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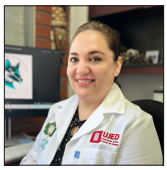
Capnocytophaga and *Neisseria* species in the Blood of Stray Dogs from Northern Mexico

Cristina García-De la Peña[1], Sergio Iván Barraza-Guerrero[2], Quetzaly Karmy Siller-Rodríguez[1], Luis Manuel Valenzuela-Núñez[1], Jesús Vásquez-Arroyo[3]

[1]Department of Conservation Medicine Laboratory, Biological Sciences Faculty, Juarez University of the State of Durango, Gomez Palacio, [2]Department of Safety and Hygiene, Faculty of Veterinary Medicine and Animal Zootechny, Antonio Narro Agrarian Autonomous University, Torreón, [3]Department of Postgraduate Studies and Research, Faculty of Chemical Sciences, Juarez University of the State of Durango, Gomez Palacio, Mexico.



***Corresponding author:**
Cristina García-De la Peña, Laboratorio de Medicina de la Conservación, Facultad de Ciencias Biológicas, Universidad Juárez del Estado de Durango, Gómez Palacio, Mexico.

cristina.garcia@ujed.mx

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ABSTRACT

Objectives: Knowledge of the diversity of bacteria that are potentially pathogenic to humans in domestic animals is important for public health. Dogs are the closest animal species to humans and carry some species of bacteria that can be transmitted by their bite. Blood is considered a tissue that can reflect an approximation of the general bacterial microbiome of an individual, including that of the oral cavity. In particular, some species of the genera *Capnocytophaga* and *Neisseria* have been documented as part of the oral microbiome of the canine mouth. There are reports that both genera cause mild or severe infections in people bitten by dogs. In Mexico, as in many developing countries, millions of stray dogs carry potentially zoonotic bacteria. Therefore, the present study aimed to determine the species of *Capnocytophaga* and *Neisseria* from blood samples of stray dogs in northern Mexico through next-generation sequencing.

Material and Methods: Deoxyribonucleic acid DNA was extracted from blood samples of 12 randomly selected dogs, the V3-V4 16S ribosomal ribonucleic acid (rRNA) region was amplified, and the Illumina NovaSeq platform was used for sequencing. The amplicon sequence variants (ASVs) sequences of both genera were compared to those in the National Center for Biotechnology Information database through the basic local alignment search tool, which considers 97% identity as the minimum percentage of identity to accept the species.

Results: The species identified were *Capnocytophaga cynodegmi*, *Capnocytophaga catalasegens*, *Capnocytophaga canimorsus, Neisseria canis*, *Neisseria perflava*, *Neisseria weixii*, *Neisseria weaveri*, *Neisseria dumasiana*, and *Neisseria zoodegmatis*.

Conclusion: Most of these species cause infections following dog bites, but others have only recently been described in hosts other than dogs. It is important to continue using sequencing technologies in animal and public health to increase information, prevention, diagnosis, and treatment.

Keywords: Next-generation sequencing, 16S rRNA, *Capnocytophaga canimorsus, Capnocytophaga cynodegmi, Neisseria canis, Neisseria zoodegmatis*

INTRODUCTION

Understanding the bacteria that inhabit the mouths of dogs is crucial for developing effective prevention and treatment strategies. This includes education on the importance of proper wound cleaning after a bite, as well as the appropriate prescription of antibiotics. In addition, the study of these bacteria allows for the development of vaccines or preventive treatments, which could be administered to people at greater risk of being bitten or to dogs to reduce the

bacterial load in their oral cavities. Recent studies have employed advanced molecular techniques to characterize oral bacterial communities in healthy dogs, revealing a more complex picture than was previously known.[1] The genus *Capnocytophaga* consists of gram-negative, facultative anaerobic bacteria known to be part of the oral microbiome of dogs, often colonizing the gingival sulcus and other mucosal surfaces.[2] However, the potential impact of these bacteria on the well-being of companion canines has been the subject of increasing research and clinical interest.[3] Human *Capnocytophaga* infections caused by dog bites have been reported in various parts of the world, primarily in regions where close contact with dogs is common and where health services allow for accurate diagnosis of bacterial infections. In the United States, the Centers for Disease Control and Prevention has documented cases across the country, especially in people with compromised immune systems, who are more susceptible to infection after dog bites.[4] In Europe, countries such as Germany, France, and the United Kingdom have reported *Capnocytophaga* infections following dog licking or bites.[5,6] In France, for example, a study conducted in several hospitals identified the presence of this bacterium in cases of serious infections in humans.[7] Infections have also been reported in Australia, where serious cases have been documented in people who have been bitten by dogs.[8] Therefore, this genus represents a significant public health concern due to its potential to cause severe infections, particularly in older and immunocompromised individuals.[9]

The genus *Neisseria* is a group of Gram-negative, aerobic, oxidase-positive bacteria that are known for their importance in human health and disease, particularly the well-known *N. gonorrhoeae* and *N. meningitidis*, which cause the sexually transmitted disease gonorrhea and severe, often fatal meningitis, respectively.[10,11] While the majority of *Neisseria* infections in humans are well documented, the presence and significance of this genus in animal populations have received less attention. Nonetheless, studies have reported the isolation of *Neisseria* from various animal species, including cattle, sheep, goats, and wildlife.[12-15] These findings suggest that *Neisseria* has a broader host range, raising concerns about its potential zoonotic implications. Dog bites, while generally not considered a significant risk factor for *Neisseria* infections, have been reported as a potential mode of transmission in a small number of cases. Therefore, the study of *Neisseria* in dogs may provide valuable insights into the epidemiology and transmission dynamics of these bacteria, as these animals often live in close proximity to humans and can act as reservoirs for zoonotic diseases.

The identification and characterization of *Capnocytophaga* and *Neisseria* species can be challenging. Conventional biochemical methods, as well as more advanced techniques such as real-time polymerase chain reaction (PCR), 16S rRNA gene sequencing, and whole-genome sequencing, have been employed to differentiate between the various species within these genera.[16,17] Blood is considered an enriched sample that can provide insight into an animal's oral, intestinal, and dermal microbiome because bacteria can translocate from organs into the bloodstream or enter through wounds;[18] therefore, this type of sample is preferable. In the present study, massive sequencing of the V3-V4 16S rRNA gene was used in blood samples from stray dogs in northern Mexico to identify *Capnocytophaga* and *Neisseria* species carried by these animals. This information will complement existing knowledge about the *Capnocytophaga* and *Neisseria* species that inhabit dogs, benefiting public health.

MATERIAL AND METHODS

In October 2023, blood was collected aseptically using 3 mL syringes (23 G) from the cephalic vein of 12 randomly selected and apparently healthy adult stray dogs in Comarca Lagunera, Mexico (25°34'45"N 103°26'49"W). Ten drops (50 mg of blood) were collected from each sampled dog and placed in BashingBead lysis tubes containing 750 µL of lysing/stabilizing solution (Zymo Research, Irvine, CA USA); then, the samples were processed in a Terralyzer (Zymo Research, Irvine, CA USA) cell disruptor. Deoxyribonucleic acid (DNA) extraction was performed through the ZymoBiomics DNA Miniprep Kit (Zymo Research, Irvine, CA USA) following the established protocol.

The samples were processed at Novogene Corporation, Inc. (Davis, CA USA), and V3–V4 16S rRNA amplicons were obtained through the 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT) primers. PCR reactions were carried out with 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 2 µM forward and 2 µM reverse primers, and approximately 10 ng of template DNA. Thermal cycling consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s. Finally, the samples were heated at 72°C for 5 min. The same volume of ×1 loading buffer [containing Synthetic, yellow, bromine (Sybr®) green] and PCR products were mixed for electrophoresis on a 2% agarose gel for detection. The PCR products were mixed in equal ratios. The mixed PCR products were subsequently purified with a Qiagen Gel Extraction Kit (Qiagen, Germany). The sequencing libraries were generated through a TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following the manufacturer's recommendations, and index codes were added. Library quality was assessed on a Qubit@ 2.0 fluorometer (Thermo Scientific, Waltham, MA USA) and an Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina NovaSeq platform, and 250 base pairs (bp) paired-end reads were generated.

Sequence analysis was performed in quantitative insights into microbial ecology 2 (QIIME2).[19] The divisive amplicon denoising algorithm 2 was used to remove low-quality sequences, filter chimeric/singleton sequences, and generate Amplicon Sequence Variants (ASVs).[20] The Greengenes2 database[21] was initially used to assign taxonomy. We then searched for *Capnocytophaga* and *Neisseria* species from the output taxonomic file. The ASV sequences of both genera were confirmed in the National Center for Biotechnology Information database using the basic local alignment search tool, considering an E value of 0.0 and a minimum identity percentage of 97% to accept the species.

RESULTS

The species of *Capnocytophaga* that was recorded from the blood samples was *Capnocytophaga cynodegmi*, *Capnocytophaga catalasegens*, and *Capnocytophaga canimorsus.* A total of six species of *Neisseria* were identified (*Neisseria canis*, *Neisseria perflava*, *Neisseria weixii*, *Neisseria weaveri*, *Neisseria dumasiana*, and *Neisseria zoodegmatis*). The total frequency of the sequences and the mean percentages of the bacterial species are shown in Table 1.

DISCUSSION

According to Iwai,[22] one of the main sources of enrichment of the blood microbiome is the oral microbiome; this enrichment is caused by the breakdown of epithelial cells or damage to gums. The oral cavity is then the route of entry of *Capnocytophaga* and *Neisseria* species into the blood of these dogs. Therefore, although the abundance of these bacteria is very low, it is important to consider their presence in terms of bites to the population. Two of them, *C. canimorsus* and *C. cynodegmi*, are particularly notable for their ability to infect humans through animal bites, scratches, or even licks, leading to potentially life-threatening systemic infections or death;[23,24] meanwhile, *C. catalasegens* was recently described as part of the oral microbiome of cats in Japan,[25] and their zoonotic potential has not yet been documented. On the other hand, epidemiological data on the incidence of *Neisseria* infections due to dog bites are limited, as these cases are relatively rare. However, the potential for these infections to occur, coupled with potentially serious consequences, underscores the importance of prompt recognition, appropriate treatment, and preventive measures. The clinical presentation and severity of these infections can vary depending on the specific *Neisseria* involved, the extent of the injury, and the host's immune response. The pathogenic potential of *N. canis* in humans is not fully understood, but it has been associated with a range of clinical manifestations, including local skin and soft-tissue infections, as well as more severe systemic infections.[26,27] Information on infections caused by *N. perflava* is scarce; it has been reported only in isolated cases of human endocarditis.[28,29] *N. weaveri* is part of the normal oropharyngeal microbiome of dogs,[30] but there are several reports of bacteremia and cellulitis in humans caused by the transmission of this species through dog bites.[31,32] On the other hand, *N. dumasiana* was recently described from the sputum of elderly people and the oral cavity of dogs.[33] *N. weixii* was recently described by Zhang *et al.*[34] from the rectal contents of the Tibetan Plateau pika (*Ochotona curzoniae*); there are no records that this bacterial species has the potential to cause infection. Finally, *N. zoodegmatis* is a common inhabitant of the oral cavity of dogs and cats but has been reported to cause infections in wounds caused by cat bites.[35-37]

In Mexico, as in many other countries in the world, available information on specific *Capnocytophaga* and *Neisseria* infections is limited, suggesting that cases may be infrequent or not systematically reported. Some factors that may influence the low frequency of reports are as follows: (1) limited diagnosis (identification of these infections

Table 1: *Capnocytophaga* and *Neisseria* species recorded in the blood of stray dogs in Comarca Lagunera, Mexico.

Bacterial species	TNS	%BM	No. Dogs	NCBI Accession	%ID
Capnocytophaga cynodegmi	377	0.248	1	NZ_CP022378.1	100
Capnocytophaga catalasegens	96	0.063	2	NZ_BQKB01000061.1	99.29
Capnocytophaga canimorsus	29	0.019	1	NZ_CP022382.1	99.76
Neisseria canis	181	0.119	3	NZ_LR134313.1	100
Neisseria perflava	47	0.030	1	NZ_JAMDHR010000063.1	99.77
Neisseria weixii	71	0.046	1	NZ_RPFM01000093.1	97.42
Neisseria weaveri	29	0.019	1	NZ_LR134533.1	99.77
Neisseria dumasiana	23	0.015	1	NZ_CP091509.1	100
Neisseria zoodegmatis	7	0.004	1	NZ_LT906434.1	99.53

TNS: Total number of sequences, %BM: Mean percentage of the bacterial species in the total microbiome, %ID: Percentage of identity according to NCBI, NCBI: National Center for biotechnology information, NZ: prefix for the accession numbers of genome assemblies that are deposited in the GenBank database and that are not completely finished genomes (draft or provisional quality).

requires specialized microbiological tests that may not be widely available in all healthcare facilities); (2) underreporting (there may be underreporting of cases due to lack of clinical suspicion, especially in patients without obvious risk factors, such as immunosuppression); and (3) epidemiological characteristics (*Capnocytophaga* and *Neisseria* infections are usually more common in people with weakened immune systems, so the prevalence of this condition in the general human population could influence the frequency of reports).

CONCLUSION

The impact of *Capnocytophaga* and *Neisseria* on public health is significant due to the morbidity they cause. Continued research is essential to address the challenges posed by these bacterial taxa. Blood offers the advantage of acting as a compiler of most of the bacteria that enter or inhabit the body of an animal; in the case of *Capnocytophaga* and *Neisseria*, the oral cavity of dogs. Hence, it is important to continue using sequencing technologies for public health concerning pathogen transmission from animals to increase information and develop better methods for prevention, diagnosis, and treatment.

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