sleman Regency, located in the Special Region of Yogyakarta, was selected as the study area due to its ecological and management conditions that may favor tick survival and the transmission of tick-borne diseases. The region is characterized by a warm tropical climate, relatively high humidity, and the widespread practice of semi-intensive and extensive sheep farming systems, which increase the likelihood of host–vector contact. In addition, reports from local farmers and field veterinarians have indicated the occurrence of clinical signs such as hematuria in several sheep, raising concerns about the possible presence of tick-borne hemoparasitic infections. Although tick infestation in small ruminants has been frequently observed, epidemiological data on blood parasite infections in sheep, particularly babesiosis, remain scarce in Sleman and the broader DIY region. To date, no published studies have provided molecular confirmation or species-level characterization of *Babesia* infecting sheep in this area, indicating a significant gap in regional parasitological knowledge.

The diagnosis of babesiosis has traditionally relied on microscopic examination of Giemsa-stained blood smears. While this method is simple and cost-effective, it has limited sensitivity in cases of low parasitemia and may lead to false-positive interpretations due to staining artifacts [6]. Molecular diagnostic techniques, such as Polymerase Chain Reaction (PCR), offer improved sensitivity and specificity and allow the detection of *Babesia* DNA even in subclinical infections. Moreover, DNA sequencing and phylogenetic analysis provide robust tools for species identification and for determining the genetic relationships between local isolates and previously reported *Babesia* species.

Therefore, this study was conducted to detect *Babesia* infection in sheep from smallholder farms in Sleman Regency using microscopic examination and PCR targeting the 18S rRNA gene, as well as to further characterize the detected isolates through DNA sequencing and phylogenetic analysis in order to determine their genetic relationship with known *Babesia* species. This study provides preliminary molecular evidence of *Babesia* infection in sheep in Sleman and contributes baseline data for future epidemiological investigations and control strategies for tick-borne diseases in small ruminants.

2 Methods

2.1 Sampling

This study was conducted with the approval of the Research Ethics Committee, Faculty of Veterinary Medicine, Universitas Gadjah Mada (Ethical Clearance No. 17/EC-FKH/int.2024). Sample collection was conducted at several smallholder sheep farms in Sleman Regency, Special Region of Yogyakarta. The study area was selected following field reports from local farmers and veterinarians regarding the occurrence of hematuria in sheep, which raised concerns about possible hemoparasitic infections. Within the selected farms, sheep were sampled using a simple random sampling approach, in which individual animals were randomly selected from the available flock at the time of sampling to minimize selection bias.

A total of 35 sheep originating from Sleman Regency were included in this study. The sampled animals consisted of Thin-Tailed Sheep, Garut Sheep, Merino Sheep, Texel Sheep, and Cross-Texel Sheep. The sample size was determined based on feasibility and accessibility under field conditions and was intended to provide preliminary data on the presence of *Babesia* infection rather than to estimate population-level prevalence. Therefore, the results of this study should be interpreted as exploratory and not necessarily representative of the entire sheep population in Sleman Regency. The inclusion criteria for sampling were sheep of any breed and sex that were present on the selected farms at the time of sampling and were clinically accessible for blood collection. Animals were included regardless of clinical status to allow detection of both clinical and subclinical infections. Sheep that were severely stressed, aggressive, or unsuitable for safe blood collection were excluded. Information on age, sex, and history of tick control was not used as a selection criterion and thus was not considered in the sampling design.

Blood samples were collected via jugular venipuncture following proper animal handling and restraint procedures. The jugular vein was occluded at one-third of the distal part of the neck, and the sampling area was disinfected using alcohol-soaked cotton. A venoject needle was inserted into the vein, and once blood flow was observed, a vacutainer tube containing EDTA was attached. Approximately 3 mL of blood was collected from each animal [7].

2.2 Preparation of Blood Smear

Blood smear preparation was carried out at the Parasitology Laboratory, Faculty of Veterinary Medicine, Universitas Gadjah Mada. The process began by preparing a clean and dry glass slide. A drop of blood was placed on the slide, and another slide was used as a spreader, positioned in front of the blood drop at an angle of 30–40°. The spreader was drawn slightly backward until the blood spread evenly along the edge, then quickly pushed forward with steady pressure to form a thin and uniform smear. Once the blood smear was formed, the next step was staining. The staining process began with fixation using methanol for 10 minutes. Fixation serves to open the erythrocyte membranes, allowing optimal absorption of the stain, improving adhesion of the smear to the slide to prevent peeling, and stopping metabolic processes without altering the actual cell structure [8]. After fixation, the blood smear was stained using 10% Giemsa solution, prepared with phosphate buffer at a 1:9 dilution ratio, and immersed for 45 minutes. The slides were then rinsed gently with water and air-dried.

2.3 Microscopic Examination

Microscopic examination of blood smears was performed using a light microscope with an objective magnification of 100×, which is appropriate for observing the morphology of endoparasites. A drop of immersion oil was applied to the stained smear to enhance image clarity during observation. Samples were considered positive when intraerythrocytic forms of *Babesia* sp. were detected within red blood cells [9]. The parasitemia level in this study was determined by counting the number of intracellular parasites in 2,000 red blood cells (RBCs). Samples identified as positive were subsequently analyzed using molecular techniques (PCR) for confirmation.

2.4 DNA Extraction

The process of DNA isolation or extraction began by adding 400 µL of lysis solution and 20 µL of proteinase K solution to 200 µL of blood sample. The mixture was homogenized using a pipette or vortex mixer. The sample was then incubated at 56°C for 10 minutes, with occasional mixing to ensure complete cell lysis. After lysis, 200 µL of ethanol was added and mixed thoroughly using a vortex mixer. The prepared lysate was transferred into a GeneJET™ Genomic DNA Purification Column placed in a collection tube, followed by centrifugation at 6000 × g for 1 minute. The flow-through in the collection tube was discarded, and the column was transferred to a new collection tube. Then, 500 µL of Wash Buffer I (with ethanol) was added, and the column was centrifuged at 8000 × g for 1 minute. The flow-through was discarded, and the column was reinserted into the collection tube. Subsequently, 500 µL of Wash Buffer II (with ethanol) was added and centrifuged at 12,000 × g for 3 minutes. After discarding the collection tube, the GeneJET™ Genomic DNA Purification Column was placed in a sterile microcentrifuge tube. Then, 200 µL of Elution Buffer was added to the center of the column membrane and incubated at room temperature for 2 minutes, followed by centrifugation at 8000 × g for 1 minute. The column was discarded, and the extracted DNA was stored at -20°C until further analysis. The DNA extraction procedure followed the manufacturer's protocol provided with the GeneJET™ Genomic DNA Purification Kit (Thermo Fisher Scientific, USA).

2.5 Molecular Detection

The molecular examination using Polymerase Chain Reaction (PCR) targeting the 18S rRNA gene of *Babesia* sp. was conducted to confirm the presence of the parasite's DNA. The reaction mixture was prepared in an Eppendorf tube containing 6.25 µL of KOD Fx Neo Buffer, 2.5 µL of Deoxynucleoside Triphosphates (dNTPs), 0.375 µL of forward primer (panpiroF), 0.375 µL of reverse primer (panpiroR), 1.75 µL of double-distilled water (ddH₂O/SPW), and 0.25 µL of Taq Polymerase. The mixture was homogenized using a vortex mixer for 30 seconds. Then, 11.5 µL of the prepared master mix was added to the PCR tube containing the DNA sample.

Each PCR run included a positive control (DNA from a previously confirmed *Babesia*-positive sample) and a negative control (double-distilled water) to ensure the validity of the amplification process. The PCR tubes were sealed and vortexed for 30 seconds before being placed in the thermocycler. The amplification program consisted of an initial denaturation at 94°C for 2 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 68°C for 75 seconds, with a final extension at 68°C for 5 minutes. After amplification, 5 µL of the PCR product was loaded onto a 1.5% agarose gel for electrophoresis to visualize the amplified fragments. The primers used in this study are presented in Table 1 [10].

Table 1. Sequences, Target Fragment Size, and PCR Amplification Conditions

Gene	Primer	Primer sequence 5'-3'	Target size (bp)	PCR Conditions
18SrRNA	<i>PanpiroF1</i>	TGGCGGCGTTTATTAGTTCG	1127	PD: 94°C 2 min, 40 cycles for D: 94°C for 30 sec, A: 55°C for 30 sec, E: 68°C for 75 sec, FE: 68°C for 5 min
	<i>PanpiroR1</i>	CCACGCTTGAAGCACAGGA		

2.6 Electrophoresis of PCR Products

A 1.5% agarose gel was used as the medium to detect PCR amplification products. The gel was prepared by dissolving 0.75 g of agarose powder in 25 mL of 1X TAE buffer. The mixture was placed in an Erlenmeyer flask and gently swirled in a circular motion to ensure even mixing. The flask was then heated in a microwave at medium-high power for approximately 4 minutes until the solution reached boiling point. Once fully dissolved, the agarose solution was carefully poured into a casting tray equipped with a comb to form wells corresponding to the number of samples. The gel was allowed to solidify at room temperature for 30–60 minutes. The agarose was poured directly without pre-cooling to prevent premature solidification. After solidification, the tray was placed in the electrophoresis chamber, and 1X TAE buffer was poured until it completely covered the gel surface. The PCR DNA products were then loaded into the wells. A mixture of 1.5 µL of blue loading dye and 5 µL of DNA ladder or marker was prepared on a parafilm strip using a micropipette, then loaded into the first well. Subsequently, the marker/ladder, sample amplicons, and positive or negative controls were sequentially loaded into the remaining wells. Electrophoresis was performed by connecting the electrophoresis tank to a power supply at 100 volts for 30 minutes. The PCR products were visualized under UV light using a transilluminator. The resulting DNA bands were compared with the DNA size marker, and samples were considered positive when a distinct band appeared at the expected fragment length. Positive *Babesia* sp. PCR products were then sent to LPPT Universitas Gadjah Mada for sequencing analysis.

2.7 Sequence Analysis

All sequencing results of PCR products from *Babesia*-positive samples, based on PCR amplification targeting the 18S rRNA gene (1127 bp), were evaluated and manually edited using BioEdit software version 7.0.5.3. A consensus sequence was constructed based on chromatograms obtained from both the forward and reverse primers. The resulting sequences were analyzed and compared with reference sequences available in the GenBank™ database using BLASTn searches, accessible online at <https://blast.ncbi.nlm.nih.gov/Blast.cgi>, with default parameters. The sequences obtained from this study were subsequently deposited in the GenBank™ database.

Sequence data were also used for phylogenetic analysis. The sequences generated in this study were combined with other *Babesia* and piroplasm sequences retrieved from GenBank to construct a phylogenetic tree. The phylogenetic tree was generated using the Maximum Likelihood (ML) method implemented in IQ-TREE version 1.6.12, available at <http://www.iqtree.org/>. The resulting consensus tree (contree file) was then visualized using FigTree version 1.4.3, which can be accessed at <http://tree.bio.ed.ac.uk/software/figtree/>.

2.8 Statistical Analysis

The number of Babesiosis-positive cases in sheep was calculated using binomial proportion analysis with the Clopper–Pearson method. Statistical analyses were performed using a freely accessible online statistical analysis platform available at <https://epitools.ausvet.com.au>. To obtain the prevalence results, the input data included the total number of samples, the number of positive samples, the confidence level, the statistical method applied, and the number of decimal places desired in the output.

3 Result and discussion

3.1 Microscopic Identification of *Babesia* sp.

Examination of blood parasites (*Babesia* sp.) was performed using microscopic and molecular methods at the Parasitology Laboratory, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Samples were collected from sheep (*Ovis* sp.) raised in several smallholder farms in Sleman Regency. A total of 35 blood samples were examined microscopically using the Giemsa-stained blood smear method.

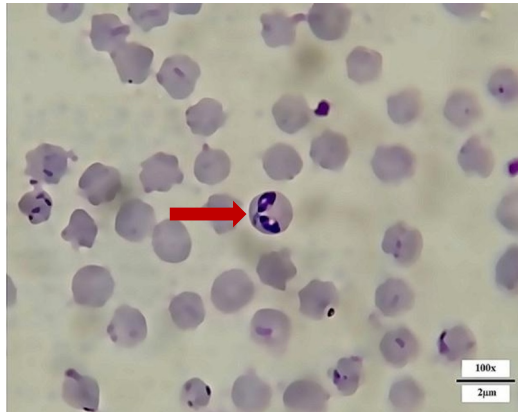


Fig. 1. Microscopic examination of *Babesia* sp. in a blood smear from a sheep positive for babesiosis in Sleman, observed under 100× magnification

Microscopic observations were carried out using a Novel XSZ-107BN microscope under 100× magnification to identify the morphology of *Babesia* sp. The blood parasite infestation was further evaluated to determine the level of parasitemia, categorized as mild, moderate, or severe. Parasitemia was calculated by counting the number of intraerythrocytic parasites per 2,000 red blood cells (RBCs). From the microscopic examination, one blood smear sample was found positive for *Babesia* sp., characterized by the distinctive pear-shaped form within red blood cells (Fig 1). This finding aligns with *Babesia* sp. characteristics which is pear-shaped morphology. In addition, red blood cells infected with *Babesia* sp. appeared larger and swollen. Measurements using ImageJ software showed that an infected erythrocyte measured approximately 8 µm, which is larger than the normal diameter of sheep erythrocytes (4–6 µm) [11].

The parasitemia level of the positive *Babesia* sp. sample was calculated at 1.2%, indicating a mild infection, which is lower than that of severe babesiosis cases, where parasitemia exceeds 10%. Babesiosis is considered severe when parasitemia exceeds 4%, often accompanied by symptoms such as chills, fever, myalgia, nausea, vomiting, diarrhea,

and acute respiratory distress [12]. Furthermore, many severe babesiosis cases with parasitemia above 10% are associated with end-organ damage, including hepatic and renal failure [12]. The positive microscopic finding was further confirmed through molecular analysis using PCR to validate the presence of *Babesia* sp. DNA.

3.2 Molecular Identification of *Babesia* sp. using PCR and Electrophoresis

Molecular screening using the Polymerase Chain Reaction (PCR) aimed to validate the presence of *Babesia* sp. blood parasite infestation previously detected through microscopic observation. The PCR method is more sensitive and specific than microscopic examination. Moreover, PCR can amplify target DNA sequences from extremely small quantities, even from a single cell [13]. Another advantage of PCR is its ability to clone DNA within a few hours, which is relatively faster than cell-based cloning methods [13].

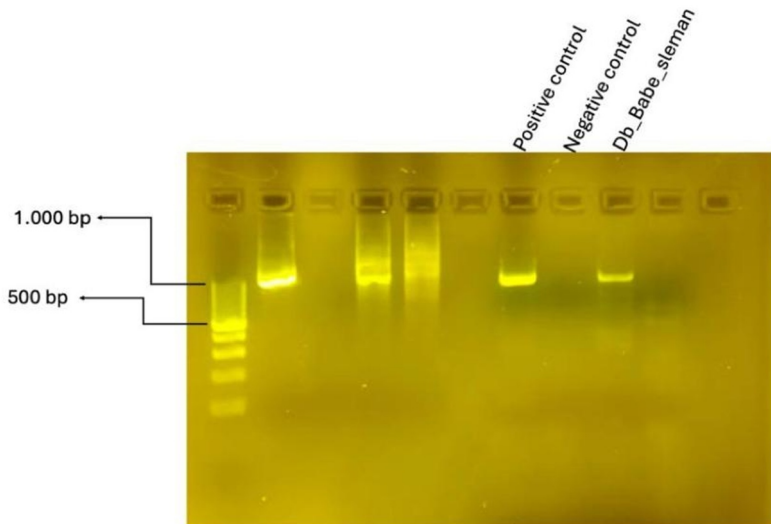


Fig. 2. Visualization of molecular detection using PCR on 1.5% agarose gel showing a band at 1127 bp.

The PCR products were visualized using a UV transilluminator. DNA fragment sizes were identified by comparing the sample bands with a standard DNA marker. In this study, a marker of 1127 bp (base pairs) was used, with fragment sizes ranging from 100–1000 bp. The gel electrophoresis results showed clear, bright DNA bands without smearing or electropherogram distortion, indicating that the isolated DNA was free from contaminants such as proteins, polysaccharides, or phenolic compounds. The molecular examination showed a positive PCR band at 1127 bp, confirming that the target DNA was successfully amplified (Fig 2).

The PCR-positive sample was then subjected to sequencing to determine the specific *Babesia* species infecting sheep in Sleman. Both microscopic and molecular examinations confirmed one positive sample, and the prevalence was calculated using the EpiTool software based on the Clopper–Pearson method. This method offers several advantages over other approaches, as it is based on the binomial distribution rather than the normal distribution, making it suitable for small sample sizes or very low proportions of positive cases. Although the Clopper–Pearson method is more conservative, it produces wider confidence intervals,

reducing the risk of under- or overestimating prevalence. The method remains valid even with very few positive cases.

In this study, the detected prevalence of *Babesia* sp. was 0.03 or 3%, based on both microscopic and PCR results. This prevalence was lower than that reported in China, who found a 12.9% prevalence of *Babesia motasi*-like infection in sheep and goats using nPCR. The present study included 35 samples with one positive result collected from smallholder farms in Sleman, whereas study in China examined 1,081 samples from seven provinces, identifying 139 positive cases [14]. The sample size, geographical differences, environmental factors, farm management practices, and vector distribution likely contributed to the variation in prevalence rates. These findings suggest that *Babesia* sp. distribution may vary according to geographic location and local epidemiological conditions.

3.3 Molecular Sequencing and Phylogenetic Analysis of *Babesia* sp.

The BLAST search results from the NCBI database revealed that the *Babesia* DNA sequence obtained from sheep in Sleman showed 97.39% sequence identity with *Babesia motasi* from Japan (accession number LC811651.1). Sequencing analysis confirmed that the *Babesia* species found in Sleman sheep was closely related to *Babesia motasi*. The BLAST results are shown in (Fig 3) while the phylogenetic tree constructed using the Maximum Likelihood method is shown in (Fig 4).

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Babesia cf. motasi GFHsRmo2022-37-2 gene for 18S rRNA, partial sequence	Babesia cf. motasi	1823	1823	100%	0.0	97.39%	1433	LC811651.1
Babesia cf. motasi GFHsRmo2022-37-1 gene for 18S rRNA, partial sequence	Babesia cf. motasi	1814	1814	100%	0.0	97.20%	1434	LC811650.1
Babesia sp. Hebei-2005 18S ribosomal RNA gene, partial sequence	Babesia sp. Heb...	1812	1812	100%	0.0	97.20%	1683	DQ159074.1
Babesia sp. KO1 18S ribosomal RNA gene, partial sequence	Babesia sp. KO1	1803	1803	100%	0.0	97.02%	1684	DQ346955.1
Babesia sp. Liaoning-2005 18S ribosomal RNA gene, partial sequence	Babesia sp. Liao...	1801	2111	99%	0.0	97.18%	1682	DQ159075.1
Babesia sp. isolate KCDC-1 small subunit ribosomal RNA gene, partial sequence	Babesia sp.	1801	1801	100%	0.0	97.01%	1685	MK930513.1
Babesia sp. China-BQ1 18S ribosomal RNA gene, complete sequence	Babesia sp. Chin...	1792	1792	100%	0.0	96.83%	1687	AY260182.1
Babesia sp. Madang-2005 18S ribosomal RNA gene, partial sequence	Babesia sp. Mad...	1781	1781	100%	0.0	96.74%	1680	DQ159071.1
Babesia sp. Tianzhu-2005 18S ribosomal RNA gene, partial sequence	Babesia sp. Tian...	1770	1770	100%	0.0	96.55%	1680	DQ159072.1
Babesia motasi 18S ribosomal RNA gene, complete sequence	Babesia motasi	1762	1762	100%	0.0	96.37%	1701	AY260179.1
Babesia sp. China-BQ1 18S ribosomal RNA gene, complete sequence	Babesia sp. Chin...	1760	1760	100%	0.0	96.36%	1683	AY260181.1
Babesia motasi 18S small subunit ribosomal RNA gene, complete sequence	Babesia motasi	1751	1751	100%	0.0	96.18%	1684	AY533147.1
Babesia sp. JL-2016a isolate DF02 18S ribosomal RNA gene, partial sequence	Babesia sp. JL-2...	1744	1744	100%	0.0	96.08%	1522	KT959230.1
Babesia sp. isolate Nipoxia-10 small subunit ribosomal RNA gene and internal transcribed spacer 1, partial sequence	Babesia sp.	1740	1740	100%	0.0	96.00%	1701	OR735156.1

Fig. 3. BLAST analysis results of *Babesia* DNA sequences from sheep samples collected in Sleman.

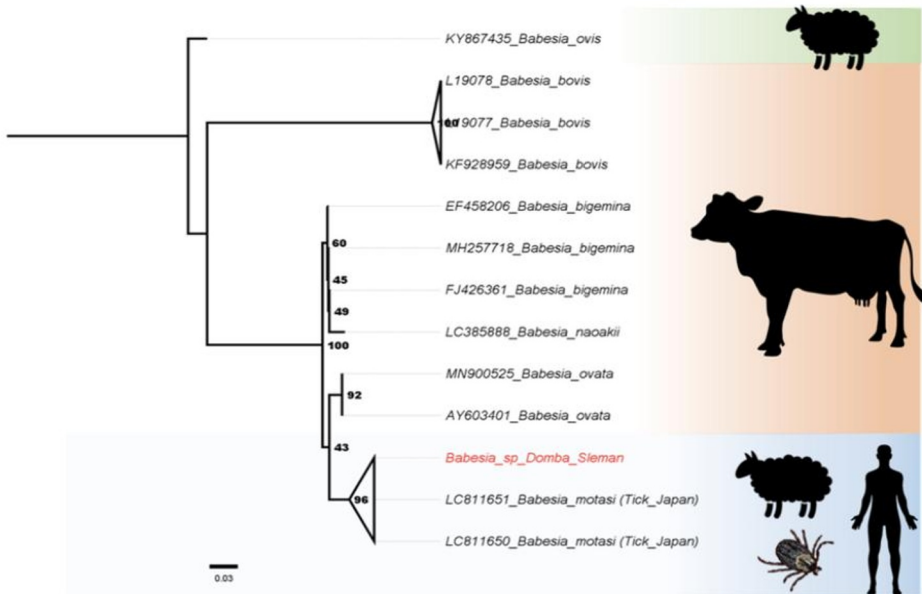


Fig. 4. Phylogenetic tree constructed using the Maximum Likelihood method based on 18S rRNA gene sequences, generated with IQ-TREE software.

Phylogenetic reconstruction was performed on the sample confirmed positive for *Babesia* sp. to determine its evolutionary relationship with reference sequences obtained from the NCBI database. The cladogram, consisting of 13 sequences, was generated using the Maximum Likelihood approach, which divided the sequences into five main clades. Based on the phylogenetic tree, the *Babesia* DNA sequence from sheep in Sleman clustered within the same clade as *B. motasi* isolates from ticks in Japan, showing 97.39% (LC811651) and 97.20% (LC811650) identity. These three organisms shared a bootstrap value of 96, indicating a close genetic relationship and a highly reliable clade.

Babesia motasi commonly infects sheep and goats [4]. The *Babesia* sp. identified in this study showed close phylogenetic relatedness to *B. motasi* isolated from *Haemaphysalis* sp. ticks in Japan. *Haemaphysalis* ticks thrive in humid habitats with dense vegetation, particularly in Eurasia and tropical Africa. They are three-host ticks, with larvae and nymphs parasitizing small mammals and birds, while adults feed on large mammals, especially livestock [4].

Haemaphysalis ticks ingest blood from infected sheep, allowing *B. motasi* merozoites or gametocytes to enter the tick's digestive system. Inside the gut, *B. motasi* develops into ookinetes, which penetrate the gut wall and migrate to the salivary glands, where they form sporozoites, the infectious stage. When the tick bites a healthy sheep, these sporozoites are transmitted through saliva into the host's bloodstream. Once inside, they invade red blood cells, develop into trophozoites, and multiply through binary fission to form merozoites, which rupture the host cells and infect new erythrocytes. This cycle continues, causing anemia, fever, and other typical signs of babesiosis [7]. Infected sheep can serve as a reservoir for other ticks, facilitating the continued environmental transmission of *B. motasi*.

Through transstadial transmission, *B. motasi* persists within the tick even as it molts from larval to nymphal to adult stages. Thus, ticks act not only as vectors but also as biological hosts supporting parasite development. Consequently, the prevention of *B. motasi* infection

should focus not only on animal health but also on improved farm management, biosecurity measures, and tick control programs to effectively reduce transmission.

Babesia motasi poses a significant risk to both animal and human health. A fatal human case caused by *B. motasi* infection was reported [7]. Blood samples from the patient were analyzed microscopically (blood smear) and molecularly (PCR), followed by phylogenetic reconstruction. The results showed that the infecting *Babesia* was closely related to *B. motasi Nungxian* (JX440507) and *B. motasi Ningxian* (JX866781). Further investigations involving tick collection and identification around the patient's residence revealed *Haemaphysalis longicornis* and *H. flava*, in which *B. microti* and *B. motasi* genes were detected [7]. This zoonotic case was likely transmitted through an infectious tick bite, highlighting the potential health threat ticks pose to humans, particularly farmers in close contact with livestock.

The findings of this study should be interpreted as preliminary molecular evidence of *Babesia* infection in sheep in Sleman Regency rather than as an estimate of regional prevalence, given the limited sample size and the detection of only a single positive case. Molecular characterization indicates that the detected *Babesia* is closely related to *Babesia motasi*, a species that has been reported in association with *Haemaphysalis* ticks and has been implicated in zoonotic infections in previous studies. Although the present study does not address zoonotic transmission, this finding highlights the importance of effective tick control to reduce the risk of babesiosis in livestock and its potential public health implications. Further studies are required to clarify the pathogenicity of *B. motasi* in humans and to develop appropriate control strategies in livestock production systems.

Babesiosis prevention can be supported through regular acaricide application, barn sanitation, adequate nutrition, and routine veterinary monitoring to reduce tick populations. Tick control strategies, including acaricides and tick vaccines, have been successfully applied in other regions such as Australia [15]. This study was limited by a small sample size (n = 35) and the detection of only one positive case, as well as potential human error during microscopic examination. Therefore, further studies involving larger sample sizes and wider geographic coverage are needed to better understand the occurrence of *Babesia motasi* and to support effective control strategies.

4 Conclusion

This study examined 35 sheep from smallholder farms in Sleman Regency and identified one sample positive for *Babesia* sp. with the characteristic pear-shaped morphology. Microscopic and molecular analyses showed a prevalence of 3% and a mild parasitemia level of 1.2%. Sequencing and phylogenetic analysis revealed that the detected *Babesia* isolate was closely related to *Babesia motasi* from Japan (accession no. LC811651.1) with 97.39% identity. These findings indicate the presence of *B. motasi* infection among local sheep, highlighting the need for tick vector control, improved farm management, and preventive measures to minimize transmission. Further research and surveillance are recommended to confirm parasite distribution and prevent its spread in the region.

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