Acta Entomology and Zoology

E-ISSN: 2708-0021
P-ISSN: 2708-0013
www.actajournal.com
AEZ 2024; 5(2): 197-201
Received: 14-08-2024
Accepted: 20-09-2024

Amanee Mohammed Radhy
Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, Baghdad University, Iraq

Saba Thamer Mosa
Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, Baghdad University, Iraq

Naseir Mohammed Badawi
Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, Baghdad University, Iraq

Corresponding Author:
Amanee Mohammed Radhy
Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, Baghdad University, Iraq

Isolation of gram-negative bacteria from respiratory infections in dogs with molecular detection of predominant bacteria in Baghdad city

Amanee Mohammed Radhy, Saba Thamer Mosa and Naseir Mohammed Badawi

DOI: https://doi.org/10.33545/27080013.2024.v5.i2c.171

Abstract
The present study aimed to isolate and detect the Gram-negative bacteria isolated from the nasal cavity of dogs in Baghdad city (100 dogs) of both sexes and different ages (46 dogs less than year and 54 dogs more than year) from different breeds examined in veterinary clinics and Baghdad veterinary hospital during the period from November 2022 to March 2023. One hundred nasal swabs were collected from the infected dogs and then analyzed by conventional bacteriological methods, Vitek analysis, and molecular detection by conventional PCR technique of predominant bacteria. The results of the clinical study of the examined dogs revealed that the most prevalent signs in dogs were difficulty breathing and fever with loss of appetite, followed by rhinitis, sneezing, and coughing. The total isolation rate in dogs of various types of bacteria was 30% without significant differences among bacteria species. The results of the bacterial isolation and identification showed that *Psedomonas aeruginosa* was the most isolated species (12%) from the nasal cavity of dogs, followed by various types of bacteria: *Psedomonas luteola, Serratia ficaris, Enterobacter cloacae, Klebsiella pneumonia,* and *Leclercia adecarboxylata*. The infection rate with various species of isolated bacteria was not affected by the sex of the examined dogs; the total rate of isolation in dogs was 23.6% in males and 37.7% in females, without significant differences. The percentage of bacterial isolation with different types was highly recorded in dogs (39.1%) in the age group less than one year as compared with the age group more than one year (22.2%). The study concluded that highly prevalence of different types and species of Gram-negative bacteria associated with respiratory infection in dogs in Iraq.

Keywords: Gram, negative, bacteria, respiratory, dogs

Introduction
Canine species are among the most common household pets globally, and their potential for transmission of infectious disease is also well known. The respiratory infections acquired from pets need to be additionally highlighted. The respiratory tract infection in dogs and cats are highly contagious and easily speared, making the prevention the first and the most important step in avoiding the risks of further complications associated with the inflection [1]. The respiratory tract infection in dogs are complex conditions and caused by different types of viruses and bacteria, and typically initiated by stress-induced immune-suppression within the infected pets. The most prevalent bacteria isolated by other researchers from lung of cats and dogs with bacterial pneumonia was the enteric organisms including: *klebsiella* sp. and *Escherichia coli* as well as *Staphylococcus* sp. *Pasteurella* sp. *Bordetella bronchiseptica* [2]. In a study performed in Egypt by Khalifa *et al*. (2021) [3] the most isolated bacterial species from respiratory infections in diseased dogs was *Enterobacter cloacae* then *Escherichia coli, Klebsiella pneumonia, Citrobacter braakii and Serratia marcescens.* The study aimed to clinical study of respiratory infections in dogs with detection of Gram negative bacteria from nasal cavity of dogs as well as the molecular detection of the predominant bacteria in Baghdad city.

Materials and Methods
Animals: The study was conducted on 100 dogs that were received in Baghdad Veterinary Hospital/Baghdad City through November 2022 to March 2023. They were examined clinically before taking the nasal swabs, recording their temperature, pulse, respiration,

previous treatment, and the presence or absence of any abnormal respiratory symptoms, coughing, and nasal discharge.

Samples collection and bacterial identification: The sterile swabs were passed directly and deeply into the nasal cavity of the dogs then inputted in transport media until reached to laboratory. All the nasal swabs were cultured on nutrient broth containing nutrients for the activation of microbes to get a pure culture, swabs cultured on MacConkey agar then petri dishes incubated at 37 °C for 24 hours. If no suspected colonies were observed, plates were kept in incubation for 48 hours before being considered negative. Isolated microorganisms were identified by colony macroscopic characteristics and Gram staining [4]. Identification of isolates by using the VITEK (VITEKVR 2 system), with its accuracy and fully automated system, allows microbial analysis and the identification of Gram-negative bacteria [5].

Molecular assay: A molecular assay was done on the five isolates of *Psedomonas aeruginosa* by PCR after the DNA extraction of the bacteria using the genomic extraction kit of the gram-negative bacteria (Geneaid™, Tawin). Specific primers of the gyr b222 gene of *Psedomonas aeruginosa* were used for the molecular confirmation of this bacteria: F: CCT GAC CAT CCG TCG CCA CAA C, and R: CGC AGC AGG ATG CCG ACG CC [6] for amplifying the 222 bp fragment of this gene. Green Master Mix (Promega, USA) total volume 12.5 µl was used out of total volume 25 µl with 1 µl for each forward and reverse primers (10 picoml) and template DNA 3 µl, and completed the volume with 7.5 deionized distal water. The thermocycle protocols included initial denaturation at 94 °C for 5 minutes, 35 cycles of denaturation at 94 °C for 1 minute, annealing at 60 °C for 1 minute, extension at 72 °C for 1 minute, and final extension at 72 °C for 5 minutes. Gel electrophoresis of 1.2% with red safe stain was run at 80A and 120V for 30 minutes and documented under the blue light gel documentation system.

Statistical Analysis: The data for the present study were carried out statistically by SPSS version 20. The chi-square test and Odds ratio were achieved to compare the percentages of the study. The estimation was measured as statistically significant at significance of $p \leq 0.05$.

Results

The results of the clinical examination of 100 dogs revealed non-significant differences between the clinical signs as follows: the most predominant signs were difficulty breathing (40/100) (Figure 1), and fever with loss of appetite (22/100), followed by rhinitis (18/100), and sneezing and coughing were reported at equal numbers (14/100) (Table 1).

Table 1: The clinical signs appeared in 100 dogs of the study.

Clinical signs	Number Of infected dogs N (%)	OR (95%Cl)
Sneezing	14 (14%)	Ref=1
Rhinitis	18(18%)	0.88 (0.21-3.66)
Cough	14 (14%)	1.28 (0.30-5.42)
Difficulty in breathing	40 (40%)	0.70 (0.20-2.44)
Fever and loss appetite	22 (22%)	1.18 (0.31-4.42)

OR: Odds ratio, CI: Confidence interval

Fig 1: Showed dog suffering from difficulty in breathing and fever.

The total percentage of bacterial isolates for different types of bacteria on MacConkey agar (Figure 2), it was 30% in dogs without significant differences (Table 2). The results of the present study revealed that *Psedomonas aeruginosa* was the most isolated species (12%) from the nasal cavity of dogs, and the results revealed that 100% of *Psedomonas aeruginosa* in all 12 isolates of dog samples were positive for PCR using the gyr b222 gene of *Psedomonas aeruginosa* (Figure 3), followed by *Psedomonas luteola*, *Enterobacter cloacae*, and *Klebsiella pneumonia* at an equal percentage (4%). *Serratia ficaris*, *Sphingomonas paucimoblis*, and *Leclercia adecarboxylata* were the least isolated bacteria, with an isolation rate of 2%.

Table 2: Types of bacteria that isolated from dogs by Vitek analysis.

Type of isolated bacteria	No. of positive isolates in dogs (%)
Psedomonas aeruginosa	12 (12%)
Psedomonas luteola	4 (4%)
Serratia ficaris	2 (2%)
Enterobacter cloacae Complex	4(4%)
Sphingomonas paucimoblis	2 (2%)
Klebsiella pneumonia	4 (4%)
Leclercia adecarboxylata	2 (2%)
Total	30 (30%)
Chi-square value=7.86, P=0.85	

Fig 2: Showed the positive culture of Gram negative bacteria from nasal swabs on MacConkey agar.

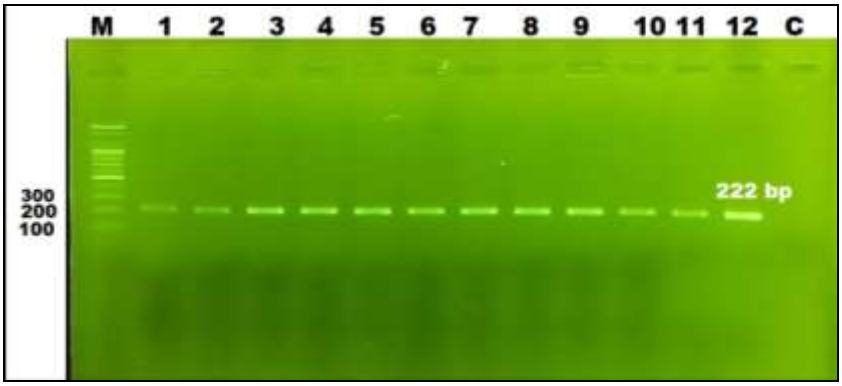

Fig 3: Amplification of 222 BP of small fragments of the GYR b222 gene of *Psedomonas aeruginosa*, M: DNA ladder, lane 1-12 the positive cases, lane 13: negative control, the gel electrophoresis 1.2 was loading with red safe stain on 100 V 80 A for 30 minutes.

The infection rate with different species of the isolated bacteria was not affected by the sex of the examined dogs (Table 3). The total isolation rate in dogs between males and females was 23.6% and 37.7%, respectively, without a significant variation. The effect of age on isolation rate is shown in Table (4). The highest percentage of bacteria isolation was recorded in dogs (39.1%) in the age group less than one year as compared with the age group more than one year (22.2%).

Table 3: The total isolation rates of bacteria in dogs according to the sex.

Sex of animals	Total no. of isolation / Total No.	Statistical values
Female	17/45 (37.7%)*	Odds ratio: 1.96
Male	13/55 (23.6%)	Confidence intervales 95%= (0.82-4.66)
*P value= 0.127, non-significant		

Table 4. Comparison of total isolation rates between dogs and cats according to the age.

Age of animals	Total no. of isolation / Total no.	Statistical values
Less than one year	18/46(39.1%)*	Odds ratio: 2.25
More than one year	12/54(22.2%)	Confidence intervales 95%= (0.94-5.99)
*P value= 0.069, non-significant		

Discussion

The present results of the clinical study revealed that the most predominant sign in dogs was difficulty breathing. This was contrary to the findings by other researchers, who recorded that nasal discharge (bloody, serous, greenish) and nasal ulcer were the most frequent signs in dogs and cats, in addition to other signs such as sneezing, conjunctivitis, and mild cough [3]. The results of bacterial isolation and identification have shown various types of gram-negative bacteria were isolated from the nasal cavity of dogs. At the same time, Lee ***et al***. (2021) [7] reported different species of bacteria from the upper respiratory tract, including *Enterobacter spp.*, *Streptococcus spp.*, *Psedomonas spp.*, *Serratia spp.*, *Micrococcus spp.*, and *Klebsiella spp.* In a study performed in America, the most prevalent bacteria isolated from the lung of dogs with pneumonia were enteric bacteria as *Klebsiella* sp. and *Escherichia coli* as well as *Staphylococcus* sp., *Pasteurella* sp., and *Bordetella*

bronchiseptica [2]. Also, Vientos-Plotts *et al.* (2019) [8] in America recorded different species of bacteria (negative and positive for Gram stain) in dogs infected with pneumonia. In a previous study in China, different types of samples were collected from various regions of dogs. Klebsiella pneumonia was highly isolated from urine and skin samples as compared with the other sites: nasal swabs, throat swabs, and tracheal lavage [9]. Li *et al*. (2020) [10] in the Iberian Peninsula isolated *Pseudomonas spp*. (16%) and *Escherichia coli* (8%) in dogs from different types of samples, including respiratory tract and concluded that *Pseudomonas aeruginosa* is an opportunistic pathogen in dogs that caused systemic and localized infections, this pathogen is commonly presented in the environment, and strong resistance to environmental conditions, and had potential to be transmitted between various mammalian species. The present findings demonstrated that all the isolates of *P. aeruginosa* detected by traditional methods were also confirmed using the PCR technique. In a study performed in Poland by Plokarz *et al*. (2022) [11] in dogs, the strain *P. aeruginosa* was isolated from the respiratory tract, skin and external auditory canal and the biofilm-forming strains associated with 90.6% *P. aeruginosa* in dogs; while the most virulence gene of this *P. aeruginosa* was ppyR at percentage 97.2%. While Moon *et al.* (2022) [12] in Korea recorded that the most isolates of respiratory system of the dogs by bacterial culture and confirmed by PCR, the results revealed that the most prevalent bacteria was *S. pseudintermedius*, then by *E. faecium*, *E. coli*, *R. nasimurium*, *K. pneumoniae*, *P. canis*, and *P. mirabilis*. The study disagrees with Meepoo *et al.* (2022) [13]. in Thailand, recorded high infection rates with *Psedomonas spp.* (32.1%), *Pasteurella spp*. (17.6%) and *E. coli* (8.4%) from chronic rhinitis cases in pets. This finding was in agreement with our results, which recorded high infection rates with *Psedomonas spp*. In a study done by Khalifa *et al.* (2021) [3], it was found that the common isolated bacteria with various species as a single or mixed infection also revealed a significant association between the older ages and longer course of the disease. In a study performed in Egypt by Khalifa *et al.* (2021) [3], *Enterobacter cloacae* was the most isolated species in diseased cats, followed by *Escherichia coli*, *Klebsiella oxytoca*, *Leclercia adecarboxylata*, *Pantoea sptica*, and *Pseudomonas aeruginosa.* While the most prevalent bacteria in diseased dogs was *Enterobacter cloacae,* then *Escherichia coli*, *Klebsiella pneumonia*, *Citrobacter braakii*, and *Serratia marcescens*. The results in the current study were different from other reports from the United Kingdom, South Africa, and Japan that reported that *Bordetela bronchiseptica* and *Pasteurella spp*. were the most main isolates [14, 15, 16]. The Guo and Lu (2020) [17] were able to isolate *Sphingomonas paucimotilis* from a cat with many abnormalities, including a wound at the base of the tail with purulent discharge, paraplegia, and hyperaesthesia. Whereas Cengiza *et al.* (2015) [18] isolated the same species from two cows, a calf and a lamb, with different clinical symptoms, including chronic pneumonia, lameness, and severe diarrhea. The bacterial infection is mainly affected by multiple factors in pet animals, such as ageing, secondary diseases, vaccination and management procedures, antibiotic treatment, and type of feeding [19]. Moon *et al.* (2022) [12] in Korea reported that the isolated bacteria species from respiratory samples of dogs showed differences in the infectious rate according the age groups; *P. aeruginosa* was most isolate in dogs less than one year but less prevalent in older age groups (6–10 years); *E. coli* infection rate showed a similar results in all age groups except in the dogs aged between 6–10 year; *Proteus mirabilis* was the most prevalent isolate in middle and old age of dogs; finally, *Klebsiella pneumonia* was high prevalent in the old age dogs when compared to the younger age dogs.

Conclusion

The highly prevalence of different types and species of Gram-negative bacteria in dogs in Iraq, especially *Psedomonas aeruginosa,* most occurrence in the young age of dogs.

Acknowledgement

The researchers are thankful to the College of Veterinary Medicine/University of Baghdad and Veterinary Bagdad Hospital for providing the facilities to conduct this study.

References

1. Weese JS, Giguere S, Guardabassi L, Morley PS, Papich M, Sykes JE. ACVIM consensus statement on the therapeutic antimicrobial use in animals and antimicrobial resistance. J Vet Intern Med. 2015;29(2):487-498.
2. Dear JD. Bacterial pneumonia in dogs and cats: An update. Vet Clin North Am Small Anim Pract. 2020;50(2):447-465.
3. Khalifa HO, Oreiby AF, Okanda T, Kato Y, Matsumoto T. High β-lactam resistance in Gram-negative bacteria associated with kennel cough and cat flu in Egypt. Sci Rep. 2021;11(1):3347.
4. Quinn PJ, Carter ME, Markey B, Carter JR. Clinical Veterinary Microbiology. 6th ed. London: Mosby Wolf; 2004. p. 130.
5. Markey B, Leonard F, Archambault M, Cullinane A, Maguire D. Clinical Veterinary Microbiology E-Book. 1st ed. Edinburgh: Elsevier Health Sciences; c2013. p. 375-86.
6. Lee CS, Wetzel K, Buckley T, Wozniak D, Lee J. Rapid and sensitive detection of *Pseudomonas aeruginosa* in chlorinated water and aerosols targeting gyrB gene using real-time PCR. J Appl Microbiol. 2011;111(4):893-903.
7. Lee K, Afiff U, Safika S, Sunartatie T. Antimicrobial sensitivity of most commonly isolated bacteria from feline upper respiratory infection. ARSHI Vet Lett. 2021;5(3):55-56.
8. Vientós-Plotts AI, Ericsson AC, Rindt H, Reinero CR. Respiratory dysbiosis in canine bacterial pneumonia: standard culture vs. microbiome sequencing. Front Vet Sci. 2019;6:354.
9. Zhang Z, Zhang L, Dai H, Zhang H, Song Y, An Q, *et al*. Multidrug-resistant *Klebsiella pneumoniae* complex from clinical dogs and cats in China: Molecular characteristics, phylogroups, and hypervirulence-associated determinants. Front Vet Sci. 2022, 9.
10. Li Y, Fernández R, Durán I, Molina-López RA, Darwich L. Antimicrobial resistance in bacteria isolated from cats and dogs from the Iberian Peninsula. Front Microbiol. 2021;11:621597.
11. Plokarz D, Czopowicz M, Bierowiec K, Rypula K. Virulence genes as markers for *Pseudomonas*

aeruginosa biofilm formation in dogs and cats. Animals. 2022;12:422.
12. Moon DC, Choi JH, Boby N, Kang HY, Kim SJ, Song H, *et al*. Bacterial prevalence in skin, urine, diarrheal stool, and respiratory samples from dogs. Microorganisms. 2022;10(8):1668.
13. Meepoo W, Jaroensong T, Pruksakorn C, Rattanasrisomporn J. Investigation of bacterial isolations and antimicrobial susceptibility of chronic rhinitis in cats. Animals. 2022;12(12):1572.
14. Morrissey I, Moyaert H, De Jong A, El Garch F, Klein U, Ludwig C, *et al*. Antimicrobial susceptibility monitoring of bacterial pathogens isolated from respiratory tract infections in dogs and cats across Europe: ComPath results. Vet Microbiol. 2016;191:44-51.
15. Qekwana DN, Naidoo V, Oguttu JW, Odoi A. Occurrence and predictors of bacterial respiratory tract infections and antimicrobial resistance among isolates from dogs presented with lower respiratory tract infections at a referral veterinary hospital in South Africa. Front Vet Sci. 2020;7:304.
16. Lavan R, Knesl O. Prevalence of canine infectious respiratory pathogens in asymptomatic dogs presented at US animal shelters. J Small Anim Pract. 2015;56:572-576.
17. Guo S, Lu D. Clinical presentation, diagnosis, treatment, and outcome of spinal epidural empyema in four cats (2010 to 2016). J Small Anim Pract. 2020;61(6):381-388.
18. Cengiza S, Seyitoglub S, Kılıç Altunb S, Dinlerb U. Detection of *Sphingomonas paucimobilis* infections in domestic animals by VITEK® Compaq 2 and polymerase chain reaction. Arch Med Vet Sci. 2015;47:117-119.
19. Greene CE, Decaro N. Infectious diseases of the dog and cat. 4th ed. St. Louis: W.B. Saunders, Elsevier; c2012. p. 67-74.