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Diagnostic Study of Pathogenic Bacterial Isolated from Camel's Pneumonic lungs Slaughtered in Al-Muthanna Province Abattoir, Iraq Using Vitek2 Compact

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ABSTRACT

The bacterial etiologies of pneumonia in camels represent a rising health concern, with camels being healthy during antemortem examination despite significant losses in production and mortality attributed to maladies identified only upon postmortem evaluation. A cross-sectional study was undertaken at an abattoir, spanning the period from October 2022 to March 2023, to examine gross pathological disorders and identify bacterial pathogens in the respiratory system (specifically the lungs) of camels (*Camelus dromedaries*) suspected of having pneumonia. A total of 100 dromedary camels slaughtered in Al-Muthanna abattoir were examined. Postmortem examination was performed to identify any gross changes in camels' lungs. Then, the swab samples were obtained from lungs and submitted to bacteriological cultures on both general and selective media. Additionally, microscopic examination of the bacteria using Gram staining followed by diagnosis with Vitek 2 system. The number of Gram-negative bacterial species isolated from camel lungs was 74 isolates, distributed as 31 (41.89%) of *Klebsiella pneumoniae*, 26 (35.13%) of *Escherichia coli* and 17 (22.97%) of *Pseudomonas aeruginosa*. This study suggests that *K. pneumoniae* exhibited a higher infection rate compared to *E. coli* and P. aeruginosa in pneumonic camels slaughtered at the Al-Muthanna abattoir.

Keywords: camels, pneumonia, *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumoniae*

INTRODUCTION

Camels are many sided animals that can pull out and burgeon in arid and semi-arid regions, where other livestock may not be able to beat (1, 2). They furnish diversified output and benefit, such as milk, meat, hides, and transportation, which are substantial for the livelihood and nourishment of the people in these towing. In Iraq, ranching camels is more than a mere seeding vigor; it is an imitative career passed down among descent. Award to neoteric statistics, the number of camels in Iraq has shown an upward orientation, altitude from 23413 heads in 2001, to 51703 heads in 2008, and then to 88282 heads in 2014 (3). Al-Muthanna province, situated in the southern part of Iraq, the third province in Iraq in terms of camel inhabitance, with 12.4% of the total camels in the country (4). However, camels are apt to various diseases that can inspire their output and benevolence, as well as pose a zoonotic risk to humans (5).

One of the most rife and withering diseases that vestige camels is pneumonia, which is a respiratory infection caused by assorted kinds of pathogens, such as bacteria, viruses, fungi, and parasites (6, 7). Amidst the bacterial pathogens, three Gram-negative species, namely *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*, are vastly spy out as the prime ground of pneumonia in

camels (8, 9). These bacteria can also cause nosocomial infections and have developed resistance to many antibiotics, making them difficult to treat and control (10-12).

Abattoir or slaughterhouses surveillance has been a critical role in the control and eradication of infectious disease programs worldwide (13). Despite the economic and social importance of camels, and the potential threat of pneumonia, there is a lack of research and development on camel health and management in Iraq, especially in Al-Muthanna province. Therefore, the aim of this study was to fill the existing knowledge gap by diagnosing the Gram-negative bacteria commonly attributed to pneumonia in camels using traditional bacteriological methods and the Vitek 2 automated system. The study also seeks to observe the associated pathological changes in the lungs of affected animals.

MATERIALS AND METHODS

Study Design and Ethical Approval

An abattoir-based cross-sectional study was conducted from October 2022 to March 2023 at the Al-Muthanna Veterinary Abattoir, Al-Muthanna, Iraq. This research sought to explore significant pathological abnormalities and identify bacterial pathogens present in the lungs of 100 suspected pneumonia-afflicted camels (*Camillus dromedaries*). The study received ethical approval from the local Animal Care and Use Committee at the College of Veterinary Medicine, University of Baghdad (Approval Number: 2390/P.G. dated October 31, 2022).

Sample Size

The samples size was determined using the StatCal feature of Epi Info™ software version 7.2.6.0. With an expected frequency of 50% (based on the absence of prior studies in Al-Muthanna province, as to the authors' best knowledge), a 10% margin of error, and a 95% confidence level, the minimum sample size was calculated as 96. To enhance the accuracy of the results, the study included 100 camels.

Sampling and Physical Examination

Camels were brought to the abattoir for routine slaughter, and those suspected of having pneumonia based on owner information (when available) and visible signs were selected. Detailed case histories, including age, breed, and sex, were recorded. Physical examinations were conducted antemortem and postmortem, focusing on clinical signs of pneumonia such as abnormal heart rate, increased temperature, and irregular respiration (14). Sampling for bacteriological examination was performed on 3–5 camels per abattoir visit, occurring three to four times a week.

Sample Collection

Lung swab and tissue samples were collected postmortem. The lung surface was sterilized by flaming before making an incision with a sterile blade. Samples from lesions or apparently affected areas were placed in sterile tubes containing nutrient broth (HiMedia, India) then transported in an icebox and subjected to bacteriological examination within two hours of collection (15).

Bacterial Culture and Identification

Collected lung swab samples were cultured on MacConkey's agar (HiMedia, India) and incubated at 37 °C for 24 h. Colonies indicative of bacterial growth were further cultured on blood agar for hemolysis assessment and on a CHROM orientation (HiMedia, India) agar and eosin methylene blue ([EMB], HiMedia, India) agar for selective isolation of *E. coli*, *P. aeruginosa*, and *K. pneumoniae* (15).

Microscopic and Vitek 2 Examination

Following bacterial culture, Gram-stained smears of the colonies were prepared and examined under an oil immersion lens (100×) at the Internal and Preventive Medicine Laboratory of the College of Veterinary Medicine, University of Baghdad.

The Gram-negative bacteria that were isolated by culture media and examined by Gram staining were further diagnosed using Vitek 2 system (BioMerieux, France) with specific Gram-negative cards. The cards contained 64 wells; each well represented a specific biochemical test (16).

Statistical Analysis

The Statistical Analysis System (SAS) program, as of 2018, was employed to analyze the impact of various factors on our study parameter. In this study, the Chi-squared (χ^2) statistical test was used to compare significance of results (0.05 probability).

RESULTS AND DISCUSSION

Description of the Sample Studied

The sample was categorized based on age and sex. In terms of sex, the studied cohort included 28 male camels and 72 female camels. Based on age, the animals were grouped as follows: 5 to 7 years, 23 camels; 8 to 10 years, 49 camels; and 11 to 12 years, 28 camels).

Physical Examination

The major clinical manifestations observed in pneumatic camels are presented in Table 1. The physical examination indicated that camels had irregular respiration and abnormal lung sounds due to pneumonia. The auscultation revealed moist crackles sound, in addition, the camels suffered from fever.

Table 1. Important clinical signs were documented in camels which were brought into Al-Muthanna abattoir

Clinical sign	Percent %
Fever	64
Nasal discharge	88
Eye lacrimation	67
Enlargement of lymph node	8
Coughing	39
Emaciated	23
Increase pulse rate	47
Rabid shallow respiration	47
Crackle sound	58
Clear loud sound	36
Grunting sound	6
P-value	0.049

Postmortem Examination

Postmortem examination of the lungs showed that there were areas of lung hepatization that were prominently firm and blunted end (Figure 1 A). Additionally, petechial hemorrhage and red consolidation were also observed (Figure 1 B).

The grossly inspection that was reported in this study is relatively convenient with (17), who found that postmortem examination of 24 camels from Al-Najaf and Al-Qadisiya had disclosed lungs with elastic texture, greyish, parts of consolidations and meaty cut-surface.

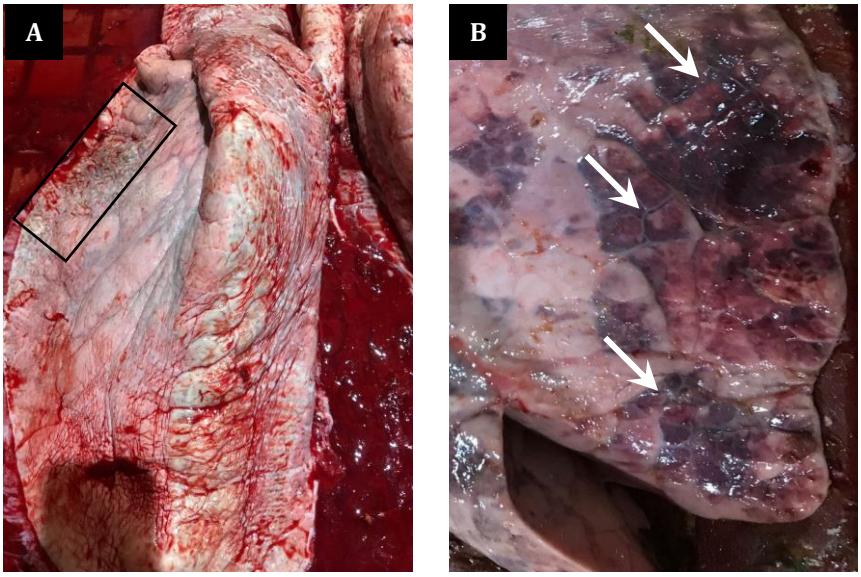

Figure 1. Postmortem examination photograph of the camel **(A)** right lung reveals pneumonic lesions with firm, blunted end (black sequare), **(B)** left lung petechial hemorrhage and consolidation (white errows)

Bacterial Culture

E. coli on MacConkey agar appeared pink to red owing to the presence of lactose fermentation. *E. coli* colonies typically appear small, round, and smooth. On the other hand, *E. coli* on blood agar exhibit gamma hemolysis (non-hemolytic). Eosin Methylene Blue (EMB) agar was used as selective media, the bacterium produced green metallic sheen (Figure 2A). The isolation of *E. coli* in this study accounted for 26 (35.13%), which relatively disagrees with the findings of (19) who reported (27.14%) from a total of 100 examined camels' lungs. In addition, (20) recorded a lower percentage of 10 (17.5%) *E. coli* from a total of 54 isolated bacterial species.

The findings showed the presence of *K. pneumoniae* on MacConkey agar. The bacterial colonies displayed a smooth, convex, and circular morphology with a moist, mucoid consistency. The colony sizes ranged from smaller to larger, and the growth displayed a pattern of expansion and contraction, as depicted in. On blood agar, *K. pneumoniae* exhibited a non-hemolytic appearance. Meanwhile, when isolated on CHROM agar, *K. pneumoniae* colonies appeared steel blue, as shown in (Figure 2B). The gram-negative bacterial isolate with the highest prevalence was *K. pneumoniae*, accounting for 31 cases (41.89%). This percentage is higher than the findings reported by (19) who identified a prevalence of 26.71% from total 97 of bacterial isolates. Furthermore, out of the total samples obtained from one hundred pneumonic camels in the abattoir, 31(41.89%) were *K. pneumoniae* isolates. This percentage is lower than that found by (21) who identified 47 (72.30%) isolates out of a total of 65 samples, 47 of which were camels diseased with pneumonia and 18 of which were healthy camels. On the other hand, our study found a higher percentage of isolates than (22) who recorded 4 of *K. pneumoniae* isolates out of a total of 100 Gram-negative bacterial isolates from pneumonic lungs.

In this study, *P. aeruginosa* appeared smooth and mucoid on MacConkey agar. On blood agar, P. aeruginosa appears to undergo β-Hemolysis and forms white colonies. On the other hand, P. aeruginosa forms blue-green colonies

under CHROM agar (Figure 2C). The number of *P. aeruginosa* isolates. The result shows 17 (22.97%) isolates out of a total one hundred pneumonic camels. This result disagrees with the findings of (23) who documented 6 (8.6%) isolates from a total of 70 examined samples collected from camel lungs. On the other hand, the incidence of *P. aeruginosa* was 17 (22.97%), which is higher than the findings of (24) who reported 10 (7.81%) out of total Gram-negative bacterial isolates.

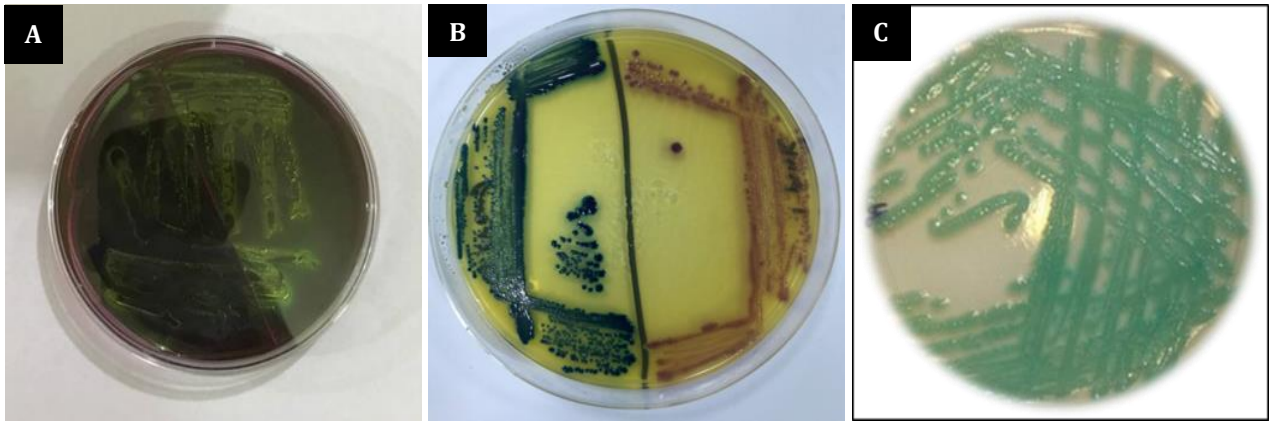

Figure 2. (A) *E. coli* on Eosin Methylene Blue (EMB) agar. **(B)** *K. pneumoniae* on CHROM agar orientation. **(C)** *P. aeruginosa* on CHROM agar orientation

Conformation of Bacteria by Vitek 2 system

The number of Gram-negative bacteria as confirmed by Vitek 2 are shown in Table 2.

Table 2. Gram-negative bacterial isolates by using Vitck 2 System

Bacterial spp	Bacterial Isolates		Probability (100%)
	Number	%	
K. pneumonia	31	41.89	99
E. coli	26	35.13	99
P. *aeruginosa*	17	22.97	99
Total	74	100	
χ^2	4.661		
P-value	0.049		

The results were represented as 41.89 % for *K. pneumoniae*, 35.13% of *E. coli* and 22.97% for *P. aeruginosa* from total 74 Gram-negative bacterial isolates. *P. aeruginosa* represented 22.97% of a total of 74 Gram-negative bacterial isolates; similar percentage was noted by (25) who reported 22% from total 100 samples of pneumonic camels were examined by Vitek 2 system technique. On the other hand, *K. pneumoniae* recorded 31(41.89%) from total 74 Gram-negative bacterial isolates examined by Vitek2 system. The results are not compatible with (7) who reported 3 (0.42%) from total 52 Gram-negative bacterial isolates diagnosed with Vitek2 system. Meanwhile, *E. coli* diagnosed by Vitek2 system technique was 26 (35.13%), lower number recorded by (26) who recorded 17 (11.3%) of *E. coli* isolates diagnosed by Vitek2 system in camels. The differences between results of this study and other studies may be associated with several factors; one important factor is the quality of the specimen collected for analysis. Adequate cultivation and incubation, transportation, and storage were essential for optimal test results. Moreover, it is essential that the specimens are collected using a standardized protocol to reduce variability (27).

Percentage of Infection

In this study, significant differences were found between male and female camels in terms of positive for Gram-negative bacterial infection (Table 3). The number of male camels that were infected with pneumonia is relatively consistent with the study by (18) which reported 29 males out of a total of 150 examined camels. On the other hand, the number of infected female camels was higher than the (18) study which recorded 16 females out of a total of 150 camels. The statistical analysis showed that there were non-significant differences between the groups of ages used in this study. These results disagree with (18) who reported a significant difference at (P<0.05) between the groups of ages that were recorded.

Table 3. The distribution of pneumonic camels recording age and sex in Al-Muthanna abattoir

Category	Subcategory	Number	%	χ^2	*P*-value
Sex	Male	21	28.38	13.84	<0.001
	Female	53	71.62		
Age (year)	5-7	19	25.67		
	8-10	32	43.24	3.638	0.162
	11-12	23	31.08		

This study suggests that *K. pneumoniae* showed a higher rate of infection than *E. coli* and *P. aeruginosa* isolated from camels that suffered from pneumonia slaughtered in Al-Muthanna abattoir. The study recommended the necessity

of conducting research studies on camels and their diseases in Iraq. In addition, the use of the Vitek2 system technique in the investigation rate of the infection.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Abdul-Rahaman YT, Mnati AA, Mohammed WM. Effect of different gestation periods on some physiological aspects of Iraqi female dromedary camel (*Camelus dromedarius*). Iraqi J. Agric. Sci. 2018; 49(4): 694. http://dx.doi.org/10.36103/ijas.v49i4.80
2. Sokkar SM, Ahmed KA, Samir A, Sultan AA. Pathological studies on lung mycoplasmosis in camels. Egypt. J. Comp. Path Clinic Path. 2014;27(1):46-54.
3. Sabri WK. Studying and Evaluation The Reality of Camelids Breeding in Al Muthanna Province. Adv. Anim. Vet. Sci. 2018;6(8):332-6. http://dx.doi.org/10.17582/journal.aavs/2018/6.8.332.336
4. Ayied AY, Al-Badran AI, Al-Zaalan AR. Detection of Intestinal Protozoa in Camels and their Breeders in Najef, Iraq. Adv Anim Vet Sci. 2018;6(7):273-277. http://dx.doi.org/10.14737/journal.rjvp/2015/3.3.53.57
5. Khalafalla AI. Zoonotic diseases transmitted from the camels. Front. Vet. Sci. 2023:10(12):44-833. http://dx.doi.org/10.3389/fvets.2023.1244833
6. Ismail ZB. Pneumonia in dromedary camels (*Camelus dromedarius*): a review of clinico-pathological and etiological characteristics. J. Camel Pract. Res. 2017;24(1):49-54. https://doi.org/10.5958/2277-8934.2017.00007.8
7. Ahmed MA, Musa MT, Mohammed AE. Bacteria associated with pneumonia in camels (*Camelus dromedarius*) in the Sudan and sensitivity of some isolates to antibiotics using Vitek 2 Compact. Glob. J. Sci. Front. Res. C Biol. Sci. 2015:2:1102-1105.
8. Ibrahim ZI. Pathological findings of respiratory diseases in one hump camel (*Camelus dromedaries*) in Wasit governate. Al-Qadisiyah J. Vet. Med. Sci. 2022;21(2).
9. Mohammed YJ, Mustafa JY, Abdullah AR. Isolation and molecular study of some bacterial urinary tract infections of sheep in Basrah province. Iraqi J. Agric. Sci. 2020;51(3):885-893. http://dx.doi.org/10.36103/ijas.v51i3.1043
10. Tawfiq SM. Bacteriologigal and Genetic study of Pseudomonas aeruginosa isolates. Iraqi J. Agric. Sci. 2018;49(1). http://dx.doi.org/10.36103/ijas.v49i1.201
11. Qasim DA. Molecular detection of Pseudomonas aeruginosa isolated from minced meat and studies the pyocyanin effectiveness on pathogenic bacteria. Iraqi J. Agric. Sci. 2019;50(4). http://dx.doi.org/10.36103/ijas.v50i4.764
12. Aziz TA, Lafta IJ. Isolation and antimicrobial resistance of Staphylococcus spp., enteric bacteria and Pseudomonas spp.associated with respiratory tract infections of sheep. Iraqi J. Vet. Sci. 2021;35:53-58. http://dx.doi.org/10.33899/ijvs.2021.131098.1917
13. Ahmed BA, Abdullah MA. Isolation and molecular diagnosis of the main bacterial species causing Pneumonia in small ruminants in the Duhok Abattoir-Kurdistan region of Iraq. mb.journals.ekb.eg. 2023;7(2):66-73. http://dx.doi.org/10.21608/MB.2023.193960.1069
14. Peter GG, Peter DC. Clinical Examination of Farm Animals. Clinical Examination of the Respiratory System. 7th ed. Malden, MA: Blackwell Science, Inc.; 2002
15. Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S, Fitzpatrick E. Veterinary microbiology and microbial disease. John Wiley & Sons. 2011.
16. David HP. Microbial identification using the BioMérieux VITEK® 2 System. Hazelwood, MO: BioMérieux, Inc.; 2009.
17. Hussain MH, Habasha FG, Mansour KA. Clinical, microbial, histopathological and molecular investigation of interstitial pneumonia in camels in Iraq. Al-Qadisiyah j. Vet. Med. Sci. 2018;17(1): 132-137. https://doi.org/10.29079/vol17iss1art488
18. Al-Hakak ZM. Measuring the Sedimentation Rate of Red Blood Cells in Infected Camels with Respiratory Disease like Pneumonia, Bronchitis and inflammation of Lung. J. Glob. Pharma Technol. 2017; 08(9):103-109.
19. Wareth G, Murugaiyan J, Khater DF, Moustafa SA. Subclinical pulmonary pathogenic infection in camels slaughtered in Cairo, Egypt. J. Infect. Dev. Ctries. 2014; 8(7): 909-913. 10.3855/jidc.4810.
20. Awol N, Ayelet G, Jenberie S, Gelaye E, Sisay T, Nigussie H. Bacteriological studies on pulmonary lesions of camel (*Camelus dromedarius*) slaughtered at Addis Ababa abattoir, Ethiopia. Afr. J. Microbiol. Res. 2011; 5(5):522-527.
21. Sharma SK, Mudgal NK, Sharma P, Shrngi BN. Comparison of phenotypic characteristics and virulence traits of Klebsiella pneumoniae obtained from pneumonic and healthy camels (*Camelus dromedarius*). Adv. Anim. Vet. Sci. 2015; 3(2): 116-122. 10.14737/journal.aavs/2015/3.2.116.122.
22. Elhilali AF. Isolation of Aerobic Bacteria from Pneumonic Lung of Camels. Doctoral dissertation, University of Khartoum. 2012.
23. Sharaf EM, El-Monem HM. Application of Electerophoresis and Immunoblot in diagnosis of Pseudomonas aeruginosa isolated from respiratory system of camels. Assiut Vet. Med. J. 2011; 57(130):258-267. 10.21608/AVMJ.2011.176876.
24. Moawad AA, Ibrahim HS, Elsherbiny MM. Characterization of Gram-Negative Bacteria Isolated from Lungs of Camels. Kafrelsheikh Vet. Med. J. 2011; 9(1):98-144. 10.21608/KVMJ.2011.113532
25. Abdelrahman AM, Mohamed SR, Soliman SM, Marouf S. Pseudomonas species isolated from camels: Phenotypic, genotypic and antimicrobial profile. Adv. Anim. Vet. Sci. 2022; 10(2): 219-225. 10.17582/journal.aavs/2022/10.2.219.225.
26. El-Ghareeb WR, Abdel-Raheem SM, Al-Marri TM, Alaql FA, Fayez MM. Isolation and identification of extended spectrum β-lactamases (ESBLs) Escherichia coli from minced camel meat in Eastern province, Saudi Arabia. Thai J. Vet. Med. 2020; 50(2): 155-161. 10.56808/2985-1130.3013.
27. Pincus DH. Microbial identification using the bioMérieux Vitek® 2 system. PDA J. Pharm. Sci. Technol. 2006; 1-32.

دراسة تشخيصية حول البكتيريا الممرضة المعزولة من الرئتين المصابتين بالالتهاب الرئوي للجمال والتي تم ذبحها في مسلخ محافظة المثنى/ العراق باستخدام جهاز Vitek2

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الخلاصة

تمثل المسببات البكتيرية للالتهاب الرئوي في الإبل مصدر قلق صحي متزايد، حيث تبدو الإبل صحية ظاهريًا أثناء الفحص ما قبل الذبح و على الرغم من الخسائر الكبيرة في الإنتاج والوفيات المنسوبة إلى الأمراض التي تم تحديدها فقط بعد تقييم ما بعد الذبح. أجريت دراسة مقطعية في المجزرة خلال الفترة من أكتوبر ٢٠٢٢ إلى مارس ٢٠٢٣ للتحري عن التغييرات المرضية الجسيمة وعزل مسببات الأمراض البكتيرية من الجهاز التنفسي (الرئتين) لـ ١٠٠ من الإبل المذبوحة المشتبه بإصابتها بالالتهاب الرئوي. تم فحص إجمالي ١٠٠ رأس من الإبل العربية المذبوحة في مسلخ محافظة المثنى. تم إجراء فحص ما بعد الذبح لتحديد أي تغيرات مرضية في رئتي الإبل. بعد ذلك، تم الحصول على عينات المسحة من الرئتين زرعها على الاوساط الرزعية البكتريولوجية على كل من الوسائط العامة والانتقائية. بالإضافة إلى الفحص المجهري للبكتيريا باستخدام صبغة جرام ومن ثم التشخيص بتقنية الفايتك. بلغ عدد العزلات البكتيرية سالبة الجرام المعزولة من رئات الإبل ٧٤ عزلة، توزعت بواقع ٣١ (٤١,٨٩٪) من الكلبسيلا الرئوية، و٢٦ (٣٥,١٣٪) من الإشريكية القولونية، و١٧ (٢٢,٩٧٪) من الزائفة الزنجارية. تشير هذه الدراسة إلى أن معدلات الإصابة بـ الكلبسيلا الرئويةأعلى من معدلات الإصابة بـ الاشريكية القولونية الزائفة الزنجارية المعزولة من الإبل الرئوية التي يتم ذبحها في مسلخ محافظة المثنى.

الكلمات المفاحية: الإبل، الالتهاب الرئوي، الزائفة الزنجارية، الإشريكية القولونية، الكلبسيلا الرئوية، نظام فايتيك