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Research Article

# The Metagenomic Analysis of Potential Pathogenic Emerging Bacteria in Fleas

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## Abstract

**Background and Objective:** At present many pathogenic microbes that cause disease in humans are transmitted through animals. *Ctenocephalides felis* is specific ectoparasites in cats. Metagenomic research on the digestive tract and body surface of *C. felis* has been conducted. DNA genomics was extracted from the body surface and digestive tract of *C. felis*. **Materials and Methods:** Metagenomic analysis has used the 16S rRNA gene (V3-V4 region). Sequencing was carried out using New Generation Sequencing at the First BASE Laboratory, Singapore. **Results:** Wolbachia has the most significant bacterial composition in *C. felis* (94.4%), we were found bacteria with a composition >1% that have never been reported to be associated with *C. felis*. Also, there were 0.2% of bacteria whose taxonomic status cannot be determined. **Conclusion:** The results of this study become a vital reference pathogenic bacteria that can be transmitted to humans and animals through *C. felis*. It is necessary to study the resistance of bacteria isolated from *C. felis* to antibiotics in the future.

**Key words:** Metagenomics, 16S rRNA, *Ctenocephalides felis*, bacteria composition, symbiotic, caulobacteriaceae, staphylococcaceae

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Ctenocephalides felis* is the most widely distributed ectoparasite worldwide[1,2]. This is due to human migration which also brings domestic cats to various regions around the world[3,4]. Insects have carried out symbiotic coevolution with various types of bacteria. Metagenomic analysis has made it possible to examine the diversity of symbiotic and non-symbiotic bacteria in insects, especially species of bacteria that cannot be cultured in a laboratory[5,6]. However, the genome and behaviour of these bacteria are the object of very deep biological studies and holds a mystery. Metagenomic exploration in insects has been carried out lately. Several insects have been performed metagenomic analysis including Apids[7], *Megaphragma amalphitanum*[8], blowflies and house flies[9,10], *Dendroctonus valens* and *D. mexicanus*[11]. All metagenomic research results on these insects are reported to be genera and bacterial species that have never been known to be associated with insects before.

However, the composition of bacteria based on genus and family needs to be mapped to determine the abundance of bacteria at the family and genus levels found in cat fleas. Recently, pathogenic microbes that come from animals and infect humans have become a topic of much research[6,9]. Identifying bacteria and the composition of bacteria in cat fleas is important to study, among others, the background that cats are the domestic pets in the world. As a pet, cats interact directly with humans, so the possibility of transmitting pathogenic microbes to humans is substantial[10-12].

Previous research found several families of potential pathogens in humans. These families include Rickettsiaceae, Burkholderiaceae, Pseudomonadaceae, Planoccocaceae, Corynebacteriaceae, Caulobacteriaceae, Isosphaeraceae and Staphylococcaceae[13]. Bacterial identification using a metagenomic approach will identify 99% of the bacteria present in samples[7,13,14]. For the identification of bacteria in this study using the 16S RNA gene. Bacterial identification using the 16S RNA gene is still empathetic to identify bacteria[15-18].

However, the composition of bacteria at the genus and family level needs to be studied in depth. This study describes the bacterial composition of *C. felis* at the family and genus levels using a metagenomic analysis approach.

## MATERIALS AND METHODS

**Study area:** Samples of *C. felis* were obtained from wild cats in several locations in Manado City, namely Karombasan Village, Paal Dua Village and Malalayang Village in Manado City, North Sulawesi, Indonesia. The blackish-brown cat fleas, complete with their organs, were then taken to the laboratory for analysis (Fig. 1). Morphological identification was carried out in the Biology Laboratory of Manado State University, Indonesia. Samples were prepared with 70% alcohol before being used for total DNA genomics extraction. Complete DNA extraction was carried out at the Laboratory of Bioactivity and Molecular Biology, Manado State University, North Sulawesi, Indonesia, from July, 2019 until March, 2020. Metagenomic sequencing was carried out at Axil Scientific Pte Ltd Laboratory (20-0200922-D) 41 Science Park Road, #04-08, The Gemini, Singapore Science Park II, Singapore. The study was carried out at the Department of Biology, Bimolecular Lab, Indonesia from July, 2019-March, 2020.

Fig. 1: *Ctenocephalides felis* samples from Manado North Sulawesi, Indonesia

**Extraction of DNA genomic:** Total DNA *C. felis* was extracted using the Bacteria Genomic DNA Kit with the manufacturer's protocol. Total DNA *C. felis* was extracted using the Bacteria Genomic DNA Kit with the manufacturer's protocol and with conventional methods (CTAB, Cetyltrimethylammonium Bromide)[19,20]. To determine the concentration and purity of DNA, the 1% gel electrophoresis method was used where based on the concentration, the DNA was diluted to 1 ng $L^{-1}$ using dd $H_2O$.

**Amplicon generation:** 16S rRNA genes of distinct regions 16S V3-V4 were amplified used a specific primer with the barcode. The primer was used as follow Table 1.

**PCR products**

**Quantification and qualification:** To identify the PCR product, mix equal quantities of 1X loading buffer (including SYB

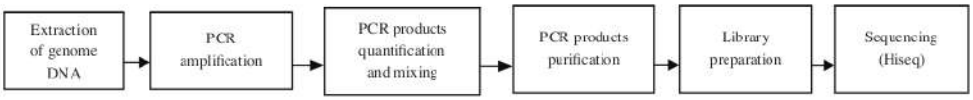


Fig. 2: Metagenomic research workflow

Table 1: Primer sequence used in the research

| Hypervariable region | Primer | References |
|---|---|---|
| V3 | 28F: 5'-GAGTTTGATCNTGGCTCAG-3' | Suchodolski *et al.*[21] |
| | 519R: 5'-GTNTTACNGCGGCKGCTG-3' | Turner *et al.*[22] |
| V4 | 515F: 5'-GTGCCAGCMGCCGCGG-3' | Weisburg *et al.*[23] |
| | 907R: 5'-CCGTCAATTCMTTTRAGTTT-3' | |

All PCR reactions were carried out with Phusion® High-Fidelity PCR master mix (NewEngland Biolabs)

green) and electrophoresis on a 2% agarose gel. Further studies were conducted on samples with bright main strips ranging from 400-450 bp. Tapestation 4200, picogreen and nanodrop were used to test the quality and quantity of the V3V4 amplicon. All of the samples passed the quality-control tests and were sent straight to production line[21].

**Mixing and purification:** PCR products were mixed in equidensity ratios. Then, a mixture of PCR products was purified with Qiagen Gel Extraction Kit (Qiagen, Germany). The libraries generated with TruSeq® DNA PCR-Free Sample Preparation Kit and quantified via Qubit and Q-PCR would be analyzed by HiSeq2500 PE250 (Fig. 2).

The quality of the libraries was measured using TapeStation4200, Picogreen and qPCR. All libraries passed the QC measurement. The library was then pooled according to the protocol recommended by Illumina and proceed straight sequencing using the MiSeq platform at 2x301PE format. The libraries were prepared using Illumina 16s metagenomics library prep kit and their quality and quantity were determined using Agilent Tapestation 4200 and Picogreen.

**New generation sequencing of DNA:** The amplicon was sequenced on the Illumina HiSeq paired-end platform, yielding 250 bp paired-end raw reads (Raw PE), assembled and processed to get Clean Tags. Raw data would be combined and filtered to provide clean data for sequencing data analysis. The effective data is utilized to create OTU clusters and species annotations for each OTU's sequence. As a result, the relativistic[24].

## RESULTS AND DISCUSSION

**Family level:** The composition of the family level results of metagenomic analysis based on the 16S rRNA gene from *C. felis* found 0.2% of the population of Unassigned bacteria, which means it has not been recorded or identified in the world bacterial taxonomic system. The bacterial database based on the 16S rRNA gene has been stored in the NCBI gene bank. As many as 0.1% belong to the family Corynebacteriaceae, 0.2% included in the Bacillales family, 1.8% included in the family Plannococcaceae, 0% included in the Staphylococcus, 0.1% belongs to the family Methylophilaceae, 0.1% belong to the family Neisseriaceae, 0.1% belong to the Moraxellaceae family and 96.4% belong to the Rickettsiaceae family (Table 2). The Rickettsiaceae family is the dominant bacterial family of bacteria on the surface of the body and the digestive tract of *C. felis* cat fleas.

**Genus level:** The composition of genus-level resulted from metagenomic analysis based on 16S rRNA, *C. felis* genes was found in 0.2% of the population of unassigned bacteria, which means it has not been recorded or identified in the world bacterial taxonomic system. The bacterial database based on the 16S rRNA gene has been stored in the NCBI gene bank. As much as 0.1% belongs to the genus Corynebacterium, 0.2% included in the genus Bacillales, 1.8% belong to the family genus Plannococcaceae not yet known, 0.8% belongs to the genus Staphylococcus, 0.1% belongs to the family of the genus Methylophilaceae unknown, 0.1% belongs to the family of the genus Neisseriaceae unknown, 0.1% belong to the family Moraxellaceae genus unknown, 1.5% belong to the genus Rickettsia and 94.4% to the genus Wolbachia (Table 3). The family Rickettsiaceae genus Wolbachia is the dominant bacterium on the body's surface and the digestive tract of *C. felis*.

The results of this study indicate that a new family and genus of bacteria were first reported in cat fleas. The genus has many pathogenic species in humans, namely the genus Staphylococcus, Corynebacterium and the family Plannococcaceae. It is necessary to study more deeply the potential of *C. felis* to transmit other pathogenic bacteria to humans.

Table 2: Composition of bacteria at the family level

| Numbers | Genus | Percentage |
|---|---|---|
| 1 | Corynebacteriaceae | 0.1 |
| 2 | Bacillales | 0.2 |
| 3 | Plannococcaceae | 1.8 |
| 4 | Staphylococcus | 0.0 |
| 5 | Isosphaeraceae | 0.3 |
| 6 | Rickettsiaceae | 0.5 |
| 7 | Rickettsiae | 96.4 |
| 8 | Methylophilaceae | 0.1 |
| 9 | Neisseriaceae | 0.1 |
| 10 | Moraxellaceae | 0.1 |

Complete composition is shown in Appendix 1

| Legend | Taxonomy | Total % | RN % |
|---|---|---|---|
| | Unassigned;Other;Other;Other;Other | 0.1% | 0.2% |
| | k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__iii1-15;f__ | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinopolysporaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Corynebacteriaceae | 12.3% | 0.1% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Dermatophilaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae] | 0.0% | 0.0% |
| | k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__;f__ | 0.0% | 0.0% |
| | k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__JG30-KF-CM45;f__ | 0.0% | 0.0% |
| | k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;Other | 0.1% | 0.2% |
| | k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae | 4.7% | 1.8% |
| | k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae | 0.4% | 0.6% |
| | k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;Other | 0.0% | 0.0% |
| | k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae | 0.0% | 0.0% |
| | k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Isosphaeraceae | 0.1% | 0.3% |
| | k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae | 0.9% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bartonellaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae | 48.2% | 96.4% |
| | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae | 32.5% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Methylophilales;f__Methylophilaceae | 0.0% | 0.1% |
| | k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Neisseriales;f__Neisseriaceae | 0.1% | 0.1% |
| | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;Other;Other | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Aeromonadaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae | 0.0% | 0.1% |
| | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae | 0.3% | 0.0% |
| | k__Bacteria;p__[Thermi];c__Deinococci;o__Deinococcales;f__Deinococcaceae | 0.0% | 0.0% |

Appendix 1: Family composition of bacteria from *C. felis*

Table 3: Composition of bacteria at the genus level

| Numbers | Genus | Percentage |
|---|---|---|
| 1 | Corynebacterium | 0.1 |
| 2 | Bacillales | 0.2 |
| 3 | Plannococcaceae-genus unknown | 1.8 |
| 4 | Staphylococcus | 0.8 |
| 5 | Isosphaeraceae genus unknown | 0.3 |
| 6 | Rickettsiaceae genus unknown | 0.5 |
| 7 | Rickettsia | 1.5 |
| 8 | Wolbachia | 94.4 |
| 9 | Methylophilaceae genus unknown | 0.1 |
| 10 | Neisseriaceae genus unknown | 0.1 |
| 11 | Acinetobacter | 0.1 |

Complete composition is shown in Appendix 2

| Legend | Taxonomy | Total % | RN % |
|---|---|---|---|
| | Unassigned;Other;Other;Other;Other;Other | 0.1% | 0.2% |
| | k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobacterium | 0.0% | 0.0% |
| | k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__iii1-15;f__;g__ | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinopolysporaceae;Other | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Corynebacteriaceae;g__Corynebacterium | 12.3% | 0.1% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Dermatophilaceae;Other | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae;g__ | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__ | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Kocuria | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Rothia | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__ | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae;g__Saccharopolyspora | 0.0% | 0.0% |
| | k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Collinsella | 0.0% | 0.0% |
| | k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae];g__Cloacibacterium | 0.0% | 0.0% |
| | k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__;f__;g__ | 0.0% | 0.0% |
| | k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__JG30-KF-CM45;f__;g__ | 0.0% | 0.0% |
| | k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;Other;Other | 0.1% | 0.2% |
| | k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus | 0.0% | 0.0% |
| | k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;Other | 0.0% | 0.0% |
| | k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__ | 4.7% | 1.8% |
| | k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Macrococcus | 0.0% | 0.0% |
| | k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Salinicoccus | 0.0% | 0.0% |
| | k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Staphylococcus | 0.4% | 0.6% |
| | k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;Other;Other | 0.0% | 0.0% |
| | k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__ | 0.0% | 0.0% |
| | k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__ | 0.0% | 0.0% |
| | k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Isosphaeraceae;g__ | 0.1% | 0.3% |
| | k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;g__ | 0.0% | 0.0% |
| | k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__ | 0.9% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bartonellaceae;g__Bartonella | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__ | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__ | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae;Other | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae;g__ | 0.2% | 0.5% |
| | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae;g__Rickettsia | 0.8% | 1.5% |
| | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae;g__Wolbachia | 47.2% | 94.4% |
| | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Kaistobacter | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Burkholderia | 12.5% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Delftia | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Methylophilales;f__Methylophilaceae;g__ | 0.0% | 0.1% |
| | k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Neisseriales;f__Neisseriaceae;g__ | 0.1% | 0.1% |
| | k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Neisseriales;f__Neisseriaceae;g__Neisseria | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;Other;Other;Other | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Aeromonadaceae;g__ | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;Other | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__ | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__Actinobacillus | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter | 0.0% | 0.1% |
| | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__ | 0.0% | 0.0% |
| | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas | 0.3% | 0.0% |
| | k__Bacteria;p__[Thermi];c__Deinococci;o__Deinococcales;f__Deinococcaceae;g__Deinococcus | 0.0% | 0.0% |

Appendix 2: Genus composition of bacteria from *C. felis*

Although the Wolbachia genus has the most total bacterial composition in *C. felis* (94.4%), there are bacteria with a composition >1% that have never been reported to be associated with *C. felis*. Furthermore, there are 0.2% of bacteria whose taxonomy has yet to be determined. The results of this study become a vital reference pathogenic bacteria that can be transmitted in humans and animals through *C. felis*. It is necessary to study the resistance of bacteria isolated from *C. felis* to antibiotics in the future.

This study confirms some bacteria associated with cat fleas such as Bartonella, Rickettsia and Wolbachia[25]. Rickettsia and Bartonella infections occur worldwide and may cause serious diseases in people[19,26,27]. Bacteria of the genera Staphylococcus and Streptococcus are found in cat fleas[28]. However, in this study, it was found that the composition of the genus Rickettsia bacteria was the bacteria with the largest composition. *Rickettsia asembonensis* and *R. felis* were reported to be human pathogens[29]. The dominance of the genus Rickettsiae in cat fleas was also reported by Douglas *et al.*[30], Billeter *et al.*[31], Roucher *et al.*[32]. The bacterial species found in *C. felis* in this study are medically relevant bacteria (MR) in humans, based on the definition of the International Statistical Classification of Diseases and Related Health Problems, WHO[33]. This study proposes a recent study of the bacterial composition of *C. felis* with a metagenomic approach. This is the basis for the study of bacteria that have the potential to infect humans in the future. However, further research is needed to identify bacteria in *C. felis* using marker genes other than 16S rRNA.

## CONCLUSION

The results of metagenomic analysis of bacteria from *C. felis* found many species of bacteria that conventional bacterial isolation methods can not isolate. Species of bacteria are found to be potentially infecting humans because they belong to medically relevant bacteria. The dominant bacterial genus in cat fleas is Wolbachia. Found a genus that has never been reported found in *C. felis*, where this genus has many species of pathogens in humans.

## SIGNIFICANCE STATEMENT

This study found the composition of the bacterial genus associated with *C. felis* which could be useful for the study of potential bacterial pathogens not only in cats but in humans in the future. This study will help researchers to uncover critical areas of metagenomic bacteria associated with *C. felis* that many other researchers have not explored. Thus, this study provides scientific information about the composition of *C. felis* bacteria through a metagenomic study approach.

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