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Molecular identification of *Capnocytophaga canimorsus* in canine oral samples

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Abstract

Fifty oral swabs (25 from Anbar and 25 from Salah Aldein) were collected from dogs of different ages, global strains, and different genders. *Capnocytophaga canimorsus* was diagnosed by PCR using the designed genes. The collected samples were first cultured on nutrient, blood, and MacConkey agar. Then the colonies were subcultured on brain heart infusion and blood agar supplemented with 2.5µg/ml trimethoprim and 2.5µg/ml amphotericin B. Gram staining and biochemical tests including catalase, oxidase and fermentation of glucose, maltose, sucrose, sorbitol and mannitol were performed. The isolate identification results showed that 22 strains (9 from Anbar and 13 from Salah Aldein) were likely *Capnocytophaga* spp. Action was taken. These putative Capnocytophaga spp. isolates. The PCR test was performed, and the results showed that 14 (6 from Anbar and eight from Salah Aldein) isolates were positive for the designed gene. In addition, the percentage results of *C. canimorsus* showed that 24% were from Anbar and 32% from Salah Aldein, while the other 36 dogs were negative for *C. canimorsus*, of which 19 (76%) were from Anbar, and 17 (68%) were from Salah Aldein. In conclusion, dogs are an essential carrier for *C. canimorsus* in Iraq.

Keywords: Dogs, *Capnocytophaga canimorsus*, PCR.

1. Introduction

Capnocytophaga canimorsus are commensal bacteria in the oral cavities of dogs, *C. canimorsus* may cause life-threatening infections in humans (Suominen *et al.*, 2024). Anaerobic bacteria is known as *capnocytophaga* typically infect dogs and may infect people via bites (Beernink *et al.*, 2016; Hess *et al.*, 2017). The bacteria cause septicemia and peripheral gangrene in humans despite adequate treatment (Brenner *et al.*, 1989; Suominen *et al.*, 2024). According to Pers et al. (1996), *C. canimorsus* was found in cats and dogs who had chronic sinusitis and rhinitis. Dogs' mouth secretions may spread infections to people. Infection is transmitted to humans through dog oral secretions, e.g., saliva, contact with the tongue and mouth, and *Capnocytophaga* infection—humans with immunocompromised patients (özavci and Kirkan, 2013).

Research has shown that macrophages taken from a patient who died of septicaemia did not secrete nitric oxide, tumour necrosis factor-alpha, interferon-gamma, interleukin-1, interleukin-6, macrophage inflammatory protein 1b, or interleukin-8. The capacity to react to C. canimorsus via Toll-like receptors 4 is a hallmark of prophytes (Shin et al., 2007). A combination of lipopolysaccharide (LPS) and a capsule effectively kills C. canimorsus (Shin et al., 2009). Evidence suggests that C. canimorsus consumes glycoproteins found on animal cell surfaces, such as macrophages (Mally et al., 2008; Manfredi et al., 2011).

In the lack of a proinflammatory response, Toll-like receptor (TLR) 4 was unable to react to Cc5. Also, according to Meyer et al. (2008), live Cc5 inhibited the release of TNF and NO caused by endotoxic lipopolysaccharide, downregulated the expression of TLR4, and dephosphorylated p38 mitogen-activated protein kinase. Human infections can only be diagnosed and treated with the help of Capnocytophaga, which was isolated (Martino et al., 2001; Sixou et al., 2006).

Microscopic features, colony morphology and biochemical tests are the main ideas for identifying *Capnocytophaga canimorsus*. (Van Samka *et al*., 2016). One member of the typical oral flora of cats and dogs is the gram-negative, facultative, slow-growing bacteria *Capnocytophaga canimorsus*, which has just been identified. It is crucial for public health and dog owners' well-being to determine the prevalence of this bacteria because of its pathogenicity in humans (Moradi *et al*., 2024). Most puppies PCR-positive *Capnocytophaga* spp. were born at 5 or 7 weeks (Roccaro & Peli, 2020). *C. canimorsus* is resistant to trimethoprim so it can be used in the isolation medium of this bacterium (Hawkey *et al*., 1987). In addition, the authors used amphotericin to isolate this bacterium and inhibit fungal infections (Erman *et al*., 2013). PCR reactions can be used to unambiguously determine the causative microorganism and increase the speed of diagnosis (Saravolatz *et al*., 2003; Beernink *et al*., 2016). Despite these encouraging findings, additional research is necessary to investigate other factors impacting the prevalence of *Capnocytophaga* spp adequately (CZEKAJ *et al*., 2024). This study aimed to isolate, identify, and molecularly characterize *Capnocytophaga canimorsus* in dog oral samples.

2. Materials and methods

Animals

Fifty pet dogs of both genders (25 from Anbar governorate and 25 from Salah Alden governorate) of different breeds and ages ranging from 2 months to 6 years were collected from February to April 2019. The animals were selected randomly and had a history of good hygienic housing.

Examination of animals

All animals were examined clinically for temperature, pulse, respiratory rate, and abnormal signs.

Collection of the Samples

Fifty oral swabs (rubbed in oral cavity wall) with transport media (nutrient broth) were collected and transported anaerobically to the College of Veterinary Medicine laboratories (Lab. Of clinical pathology) at the universities of Fallujah and Tikrit for diagnosis.

Bacteriological examination

The isolation and identification of bacteria in the samples were conducted according to (Markey *et al*., 2013); all of the swabs were cultured on MacConkey agar, blood agar, and

nutrient agar, and the temperature was incubated at 37°C for 48 hours in anaerobic conditions. Colonies were Gram-stained, and presumptive bacteria were cultivated on MacConkey, blood, and nutrient agar that were additionally coated with 2.5 µg/ml trimethoprim and 2.5 µg/ml amphotericin B as described in (Rummens *et al.*, 1985) and the further biochemical test was done including the fermentation of carbohydrates like glucose, maltose, sucrose, mannitol and sorbitol.

Molecular identification:

DNA extraction

The genomic DNA extraction was done using the Promega Genomic DNA Kit, and the extraction method was done according to (Hamzah *et al.*, 2020).

Molecular characterization by using PCR

The PCR assay was achieved in the Central laboratory at the University of Tikrit.

a. **Primers:**

Wahj Aldna company supplied the primer in Korea (Table 1).

Table (1) The primer with their sequence and the size of the product

Sequence of primer (5- to 3-)	bp
F TCA TTA ACC ACC CCC TGT GC	1014
R TGA CGT GGC TCC GAA TCA AA	

B. PCR mixture components for gene:
Two µL DNA+ 12.5 µL master mix+ two µL of each primer + 6.5 µL PCR water = 25 µL.

C. Thermo-cycler program: The program of thermo-cycle for the detection of genes as follows (35 cycles):
Initial denaturation= 95°C for 5 min
Denaturation= 95°C for 30s ,
Annealing= (57) C for 30s
Extension= 72°C for 30s
Final extension= 72°C for 7 min

D. Analysis of PCR product (Agarose Gel Electrophoresis 2%)

3. Results

After clinical examination, all dogs showed average temperature, pulse, and respiratory rate and appeared healthy.

***Capnocytophaga* spp. Isolation:**

The bacterial cultivation revealed yellow to orange colonies on nutrient, brain heart infusion, and blood agar supplemented with trimethoprim and amphotericin for all suspected isolates; they do not grow on MacConkey.

The isolated bacteria were seen under a light microscope as gram-negative bacilli. The results of the biochemical tests included catalase-negative and oxidase-negative results, as well as positive results for glucose, maltose, and sucrose fermentation. Also, they showed negative results

with mannitol and sorbitol. Twenty-two (9 from Anbar and 13 from Salah Aldein) isolates were suspected to be *Capnocytophaga* spp. (Fig. 1, 2).

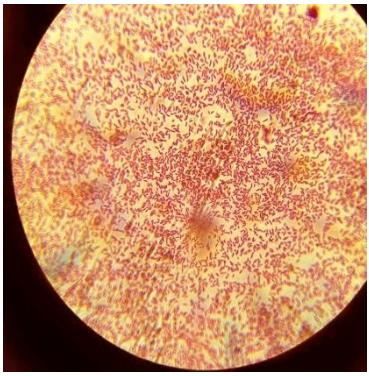

Figure 1. Gram stain

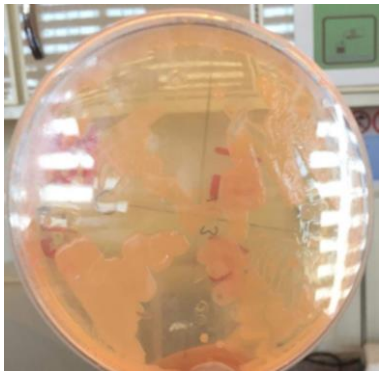

Figure 2. suspected *Capnocytophaga* spp. on brain heart infusion agar supplemented with trimethoprim and amphotericin

Molecular Characterization:

The results presented that the amplified PCR output was 1014bp (Fig. 3). The PCR assay results on 22 suspected *C. canimorsus* isolates revealed that fourteen isolates (6 from Anbar and eight from Salah Aldein) were positive for this designer gene.

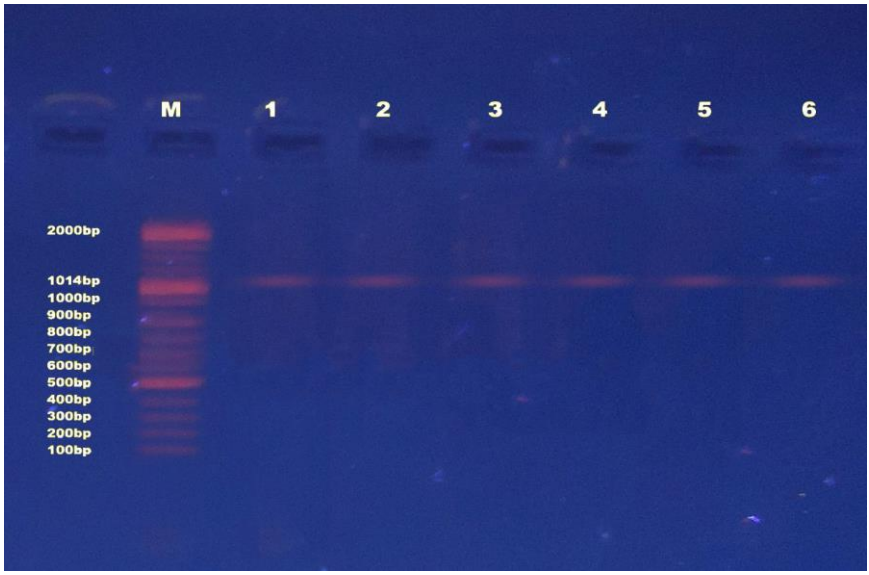

Figure 3. electrophoresis of (2%) agarose gel showing 1014bp of amplification fragments

Percentages of *C. canimorsus* in dogs:

The results showed that 14 {6 (24%) from Anbar and 8 (32%) from Salah Aldein} dogs carried the *C. canimorsus,* which PCR confirmed. In comparison, the other 36 dogs showed negative results for *C. canimorsus* (19 (76%) from Anbar and 17(68%) from Salah Aldein) (Table 2). Since the p-value (0.753) is greater than 0.05, there is no statistically significant difference in the positivity rates of *C. canimorsus* between Anbar and Salah Aldein (Table 3).

Table 2. Percentages of *C. canimorsus* in dogs

Province	Positive	Percentages	Negative	Percentages
Anbar (25 samples)	6	24%	19	76%
Salah Aldein (25 samples)	8	32%	17	68%
Total	14	28%	36	72%

Table 3. Statistical analysis of *C. canimorsus* in dogs

Province	Observed Positive	Observed Negative	Chi-square Value	p-value
Anbar	6	19	0.099	0.753
Salah Aldein	8	17		
Total	14	36	Chi^2=0.099	p=0.753

4. Discussion

The oral cavity of dogs and cats usually contains a community bacterium called *Capnocytophaga canimorsus*. Although this organism rarely causes infection, prompt diagnosis is crucial for the survival of these patients (Ahmad *et al.*, 2019).

The current results showed a yellow to orange color on nutrient and blood agar supplemented with trimethoprim and amphotericin. Also, the results showed a fermentation of glucose, maltose, and sucrose. These results were compatible with Socransky *et al.* (1979), who found that the colonies of *Capnocytophaga spp.* It appeared predominantly after incubation in 5 to 10% carbon dioxide or anaerobic glycolysis for 48 to 72 hours. Most organisms have an orange-yellow distinctive brown structure and smell; the slip is evident on some media. There is a big difference between the three types of carbohydrate reactions. All strains, including glucose, maltose, and sucrose, produce acids. Mannitol, sorbitol, ribose, and xylose have no acid production. Also, these results agree with the results of (Rummens *et al.*, 1985).

The presented results showed that the percentage of *Capnocytophaga canimorsus* in oral swabs of dogs was 28%. Several researchers confirmed the *C. canimorsus* in dogs, cats, and humans by using PCR. Currently, to determine the prevalence of *C. canimorsus* in dogs and cats, the bacteria was diagnosed by using the PCR method, and the prevalence of this bacteria was 74% of the sample in canine (out of 325 canines) sheltered *C. canimorsus* (Suzuki *et al.*, 2010). Van Dam et al. (2009) used the PCR to confirm the suspected isolates of dogs, cats, and humans taken from blood and oral swabs; they found that the *C. canimorsus* percentage was 25% in dogs; this result was in agreement with our results.

A swab of oral samples (200 samples) was taken from dogs to display the incidence of *Capnocytophaga* spp. and the risk of this to people's health. The results showed that *Capnocytophaga* spp. was identified from eleven (5.5%) of the tested 200 specimens; they concluded that *Capnocytophaga* spp. was a risk to human health, and it was difficult to diagnose these

agents due to a lack of information about the disease (özavci and Kirkan, 2013). Also, Blanche *et al.* (1998) reported that the results of culturing and PCR for *C. canimorsus* was 26% of the dogs that tested for the presence of this organism.

4. Conclusions

Capnocytophaga canimorsus is a severe danger to human health, and dogs can be regarded as a source for transmitting this bacterium to humans. In Iraq, this strain was isolated from the oral flora of dogs at a rate of 28%, so caution should be regarded when dealing with dogs, especially veterinarians and owners who were immunocompromised.

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Conflict of interests

The authors declare that they have no competing interests.

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