[biodiv] New notification from Biodiversitas Journal of Biological Diversity

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DEWI NUR PRAT... 2/12/2022

kepada saya

You have a new notification from Biodiversitas Journal of Biological Diversity:

You have been added to a discussion titled "Uncorrected Proof" regarding the submission "Molecular Detection Of Pathogenic Bacteria In Rhipicephalus sanguineus sensu lato Ticks From Bitung, North Sulawesi, Indonesia.".

Link: https://smujo.id/biodiv/authorDashboard/submission/11776

Ahmad Dwi Setyawan

Biodiversitas Journal of Biological Diversity

Smujo Editors 1/11/2022
kepada saya

Jane Tahulending:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Molecular Detection Of Pathogenic Bacteria In Rhipicephalus sanguineus sensu lato Ticks From Bitung, North Sulawesi, Indonesia.".

Our decision is: Revisions Required

Reviewer A:

Dear Author,

The manuscript "Molecular detection of pathogenic bacteria in *Rhipicephalus sanguineus* sensu latoticks from Bitung, North Sulawesi, Indonesia" has been revised but still there are few corrections

Major comments

- I have corrected the manuscript and give some comments for correction as sticky notes
- In text author have mentioned 27F. Check The table 1 may be deleted and Only citation may be given in text where the primers have been given
- No need of Table 2 . All details are mentioned in manuscript already

26 Agustus 2022

Reviewer A:

Dear Author,

The manuscript "Molecular detection of pathogenic bacteria in *Rhipicephalus sanguineus* sensu latoticks from Bitung, North Sulawesi, Indonesia" needs revision for English language and comments mentioned below

Major comments

- Revise the manuscript for the English
- Check the sentences for the correctness
- For citation of Table and Figure……Give as (Table x) or (Figure x) at end of the sentences
- Scientific words should be italic.
- Don't start the sentence with preposition words.
- The "Citations" and "References" should be as per the Instruction of the journal.
- At least 80% of references must be from scientific journals published in the last 10 years (2012-2022).
- Add more relevant and recent references to the manuscript. See the related published article in this journal as discussed with the present investigation
- The revised manuscript should be shown as changes in form of highlights the modification with another color (Red color font with yellow highlight)

19 Oktober 2022

Major comments

- I have corrected the manuscript as "Track change" and given some suggestions as "Sticky note" for revision of manuscript for further consideration.
- Revise the manuscript for the English.....Check the sentences for the correctness
- Heading and sub-heading in <Materials and Methods> and <Results and Discussion> section are not clear. .....Heading in <Materials and Methods> should be there in "Result Section" almost
- There should not be heading in "Discussion" Section
- Author have presented most of results in "Discussion" section.....Revise it carefully
- I have corrected 5 references. Please revise remaining. Reference should be as per journal. See the journal guidelines. Complete in all form. Each reference with DOI. Scientific name should be italic. Names of journals should be abbreviated according to the ISSN List of Title Word Abbreviations (www.issn.org/2-22661-LTWA-online.php).

31 Oktober 2022

Major comments

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Molecular detection of pathogenic bacteria in *Rhipicephalussanguineus* (sensulato) ticks from Bitung, North Sulawesi, Indonesia.

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Abstract.*Tahulending J, Tulung M, Pelealu J, Doda DV. Molecular detection of pathogenic bacteria inRhipicephalussanguineus(sensulato) ticks from Bitung, North Sulawesi, Indonesia.* *Rhipicephalussanguineus*is a vector of manypathogens caused diseases associated with the species of ticks that may feed on domestic animals and humans.This study aims to investigate bacteria in the digestive tract of ticks using the metabarcode approach. The DNA barcode region used in this approach was V3-V4 which is a hypervariableregion of 16S rRNA. *R. sanguineus* (sensulato) ticks has been taken from dogs inMatuari, Girian and Aertembaga, Bitung, North Sulawesi, Indonesia. There were 9 species bacterial identified from the digestive tract of ticks.The most detected pathogens were *Pseudomonas stutzeri* (35%), *Brevundimonas*sp. (21%), and *Aquamicrobiumlusatien*(17%).These results extend our knowledge of the potential pathogenic bacteria affecting human and animal health in the future.

Keywords: Bacteria, digestive tract, *Rhipicephalussanguineus*(sensulato), ticks, V3-V4 region, 16S rRNA

Abbreviations:

Running title:

Commented [Ajar Nath1]: Abstract should be (± 200 words)

Commented [Ajar Nath2]: Keywords is about five words, covering scientific and local name (if any), research theme, and special methods which used; and sorted from A to Z.

Commented [Ajar Nath3]: **Abbreviations** (if any): All important abbreviations must be defined at their first mention there. Ensure consistency of abbreviations throughout the article.

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INTRODUCTION

R. sanguineus (sensulato) was reported in Indonesia as a common parasite of dogs (Hadi et al. 2016). Dogs have been in close contact with humans for a long time; thus, humans are at risk particularly for zoonotic tick-borne pathogens (TBPs) (Corales et al. 2014). The tropical climate of Southeast Asian countries, which include Bitung, North Sulawesi, Indonesia, the presence of stray or neglected companion animals, and the high popularity of dog ownership all contribute to favorable conditions for tick survival and reproduction. Ticks are known to be capable of transmitting more pathogens to humans and animals than any other arthropod (Baneth 2014). The incidence of tick-borne diseases (TBDs) has been reported to have increased worldwide in recent years. Ticks are the most important vectors of vector-borne diseases in terms of human and animal health, responsible for the transmission of many infectious agents such as bacteria (*Borrelia, Anaplasma*), viruses (tick-borne encephalitis), and even parasites (*Babesia, Theileria*) (Dantas-Torres et al. 2012). Ticks are vectors of various pathogenic bacterial (Sperling et al. 2017).

In the last decade, studies on the molecular detection of TBPs in dogs through PCR have been increasing.Dogs have been in close contact with humans for a long time; thus, humans are at risk particularly for zoonotic TBPs (Galay et al. 2018). Microbes transmitted from animals to humans and cause many new diseases have been reported recently (Estrada-Peña 2015). However, some zoonotic diseases must be wary of for public health. Most information available in the scientific literature on bacterial transmission between pets and humans relates to human pathogenic bacteria. Dogs and cats are potential sources of various zoonotic bacteria that can be transmitted via vectors (i.e. ticks). Pet-associated zoonoses are usually sporadic and their frequencies are not easily determined because of the difficulty in recognizing and validating disease transmission from pets (Tan 1997). However, epidemiological data in Bitung are clearly needed because surveys were limited to small areas of the city. Furthermore, previous studies mostly included pet dogs that showed clinical signs and presented in a few veterinary clinics.

R. sanguineus, the brown dog tick is the most common ectoparasites of dog in the world (Dantas-Torres 2010) and also in Indonesia (Hadi and rusli 2006). *R. sanguineus*tick is a vector of many disease agents, such as *Coxiellaburnetii* (Khalili et al. 2018), *Ehriliciacanis* (Rochelle et al. 2018), *Rickettsiaconorii* and *Rickettsiarickettsia* (Solomon et al. 2022). The tick can carry and spread a range of blood-borne diseases that can affect both animals and humans. These include Q fever as one of the tick-borne zoonotic diseases (Norris et al. 2013). Moreover, in the era of globalization and climate changes, the brown dog tick has becoming increasingly relevant from a public health perspective. This tick has also been implicated in the transmission of pathogens of zoonotic concern. *R. sanguineus* is the most widely distributed tick, prevalent throughout the year in tropical and subtropical areas. It is a three-host tick, dropping from its host after each blood meal and molting

in the environment to the next stage. Thus, this tick can utilize a different host for every blood meal, and therefore has a higher chance of spreading pathogens it might carry to other hosts (Dantas-Torres and Otranto 2015). Tick-borne diseases are among the most prevalent problems diagnosed in both the medical and veterinary medicine and their spectrum has been recently increased (Dantas-Torres et al. 2012). Ticks are obligate hematophagous arthropods which can be transported over large distances by animals and serve as vectors of pathogens, they have short feeding structures (Bonnet et al. 2016).

The identification of bacteria using a metagenomic approach with 99% of accuracy in samples (Trinh et al. 2018) and the 16S RNA gene empathetic to identify bacteria (Koo et al. 2018). However, data on ticks are clearly needed for investigationof pathogenic bacteria as well as for preventing the risk of transmission to humans.This research using metagenomic approach to identified the bacteria on the digestive tract of *R. sanguineus*(sensulato) in Bitung. To our knowledge, based on the analysis using metagenomics approach, have not previously been reported. Thus, this study was conducted to further establish the topic. These results extend the knowledge of the potential pathogenic bacteria affecting human and animal health in the future.

MATERIALS AND METHODS

Study sites

This study was conducted at three areas in the city of Bitung, namely Matuari (M), Girian (G), and Aertembaga (A)(Figure 1). Located between:Matuari(1º26'15.5"N and 125º06'25.7"E), Girian(1º26'32.2"N and 125º07'58.1"E), and Aertembaga(1º28'13.7"N and 125º12 '02.7"E). We sampled domestic dogs from three areas in each plain. Areas were selected based on increasing dog population and the number of infected dogs.

Figure 1. The locations used for sampling *R. sanguineus* (sensulato) in Matuari, Girian and Aertembaga(Bitung, Indonesia Travel Weather Averages (Weatherbase)

Smaples collection

We sample a total of 15 dogs, 5 dogs were sampled per area. Dog ticks were sampled directly from using a sterile gloves and placed in a clean sample bottle. Each bottle was filled with 70% ethanol and placed on ice.Samples, then transported to the Microbiology and Chemistry of Pharmaceutical Analysis Laboratory at Sam RatulangiUniversity, North Sulawesi, Indonesia.Samples were stored in a refrigerator at -20 °C before being used for total DNA genomics extraction.

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Molecular characterization

The total genomic DNA was extracted fromthe digestive tract of ticks.DNA extraction steps are lysis, binding, washing, and elution, then coded for the tick DNA. Ticks wasextracted using the CTAB/SDS method.The extraction results become a template for the CO1 gene amplification stage.DNA isolation was carried out using the fungal/bacterial quick-DNA and Quick-DNA plant/seed mini kit (Zymo Research, D6005) and Quick-DNA Plant/Seed Miniprep Kit (Zymo Research, D6020). The DNA isolation steps follow its manufacturer

protocol.DNA Amplification was performed with PCR Thermocycler using two CO1 primers. We amplified and sequenced the mitochondrial DNA specifically the CO1 gene using following primers: 27F: 5' AGAGTTTGATCMTGGCTCAG 3' and 1492R : 5' TACGGYTACCTTGTTACGACTT 3'.The V3-V4 hypervariable regions were selected from the 16S rRNA gene for this metabarcoding process. The area was amplified usingMyTaq ™ HS Red Mix (HS Red Mix, 2X (Bioline, BIO-25048) & KOD FX Neo (Toyobo, KFX-201). The amplifications were carried out under the following condition: 1 cycle of initial denaturation at 95 °C for 60 seconds, then followed by 35 cycles of denaturation at 95°Cfor 15 seconds, annealing at 55 °C for 15 seconds, and a final extension to complete the process at 72°C for 10 seconds (Table 2). The electrophoresis of PCR products was run on a 0.8% and 1% agarose gel centrifugation with Red Mix (Bioline) and buffered with BashingBead™. PCR products were The 16S rRNA gene from different regions 16S V3-V4 was amplified using specific primers with barcodes (Table 3). All amplification samples were sent to First Base Singapore through PT. GenetikaScience Jakarta (Indonesia) for purification and sequencing.

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Table 1.16S rRNA gene-targeted PCR primers used in this study (Development of 16S rRNA gene-targeted primers for detection of archaeal anaerobic methanotrophs (ANMEs) | Oxford Academic (oup.com)

Primer name	Target group	Sequence (5′ to 3′)	*E. coli* position	References
8F	Bacteria	AGA GTT TGA TCC TGG CTC AG	8–27	Weisburg et al. (1991)
1492R	Archaea and Bacteria	GGH TAC CTT GTT ACG ACT T	1492–1510	Weisburg et al. (1991)

Table 2. PCR conditions(www.bioline.com)

Phase	Temperature(ºC)	Duration (Seconds)	Cycle
Initiation Denaturation	95	60	
Denaturation	95	15	
Annealing	55	15	35 x
Final Extension	72	10	

Based on the results of genomic DNA extraction of ticksusing nanodrop conducted a concentration of 72.4 ng/µl, 63.8 ng/µl, and 127.9 ng/µl. Furthermore, DNA purity detected was 1.97, 1.99 and 1.97 (A260/A280) while with Nanodrop 2.23, 2.29 and 0.45 (A260/ A230). The results obtained can be continued for amplification of the 16S rRNA gene was carried out in regions V3 and V4 (Table 3).

Table 3. Genomic DNA extractionof *R. sanguineus* (sensulato)

Nucleic Acid	Nanodrop (ng / µl)	A260/A280	A260/A230	Volume (µl)
Isolate A	72.4	1.97	2.23	35
Isolate M	63.8	1.99	2.29	35
Isolate G	127.9	1.97	0.45	35

Data analysis

Raw nucleotide sequences and chromatograms were viewed using BioEdit. Additional sequences from GenBank were added as a dataset. The genetic distance was calculated by the Kimura 2-parameter method. The neighbor-joining (NJ) method was used togenerate the phylogenetic tree using MEGA software (Kumar et al. 2018). BLAST searches were used to compare the DNA sequences in this study with GenBank database to determine the closest matches.

RESULTS AND DISCUSSION

Results

In this study,a total of 150 tickwere randomly collected from 15 dogs. All ticks were morphologically identified to be *R. sanguineus* (sensulato)regarding the standard characteristics including red-brown color, elongated body shape, and hexagonal basis capituli (Figure 2). Ticks samples were prepared for DNA extraction after identification and sorting.

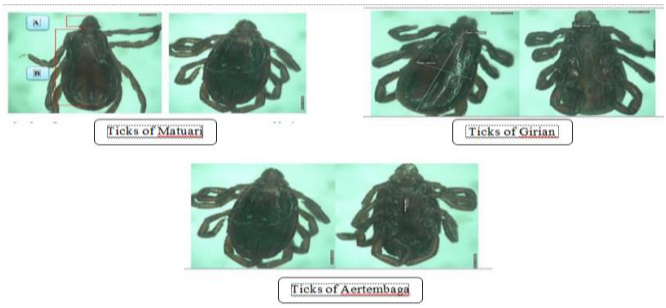

Figure 2. The *R. sanguineus* (sensulato) from Bitung, North Sulawesi, Indonesia

The control genes actin and mt-rrs were successfully detected in all tick DNA samples, respectively. Following nested PCR using screening primers, the DNA of bacterial pathogens of *P. stutzeri*,*Brevundimonas*sp., and *A. lusatience.*

All obtained sequences for each pathogen were 100% homologous and were found to share99.86-99.93% identity to*P. stutzeri*(Accession number: KM278988.1, CP025149.2, CP027664.1, CP011854.1, CP062162.1, CP091174. 1, OK618380.1, OK618369.1, MZ596191.1, MZ508319.1). All positive amplicons share 99.92-100% sequence identity to *Brevundimonas* sp. (Accession number: KY486816.1, CP039382.1, LC324685.1, LC3246841), and 97-98% identity to*A. lusatience* (Accession number: KU525645.1, KU525640.1, AM884147.1, KM210272.1, MK396598.1).

Discussion

The different microbiomes composition found comparatively in the samples can be explained by tick's intrinsic mechanisms and environmental factors, like life stage and the collection site (Kueneman et al. 2021). However, this difference was not greatly expressed comparing the number of species in the samples. Three phyla found in our samples (*Pseudomonadaceae,Phyllobacteriaceae, Caulobacteraceae*) composed by adult ticks. Members of this phylum are gram-negatives,compose animal and the human microbiome from the urinary tract infections. Ticks acquires different bacteria in the microbiome depending on the environment, in the nest or affected by blood diet, the bacterial presence in the DNA could be originated from the tick contact with the animal-host skin (Jose et al. 2021). Compared with the results of other studies, that *Coxiella, Rickettsia* and *Bacillus* are the most pathogens in ticks from France, Senegal and Arizona (René-Martelle et al. 2017).Similar results were also reported by Khalili et al. (2018), that *Coxiellaburnetii*is the most pathogen in ticks collected from infested dogs in Iran. Geurden et al. (2018), the *Rickettsia* is the most pathogen in ticks from dogs and cats in the different European countries. The most common bacteria found in general in the microbiome of adult ticks are *Coxiella, Francisella, Anaplasma, Borrelia, Ehrlichia* and *Rickettsia* (Greay et al. 2018b). Meanwhile, the test results of molecular detection of tick-borne pathogens in stray dogs and *R. sanguineus* (sensu lato) ticks from Bangkok found that *Babesiavogeli*is the most pathogen (Thom et al. 2021). Tick microbiome can be different depending on tick genotype, biogeographical area, and if the tick is male or female (Luzzi et al. 2021). The environmental microorganisms can be found in the tick microbiome and may be difficult to separate from where they belong (O'Neal et al. 2021) because these bacteria might have originated from dogs' skin, blood or even saliva because it is known that dogs lick where ticks have bitten (Luzzi et al. 2021).

The presence of symbiont bacteria communities in the digestive tract of *R. sanguineus*(sensu lato) tick was analyzed using the metabarcoding approach. The DNA barcode region used in this approach was V3-V4 which is a hypervariable region of 16S rRNA. The result of this metabarcoding shows a total of 3 phyla that can be identified from the digestive (Table 4). The most abundant phyla are originated from *P. stutzeri*(35%), followed by *Brevundimonas*sp. (21%) and *A. lusatience*(17%) (Figure 4).

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Table 4. The bacteria species in *R. sanguineus* (sensulato) ticks

Location	Family	Genus	Species	Group
Matuari	*Pseudomonadaceae*	*Pseudomonas*	*Pseudomonas stutzeri*	Gram- negative bacteria
Girian	*Phyllobacteriaceae*	*Aquamicrobium*	*Aquamicrobiumlusatience*	Gram- negative bacteria
Aertembaga	*Caulobacteraceae*	*Brevundimonas*	*Brevundimonas* sp.	Gram- negative bacteria

Based on this table, the most common bacterial detected belongs to the group of Gram-negative bacteria. Other researchers have also agreed that the most of symbiotic/pathogenic bacterial included of Gram-negative group (Boulanger et al. 2019).This obligate intracellular Gram-negative bacterium protects itself in hostile environments by forming spores which can survive for long periods, for example 586 days in tick feces at room temperature (Philip 1948). This pathogen can be transmitted vertically between invertebrates through life stages or be transmitted horizontally from invertebrates to vertebrates or vice versa during feeding of the tick on its host (Weinert et al. 2009).

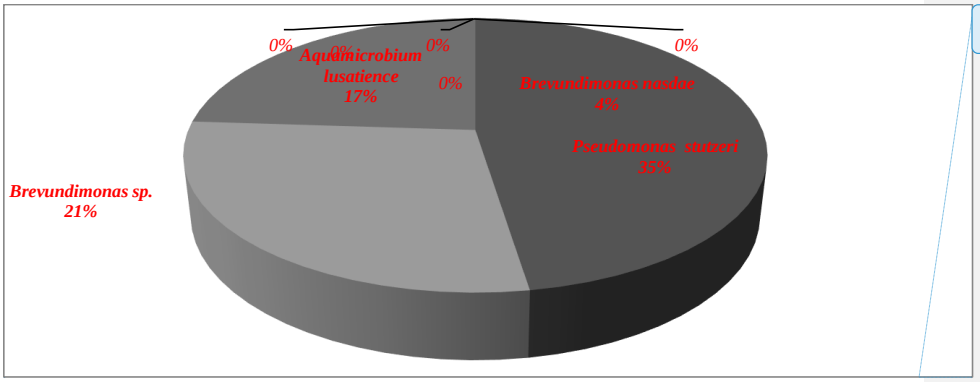

Figure 4. The percentage of bacterial in the digestive tract of *R. sanguineus* (sensulato) ticks

Based on this diagram, it is confirmed that there are different of species.The *P. stutzeri*is responsible for 35% of the total bacterial population, followed by *Brevundimonas*sp.(21%), and *A. lusatience*(17%).Previous surveys have shown that *R. sanguineus* (sensulato) ticks were mainly infected by the pathogenic bacterial genera *Coxiella*and *Rickettsia* and *Bacillus*(René-Martelle et al. 2017), *Francisella*, *Anaplasma*, *Borrelia*and*Ehrlichia* (Greay et al. 2018b). The abundance of pathogenic bacterial in the digestive tract of ticksis presumably related to the differences on structure of the microbiota, based on the tick's genotype, geographical origin and life cycle of ticks (nymphs, adult males and females) (René-Martellet2017).

P. stutzeri is a species strain of the genus *Pseudomonas*, rod-shaped cells, 1-3 µm long and 0.5 µm wide with a single flagella. The widespread geographic distribution in nature, moist areas found in water and soil, occupies diverse ecological niches, and has been isolated as an opportunistic pathogen of humans(Lalucat et al. 2006).Osteomyelitis, arthritis, bacteremia, endocarditis, endophthalmitis, pneumonia, empyema, urinary tract infections and meningitis are human infections caused by *Pseudomonas* species (D'Agata 2015, ; Goldman 2020). Human can be exposed in various ways, because these bacteria can also grow on fruits and vegetables, or in humid public areas such as public baths, bathrooms, kitchens, and sinks (Bhargav2016).

The genus *Brevundimonas* was first proposed by Segers et al. *Brevundimonas* species are aerobic Gram-negative, oxidase and catalase positive, non-fermenting rods 1 to 4 mm in length and 0.5 mm in width, they are aerobic with optimal growth temperatures of between 30–37 °C, belonging to the *Alphaproteobacteria*class and *Caulobacteraceae* family (Segers et al. 1994). The group can survive in a wide variety of environments including different water sources (aircraft water, bottled water, hospital water, purified water) (Handschuh et al. 2017), and are usually resistant to a wide array of antimicrobials (Flores-Trevino et al. 2014). Bacteria such as these have the ability to infect patients/individuals with underlying medical conditions and diseases(co-morbidity), *Brevundimonas*-associated bacteriemic conditions (Swain and Rout 2017), urinary tract infections, empyema, and foot ulcers (Chandra et al. 2017; Sánchez-Montes et al. 2021). Examination of the scientific literature showed multiple types of infections resulting from *Brevundimonas* spp. This indicates that the genus may be a more widespread pathogen with infections caused by *Brevundimonas* spp. being invasive and severe (Ryan and Pembroke 2018).

The genus *Aquamicrobium* was first proposed by Bambauer et al. (1998). In comparison with *Phyllobactrium*, in *Phyllobacteriaceae,* it is still a relatively new bacterial species. Currently, 7 species in the genus were isolated and taxonomically identified, which are*A. defluvii*, *A. aerolatum*, *A. lusatience*, *A. ahrensii*, *A. segne*, *A. aestuarii*, *A. soli*, and *A. terrae.*Interestingly, all members of the genus *Aquamicrobium* have been isolated from pollutant-loaded environments such as wastewater-treatment plants, activated sewage sludge and

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biofilters. Itis a gram-negative bacteria, aerobic bacteria, this genus is found in the activated sludge, air, chemical industry center and Bohai Sea, can degrade thiophene-2-carboxylate, petroleum, polychlorinated biphenyls (PCB). The species of the *Aquamicrobium* genus have been isolated from polluted environments such as sewage treated factory, active sewage sludge and biological filters. The degradation abilities of some strains have been investigated in detail for specific pollutants, suggesting that members may have uses in the degradation of pollutants (Wu et al. 2021).

The habitat of *R. sanguineus*tickis endophilic (living indoors), but this tick also survive in environment(Dantas-Torres 2010). A tick's habitat is composed of the variety of living and non-living things in the space in which it lives. Ticks are adapted to two contrasting components of their habitat: the physical environment and their host (Barker and Walker 2014).

PCR amplification and sequence identification

After obtaining the sequence readings, sequences were compared to reported isolates using the Basic Local Alignment Search Tool (BLAST) of the U.S. National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

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Table 5. Species identification based on GenBank database using BLAST identification

Identified species from GenBank	Similarity (%)	Accession number	Location	References
Pseudomonas stutzeri strain SGAir0442	99.93	CP025149.2	Singapore	Direct submission
Pseudomonas stutzeri strain 1W1-1A	99.93	CP027664.1	China	Direct submission
Pseudomonas stutzeri strain SLG510A3-8	99.93	CP011854.1	China	Direct submission
Pseudomonas stutzeri strain 2020WEIHUA_G	99.93	CP062162.1	China	Direct submission
Pseudomonas stutzeri strain XX1.	99.93	CP091174.1	China	Direct submission
Pseudomonas stutzeri strain B-38-7.	99.93	OK618380.1	India	Direct submission
Pseudomonas stutzeri strain B-34-6.	99.93	OK618369.1	India	Direct submission
Pseudomonas stutzeri strain GR1.4.	99.93	MZ596191.1	Indonesia	Direct submission
Pseudomonas stutzeri strain GR 114.	99.93	MZ508319.1	Indonesia	Direct submission
Pseudomonas stutzeri strain ZH-1.	99.86	KM278988.1	China	Direct submission

BLAST analysis indicated that isolates samples from Matuaribelong to *Pseudomonas* genus. The 16s rRNA sequence of isolate Matuari is 99.86-99.93% identity to that of *P. stutzeri* from Singapore, China, India and Indonesia.

Table 6. Species identification based on GenBank database using BLAST identification

Identified species from GenBank	Similarity (%)	Accession number	Location	References
*Aquamicrobiumlusatiense*strain XJ-9.	98	KU525645.1	China	Direct submission
*Aquamicrobiumlusatiense*strain XJ-3.	97	KU525640.1	China	Direct submission
*Aquamicrobiumlusatiense*strain 854/1.	98	AM884147.1	Germany	Direct submission
*Aquamicrobiumlusatiense*strain ADC-22.	98	KM210272.1	Greece	Direct submission
*Aquamicrobiumlusatiense*strain P4N-04.	98	MK396598.1	Korea	Direct submission

Based on data in Table 6, thus it can be concluded that the bacterial isolates ofGirian samples showed 97-98% homologouswith *Aquamicrobium*sp. from China, Germany, Greece and Korea.

Table 7. Species identification based on GenBank database using BLAST identification

Identified species from GenBank	Similarity (%)	Accession number	Location	References
Brevundimonas sp. strain EN14ES7.	99	KY486816.1	Poland	Direct submission
Brevundimonas sp. 266XY5.	100	KF818659.1	China	Direct submission
Brevundimonas sp.	99	CP039382.1	China	Direct submission
*Brevundimonas*sp. FrW-Asx16.	99	LC324685.1	Egypt	Direct submission
Brevundimonas sp. FrW-Asx5.	99	LC324684.1	Egypt	Direct submission
Brevundimonas sp. GW460-12-10-14- LB2.	99	KY486816.1	Poland	Direct submission

Based on the BLAST analysis, it was concluded that the bacterial isolate from Aertembaga showed 99-100% homologous with *Brevundimonas* sp. from Poland, China, and Egypt. The species found in this study

have never been reported from *R. sanguineus* ticks. *P. stutzeri*, *Brevundimonas*sp., and *A. lusatience*are pathogens potentially infected humans.

In conclusion, this study shows the bacterial community's composition in the digestive tract of *R. sanguineus* (sensulato) ticksusing the V3-V4 hypervariable region of 16S rRNA gene marker. *P. stutzeri*, *Brevundimonas sp., and A. lusatience*dominated most of the species. This study can provide data on the microbiome's diversity in the digestive tract of *R. sanguineus* (sensulato). As a result, this study provides scientific information of bacterial in ticks through the metagenomic approach. However, further research will be required to determine the specific relationship between the digestive tract of *R. sanguineus* (sensulato), its host, and its ecological niche.

ACKNOWLEDGEMENTS

This research is part of the Doctoral Dissertation Research, and the authors would like to thank to NurmiatyKarim, M.Si and the team at the Laboratory of Microbiology and Chemistry of Pharmaceutical Analysis, Sam Ratulangi Universityfor their assistance during the research.

REFERENCE

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