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Molecular Detection of Clostridium-Related DNA in Bats from Soppeng, Indonesia

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ABSTRACT

Introduction: Clostridium spp. are anaerobic, spore-forming bacteria widely distributed in soil, water, and the gastrointestinal tracts of humans and animals. Several species are associated with important human and veterinary diseases. Information regarding Clostridium-related bacteria in bats remains limited in Indonesia. This study aimed to generate preliminary molecular evidence of Clostridium-related bacterial DNA in bats from Soppeng Regency, South Sulawesi, Indonesia.

Methods: Bat samples were collected in Soppeng Regency, South Sulawesi, Indonesia. Ten fruit bats (Cynopterus brachyotis) were captured near roosting and foraging sites, and three intestinal tissue samples were selected for molecular analysis based on sample integrity and DNA preservation quality. Genomic DNA was extracted using a commercial extraction kit following aseptic sample processing. Molecular detection was performed using PCR targeting the bacterial 16S rRNA gene, followed by agarose gel electrophoresis. PCR-positive amplicons were subjected to Sanger sequencing and analyzed using BLAST against the NCBI/GenBank database based on sequence identity, query coverage, and E-value.

Results: All three analyzed samples produced positive amplification bands at the expected amplicon size of approximately 1,465 bp. BLAST analysis showed the closest sequence matches to Clostridium septicum (K1; 94% query cover; 97.57% identity; E=0.0), Paraclostridium sordellii (K3; 97% query cover; 96.64% identity; E=0.0), and Clostridium haemolyticum (K5; 95% query cover; 98.06% identity; E=0.0).

Conclusion: This study provides preliminary molecular evidence of Clostridium-related bacterial DNA in fruit bats from Soppeng Regency. However, DNA detection alone does not confirm bacterial viability, pathogenicity, transmission potential, or reservoir status. Further studies involving culture confirmation, toxin gene characterization, environmental sampling, and broader wildlife surveillance are needed to clarify the ecological and public health significance of these findings.

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INTRODUCTION

Zoonotic diseases remain a major global public health concern because they can trigger outbreaks with substantial health, economic, and ecological consequences. A large proportion of emerging infectious diseases in humans originate from zoonotic spillover, in which wildlife plays a critical role in pathogen maintenance and transmission (1–3). Within wildlife hosts, bats have received sustained scientific attention due to their distinctive ecological and biological traits—including long lifespan, social roosting, flight capability, and broad geographic distribution—which collectively facilitate the maintenance and dissemination of diverse microorganisms (4–6). Importantly, bats can harbor a wide range of pathogens (viruses, bacteria, fungi, and parasites) often without apparent clinical disease, suggesting an effective reservoir capacity and a complex host–pathogen balance that may support silent circulation in nature (7,8).

Although bat-associated zoonotic research has frequently centered on viral threats, bacterial pathogens carried by bats remain comparatively under-characterized in many settings (9). This evidence gap is especially relevant at human–wildlife interfaces, where agricultural activities, settlement expansion, and wildlife habitat overlap can increase opportunities for indirect exposure through environmental contamination. In Soppeng Regency, South Sulawesi, such interfaces are plausible given the close proximity between human activities and bat foraging/roosting environments, making this area epidemiologically meaningful for baseline zoonotic surveillance (10).

Among bacterial hazards, the genus Clostridium comprises anaerobic, spore-forming organisms widely distributed in soil and water and commonly present in the gastrointestinal tracts of humans and animals (11,12). Several species are clinically important pathogens capable of causing severe conditions, including gas gangrene, enteritis, and toxemia in humans and animals. A defining characteristic of Clostridium spp. is their ability to form endospores, enabling persistence under harsh environmental conditions and thereby increasing the likelihood of environmental survival and potential exposure across the human–animal–environment continuum (13,14).

From a One Health perspective, this environmental durability strengthens the plausibility that wildlife-associated shedding or contamination pathways could contribute to repeated human and livestock exposure in settings where sanitation, water safety, and animal–human contact are not fully controlled. Despite this relevance, the potential role of bats in harboring and possibly disseminating pathogenic Clostridium spp. remains insufficiently studied, particularly in Indonesia. Available Indonesian work on bats has largely emphasized viral pathogens, while investigations focused on bacterial agents—especially Clostridium spp.—are still scarce (15,16). Furthermore, molecular-based evidence documenting Clostridium spp. in bats is limited, especially in rural or peri-rural contexts where interactions among humans, wildlife, and the environment may be frequent and bidirectional. This gap constrains risk assessment because it leaves uncertainty about whether bats merely encounter Clostridium spores environmentally, transiently carry organisms in the gut, or potentially contribute to broader ecological maintenance through shedding.

Molecular identification approaches based on 16S rRNA gene amplification and sequencing are well established for bacterial detection and taxonomic assignment, particularly when culture-based recovery is challenging or when organisms are anaerobic and environmentally persistent (17–19). In this context, sequencing-supported identification, followed by similarity searches against curated databases (e.g., BLAST against NCBI resources), provides a practical and robust framework for generating baseline evidence on Clostridium presence in wildlife samples and for informing subsequent work on viability, toxin gene profiles, and transmission pathways (20).

Therefore, this study aimed to generate molecular evidence of zoonotic Clostridium spp. in bats from Soppeng Regency by applying PCR targeting the bacterial 16S rRNA gene followed by sequencing-based identification. By addressing the current scarcity of Indonesian data on bacterial zoonoses in bats, the findings are expected to strengthen baseline One Health surveillance and guide future investigations that integrate environmental sampling, broader bat population coverage, and higher-resolution characterization of pathogenic potential.

Clostridium spp. (including closely related genera within Clostridiaceae and Peptostreptococcaceae) are spore-forming anaerobes with major relevance to public and veterinary health because several species cause severe enteric and systemic disease, and their spores can persist in the environment. Evidence from bat-associated studies supports the plausibility of bats (and especially bat guano) acting as a One Health–relevant reservoir or dissemination medium for zoonotic clostridia. For example, Clostridium perfringens has been molecularly characterized from Egyptian fruit bats, including toxin gene profiling with epidemiological implications (21), while Clostridioides

difficile—a major human enteric pathogen—has been detected in bat guano, highlighting that guano can carry clinically important clostridial organisms (22). A recent synthesis of bat-associated bacteria further underscores that bats may harbour diverse bacterial taxa of potential human-health concern, particularly where roosting and foraging overlap with human activities (23).

Beyond "presence/absence," newer microbiome studies suggest that clostridia can be structured by ecology, host traits, and co-infections, which is directly relevant to interpreting molecular detection data from bat intestines (24). Multi-compartment profiling (e.g., blood, faeces, oral secretions) has shown that faecal bacterial communities can include Clostridium sensu stricto among the detectable taxa, with community composition varying by sample type and context (25). Importantly, non-invasive, guano-based molecular surveys in bats have reported enrichment of Clostridium spp. in parasite-infected individuals, including lineages related to C. perfringens, suggesting that gut parasite status and dysbiosis may influence clostridial signals in sequencing datasets (26). Complementary bat gut research using 16S rRNA high-throughput sequencing plus cultivation also emphasizes that bat intestines contain substantial uncharacterized bacterial diversity, and that culture-independent approaches can reveal taxa with potential relevance to host and human health (27).

Methodologically, positioning your study as "16S rRNA PCR and sequencing evidence" is well aligned with how many infection journals frame culture-independent bacterial detection, but it is important to contextualize what 16S can—and cannot—resolve for zoonotic clostridia at species/strain and toxigenicity levels. Clinical evaluation work shows that direct 16S rRNA PCR (followed by sequencing) can be effective when routine cultures fail, supporting its utility as a sensitive, broad-range detection strategy (28). More recent evaluations indicate that 16S-targeted metagenomics (including Nanopore workflows) can be feasible and informative for culture-negative specimens, while also requiring careful contamination control and interpretation thresholds (29). At the same time, multi-laboratory comparisons demonstrate that methodological variation can reduce cross-study comparability for 16S-based profiling, reinforcing the need for standardized protocols, rigorous controls, and (when claiming "zoonotic Clostridium") ideally species-level confirmation plus targeted assays for toxin genes or virulence determinants (30). This framing naturally supports a strong rationale for baseline surveillance in Soppeng: 16S rRNA PCR/sequencing provides an efficient first-line map of clostridial presence, which can then be extended to higher-resolution characterization to strengthen One Health risk inference.

METHODS

This study employs a clear and systematic approach to ensure the reliability and validity of the findings. Below are the components of the methodology:

This study was conducted as a laboratory-based observational investigation with a descriptive–analytic approach to detect bacterial DNA in bats and interpret molecular findings without experimental intervention. Field sampling was undertaken in selected locations in Soppeng Regency, South Sulawesi, Indonesia, focusing on areas near bat roosting and foraging sites that represent relevant human–wildlife interface contexts.

Bat capture, handling, and sample selection

A total of ten (10) bats were captured using hand nets by intercepting individuals at their roosting sites or during low-flight activity. This active capture technique ensured direct and immediate retrieval of the specimens. Captured bats were handled carefully using puncture-resistant gloves to minimize stress and injury. All captured bats were morphologically identified as *Cynopterus brachyotis*, a fruit bat species, using standard taxonomic identification keys. For molecular analysis, intestinal tissue samples from three (3) bats were selected based on sample integrity and DNA preservation quality. Due to financial limitations associated with sequencing analysis, only three intestinal samples were processed as part of this preliminary surveillance study. The intestinal tissues were collected aseptically, placed in sterile microtubes, and transported to the laboratory under cold-chain conditions prior to DNA extraction.

DNA extraction

Bacterial DNA was isolated from selected bat samples under aseptic conditions. Samples were homogenized prior to extraction, and genomic DNA was extracted using a commercial DNA extraction kit following the

manufacturer's protocol. DNA quality and concentration were assessed before downstream molecular procedures (31).

PCR amplification of the 16S rRNA gene and gel electrophoresis

Molecular detection targeted the bacterial 16S rRNA gene using PCR primers. Each PCR reaction contained extracted DNA template, forward and reverse primers, PCR master mix, and nuclease-free water, and amplification was performed in a thermal cycler using optimized cycling steps (initial denaturation, denaturation–annealing–extension cycles, and final extension) (32). PCR products were visualized by agarose gel electrophoresis stained with an appropriate nucleic-acid dye; samples showing bands of the expected size were categorized as PCR-positive and progressed to sequencing (33).

Sanger sequencing and BLAST-based identification

PCR-positive amplicons were purified and subjected to Sanger sequencing to obtain nucleotide sequences of the amplified 16S rRNA fragment (34). Sequence similarity was assessed using BLAST against the NCBI/GenBank database, and identification was interpreted using percentage identity, query coverage, and E-value (35).

Laboratory site and ethical approval

All laboratory procedures were conducted at the Hasanuddin University Medical Research Center (HUMRC), Universitas Hasanuddin, and ethical clearance for animal handling and sample collection was obtained from the relevant institutional ethics committee prior to study implementation.

Population and Sample/Informants

The population of this study included bats inhabiting selected areas in Soppeng Regency, Indonesia. Samples were obtained using purposive sampling, considering ecological characteristics and potential zoonotic risk areas.
A total of 10 bats were collected. Ectoparasites were identified through microscopic examination, and molecular analysis of *Clostridium spp.* was conducted using PCR targeting the 16S rRNA gene.

Research Location

This study was conducted in Soppeng Regency, South Sulawesi, Indonesia. Sampling was carried out in selected locations with high potential for human–bat interaction, including residential areas and agricultural environments, to assess zoonotic risk and its relevance to public health.

Instrumentation or Tools

The instruments used in this study included bat capture equipment, sample collection tools, and laboratory instruments. Bat capture was performed using mist nets, while ectoparasite identification was conducted using a light microscope.

Molecular analysis of *Clostridium spp.* was carried out using polymerase chain reaction (PCR) equipment, including a thermal cycler, micropipettes, and electrophoresis apparatus. DNA sequencing was performed using Sanger sequencing methods to confirm species identification.

Data Collection Procedures

Data were collected through a combination of field sampling and laboratory analysis. Bats were captured using mist nets in selected areas of Soppeng Regency, Indonesia, representing environments with potential zoonotic risk. All captured bats were handled following biosafety and ethical procedures. Ectoparasites were collected and identified using microscopic examination. Samples were then subjected to molecular analysis, including DNA extraction and PCR targeting the 16S rRNA gene for the detection of *Clostridium spp.* PCR products were analyzed by gel electrophoresis and confirmed using Sanger sequencing. The data collection process was designed to support the identification of zoonotic potential and provide evidence for public health and health promotion strategies.

Data Analysis

The data were analyzed using descriptive methods. The presence of ectoparasites and *Clostridium spp.* in bat samples was identified and presented in tables and figures.

Molecular data obtained from PCR and sequencing were analyzed to confirm the presence of *Clostridium spp.* based on 16S rRNA gene sequences. The molecular results were interpreted to determine the presence of Clostridium-related bacterial DNA and to explore their potential relevance within a One Health surveillance framework.

All findings were further analyzed within a One Health framework to support public health insights and the development of health promotion strategies.

Ethical Approval

This study was approved by the Ethics Committee of the Faculty of Public Health, Universitas Hasanuddin (Approval No. 1174/UN4.14.1/TP.01.02/2025). All research procedures were conducted in accordance with ethical principles and biosafety guidelines. Research permission was obtained from the relevant local authorities prior to data collection.

RESULTS

Molecular testing was performed on three selected bat samples using PCR targeting the 16S rRNA gene. All three samples produced clear amplification bands at the expected target size, indicating positive bacterial DNA amplification. The assay controls supported result validity: the positive control (C+) showed a distinct band, while the negative control (C−) showed no amplification, indicating that the PCR run performed appropriately and suggesting absence of detectable contamination.

Sequencing And BLAST-Based Species Identification

All PCR-positive amplicons were subsequently processed for Sanger sequencing, and the resulting sequences were analyzed using BLAST against the NCBI/GenBank database for taxonomic assignment. BLAST analysis indicated that the obtained sequences showed the closest matches to Clostridium/Clostridia-related taxa, with high query coverage, high percentage identity, and E-values of 0.0, supporting reliable molecular identification. Taxonomic assignment was based on percentage identity, query coverage, and E-value obtained from BLAST searches against the NCBI/GenBank database. Sequences were interpreted as the closest matches and not as definitive species identifications.

Detected Taxa and Sequence Similarity Metrics

The obtained sequences showed the closest BLAST matches to Clostridium septicum, Paraclostridium sordellii, and Clostridium haemolyticum: BLAST analysis showed that the obtained sequences had the closest matches to Clostridium septicum (K1), Paraclostridium sordellii (K3), and Clostridium haemolyticum (K5). These findings should be interpreted as closest sequence matches rather than definitive species identifications because 16S rRNA sequence similarity alone may not provide sufficient taxonomic resolution among closely related clostridial taxa.

DISCUSSION

This study provides preliminary molecular evidence of Clostridium-related bacterial DNA in intestinal samples collected from fruit bats (Cynopterus brachyotis) in Soppeng Regency, Indonesia. BLAST analysis showed the closest sequence matches to Clostridium septicum, Paraclostridium sordellii, and Clostridium haemolyticum. These findings contribute baseline information for wildlife pathogen surveillance within a One Health context.

The results should be interpreted cautiously. Detection of bacterial DNA through 16S rRNA gene amplification does not confirm bacterial viability, active infection, shedding, pathogenicity, transmission potential, or reservoir status. Therefore, the present findings should not be interpreted as evidence that bats are confirmed reservoirs of zoonotic Clostridium species.

Alternative explanations should also be considered. The detected DNA may reflect environmental exposure, transient passage through the gastrointestinal tract, ingestion of contaminated food resources, or contact with environmental spores. In addition, the reported sequence identity values are insufficient for definitive species-level identification; therefore, the detected taxa should be regarded as the closest BLAST matches rather than confirmed species.

Several limitations should be acknowledged. Only three bat intestinal samples were analyzed, preventing prevalence estimation and limiting broader ecological interpretation. Furthermore, no culture-based isolation, toxin gene analysis, environmental sampling, or assessment of bacterial viability was performed. Future studies should include larger sample sizes, phylogenetic analysis, toxin gene characterization, and integrated One Health investigations to better understand the ecological and public health significance of Clostridium-related bacteria in bats.

Interpretation of Key Findings

This study provides preliminary molecular evidence of Clostridium-related bacterial DNA . These findings highlight the risk of transmission to humans, especially in areas with close human–bat interaction. From a public health perspective, this underscores the importance of health promotion and a One Health approach to prevent zoonotic disease transmission.

Comparison with Previous Studies

The findings are consistent with previous studies identifying, including *Clostridium spp.*. Differences across studies may be influenced by geographic and methodological variations. These results support the importance of a One Health approach in zoonotic disease research.

Limitations and Cautions

This study has several limitations. The sample size and geographic coverage were limited, which may affect the generalizability of the findings. In addition, the cross-sectional design restricts the ability to determine causal relationships. Future studies are recommended to include larger sample sizes and broader locations to better understand zoonotic risks. Several limitations should be acknowledged. First, only three bat intestinal samples were subjected to molecular analysis, limiting representativeness and preventing prevalence estimation. Second, DNA-based detection does not confirm bacterial viability, active infection, pathogenicity, toxin production, shedding, or transmission potential. Third, no culture-based isolation, toxin gene characterization, or environmental sampling was performed. Finally, data regarding human, livestock, and environmental exposure were not available, limiting interpretation within a broader One Health context.

Recommendations for Future Research

Future studies should include larger sample sizes and wider geographic coverage to better understand the distribution of zoonotic pathogens in bats. Further research integrating ecological and epidemiological data is needed to strengthen the One Health approach and support effective health promotion strategies.

CONCLUSION

This study provides preliminary molecular evidence of clostridial DNA in three intestinal samples collected from fruit bats (Cynopterus brachyotis) in Soppeng Regency, Indonesia. The obtained sequences showed the closest similarity to Clostridium septicum, Paraclostridium sordellii, and Clostridium haemolyticum. These findings support the need for broader wildlife surveillance, culture-based confirmation, toxin gene characterization, environmental sampling, and integrated One Health investigations. However, the present results do not demonstrate bacterial viability, transmission potential, or reservoir status and should therefore be interpreted cautiously.

AUTHOR CONTRIBUTIONS

Muhammad Erwan Arifin contributed to the conceptualization of the study, data collection, laboratory analysis, data interpretation, and drafting of the manuscript. Syamsuar Manyullei contributed to the study design, provided supervision throughout the research process, and critically revised the manuscript. Anwar Mallongi contributed to data analysis, interpretation of results, and provided substantial intellectual input. Erniwati Ibrahim contributed to laboratory work and validation of the experimental results. Apik Indarty Moedjiono contributed to statistical analysis and interpretation of the data.

All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The authors used generative AI tools (e.g., ChatGPT) to assist in language editing, grammar correction, and improving the clarity of the manuscript. The authors take full responsibility for the content of the manuscript and have thoroughly reviewed and verified all information to ensure its accuracy and integrity.

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