Research article

Clinical, microbial, histopathological and molecular investigation of interstitial pneumonia in camels in Iraq

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(Received 8/8/2017, Accepted 27/12/2017)

Abstract

*In this study, twenty-four camels in abattoirs of Al-Najaf and Al-Qadisiyah provinces were suspected to have interstitial bronchopneumonia. Clinical signs revealed protracted neck, misery, in appetite, heart rate was elevated (44 ± 0.29 bpm) and breathing was irregular and rapid (23 ± 0.27 bpm) and fever (39.2 ± 0.1). Moist crackles heard at auscultation. Transtracheal wash (TTW) were sampled for cytology and bacteriology to make the diagnosis; white blood cells count (WBCc) was (1420 ± 5.95 cells/ μ l) with neutrophilia (48%), total protein (TP) records (355 ± 4.29 mg/dl). Bacterial culture of the TTW revealed pure colonies on blood agar; which were recognized by the VITEK 2 compact device and confirmed using the conventional polymerase chain reaction (PCR) as *Klebsiella pneumoniae* ssp *pneumoniae*. Postmortem specimen gave three kinds of colonies: the same one in TTW, which was the causative pathogen, two others; *Staphylococcus lentus* and *St. vitulinus*; diagnosed biochemically by VITEK 2 compact. Histopathological dissections on postmortem samples found in the lungs of camels ranged only in 4-8 years old, discovered the presence of interstitial bronchopneumonia.*

Keywords: Camel, Pneumonia, Interstitial, *Klebsiella*, PCR

Introduction

The most common respiratory disease in camels is pneumonia, which is defined as an inflammation of the lungs. There are several systems for classifying the various kinds of pneumonia. One useful way is to classify according to the appearance or etiology of a particular pneumonia. (1). The causal bacterial agents of Camelids pneumonia are similar to those causing pneumonia in livestock and horses. Most infectious cases result from opportunistic bacteria. Septicemic animals usually develop pneumonia, and the most common agent isolated by (2) has been *Klebsiella*. (3) diagnosed two dromedaries with suppurative pneumonia caused by *Kl. pneumoniae* in India. (4) Had recorded camels with pneumonia caused by *Klebsiella*

ozaenae. (5) Uncovered six dromedaries with mixed pneumonia in which *Klebsiella* was one causative agent. Interstitial pneumonia caused by *Klebsiella* and *E. coli* was 58.6% prevalence with higher incidence in young camels 6 months to 4 years (2). *Klebsiella* was found in a study on diagnosis of some respiratory diseases in Camel calves (6). *Klebsiella pneumoniae* was detected from several cases within 232 camels with pulmonary lesions after slaughter in Nigeria (7). (8) Had isolated two cases of pneumonia in camels caused by *Klebsiella*. The collection and evaluation of tracheobronchial secretions is useful for assessing lower airway diseases. Although detection of these secretions is a very sensitive indicator of

pulmonary disease, cytological and bacteriological analysis is usually required to determine its etiology. Bacteriological evaluation of a TTW may provide useful information on antimicrobial sensitivity and aid in the selection of appropriate drugs. (9). Transtracheal wash collected using the percutaneous transtracheal technique is preferred for bacterial culture because these are not contaminated by oropharyngeal organisms. (10). Cytology can be a useful diagnostic tool. Inflammation, neoplasia and specific pathogens can be differentiated with

cytologic procedures. Ideally, cytology smears should be one cell layer thick to allow for adequate staining and visualization. (11). Complete Genome Sequence of *Kl. Pn. ssp. pneumoniae* was read by (12), Definition of *Kl. Pn. ssp. pneumoniae* 16S rRNA gene also made by (13). A Light Cycler real-time PCR hybridization probe-based assay which detects a partial *Kl. Pn. ssp. pneumoniae* 16S rRNA gene was developed for the rapid identification of *Kl. Pn. ssp. pneumoniae* directly from growth-positive blood culture (14).

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 421

Animals: One hundred fifty camels in abattoirs of Al-Najaf and Al-Qadisiyah Provinces were suspected to have pneumonia from which twenty four camels were diagnosed with interstitial bronchopneumonia.

Methods:

1- Physical examination:

In regard to respiratory system, an examination has been done on suspected animals; those suffering irregular respiration, depression and extended neck; include body condition, hydration, nares, body temperature, heart rate, breathing rate and rhythm, mucus membrane, lung sounds and palpable lymph nodes after taking the possible case history (15).

2- Sampling:

Transtracheal wash:- Adequately restrain animal with intramuscular injection of 0.25 ml/100kg xylazine was useful as a sedative while the local anesthetic drug was 2% lidocaine (16). The skin over the selected site (about 10 cm²) at the mid ventral aspect of the neck, where trachea can be grasped and the rings easily palpated, clipped and surgically prepared. Skin was penetrated with a stab blade and a trocar and cannula of suitable size (5 mm) was pushed firmly

between two tracheal rings ventricular to the long axis of trachea. The trocar is withdrawn to push the cannula down the tracheal lumen and imbed the catheter distally to the thoracic inlet. A 50 ml syringe filled with sterile, antibiotic-free and pre-warmed normal saline to be injected 1-3 times and immediately aspirated carrying the respiratory secretions from the lowest point of the trachea to be stored in the EDTA tubes at 4°C (11).

3- Laboratory Diagnosis

A- Total protein: Spectrophotometer (CT Chrome Tech) was used to find the total protein according to the protocol administrated by the kit (17). This method is accurate and the assay depends on the presence of amino acids which absorb UV light (18).

The respiratory secretions in the TTW are diluted with normal saline so it should be concentrated by getting rid of supernatant after centrifuge at 2500 rpm for 5 min in order to find the WBC count and differential WBC count. (19)

B- Smear of TTW:

Small drop of well-mixed TTW placed on a clean, grease-free slide, by an applicator stick or capillary tube. The greater the angle the thicker and shorter the TTW smear, and the smaller the angle the thinner and longer the smear.

Drying the film was quickly done; whenever possible TTW films were fixed and stained

immediately, otherwise they were fixed in absolute methanol for 3-5 minutes and then store in a clean box until they can be stained. Geimsa stain is the choice to be done by sinking the slide for 30-60 minute in the stain to be examined under oil immersion objective to see its contents (20).

C- White Blood Cells count (WBCc): Hemocytometer was used to enumerate total leukocytes according to (17).

D- Differential WBCc: Differential leukocytes were counted by TTW film. Best results were obtained if EDTA is used as the anticoagulants (17).

E- Bacteriological evaluation: Blood agar is the best choice for the cultivation of a variety of microorganisms but mycobacterium is well identified on Lowenstein-Jensen Medium (21).

F- Biochemical identification: The card of VITEK 2 Compact has 64 wells that contain an individual test substance. Substrates measure various metabolic activities such as acidification, alkalization, enzyme hydrolysis, and growth in the presence of

inhibitory substances. Cards have bar codes that contain information on product kind, lot number, expiration, and a unique identifier that can be linked to the sample either before or after loading the card onto the system (22).

4- Postmortem exam: Parts of the affected lungs for culture and histopathology, about a half cm³ from the edge of the obvious lesion was taken into the thioglycolate broth to promote the growth of aerobic and anaerobic bacteria (23) Another piece of 2 cm³ from the same site was persevered in 10% formalin for the histopathological dissection according to (24).

5- Molecular Identification: Conventional polymerase chain reaction: A specific primer of *Kl. pn. ssp pneumonia* 16S rRNA gene, strain ATCC13884T, partial GenBank: Y17657.1, was designed according to NCBI Gene-Bank and Primer3 plus program online and provided by (Bioneer Company, Korea) as following in table (2): (14). Deoxyribonucleic acid (DNA) was extracted from the bacteria by Genomic DNA Mini Kit, according to (25).

Table (2): specific primer of *Kl. pn. ssp pneumonia*; F (Forward) and R (reverse).

Primer	Sequence		Amplicon
<i>Kl. pn. ssp pneumonia</i>	GGAAGTGAACACCGGTCCAG	F	671bp
	CCAGGTAAGGTTCTTCGCGT	R	

Results

Physical examination of 24 camels revealed an extended neck, rapid shallow breathing rate (23 ± 0.27), an elevated heart rate (44 ± 0.29) and fever (39.2 ± 0.1). Congested mucous membrane was seen in all emaciated cases, moist crackles heard in auscultation and no enlargement in palpable lymph nodes. Cytological parameter of the TTW referred to an increase of TP (355 ± 4.29) mg/dl and WBC count (1420 ± 5.95) cell/ μ l with an obvious neutrophilia (48%) There was no statistical significance ($p > 0.05$) of gender with these indices (27). Culture formed on blood agar from the TTW, indicated Grey, round, shiny and mucoid colonies with no hemolysis on blood agar after 36-48 hr. at 37.5.C° as in Figure (1). These bacteria were gram-

negative rod as in Figure (2), consequently, GN Cards (gram negative cards) were used in VITEK 2 system which made the biochemical diagnosis as *Kl. Pn. ssp. Pneumonia*. In other hand, growth in thioglycolate broth revealed aerobic colonies from which three colonies were seen next step on blood agar. After gram staining, a gram-negative rod indicates the presence of *Kl. Pn. ssp. Pneumonia* with two-gram positive cocci *Staphylococcus lentus* and *St. vitulinus*, which all were identified biochemically. It is clear that *Kl. Pn. ssp. Pneumonia* is the causative agent of pneumonia and the others are postmortem contaminants. Postmortem examination had discloses lungs with elastic texture, greyish, parts of consolidations and meaty cut-surface

as in Figure (3). Histopathological dissection discovers the damaged alveolar walls due to excessive influx of inflammatory cells and fluids into the interstitium leading to lose air

spaces as in Figure (4). There was no statistical significance ($P > 0.05$) of gender with this infection.

Figure (1): Grey, round, shiny, mucoid and non-hemolysis colonies, on blood agar

Figure (2): Gram-negative rods of *Kl. Pn. ssp. Pneumonia* X (3000)

Figure (3): Left lung of camel with diffused interstitial bronchopneumonia

Figure (4): Interstitial pneumonia of camel lung; A: damaged alveolar walls, B: influx of inflammatory cells, (Histopathological examination) H&EX800

Figure (5): Agarose gel electrophoresis of 16S rRNA gene of *Kl. Pn. ssp. Pneumonia* from pure culture isolates M: marker (100 bp), lane (1-10) positive samples at 671 bp PCR product

All samples of the TTW; after DNA extraction; showed positive response in the conventional PCR containing the specific gene referred in (14) as in figure (5), in which they present the molecular weight of

671bp formerly administered with the imported primers affirming the diagnosis of the *Kl. Pn. ssp. Pneumonia* by the VITEK 2 Compact technique and came similar with results of (5, 6 and 7).

Discussion

The results of physical examination seem to be acceptable concerning (1) who had recorded several cases of the same complain. In the same time; results of cytological parameter of the TTW were logical as compared with (26) due to the inflammation of the lung. There was no statistical significance ($p>0.05$) of gender with these indices (27). Culture formed on blood agar from the TTW, with an agreement with (13), indicated the basic features of the suspected bacteria; *Kl. Pn. ssp. Pneumonia*. These bacteria were gram-negative rods; this result resembles results found in (13). It's clear that *Kl. Pn. ssp. Pneumonia* is the causative agent of pneumonia and the others are postmortem contaminants which seemed acceptable with (3,4,5,6,7 and 8). Postmortem examination, likewise (28), discloses lungs with elastic texture, greyish, parts of consolidations and meaty cut-surface as in figure (3). Histopathological dissection discovers the damaged alveolar walls due to excessive

influx of inflammatory cells and fluids into the interstitium leading to lose air spaces as in figure (4). These results came similar to the classification of pneumonia according to (29). It was, contrary to (30), highly significant to age 4-8 years because no cases were recorded in camels less than 4 years older than 8 years. Probably it came true with (31) who referred that most pastoralists never want to slaughter younger camels. This may be one reason for the high prevalence rate of pulmonary lesions recorded in adults, similar to observations recorded in (32). All samples of the TTW; after DNA extraction; showed positive response in the conventional PCR containing the specific gene referred in (14) in which they present the molecular weight of 671bp formerly administered with the imported primers affirming the diagnosis of the *Kl. Pn. ssp. Pneumonia* by the VITEK 2 Compact technique and came similar with results of (5, 6 and 7).

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