

First metagenome report of *Haemaphysalis bispinosa* ticks of Moa buffalo from Southwest Maluku District, Indonesia

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Abstract. Utami P, Kunda RM, Anaktototy Y. 2024. First metagenome report of *Haemaphysalis bispinosa* ticks of Moa buffalo from Southwest Maluku District, Indonesia. *Nusantara Bioscience* 16: 130-138. Ticks are vectors of pathogenic organisms such as bacteria, protozoa, and viruses, which are potentially fatal to humans and livestock. *Haemaphysalis bispinosa* Neumann, 1897 is a tick species with three mammalian hosts in Asia and Australia, including Indonesia, with the highest infestation in cattle (*Bos taurus* Linnaeus, 1758) and sheep (*Ovis aries* Linnaeus, 1758). *H. bispinosa* is known to transmit many pathogens, but studies on the profile and structure of the microbiota are still very limited. This study aims to investigate the abundance and diversity of microbiota in *H. bispinosa* to evaluate the bacterial community's structure and to identify and examine potential zoonotic agents from Moa buffalo (*Bubalus bubalis* (Linnaeus, 1758)). Amplification in this study used primers from the region (V1-V9) of the 16S rRNA gene. Metagenomic analysis shows that the microbiota community structure is dominated by non-zoonotic bacteria (96.83%), and zoonotic bacteria are found in the percentage (3.17%). Zoonotic agents were dominated by members of the genus *Rickettsia* (71.82%), *Ehrlichia* (19.19%), *Romboutsia* (3.16%), *Anaplasma* (2.43%), *Coxiella* (2.24%), *Staphylococcus* (0, 48%) and *Streptococcus* (0.43%). Overall, 16 species were found in *H. bispinosa*, i.e., 11 species belonging to the genus *Rickettsia*, 2 species from the genus *Ehrlichia* (i.e., *E. canis* and *E. minasensis*), and 1 species each belonging to the genera *Anaplasma*, *Coxiella*, and *Romboutsia*. It was concluded that the abundance of the microbial community in *H. bispinosa* based on metagenome analysis using the 16S rRNA gene from Moa buffalo in the Southwest Maluku District was classified as having a diverse abundance of species.

Keywords: *Haemaphysalis bispinosa*, microbiome, Moa buffalo, tick, vector-borne disease

INTRODUCTION

Ticks are vectors for pathogenic organisms such as bacteria, protozoa, and viruses that cause great suffering and potentially fatal human diseases (Khoo et al. 2016; Utami et al. 2021). Ticks also cause considerable losses to the livestock industry as they cause skin irritation and blood loss to the host (Muhammad et al. 2021). This reduces the elasticity of the skin epidermis and, at the same time, acts as a vector for many pathogens (Roy et al. 2018; Kim et al. 2021). *Haemaphysalis bispinosa* Neumann, 1897 is a tick species with three mammalian hosts found on the continents of Asia and Australia, with the highest infestation in cattle (*Bos taurus* Linnaeus, 1758) and sheep (*Ovis aries* Linnaeus, 1758). Petney et al. (2019) reported that in South Asia, there are about 97 tick species dominated by members of *Haemaphysalis*. Several field studies have shown that members of *Haemaphysalis* are commonly found on cattle (*B. taurus*) (Sahara et al. 2023) and recently reported on Moa buffalo (*Bubalus bubalis* (Linnaeus, 1758)), Southwest Maluku District (Utami and Kunda 2023). *Haemaphysalis* is the second largest genus of the Ixodidae family, distributed in Australia, China, Indonesia, Japan, Malaysia, Nepal, New Zealand, Pakistan, Sri Lanka, Thailand, Myanmar, and Vietnam (Sahara et al.

2019; Utami and Kunda 2023). Sahara et al. (2019) reported that *H. bispinosa* were mostly reported in Java Island, while in other parts of Indonesia, they were rarely reported, including buffalo.

Several studies reported that tick species that most frequently infest Asian buffalo in Pakistan, i.e., *Rhipicephalus* (*Boophilus*) *microplus*, *R. turanicus*, *R. haemaphysaloides*, *R. annulatus*, *R. sanguineus* (Sensu Lato), *Hyalomma anatolicum*, *H. hussaini*, *H. isaaci*, *H. scupense*, *H. dromedarii*, *H. bispinosa*, *H. montgomeryi*, *H. cornupunctata*, *H. kashmirensis*, and *H. sulcata* (Karim et al. 2017; Ali et al. 2019; Rehman et al. 2019; Ghafar et al. 2020; Aiman et al. 2022). Corrêa et al. (2012) found 19 species of ticks that are parasitic on buffalo in India, i.e., *Amblyomma testudinarium*, *Nosomma monstrosus*, five species from the genus *Hyalomma*, nine species from the genus *Haemaphysalis* and three species from the genus *Rhipicephalus*.

Pathogenic bacteria transmitted by members of *Haemaphysalis* spp. were found in 946 bacterial genera with the highest abundance, i.e., *Lactobacillus*, *Coxiella*, *Rickettsia*, and *Muribaculum*. Moreover, *Rickettsia rickettsii*, *Rickettsia japonica*, *Candidatus Rickettsia jingxinensis*, *Anaplasma bovis*, *Ehrlichia ewingii*, *Ehrlichia chaffeensis*, *Coxiella* spp. and *Coxiella*-like endosymbiont

were detected in *Haemaphysalis* spp. (Zhao et al. 2021; Zeng et al. 2022a). These pathogens cause diseases, i.e., Rocky Mountain spotted fever, Siberian or North Asian typhus, Japanese spotted fever, and Australian spotted fever, human monocytic ehrlichiosis and canine ehrlichiosis; human granulocytic anaplasmosis, and bovine anaplasmosis; tularemia; Q fever, rabbit fever, Taylor disease, Crimean Congo hemorrhagic fever, and Lyme disease and tick-borne relapsing fever (Wu et al. 2013; Fang et al. 2015; Zhao et al. 2021). Besides carrying various disease-causing pathogenic bacteria, members of *Haemaphysalis* spp. also transmit several pathogenic parasites, i.e., *Theileria* spp, *Babesia* spp, and *Hepatozoon* spp. that cause tropical theileriosis, babesiosis in cattle, dogs, and sheep; and hepatozoonosis (Chen et al. 2019).

Currently, research results on the microbiome in Indonesia are rarely reported from various samples, including from ticks, even though the potential for ticks as a vector for transmitting various zoonotic agents in humans is very high (Levytska and Mushynskiy 2020). Although it is known that *H. bispinosa* ticks play an important role as pathogen vectors of various microbes, there is very little information about the profile and composition of the microbiota contained in these ticks. Studying the profile and composition of the microbiota found in *H. bispinosa* is interesting. In this study, we used high-throughput sequencing of the intact regions V1-V9 of the 16S rRNA gene to investigate the abundance and diversity of microbiome in *H. bispinosa* to evaluate the structure of the bacterial community to the identification and potential discovery of zoonotic pathogenic bacteria in *Moa buffalo*. This metagenomic data will be very helpful in mapping and tracing the potential pathology that these zoonotic agents will cause.

MATERIALS AND METHODS

Ethical approval

This study received approval from the Animal Ethics Committee at the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia, following the procedures for using animal models for research purposes.

Study area

Tick specimens were collected from 50 local *Moa buffalo* (20 male and 30 female) in four different locations, i.e., Werwaru, Tounwawan, Klis, and Moain villages of Southwest Maluku District, Maluku Province, Indonesia (Figure 1). The buffalo were selected based on age and categorized as adults (3-4 years), which were found in local farmers. The sampling process was carried out from March until October 2023. Tick collection activities are carried out in the morning (07.00-08.30 WIT) and afternoon (17.30-18.30 WIT) following the time buffalo entered and left *Lutur* (stone cage). Farmers assist the sampling process because it avoids aggressive activities from buffalo.

Procedures

Sample collection, DNA preparation and extraction

A total of 85 *Moa buffalo* ticks (52 females and 33 males) were collected between March, May, and October 2023 (3 seasons of tick activity) using the flagging method in the afternoon during summer. Each tick was preserved in 70% alcohol solution in the Eppendorf tube, and samples were brought to the laboratory under cold conditions. The humidity and air temperature were measured in the morning and afternoon by using the temperature and humidity meter HTC-2. The ground surface was measured using GPS Garmin 11. Each tick was examined with a stereomicroscope (Olympus, Germany) using a dichotomous key and character matrix containing growth phase and sex by Anastos (1950).

Pre-PCR (Polymerase chain reaction) and PCR procedures are prepared with sterile equipment and sterile laboratory to avoid cross-contamination. Ticks were cleaned up for one minute in 70% ethanol to remove all microorganisms from the tick body surface. Then, the next process is homogenization by grinding each tick with phosphate-buffered PBS saline (without Ca²⁺ and Mg²⁺, pH=7.4). In the next step, samples were centrifuged, and the supernatant (300 µL) was used for DNA extraction. DNA Extraction was done with a spin column kit (EURx, Poland), according to the manufacturer's protocol with modifications. The quality and quantity of DNA were checked using a WPA UV1101 spectrophotometer (WPA The Old Station, Linton, Cambridge, UK) to ensure the presence of a minimum standard DNA concentration (10 ng/µL DNA). The further step was the DNA extract sample (100 µL) has been stored at -20°C.

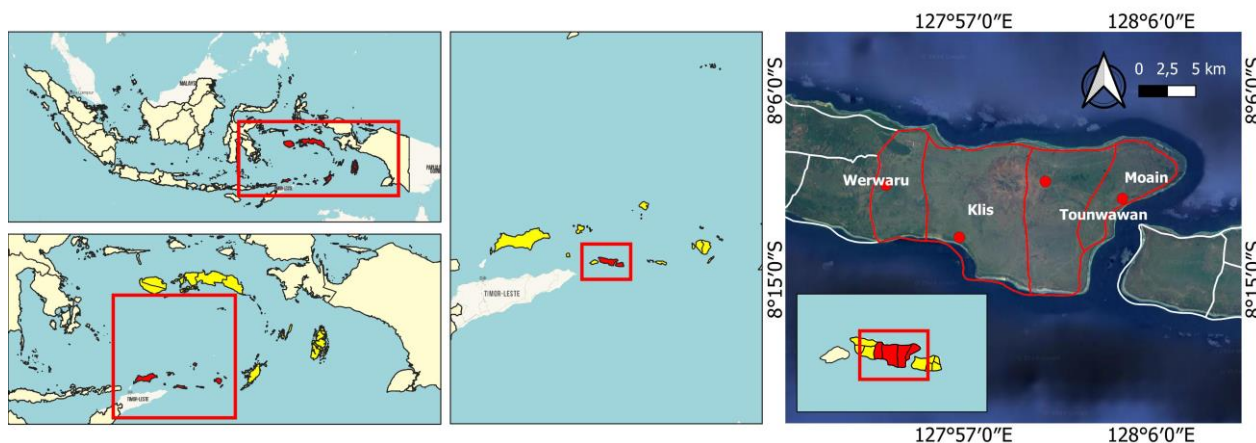


Figure 1. Map of tick sample collection in Southwest Maluku District, Maluku Province, Indonesia

Molecular analysis (PCR) for metagenomics uses 16S rRNA

Amplification of the 16S rRNA gene sequence was performed with Start-Warm HS-PCR Mix (A&A Biotechnology, Gdynia, Poland), ddWater (aseptic, free from nuclease-free water). The primer sequence used for amplifying the 16S rRNA gene is based on primer sequence 16S, full length (Nanopore). The primer sequence is the most suitable primer pair for NGS (27F: 5'-AGA GTT TGA TCM TGG CTC AG -3'; 1492R: 5'-GGT TAC CTT GTT ACG ACT T- 3'). The PCR protocol used in NGS analysis is as follows: pre-denaturation 95°C for 3 minutes, denaturation 25 x (95°C for 30 seconds, annealing 55°C for 30 seconds, elongation 72°C for 30 seconds), and post elongation 72°C for 5 minutes), then electrophoresed in a 2% agarose gel (Sigma-Aldrich, Germany) stained by using Midori Green (Nippon Genetics Europe GmbH, Germany) with electrophoresis at 90 volts for 45 minutes. The results of PCR amplicons were visualized using UV light in a 100 Gel Logic System (Kodak Imaging System, Inc., USA). The amplification product, which is 1500 bp in size, was selected for further metagenomic investigation (Nanopore MySeq).

NGS-metagenomic library preparation and sequencing

The NGS process was followed by using the Nanopore 16S metagenomic protocol (Nanopore MySeq, Inc., San Diego, California, USA). A DNA paired with the end library was created with an insert size (\pm 1500 bp) using a series primer for the variables V1 to V9 in the 16S rRNA region. The quantity and quality of the metagenomic libraries were evaluated by electrophoresis on 2200 Agilent TapeStation Instrument with Genomic DNA ScreenTape Assay (Agilent Technologies Inc., St Clara, CA, USA). Samples were pooled in equal proportions and sequenced for 600 cycles using the MiSeq Platform (Macrogen, Seoul, Korea) with v3 reagent (2 x 300 bp paired-end reads). 10% PhiX bacterial meta biome DNA was added to the sample as an internal control. Paired-end reads, recorded in FASTQ format. The FASTQ data was automatically demultiplexed and Macrogen conducted Nanopore adapters.

Data analysis

Data were analyzed using QIIME with high-throughput community sequencing data (Caparaso et al. 2010). The obtained Nanopore MySeq 16S rRNA sequences were clustered at 97% sequence similarity and analyzed with the Quantitative Insights into Microbial Ecology 2 (QIIME2) software package version 3.5.3 (Swei and Kwan 2017). The zoonotic profile was classified according to the etiological agent and divided into zoonotic and non-zoonotic microbe groups (Rahman et al. 2020).

RESULTS AND DISCUSSION

Metagenomic profile of *H. bispinosa* tick

A total of 85 adult *H. bispinosa* ticks were found in four villages, i.e., Werwaru (n=21), Tounwawan (n=22), Klis

(n=21), and Moain (n=21) individuals. Molecular analysis showed that the relative abundance of the *Rickettsia* group reached \geq 50% (very high) (Figure 4), which infested the bodies of *H. bispinosa* ticks. The *Paenibacillus* group has a lower relative abundance percentage than *Rickettsia*, i.e. \leq 10%. The *Ehrlichia* and other microbial groups have a relatively small abundance percentage \geq 1% (Figure 2).

Heatmap analysis

A total of 34 species of microbes were found in the results of this study based on heatmap analysis (Figure 3). Based on the heatmap results, three categories of microbial groups were found with high, medium and low abundance (Table 1). The abundance of microbial species that are categorized as high has a heatmap score $>$ 15. The results of the heatmap analysis show that there are two microbes with a high heatmap category, i.e., *Ehrlichia minasensis* and *Rickettsia prowazekii*. The abundance of medium-category microbial species was found in species with a score of 5-10, i.e. *Coxiella burnetii*, *Ehrlichia canis*, *Rickettsia akari*, *Rickettsia australis*, *Rickettsia conorii*, *Rickettsia hoogstraalli*, *Rickettsia hulinii*, *Rickettsia tamurae*, and *Romboutsia timonensis*. The low scores (\leq 5) microbial groups were *Bacillus anthracis*, *Bacillus capparidis*, *Bacillus cytotoxicus*, *Bacillus licheniformis*, *Bacillus marcorestrictum*, *Bacillus oleivorans*, *Bacillus pseudoflexus*, *Bacillus pseudomycoides*, *Bacillus rhizoplanae*, *Bacillus tianmuensis*, *Bacillus laterosporus*, *Rickettsia asiatica*, *Rickettsia monacensis*, and *Rickettsia slovacica* (Figure 3; Table 1).

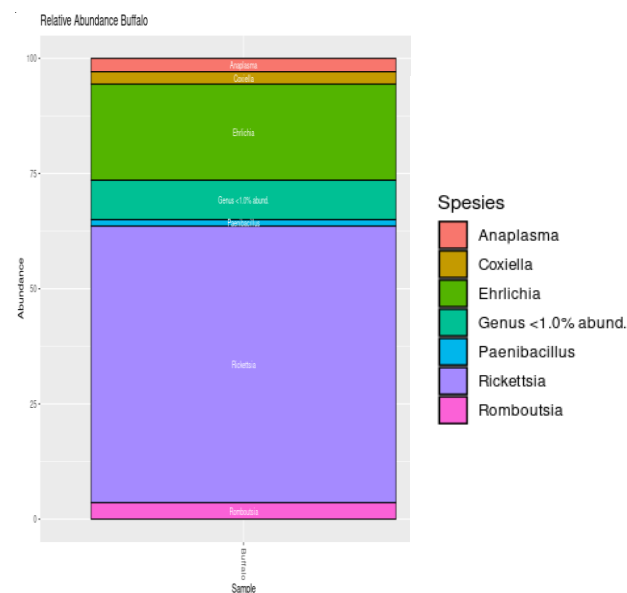


Figure 2. Relative abundance of potential pathogens at genus level

Table 1. Microbiome communities found in *H. bispinosa* (General explanation of microbiome characters and heatmap scoring)

Species name	Microbiome characters	Heatmap analysis range based on present study
<i>Anaplasma phagocytophilum</i> (Dixon and Bedenice 2019)	Human granulocytic anaplasmosis, tick-borne fever, equine ehrlichiosis.	< 5
<i>Bacillus anthracis</i> (Carlson et al. 2019)	Cutaneous, Inhalation, Gastrointestinal disease in animal and human	< 5
<i>Bacillus capparidis</i> (Wang et al. 2017)	Isolated from the surface-sterilized roots of a medicinal plant	< 5
<i>Bacillus cytotoxicus</i> (Cairo et al. 2021)	Member of the <i>Bacillus cereus</i> group with bacterial abilities to grow in high temperatures (> 52°C)	< 5
<i>Bacillus licheniformis</i> (Zeng et al. 2022b)	Found in the soil, on bird feathers, especially chest and back plumage, and most often in ground-dwelling birds and aquatic animal	< 5
<i>Bacillus marcorestinum</i> (Zhong et al. 2022)	A Gram-positive, facultatively anaerobic, endospore-forming, and rod-shaped bacterium was found in soil.	< 5
<i>Bacillus oleivorans</i> (Azmatunnisa et al. 2015)	Two Gram-stain-positive, diesel oil-degrading, solvent-tolerant, aerobic, endospore-forming, rod-shaped bacteria were isolated from a contaminated laboratory plate.	< 5
<i>Bacillus pseudoflexus</i> (Chandna et al. 2016)	A Gram-stain-positive, motile, rod-shaped, endospore-forming moderately halophilic bacterium was isolated from compost.	< 5
<i>Bacillus pseudomycooides</i> (Elsharawy et al. 2023)	Members of the <i>Bacillus cereus</i> group species include <i>B. cereus</i> , <i>B. anthracis</i> , <i>B. thuringiensis</i> , <i>B. mycooides</i> , <i>B. pseudomycooides</i> , and <i>B. eihenstephanensis</i>	< 5
<i>Bacillus rhizoplanae</i> (Kampfer et al. 2022)	Isolated from the wheat rhizoplane, including aquatic bacteria.	< 5
<i>Bacillus tianmuensis</i> (Théâtre et al. 2021)	Isolated from a soil sample, a gram-negative, endospore-forming, rod-shaped strain	< 5
<i>Brevibacillus laterosporus</i> (Ruiu 2013)	A unique canoe-shaped lamellar body attached to one side of the spore is a natural inhabitant of water, soil, and insects.	< 5
<i>Coxiella burnetii</i> (Mobarez et al. 2014)	An obligate intracellular, pleomorphic gram-negative rod-shaped bacteria that causes Q fever	5-10
<i>Ehrlichia canis</i> (Hmoon et al. 2021)	An obligate intracellular bacterium that acts as the causative agent of ehrlichiosis	5-10
<i>Ehrlichia minasensis</i> (Moura et al. 2019)	Tick-borne obligate intracellular gram-negative alphaproteobacteria of the family Anaplasmataceae	>15
<i>Rickettsia akari</i> (Szakacs et al. 2020)	An intracellular, gram-negative pathogen is the etiologic agent of rickettsialpox.	5-10
<i>Rickettsia asiatica</i> (Thu et al. 2019)	A tick-borne pathogenic species borne by <i>Ixodes ovatus</i>	< 5
<i>Rickettsia australis</i> (Stewart et al. 2017)	The etiologic agent of Queensland tick typhus (QTT)	5-10
<i>Rickettsia conorii</i> (Kamani et al. 2017)	Group of endotheliotropic infectious diseases caused by different species of genera <i>Rickettsia</i>	5-10
<i>Rickettsia montanensis</i> (Snellgrove et al. 2021)	Members of the genera <i>Rickettsia</i> range from nonpathogenic endosymbionts	< 5
<i>Rickettsia honei</i> (Parte et al. 2020)	A unique spotted fever group (SFG) agent that is pathogenic for humans	5-10
<i>Rickettsia hoogstraalli</i> (Reeves et al. 2020)	Substantial risks to both human and animal well-being	5-10
<i>Rickettsia hulinii</i>	A rickettsia pathogenic in humans	5-10
<i>Rickettsia monacensis</i> (Burkhardt et al. 2022)	Arthropod-associated gram-negative prokaryotes that reside within the cytoplasm and sometimes nuclei of eukaryotic host cells	< 5
<i>Rickettsia parkeri</i> (Lackman et al. 1965)	<i>R. parkeri</i> is closely related to <i>R. rickettsii</i> , the causative agent of Rocky Mountain spotted fever (RMSF)	< 5
<i>Rickettsia prowazekii</i> (Khan et al. 2023)	An obligate, intracellular, gram-negative coccobacillus belonging to the genera <i>Rickettsia</i>	>15
<i>Rickettsia rhipicephali</i> (ex Burgdorfer et al. 1978) Weiss and Moulder 1988	Obligate intracellular bacteria belonging to the spotted fever group of the genera <i>Rickettsia</i>	< 5
<i>Rickettsia rickettsii</i> (Brumpt 1922)	Rocky Mountain spotted fever (RMSF) is an acute febrile tick-borne illness caused by <i>Rickettsia rickettsii</i>	< 5
<i>Rickettsia sibirica</i> (Li et al. 2017)	The causative agent of Siberian tick typhus	< 5
<i>Rickettsia slovaca</i> (Sekeyová et al. 1998)	A pathogenic, tick-borne, spotted fever group (SFG) rickettsiae.	< 5
<i>Rickettsia tamurae</i> (Seo et al. 2021)	Registered spotted fever group rickettsiae.	5-10
<i>Rickettsia tillamookenis</i> (Gauthier et al. 2021)	Recognized typhus and spotted fever group <i>Rickettsia</i> species.	< 5
<i>Romboutsia timonensis</i> (Ricaboni et al. 2016)	A new bacterium isolated from the right human colon	5-10

Note: Heatmap score < 5: Low, 5-10: Moderate, > 15: High

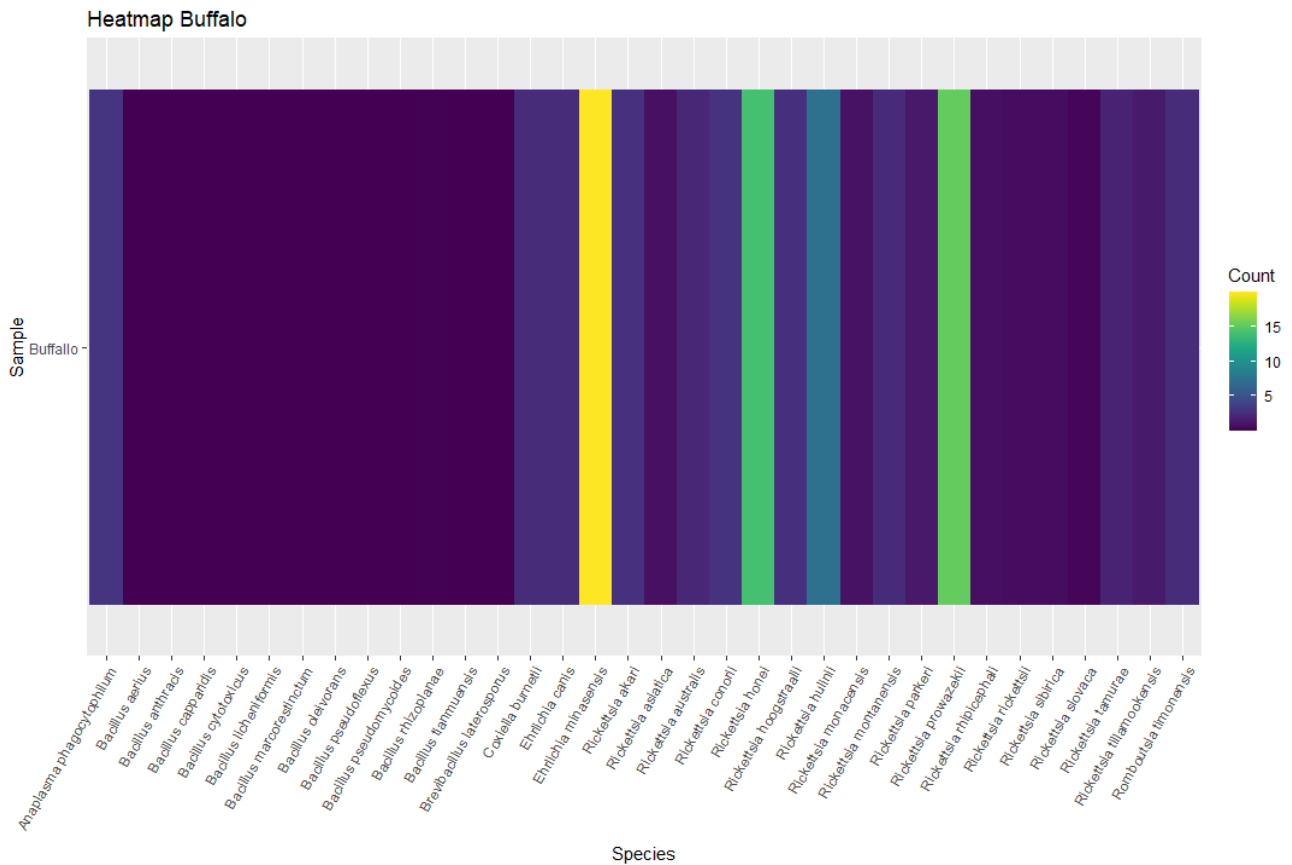


Figure 3. Heatmap of bacterial meta biome at the species level in Moa buffalo tick samples

Zoonotic profile in *H. bispinosa* tick

Analysis of zoonotic potential using the 16S rRNA gene shows that the highest percentage is found in the group of non-zoonotic bacteria. The group of bacteria classified as non-zoonotic reached 96.83%, while the group of zoonotic bacteria was 3.17% (Figure 5). Non-zoonotic microbial populations are quite high compared to the zoonotic microbial group. Zoonotic microbial population figures are dominated by the genus *Rickettsia* (71.82%), *Ehrlichia* (19.19%), *Romboutsia* (3.17%), *Anaplasma* (2.43%), *Coxiella* (2.24%), *Staphylococcus* (0.48%) and *Streptococcus* (0.43%) (Figure 6). The results of this study indicate that members of the genus *Rickettsia* are the most dominant group of microbes found in *H. bispinosa* and have a high abundance.

Discussion

Utami and Kunda (2023) reported that the ticks found on Moa buffalo were dominated by the species *Dermacentor (Indocentor) auratus* (Supino, 1897) and *H. bispinosa* (Neumann, 1897). Research conducted by Jiao et al. (2021) shows that the genera *Rickettsia*, *Anaplasma*, and *Coxiella* are most commonly found compared to the species *Dermacentor nuttalli* and *Ixodes persulcatus*, which attack the bodies of cattle in Mongolia. These scientific findings are a preliminary study of metagenome community analysis of ticks that attack livestock in ecosystems with limited rainfall and green food sources,

such as in the Moa buffalo habitat, and the sample identification in this study found only *H. bispinosa* ticks and no *D. auratus*. It is strongly suspected that the sampling period in the field was during the life cycle phase of *H. bispinosa*, so this species was often found.

The results of analysis at the species level showed that around 16 species of bacteria were found in Moa buffalo ticks, including 11 species from the genus *Rickettsia*, 2 species from the genus *Ehrlichia* (i.e., *E. canis* and *E. minasensis*), 1 species from the genus *Anaplasma* (*Anaplasma phagocytophilum*), 1 species belonging to the genus *Coxiella* (*C. burnetii*), and 1 species of the genus *Romboutsia* (*R. timonensis*). The relative abundance of *E. minasensis* species in Moa buffalo ticks indicates a high population. The relative abundance for the three species (*Rickettsia hoonei*, *R. hulinii*, and *R. prowazekii*) shows that the population figures for these three microbes are quite high after *E. minasensis* (Figure 4). The microbiota group that infests *H. bispinosa* ticks consists of various microbial genera and is non-zoonotic, while zoonotic ones are found in small numbers. Relative abundance values that vary in each genus and species of microorganisms are caused by climate and environmental factors, as well as interaction patterns of microbes with the environment (Cabezas-Cruz 2021).

Metagenome-based molecular analysis of the zoonotic potential of microbial communities will provide comprehensive basic data regarding the profile of the tick

H. bispinosa (Neumann 1897) as an ectoparasite of the Moa buffalo. The results obtained from this research are very useful for analyzing and investigating the potential of the Moa buffalo tick as a vector for carrying zoonotic agents. In addition, precautions need to be taken to identify potential risks of cross-infection. This cross-infection occurs from *Coxiella*, *Rickettsia*, *Anaplasma*, *Staphylococcus*, and *Streptococcus* members. The zoonotic agents can spread from ticks to humans or other animals. Molecular approaches to the microbial ecology of livestock origin provide a broad perspective on the application of epidemiological science (Riley and Blanton 2018). Metagenome analysis of the Moa buffalo tick identified it at the species level. The results of this research are very useful in studying epidemiological approaches, especially strategies for controlling microbial manifestations carried by Moa buffalo ticks (*H. bispinosa*). The results of metagenome analysis have identified the bacterial species *A. phagocytophilum* as a pathogen that weakens host cells immune system, especially the antimicrobial mechanism of neutrophils (Rikihisa 2011), granulocytic anaplasmosis fever in humans, and ehrlichiosis in horses (*Equus caballus* Linnaeus, 1758). The presence of *Bacillus* groups, including *Bacillus aerius*, *B. anthracis*, *B. capparidis*, *B. cytotoxicus*, *B. licheniformis*, *B. marcorestinum*, *B. oleivorans*, *B. pseudoflexus*, *B. pseudomycoides*, *B. rhizoplanae*, and *B. tianmuensis* were classified into metabolically diverse groups. The *B. licheniformis* is a hematogenous bacterium that infects after entering the tick body from the digestive tract (Ramirez-Olea et al. 2022). The mechanism for microbial entry through the tick body starts from the opening of the mouth, which is accompanied by bleed feeding into the midgut. The virus or bacteria multiply and move to the ovaries and salivary glands and settle at the anus opening (Maqbool et al. 2022).

The *Bacillus* genus member in this study's results is *B. anthracis*. The results of this study show that the *B. anthracis* species can be found in *H. bispinosa*. Generally, *B. anthracis* is a pathogenic microbe that can damage the skin or enter through the mucosa (gastrointestinal anthrax) in livestock. The *B. anthracis* can carry out extracellular multiplication with simultaneous production of capsules and toxins (Spencer 2003). Generally, buffalo is one of the opportunistic hosts of *B. anthracis* (Bakhteeva and Timofeev 2022). There has never been any reported incidence of anthrax in Moa buffalo in Southwest Maluku District and in Maluku Province at large, but the results of metagenomic analysis prove the presence of *B. anthracis*, which was detected in *H. bispinosa*.

Members of *Bacillus* spp. i.e., *Bacillus cereus*, *Bacillus subtilis*, and *B. licheniformis* are associated with septicemia, endocarditis, meningitis, and infections of wounds, ears, eyes, respiratory tract, urinary tract, and gastrointestinal tract. *B. cereus* can also cause two different food poisoning syndromes, including vomiting syndrome with rapid symptoms characterized by nausea and vomiting and diarrhea syndrome with slow symptoms (Senesi and Ghelardi 2010).

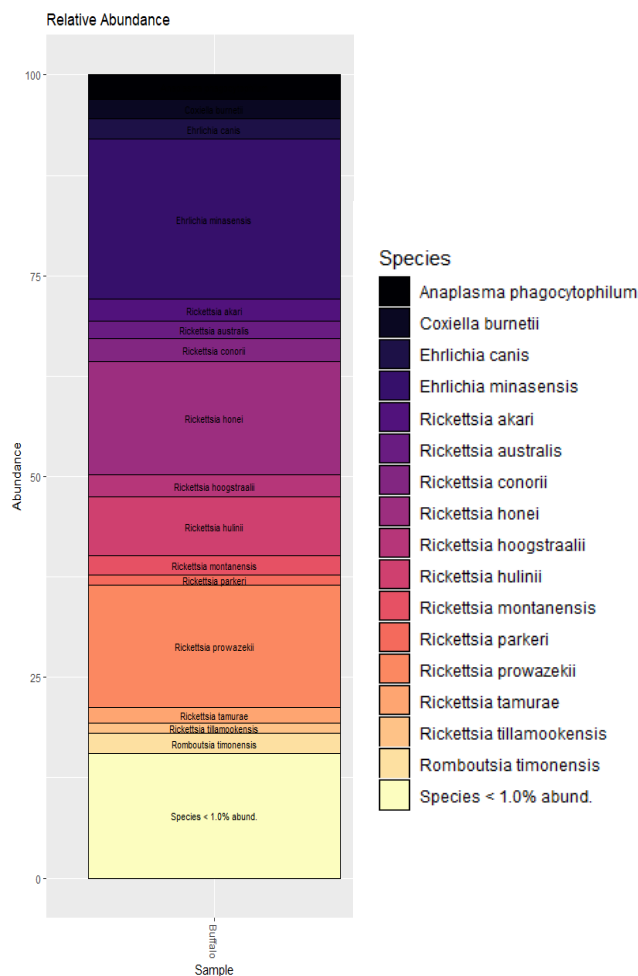


Figure 4. Relative abundance of meta biome bacteria at the species level

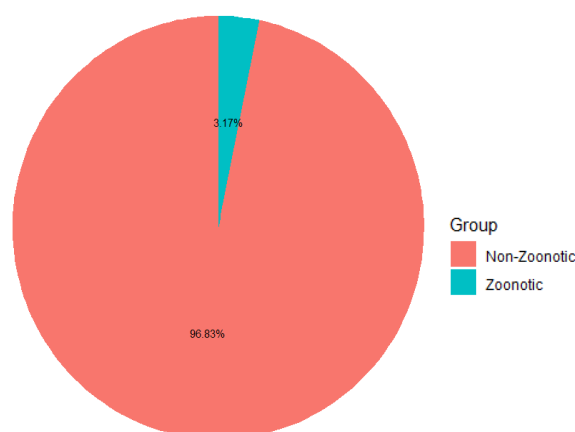


Figure 5. Percentage of the microbiome population zoonotic and non-zoonotic

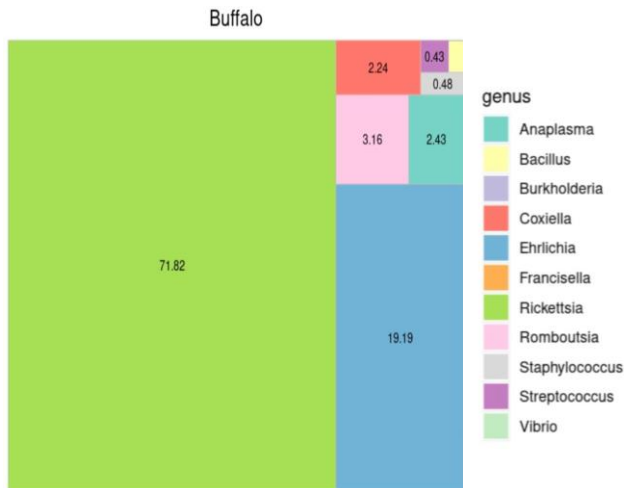


Figure 6. Profile of zoonotic agents of *H. bispinosa*

The *R. prowazekii* and *E. minasensis* are 2 species found in high percentages of *H. bispinosa* from buffalo farms on Moa Island, Southwest Maluku District. The species *E. minasensis* is a new pathogen that causes fever, lethargy, thrombocytopenia, and depression in cattle (Aguilar et al. 2014), while little is known about the biology of the rickettsial disease vector. Research by Kazimirova and Stibraniova (2013) reported that 100% of ticks infected with *R. prowazekii* will die from rickettsiae. The bacterial species *R. rickettsii* was found in less than 0.1%, with the dominant vector found in the tick *Dermacentor variabilis* compared to *Rickettsia africae* and *Rickettsia amblyommatis* (Kazimirova and Stibraniova 2013).

This study revealed that *H. bispinosa*'s role in microbial transmission is very important. Pathogen-host interactions are established through coevolution of microbes with arthropods. These interactions also include the process of pathogen replication, maintenance of persistent infections, and cross-transmission (Ravindran et al. 2023). It has been well documented that several species of ticks belonging to the Ixodidae act as vectors for carrying pathogenic agents, i.e., *Rickettsia* spp., *Anaplasma* spp., and *Ehrlichia* spp. (Barbieri et al. 2023).

The microbial groups Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria are the three groups of bacteria that most often live on the bodies of *Haemaphysalis* spp. ticks (Zeng et al. 2022a). It was found in another study on species related to the tick *H. bispinosa*, including *Haemaphysalis longicornis* and *Haemaphysalis flava*, which are living hosts for 946 genera of microbes (Zeng et al. 2022a). High abundance was found in the genera *Lactobacillus*, *Coxiella*, *Rickettsia* and *Muribaculaceae* (Zeng et al. 2022a). Other species not found in this study but found in *Haemaphysalis* spp. from Shanghai, i.e., *Rickettsia japonica*, *Candidatus Rickettsia jingxinensis*, *Anaplasma bovis*, *Ehrlichia ewingii*, *Ehrlichia chaffeensis*, and *Coxiella*-like endosymbionts (Zeng et al. 2022a).

The results of this study show the high diversity of rickettsial organisms in *H. bispinosa*. They are the first report on metagenomic data on *H. bispinosa* in Moa

buffalo from the Southwest Maluku District. This research contributes to a better understanding of the distribution of microbes of the genus *Rickettsia*, *Ehrlichia*, *Anaplasma*, *Coxiella*, and *Romboutsia* in Moa buffalo. The results of this study have implications for scientific aspects, i.e., knowing the profile of the microbiota community in Moa buffalo ticks, as well as the division of zoonotic and non-zoonotic bacterial groups as a scientific reference in anticipating zoonotic events in buffalo. In addition, this metagenomic analysis produced a specific molecular database for microbiota collected from ticks that had never been reported before.

In conclusion, the microbial community structure in *H. bispinosa* from Moa buffalo is dominated by non-zoonotic (96.83%) and zoonotic (3.17%) microbes. Zoonotic microbes are dominated by members of the genus *Rickettsia* (71.82%), *Ehrlichia* (19.19%), *Romboutsia* (3.16%), *Anaplasma* (2.43%), *Coxiella* (2.24%), *Staphylococcus* (0.48%) and *Streptococcus* (0.43%) respectively. Overall, 16 species of microbes were found on *H. bispinosa*, i.e., 11 species belonging to the genus *Rickettsia*, 2 species from the genus *Ehrlichia* (i.e., *E. canis* and *E. minasensis*), and 1 species each belonging to the genus *Anaplasma*, *Coxiella*, and *Romboutsia*. It was concluded that the abundance of the microbial community in *H. bispinosa* based on metagenome analysis using the 16S rRNA gene from Moa buffalo in the Southwest Maluku District was reported.

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