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# Molecular and hematological study in cats infected by *Pasteurella multocida* in Kerbala province

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***Abstract*—** The recently study has been conducted to detect *Pasteurella multocida* in cats in Kerbala province by molecular technique conventional PCR and to evaluate the alterations in hematological parameters in infected cats. A total of 200 cats from Kerbala privet clinical room in Kerbala province were clinically examined for respiratory signs or history of bite wounds. Throat, nasal, and skin wound swabs were collected for molecular detection, beside blood samples for hematological analysis. PCR targeting the kmt1 gene (460 bp) was used for *Pasteurella multocida* identification. Out of 200 samples, 32 (16%) tested positive for *Pasteurella multocida* :27/188 (14.3%) from respiratory cases and 5/12 (41.6%) from skin wound cases. Hematological findings in positive cases showed significant increases in RBCs, hemoglobin, hematocrit, MCHC, total leukocyte count, neutrophils, monocytes, and lymphocytes in both acute and chronic conditions compared to normal values, while MCV showed no significant difference. Platelet counts were markedly elevated in acute cases but decreased in chronic cases. These alterations reflect the systemic inflammatory response to infection. The study concludes that conventional PCR is a ideal diagnostic tool for diagnosis of *Pasteurella multocida*, and that infection causes notable hematological changes in cats.

***Keywords —*** *Pasteurella multocida*, PCR, hematology.

## INTRODUCTION

*Pasteurella multocida* is a common commensal or opportunistic pathogen and part of the natural flora of the oral cavity, nasopharynx and upper respiratory tract of cats (1,2,3,4). *Pasteurella multocida* is one of the most frequent pathogens in infected skin wounds, subcutaneous abscesses and also a common bacterium involved in respiratory tract infections (5).
Most pathogenic members of *Pasteurellaceae* reside on the mucus membranes of the respiratory, oral and genital tracts, in various species, including dogs, cats and ruminants. Transmission of *Pasteurella multocida* occurs through inhalation, scratches, bites or contact with carriers or infected animals (6).

(7) has been reported *Pasteurella multocida* was isolated from approximately 50% of dog bite wounds, 75% of cat bite wounds or scratches, and, less frequently, from licks.
A cat bite infection with P. multocida can lead to lymphangitis, cellulitis, peritonitis, abscesses and septic arthritis in humans (8).

In previous study have been showed *Pasteurella multocida* produces many virulence factors, such as lipopolysaccharide (LPS), PMT (P. multocida toxin), capsules and adhesins. According to their capsular polysaccharide antigens, isolates can be differentiated serologically into serogroups A, B, D, E and F (9).

In previous study to (10) have been showed the prevalence of P. *multocida* in cats was 29%. Among the isolates, the majority were capsular type A (96.5%) and type D (3.4%), with a predominant presence of type A.(11) were mentioned Molecular characterization of *Pasteurella multocida* by PCR analysis is a more sensitive and specific method for detecting and identifying of *Pasteurella m*ultocida.

In another hand they also found alterations in hematology, blood chemistry, oxidant-antioxidant status, proinflammatory cytokines, and APPs. These parameters are considered useful tools that could assist in clarifying the pathogenesis of the disease and inform efforts to improve diagnosis, prognosis, and efficient treatment of *Pasteurella multocida* infection (12).

## MATERIALS AND MTHODS

The study was included 200 cats from different breed, both sex and aged less 6 months to 4 years old brought to clinical rooms in the center of Kerbala governorate in the period from October (2025) to April (2026). 188 cats have been examined for the presence of abnormal respiratory signs while 12 cats were suffered from history of biting or scratching from another cats. The samples have been collected from infected cats, throat, nasal and skin wound swabs for molecular detection an another hand 10 ml of blood samples were collected from radial Vein, then samples divided equally 5 ml add to EDTA tube used to hematological study while 5 ml were added to plan tube for serum biochemical analysis, the analysis of blood and serum samples for hematology and biochemical study have been

conducted by Hb and Dry chemistry analyzer (Human. Germany) in central laboratory in Collage of veterinary medicine/Kerbala University. The study has ethical approval at number UOK.VET.ME.2026.181.

**DNA Extraction:**

Molecular methods included the extraction and amplification of *Pasturela multocida* DNA. Methods were used for extraction of DNA, by using commercial kits for DNA extraction from swabs (Genomic DNA Mini Kit).

**Primers**

Convential PCR assay has been done for detection of *Pasteurella multocida* by amplificated of universal primers of conserved region in (KMT-1) gene in *Pasteurella multocida* bacterium, it was designated by (13).

PCR products along with 1000 bp DNA ladder electrophoresed in 2 per cent

**Table 1.** Nucleotide sequences *Pasteurella multocida* primers

| Organism | Primer Sequence (5'-3') | Size of amplified product (bp) | Target gene |
|---|---|---|---|
| *Pasteurella multocida* | F (ATCCGCTATTTACCCAGTGG)<br>R (GCTGTAAACGAACTCGCCAC) | 460 pb | KMT1T7<br>KMT1SP6 |

The PCR master mix was prepared by using Accupower PCR Premix kit and done according to company instruction as the following:

| No | PCR mix | Volume (ul) |
|---|---|---|
| 1 | Genomic DNA | 5 ul |
| **2** | **Forward** primer 10 pmol | 1.5 ul |
| 3 | **Reverse** primer 10 pmol | 1.5 ul |
| 4 | PCR water | 12 ul |
| Total | | 20 ul |

| Step | Repeat cycle | Temperature | Time |
|---|---|---|---|
| Initial Denaturation | 1 | 95C° | 5 min |
| Denaturation | 30 | 95C° | 30sec |
| Annealing | | 55C° | 30sec |
| Extension | | 72C° | 50sec |
| Final Extension | 1 | 72C° | 5 min |
| Hold | 4C° forever | | |

The master mix reaction components were added to the standard PCR tube that containing the PCR Premix for *Pasteurella multocida*

The PCR product was analyzed by preparation of 1% of Agarose gel electrophoresis in 1x TBE buffer and 3ul of Ethidium bromide dye. Then the Agarose gel running into an electrophoresis chamber in 100v and 80AM for 1 hour (14).

## STATISTICAL ANALYSIS

The statistical analysis program Graph Pad Prism 8.0 has been applied $P \leq 0.05$ was chosen as the standard of significant. the data points were revealed as mean ±SD.

## RESULT AND DISCUSSION

**Result of polymerize chain reaction (PCR)**

The results of PCR on 200 sample from respiratory cases from throat, nasal and skin wounds swabs have been showed the amplification, which was performed on the DNA, extracted from all the studied isolates confirmed by electrophoresis analysis. By this analysis the strands of DNA which are resulted from the successful binding between specific primer for *Pasteurella multocida* , specific primers use to targeting a particular fragment of the kmt1 gene at 460 pb .These successful binding appear as single band under the U.V light using ethidium bromide as a specific DNA stain. The electrophoresis also used to estimate DNA weight depending on DNA marker (1000 bp DNA ladder) and the result of this estimation revealed that the amplified DNA of all *Pasteurella multocid*a isolates..

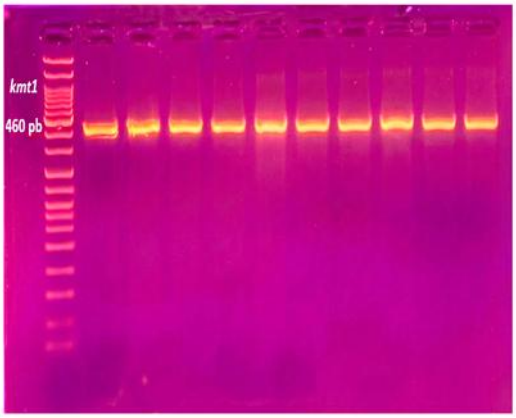


**Figure 1.** PCR amplification of (kmt1) of *Pasteurella multocida* DNA using PCR 1% agarose (460bp) (1-10) represent positive samples

*Pasteurella m*ultocida have been isolated by routine culture from cats suffering from respiratory illness ,188 case of respiratory cases ,27 cases (14.3%) were gave positive results while 161 (85.7%) cases were revealed as negative results.
In another hands 12 cases of skin scratching infection 5 cases (41.6%) have been showed as positive while 7 cases (58.4) were give negative for isolation of *Pasteurella multocida* as in table (2).

The total positive results of isolates of *Pasteurella multocida* from total 200 samples were 32 (16%) while negative 168 (84%) respectively as in table (3).

**Table 2.** Isolation of *Pasteurella m*ultocida from clinical cases of Pasturellosis in cats.

| Type of sample | No. | positive | Negative |
|---|---|---|---|
| Respiratory | 188 | 27 | 161 |
| Skin wound | 12 | 5 | 7 |
| Total | 200 | 32 | 168 |

**Table 3.** Percentage of Isolation of *Pasteurella multocida* from clinical cases of *Pasturellosis* in cats.

| No. samples | Positive | Negative |
|---|---|---|
| 200 | 32 (16%) | 168 (84%) |

**Result of hematology**

The results of red blood cell count have been showed increased the number of BRCs in acute and chronic cases with the presence of significant differences with the normal value while there is no significant between acute and chronic as in figure (2.A). in another hand the hemoglobin values were revealed raises in acute and chronic cases with the presence of significant differences among them. Hematocrit values appeared increased in acute and chronic when compared with normal value with presence of significant differences among them while no significant were showed between acute and chronic as in figure (2.C)

The results of MCV have been showed no significant differences among acute, chronic and normal as figure (2.D) while the values of MCHC were observed presence of significant differences among them as in figure (2.E).

As well as the results of platelets estimation were represented highly increased in acute cases with present of significant differences when compered normal values while the chronic cases showed decreases in platelets count with presence of significant differences with acute and normal values as in figure (2.F).

The results of total leukocytes count have been showed increased the total leukocytes between acute and chronic and acute and normal with the presence significant differences while there is no significant between chronic and normal value figure (3). As well as the differential leukocytes count have been revealed elevated the number of neutrophils monocytes and lymphocytes in acute and chronic cases compared with normal value with the presence significant differences among them while there is no significant between chronic and normal value figure (3). In another hand no significant values were occurred in eosinophils, basophils and monocytes as in figure (3).

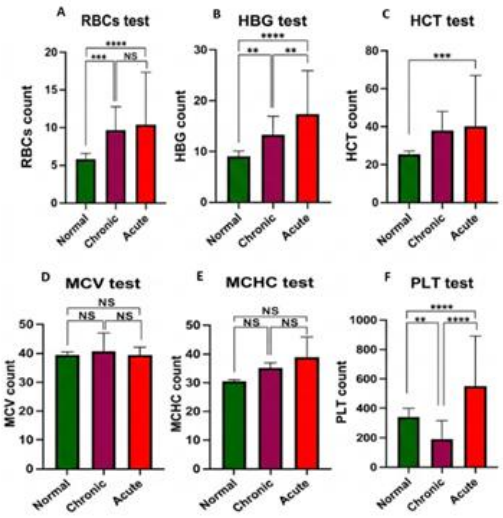


**Figure 2.** Alteration in hematology (erythrocytes and thrombocytes) in infected and non-infected cats with *Pasteurella multocida* Green bar: Normal group. Purple bar: Chronic condition. Red bar: Acute condition. Statistical Significance Indicators ****: p<0.0001,**: p<0.001,* p<0.01. NS: Not Significant

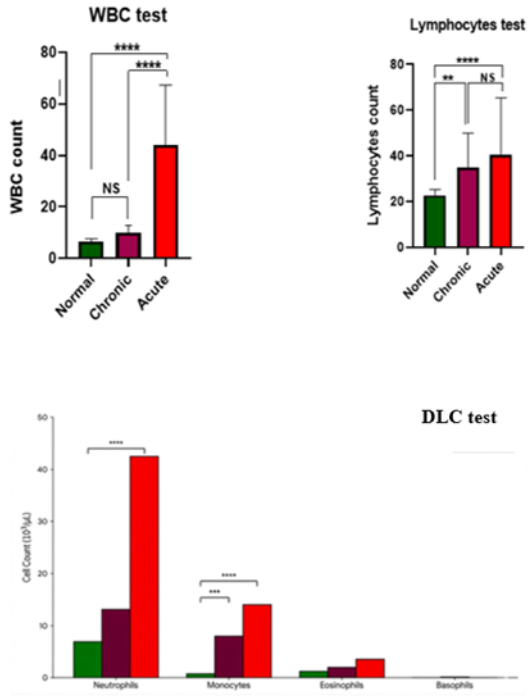


**Figure 3.** Alteration in hematology (leukocytes) in infected and non-infected cats with *Pasteurella multocida* Green bar: Normal group. Purple bar: Chronic condition. Red bar: Acute condition. Statistical Significance Indicators ****: p<0.0001,**: p<0.001,* p<0.01. NS: Not Significant

The results of recently study have been showed *Pasteurella multo*cida was detected by conventional PCR, the results of PCR on 200 sample from respiratory cases from throat, nasal and skin wounds swabs have been showed the amplification, which was performed on the DNA, extracted from all samples at 460pb confirmed by electrophoresis analysis these results were agree with previous study to (15) were mentioned polymerase chain reaction (PCR) assays targeting species-specific genes, such as kmt1, allow rapid confirmation of suspected isolates.

The specificity of the primer (kmt1) has been confirmed by positive amplification of the fragment with the extracted DNA of *Pasteurella m*ultocida isolates from rabbits at the band size of 460 bp (16, 17).

Bacterial identification by a species-specific polymerase chain reaction (PCR) using oligonucleotides designed for the unique (kmt1) gene is a fast and reliable method. Additional PCR and sequence-based technics can be used to differentiate strains, to investigate phylogenetic relationships, and to epidemiological research (18).

(19) have been showed the use of (kmt1) gene is batter to detected *Pasteurella multocida* from cats and human. A multiplex PCR reaction was used to confirm the species classification of the strains. With this method, we were able to simultaneously detect the species-specific gene (kmt1), the toxin gene, and the gene encoding capsular type A. (13,20,21). The results of our study have been showed significant increase of RBCs ,Hb , HCT and MCHC while MCV was not showed significant , these results might attributed to increase the demand of body to O2 during the acute stage of infection due to the *Pasteurella multocida* infected the respiratory system of the cat specially the throat region and causes stenosis or obstruction of air passages or infected the lower respiratory parts which lead to distraction of lung tissue this lead to hypoxia so that lead to activation of respiratory compensatory mechanism which lead to activation of erythroposis or splenic contraction to support the O2 deficiency.These results were supported by previous study to (22) where reported that intra-nasal inoculation of rabbit with *Pasteurella multocida* resulted into marked inflammation in respiratory tract leading to congestion, hemorrhages, edema, and infiltration of inflammatory cells with fibrin deposition resulting in to fibrino-suppurative bronchopneumonia.

Our results agreement with study to (15) were revealed that *Pasteurella m*ultocida infection in rabbits cause significant changes only in erythrocytes, hemoglobin, hematocrit and mean concentration of corpuscular hemoglobin.

(12) have been reported cattle infected with *Pasteurella multocida* exhibited a significant reduction in RBCs, Hb, PCV, MCV, MCH, and MCHC values compared with the apparently healthy animals that disagree with our results.

(9) study hematology and biochemistry responses towards *pasteurella multocida* Type B:2 via oral and subcutaneous route of infections were found the blood results obtained from buffaloes infected subcutaneously were having erythrocytosis and leukopenia, were study clinicopathological and Hematological Changes in Consequence to Experimental Infection of Rabbits with *Pasteurella Multocida* and they have been reported a non-significant difference between challenged and control animals in MCV while the MCHC, seem significantly high value in challenged when compared with control group (23).

The results of leukocytes alteration have been matched with (24) were reported the elevation of WBCs, mainly neutrophils and monocytes were observed in many diseases because of acute inflammatory response due to presence of bacterial infection. have been found neutrophil and monocytes count rises after *Pasteurella m*ultocida inoculation in rabbits also both the eosinophil count and basophil count showed slight decrease at the start of the infection (25). In another hands (26) were mentioned decrease in monocyte count in goat infected with pasturellosis. The rises of neutrophils in a cat infected with *Pasteurella m*ultocida represents regular acute cellular defense mechanism of the innate immune system against invasive Gram-negative pathogens (27), was revealed an acute *Pasturela multocida* infection in rabbits characteristically presents on a complete blood count (CBC) as a marked leukocytosis characterized by a significant neutrophilia (10). In our results the lymphocytes have been showed marked significant increase in acute and chronic cases due to the presence of lipopolysaccharides and surface membrane proteins make adaptive immune system activated with proliferation of clonal B-lymphocytes and T-helper cells within peripheral lymphoid tissues to produce reactive lymphocytes released to circulation, causes elevated absolute lymphocyte count. In chronic cases of pasturellosis specially wound bite the presence of abscess causes reactive lymphocytosis which attributed to active dermal and subdermal infiltrates of mature T- and B-lymphocytes mixed with degenerative neutrophils and macrophages were triggered by persistent, localized bacterial loads this agreement with (28).

The study has been concluded the PCR was considered the accurate diagnostic procedure to detection of *Pasturela multocida* also causes alteration in the hematological values in cats with specific infection by *Pasturela multocida* .

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**N/A**

**Conflict of Interest**

The authors declare no conflict of interest.

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