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# Detection and isolation of some *Pasteurella* spp. in dogs by using bronchial alveolar lavage and molecular techniques

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This study was conducted to detect and isolate *Pasteurella* spp. and to identify abnormalities in some blood traits, cellular changes, and some biochemical values detected in the respiratory tract of infected dogs using bronchial alveolar lavage (BAL) technique and polymerase chain reaction test to confirm infection. The study included a collection of BAL fluid by bronchial alveolar lavage technique from fifty-seven dogs of both sexes in which the signs of respiratory disease cough, dyspnea, sneezing, and nasal discharge were revealed during the period from September 2021 to December 2022. The clinical signs (respiratory rate, pulse rate, and body temperature) were various depending on the stage of the disease. Hematological and biochemical analysis results revealed significant differences between healthy and infected dogs. Compared to healthy dogs, the infected animals showed significant increases in the following parameters: total leukocytes, neutrophils, eosinophils, and lymphocytes (by 2.4, 1.9, 1.7, and 3.8 times, respectively, P≤0.01). Conversely, total protein levels tended to decrease, being 1.3 times lower (P≤0.01) than those in clinically healthy dogs. The BALF cytological study showed significant differences in the number of cytological cells among the infected and healthy dogs. Highly significant differences (P≤0.01) were found in the counts of neutrophils, eosinophils, lymphocytes, epithelial cells, and bacterial cells (respectively, by 3.5, 45, 2.8, 2.2, and 12.0 times higher compared to the healthy animals). With a total nucleated cell count of 490 cells, while in healthy dogs 188 cells were recorded, in the diseased dogs those were 2.6 times higher compared to the healthy animals. The results of bacteriological examination and molecular testing using PCR standard showed high effectiveness, allowing the isolation and identification of *Pasteurella canis* in 17 samples (29.82 %) and *Pasteurella stomatis* in 5 samples (8.77 %) of the diseased animals.

**Keywords:** bronchial alveolar lavage technique, PCR, *Pasteurella canis*, *Pasteurella stomatis*, dog.

# Виявлення та ідентифікація *Pasteurella* spp. у собак з використанням бронхоальвеолярного лаважу та молекулярного методу дослідження

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У роботі наведено результати досліджень щодо виявлення та ідентифікації *Pasteurella* spp. за респіраторних захворювань собак. Представлено дані щодо змін у показниках крові хворих тварин, висвітлено дані щодо цитологічного та протеїнового складу рідини, отриманої за допомогою техніки бронхоальвеолярного лаважу (БАЛ), а також результатів полімеразної ланцюгової реакції з метою встановлення видової належності збудників роду *Pasteurella*. Виконання досліджень проводили упродовж періоду з вересня 2021 року по грудень 2022 року. У цей період досліджено 57 собак різного віку та статі з ознаками респіраторних захворювань. Із загальної кількості досліджених тварин у 22 собак виявлено наявність збудників роду *Pasteurella*. Аналізуючи показники крові від тварин, що виявилися інфікованими *Pasteurella* spp., виявлено вірогідне (P≤0,01) збільшення як загальної кількості лейкоцитів (у 2,2 раза), так і окремих форм лейкоцитів. Зокрема, кількості нейтрофілів – у 1,9 раза (P≤0,01), еозинофілів – у 1,7 раза (P≤0,01) та лімфоцитів – у 3,8 раза (P≤0,01) порівняно з аналогічними показниками у клінічно здорових собак. На фоні виявлених змін у кількісному складі лейкоцитів у хворих собак встановлено протеїнемію, де вміст загального білку порівняно з клінічно здоровими тваринами був нижче у 1,3 раза (P≤0,01). Аналізуючи показники цитологічного дослідження рідини, отриманої за допомогою техніки БАЛ, встановлено значні відмінності в клітинному складі бронхоальвеолярної рідини клінічно здорових собак та собак інфікованих *Pasteurella* spp. Зокрема, в рідині хворих тварин виявлено вірогідно (P≤0,01) більшу кількість нейтрофілів, еозинофілів, лімфоцитів, епітеліальних та бактеріальних клітин (у 3,5, 45, 2,8, 2,2 та 12,0 раза) порівняно з показниками у клінічно здорових собак. Також в рідині, отриманій за допомогою БАЛ, у собак інфікованих *Pasteurella* spp. виявили збільшення кількості (у 2,6 раза, P≤0,01) клітин з ядром порівняно з клінічно здоровими собаками. Застосування бактеріологічного дослідження та молекулярного тестування з використанням ПЛР дали змогу виділити та ідентифікувати до виду *Pasteurella canis* (17 зразків / 29,82 %) та *Pasteurella stomatis* (5 зразків / 8,77 %).

**Ключові слова:** бронхоальвеолярний лаваж, ПЛР, *Pasteurella canis*, *Pasteurella stomatis*, собака.

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## Introduction

Large Gram-negative bacteria belong to the Pasteurellaceae family. Their members live commensally on mucosal surfaces of the upper respiratory tract in mammals [1]. Several members of Pasteurellaceae, like *Pasteurella canis, Pasteurella stomatis*, and *Pasteurella dagmatis* can be included in a wide range of disease conditions in dogs and may represent as primary or opportunistic pathogens [2].

*Pasteurella* spp can be spread among animals and humans. They have similarities with a number of species and sub-species, and naturally inhabit the oral cavity, skin and the digestive tract of the dog, and can cause diseases under specific conditions, and spread from dog to dog by sneezing or coughing and bite wounds when saliva enters open wounds, Infecting with *Pasteurella* can cause abscesses or septicemia, serious and fatal infections such as nasal and sinus infections, pneumonia and pus inside the chest cavity [3].

A wide variety of disease occurrence which involved from the secondary to viral infections or chronic rhinosinusitis which represent the upper respiratory tract infections and pyothorax, bronchopneumonia, otitis externa and bacteremia may occur due to the species *P. canis*, *P. dagmatis*, and *P. stomatis* and may be found as individual infection or mixed with other bacteria [4].

The technique of bronchial alveolar lavage (BAL) is used to collect samples to detect fluids and cell populations within the lower respiratory tracts that have allowed the identification and characterization of respiratory diseases and disorders in humans and animals. Therefore, sequential BAL was used to detect and evaluate pathological changes in the respiratory tract [5].

Structural changes in the bronchi occur when there is disease of the lower respiratory tract in dogs such as increased secretions, thick irregular mucosa, and alterations in a very low normal cell count [6].

BAL has been used to evaluate healthy status of the canine lower respiratory tract [7]. The veterinary referral centers use BAL technique widely, but there is little information available assessing the effect of diagnostic yield of bronchial alveolar lavage fluid (BALF) and BAL technique on sample quality [8].

The collection of cellular components of lung airway surface fluid by bronchial alveolar lavage (BAL) is a valuable method aimed at assessing differential cell profiles and is useful in diagnosing suspected clinical cases in veterinary medicine, and the biggest challenges facing veterinarians are determining the underlying cause of lower respiratory tract disease, although the results of the clinical examination and history also provide valuable information [9].

Models for sampling the respiratory tract include trans-thoracic aspiration, lung biopsies, trans-tracheal wash, bronchoscopy bronchial alveolar lavage, and bronchial brushings and are considered harsh techniques due to using a sterile catheter which is inserted into the trachea [10, 11].

Moreover, the use of a non-invasive method to collect samples from the respiratory tract and alveolar spaces such as the BAL provides a safe manner for microbial, biochemical, cytological, and immunological analyses [12].

In some cases, with no growth of pathogens and the absence of intracellular bacteria, an increase in neutrophils can be observed in the BAL fluid and this usually accompanies chronic bronchitis and may be associated with airway collapse and bronchiectasis [13].

Many reports recorded airway collapse in the presence or absence of the left atrial enlargement in dogs with airway inflammation [14].

The molecular technique is one of the most accurate methods to diagnose and confirm the *Pasteurella* infection; polymerase chain reaction assay is widely used for the identification of the most important species of Pasteurellaceae that may infect dogs. Primers designed for *Pasteurella* species like *P. canis* and *P. stomatis* were used by many researchers to identify and confirm the infection diagnosis. These species-specific PCR assays were shown to be providing an accurate and fast technique for phenotypic species detection of these bacteria [15].

In Iraq, there are many studies that deal with dog diseases [16–18], but more studies are needed to reveal respiratory diseases that pose a threat to canine and public health.

## The aim of the study

Therefore, this study was aimed at finding the prevalence of infection with some respiratory pathogens of *Pasteurella* species, conducting tests and detecting them.

## Materials and methods

*Animals*

Fifty-seven dogs of both sexes, between the ages of 1–4 years were examined in the study in Karbala province; the clinical cases brought to the special clinical room were examined. In all the cases the signs of respiratory infection such as coughing, sneezing, and running nose were registered. The study lasted from September 2021 to December 2022.

The BALF was collected from the dogs according to the clinical procedure previously described by [11]. The anesthesia (IM) with xylazine at a dose of (1 ml/kg) and ketamine at a dose of (5 ml/kg) was conducted in a standing position after which the dog received intravenous fluids, the nostrils and external were cleaned and the long catheter was passed into the nostrils.

A sterile catheter was gently introduced through the nostrils into the lower respiratory tract. A measured volume of sterile normal saline (0.9 % NaCl) was instilled and then immediately aspirated using a sterile suction device.

The recovered lavage fluid was transferred into sterile containers and transported to the laboratory in an ice box for further microbiological and molecular analysis.

Devices and materials used:

Sterile BAL catheter;

Sterile normal saline (0.9 %);

Medical suction apparatus;

Sterile sample collection tubes;

Veterinary sedative preparations and equipment.

*Bacteriological isolation and phenotypic identification*

BAL samples were cultured on blood agar and chocolate agar plates. The inoculated media were incubated aerobically at 37°C for 24–48 hours. Suspected colonies were examined for their morphological characteristics, including the size, shape, and hemolytic activity.

Gram staining was performed on representative colonies to observe cellular morphology and Gram reaction. The colonies showing characteristics consistent with *Pasteurella* species were further subjected to biochemical testing.

*Biochemical characterization*

Presumptive identification of *Pasteurella* spp. was carried out using standard biochemical tests, including oxidase, catalase, indole production, and carbohydrate fermentation tests. The obtained results were interpreted according to established bacteriological identification keys.

Blood samples were collected from the animals under aseptic conditions using sterile disposable syringes. Approximately 5 ml of venous blood was drawn from each dog. A portion of the sample was transferred into EDTA-containing tubes for hematological analysis, while the remaining blood was placed in plain tubes and allowed to clot at room temperature. The clotted samples were then centrifuged at 3,000 rpm for 10 minutes to obtain serum, which was used for biochemical assays.

Hematological parameters, including the total leukocyte count and differential leukocyte count, were measured using an automated hematology analyzer following the manufacturer's instructions. All the analyses were carried out under strict quality control procedures to ensure accuracy and reliability of the results.

*Bronchial alveolar lavage technique*

BALF was collected by inserting a flexible polyethylene tube, initially the end of which was sterilized with alcohol (70 %) and then gently inserted through the nose down to the lung and the presence of a cough reflex gave a good indication of the correct way to tube afterwards. Warm PBS was infused with a sterile syringe and then withdrawn with the same syringe and BALFs were collected in sterile beakers and sent to the laboratory under aseptic conditions.

Microscopic examination of the BALF was made immediately after the sample collection, and the volume of bubbles was immediately measured to indirectly assess the surfactant in each sample (***Table 1***) according to the surfactant score described by [8].

***Table 1***

Conducting surfactant evaluation for BAL

| Surfactant score | Surfactant |
|---|---|
| 0 | No bubbles |
| 1 | 0.10 to 0.70 ml of bubbles |
| 2 | 0.71 to 1.50 ml |
| 3 | >1.51 ml |

(200 µl) of BALF per sample was centrifuged at 180 × g for 6 min, and additional slides were prepared from centrifuged liquid at 500 × g for 5 min. A differential cell count of 400 leukocytes for each slide was performed after staining with Giemsa stain, and the presence of bacteria and epithelial cells was examined at 40x magnification. The loop full of BAL fluid was used for culturing, isolation, and identification of bacteria.

The smear of the differential cell counts and from BALF was examined under 100x magnification, the number of clusters, sheets of epithelial cells and cellularity were assessed and evaluated.

The 1 ml fresh 24-hour culture broth was used for DNA extraction (Genaid /Korea) according to manufacturing company. The polymerase chain reaction test was used to confirm *Pasturella canis* and *Pasturella stomatis* infections. Oligonucleotides sequences primers were performed for the detection of *sod A* genes primer of *Pasteurella canis*, Forward primer 5'GTAAATAATGCAAATGCGG3' and reverse primers 5'GCCTTGCAAAGTAGTAC3', and *Pasteurella stomatis* forward primers 5'CTCAGCAAATTATCGCTCGTC3' and reverse primers 5'TGCCCAGCCTGAACCGAAACGA3', which were used to detect the infection by these bacteria in the samples and the steps with these species specific primers were performed as described by [19] as the following steps: initial denaturation 94°C for 5 min. following by 35 cycles of denaturation 94°C for 30 sec. annealing at 50°C for 30sec. and extension 72°C for 120 sec. and final extension 72°Cfor 7 minutes. Statistical analyses of the study results were performed using the chi-square test, with $P<0.01$ as a criterion for significance [20].

*Ethic Statement*

The research was approved by the Ethics Committee of the University of Kerbala, College of Veterinary Medicine, under the number UOK.VET.ME.2022.061.

## Results and discussion

*Result of clinical study*

The study involved fifty-seven dogs that were examined, as a result of the culture of 57 BAL fluid samples from the dogs with abnormal respiratory symptoms. 17 samples (29.82 %) gave positive results to *Pasteurella canis* and 5 samples (8.77 %) gave positive results to *Pasteurella stomatis* (**Table 2**).

**Table 2**

The number of positive results for *Pasteurella* spp. in dog samples

| Number of examined cases | Number of positive results for *Pasteurella* spp. | Number of negative results for *Pasteurella* spp. | *Pasteurella canis* | *Pasteurella stomatis* |
|---|---|---|---|---|
| 57 | 22 (38.59 %) | 35 (61.40 %) | 17 (29.82%) | 5 (8.77%) |

*The result of hematology and biochemical values*

The hematology and total protein values showed significant differences between healthy and infected dogs, with considerable differences in total leukocyte count, neutrophils, lymphocytes, eosinophils, and total protein (***Table 3***).

**Table 3**

The results of the dogs' hematological study, mean

| Leukocyte values | Infected, n=22 | Healthy, n=35 |
|---|---|---|
| TLC, $10^9$/l | 19.4 [a] | 8.2 [b] |
| Neutrophil, cells/µl | 13065 [a] | 6883 [b] |
| Basophil, cells/µl | 44 [a] | 48 [a] |
| Eosinophil, cells/µl | 138 [a] | 118 [b] |
| Lymphocytes, cells/µl | 8034 [a] | 2117 [b] |
| Monocytes, cells/µl | 473 [a] | 417 [a] |
| Serum biochemistry | | |
| Total protein, g/l | 92 [a] | 70 [b] |

*Note*: different letters indicate a significant difference between the infected and healthy groups at P ≤ 0.01.

The diseased animals showed a significant increase in total white blood cell count, 2.4 times higher than that of clinically healthy dogs (P≤0.01). Also, in the group of infected dogs, a significant (P≤0.01) increase in the number of neutrophils, eosinophils and lymphocytes was found by – 1.9, 1.7 and 3.8 times, respectively, compared to the similar indicators in the group of clinically healthy dogs. It should be noted that the level of total protein in the blood serum of infected dogs, on the contrary, had the tendency to decrease. Thus, the level of total protein was 1.3 times lower (P≤0.01) than the indicator in clinically healthy dogs.

*The results of bronchial alveolar lavage cytology*

The results of the cytological study of bronchial alveolar lavage (BAL) fluid in the dogs revealed the presence of surfactant, sample cellularity, the presence of RBCs and epithelial cells which gave good indicators of BAL quality (***Table 4***).

**Table 4**

The results of the cytological study of bronchial alveolar lavage fluid in dogs, mean ± SEM

| BAL (differential count) | Infected, n=22 | Healthy, n=35 |
|---|---|---|
| Neutrophils | 18.2±2.0 [a] | 5.2±1.0 [b] |
| Eosinophils | 5.4±2.0 [a] | 1.2±1.0 [b] |
| Basophils | 0.2±0 [a] | 0 [a] |
| Lymphocytes | 50.0±6 [a] | 17.8±1.0 [b] |
| Monocytes | 0.6±0 [a] | 0 [a] |
| Epithelial cells | 37.0±5.0 [a] | 16.8±3.0 [b] |
| Bacteria | 9.6±0 [a] | 0.8±0.0 [b] |
| Cellularity | 12.0±5.0 [a] | 5.4±1.0 [b] |
| Total nucleated cell count (TNCC) | 490 [a] | 188 [b] |
| Total protein, g/l | 10.4±5.0 [a] | 5.1±2.0 [b] |
| Cytological score | 2 | 0 |

*Note*: different letters indicate a significant difference between the infected and healthy groups at P≤0.01.

After the sample collection, the volume of bubbles was immediately measured to indirectly assess the surfactant and the surfactant score of all the BAL samples.

The results revealed significant differences in the quantitative parameters of isolated cells among the infected and healthy dogs. Highly significant differences (P≤0.01) were found in the counts of neutrophils (3.5 times higher), eosinophils (45 times higher), lymphocytes (2.8 times higher), epithelial cells (2.2 times higher), and bacteria (12.0 times higher). A significant increase in total protein (2.6 times higher) (P≤0.01) was also recorded compared to the healthy animals. No significant differences were observed in the counts of basophils and monocytes.

The score of the BAL from the infected dogs was revealed at degree 2 with total nucleated cell count of 490 cells while in the healthy dogs there were 188 cells at 0 score.

*The results of bacteriological study*

After 48 hours of incubation at 37 Cº, the colonies of isolates appeared as the following ones: the colony of *P. canis* and *P. stomatis* on blood agar appeared as grayish-white, rough and small discrete tear shaped colonies. Other colonies were mucoid and large. There was no hemolysis on the blood agar and no growth on MacConkey agar.

After staining by Gram stain and methylene blue stain, the colonies appeared as Gram-negative and bipolar in shape in methylene blue stain, coccobacilli or short-rod single or in pairs.

The biochemical tests used for the differentiation between *Pasteurella canis* and *P. stomatis* revealed the same results, except that ornithine decarboxylase gave the positive reaction in *P. canis*, while it gave the negative reaction in *P. stomatis*, as shown in ***Table 5***.

**Table 5**

The results of biochemical reactions of *Pasteurella canis* and *Pasteurella stomatis*

| Test | *Pasteurella canis* | *Pasteurella stomatis* |
|---|---|---|
| Hemolysis on blood agar | – | – |
| Growth on MacConky agar | – | – |
| Indole | + | + |
| Urease | – | – |
| Catalase | + | + |
| Ornithine decarboxylase | + | – |
| Sugars fermentation | | |
| Glucose | + | + |
| Lactose | – | – |
| Sucrose | – | – |
| Maltose | – | – |
| Mannitol | – | – |
| Terihalose | V | – |

In the PCR technique (***Fig. 1***), an amplicon was observed from all the isolates in agarose gel electrophoresis.

In the conventional PCR technique, the dogs showed 17 (29.82 %) and 5 (8.77 %) cases of being infected with *Pasteurella canis* and *Pasteurella stomatis*, respectively. With 186 bp and 218 bp which revealed *P. canis* and *P. stomatis* respectively; there was no record that the two types were found in the same animal.

The dogs with respiratory disease showed such clinical signs as mild fever, lethargy, dyspnea, acute productive cough, chronic nasal discharge, stertorous breathing, hemoptysis, sneezing, and exercised intolerance [21].

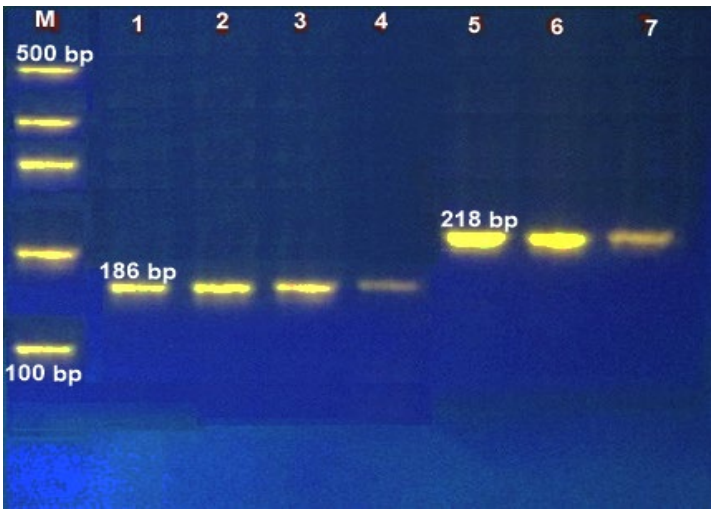


**Fig. 1.** The results of agarose gel electrophoresis of PCR product analysis on agarose gel. Line 1 is the M DNA marker; lines 1, 2, 3, and 4 represent 186 bp for *Pasteurella canis*; lines 5, 6, and 7 represent 218 bp for *Pasteurella stomatis*

In the previous study [22], the ocular and nasal discharge, sneezing, dyspnea, tachycardia were reported and depression developed in dogs within 1 to 4 days post infection, also the fever was uncommon but when present, it was transient. Besides, [23] mentioned that most infected dogs initially developed fever followed by mild clinical signs of respiratory disease (especially cough, nasal and ocular discharge, dyspnea and the elevation of heart rate).

The hematological and serum biochemical results in the present study revealed the agreement with the previous study [24], in which it was mentioned that the hematological values significantly increased total neutrophils, leucocyte and eosinophil's count in the dogs with respiratory diseases when compared to the healthy dogs.

It was reported that in healthy dogs the number of inflammatory cells encountered was fewer than those in the diseased dogs. The respiratory tract diseases due to bacterial infection lead to the increase in leukocytes [25]. Blood spotting has been reported at respiratory diseases accompanied by eosinophilia and leukocytosis [26, 27]. In hematological findings, neutrophilia of dogs infected with respiratory diseases is observed [28].

It was mentioned that eosinophilia occurring in dogs suffering from respiratory tract diseases might be due to the raised plasma histamine level [29]. In the present study, the results revealed significant differences in lymphocytes between the diseased and healthy dogs; these results disagreed with many researchers who mentioned that the hematological studies of dogs affected with respiratory disease showed no significant change in lymphocyte count, monocytes and hemoglobin concentration and significant increase in total eosinophils, neutrophils and leucocyte count of the blood [24].

In inflammatory responses to acute airway infection, lymphocytes play a major role at all stages and at all phases [30, 31].

In BALF sample obtained from the alveoli, the presence of foam is important for assessing the presence of surfactant [10]. The reduction of alveolar surface tension is caused by the action of type II pneumonia cells that secrete surfactant into the alveoli [32].

A simultaneous increase in the total number of neutrophils, lymphocytes, and macrophages occurred in airway fluid detected in BAL fluid that appeared markedly hypercellular pathogen identified by macrophages [33].

BAL lymphocytosis in dogs indicates the occurrence of a variety of pathological processes, suggesting that lymphocytes have an important role in the response to respiratory airway infection regardless of the pathogen [34].

In the surface epithelium of the respiratory tract, lymphocytes are involved in both immune responses to inhaled antigens and protective immunity against infectious agents [14].

In a study of bacterial pneumonia in dogs, the most common presentation was neutropenia, with BAL fluid neutrophils reaching 45 % and lymphocytes approaching 20 % of the differential cell counts [35]. The inflammatory pulmonary disease leads to a marked increase in the number of neutrophils in BALF [36].

The results of this study showed significant differences in the cellularity of BALF in healthy and diseased dogs. This occurs due to high percentage of lymphocytes, neutrophils, and other cells which lead to an increase in total cell count and increased cellularity score, which agrees with [8]. It has been mentioned that large numbers of epithelial cells present in the BALF may be caused by abnormal exfoliation, excessive suction, or trauma [21]. *P. canis* appeared as grayish-white, rough and small discrete tear shaped colonies of Gram-negative, coccobacilli, short-rod single or in pairs; *Pasteurella* spp. are aerobic, facultative anaerobic, and grow well at 37ºC on 5 percent sheep blood, and the growth is uncommon on MacConkey agar, the bipolar staining of *Pasteurella* species from bacterial or blood smears when stained by methylene blue [37, 38].

It was also mentioned that the isolates of *Pasteurella canis* gave the negative urease test. When ornithine decarboxylation test is positive, the acid is not produced

from L-arabinose, raffinose, D-lactose, maltose, mannitol, sorbitol, or dulcitol [37, 39].

The molecular test can also be applied in the specific PCR technique for the detection and diagnostics of *Pasteurella* spp. by using the template as a bacterial colony or by using direct clinical samples. A multiplex PCR is a highly susceptible and sensitive rapid technique used for the identification of capsular types of the conventional capsular serotyping system. The primers of specific serotypes used in the PCR technique were designed by the development of molecular tools and following sequence identification, the capsular biosynthetic analysis section of each capsular group [19, 40]. This technique has paved the way for rapid and accurate tests to detect infectious agents and has been conducted for the detection of *Pasteurella* spp. [41].

PCR is considered the fingerprinting technique and the present study uses this technique by utilizing primers specially designed for the identification of some species that infect dogs and confirm the diagnosis of *P. stomatis* and *P. canis* infection. Similarly, after the isolation and biochemical identification, the PCR has been used for the detection and diagnostics of those and other species in other studies by other researchers. This technique has been particularly designed for the identification of *Pasteurella* species in clinical samples and mixed cultures [19, 42].

It was mentioned that by using smears and lavage samples and examining respiratory tract samples of 18 pneumonic and septicemic dogs (the most commonly diagnosed conditions), the most frequent isolate from dogs was *P. canis* – 9, followed by *P. dagmatis* – 4 and *P. stomatis* – 2 [43].

Several species of *Pasteurella* such as *P. canis* and *P. dagmatis* have been reported to be transmitted to humans through animal bites [44].

It was revealed that the most common isolate by using bronchial alveolar lavage fluid from dogs was *Mycoplasma* spp. followed by *Pasteurella* spp., *Bordetella* spp, Enterobacteriaceae, and anaerobes [13].

## Conclusions

It is indicated in this study that BAL is an important clinical procedure for diagnosing respiratory diseases in dogs. It is also a technique that provides medical information about the cellular change in case of respiratory diseases in dogs and the detection of the prevalence of *Pasteurella canis* and *Pasteurella stomatis* among pet dogs in Karbala city, Iraq, diagnosed by clinical signs combined with BAL and polymerase chain reaction techniques. Cytological examination of bronchial alveolar fluid revealed highly significant differences ($P \leq 0.01$) in the counts of neutrophils, eosinophils, lymphocytes, epithelial cells, and bacterial cells (3.5, 45, 2.8, 2.2, and 12.0 times higher than in healthy animals). The total count of nucleated cells was 2.6 times higher than in healthy animals. A combination of bacteriological and molecular testing using PCR standard allowed the isolation and identification of *P. canis* in 29.82 % of samples and *P. stomatis* in 8.77 % of the samples from the diseased animals.


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## Conflict of interest

The author (s) state that there is no conflict of interest.

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