Clinical & Diagnostic Study of *E-coli* from Camels with Pneumonia using VITEK 2 Compact, Histopathology & Conventional PCR

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Summary

Twenty two camels in abattoirs of Al-Najaf & Al-Qadissiya provinces were suspected to have interstitial bronchpneumonia in this study. Clinical signs; after physical examination; revealed extended neck to inhale a lot of air due to lung insufficiency, no cough, rapid shallow respiratory rate (24±0.46) because of decreased lung volume, an elevated heart rate (44±0.75) to compensate the blood supply of lungs, fever (39.8±0.12); (it was done in winter) and congested mucus membrane, as a result of the inflammation; moist crackles heard in auscultation; which indicates the presence of acute inflammation; and no enlargement in palpable lymph nodes. In order to make the diagnosis, transtracheal washes (TTW) were sampled for cytology & bacteriology; white blood cells count (WBCc) was (1318±12.5cells/µl) with neutrophilia (51%), total protein (TP) records (308±3.6 mg/dl). Bacterial culture from the TTW showed pure colonies on blood agar; which were identified by the VITEK 2 compact technique & then confirmed by the conventional polymerase chain reaction (PCR) as *Escherichia coli*. In the same time, postmortem samples have given three types of colonies: the first was the same one as in the TTW which was the causative agent, and two others; *Staphylococcus lentus & St. vitulinus*; diagnosed biochemically by VITEK 2 compact technique. Histopathological dissections on the postmortem samples found in both lungs of camels discovered the presence of interstitial bronchopneumonia. Infection

involved both genders of camels ranged only in 4-8 years old.

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 types of pneumonia. One useful method is to classify according to the appearance or etiology of a particular pneumonia (1). Based on texture, distribution and type of exudates, pneumonia in domestic animals can be grossly classified into five morphologically distinct types; suppurative bronchopneumonia, fibrinous bronchopneumonia, interstitial pneumonia, embolic pneumonia & granulomatous pneumonia (2). Pneumonia can be caused by direct infection with bacteria by arriving haematogenously or by inhalation. In much pneumonia, a sudden alteration in the normal nasal bacterial flora with a dramatic increase in one or more species is the trigger for a lung infection. The bacteria are inhaled into the lungs in large numbers where they multiply after they have overwhelmed defense mechanisms, exposure to cold & wet weather & debility from any cause can predispose to 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 (1) has been *E. coli*.

Shigidi, (1973) and Chauhan *et.al.* (1987) found *E. coli* as a result of nasal swabs and bronchial lymph nodes culture from Sudanese and Indian dromedaries. Abdurahman (1987) found *E. coli* and other bacteria in the pathoanatomically altered lungs in 6 (3%) of 200 slaughtered Somali dromedaries. Al Darraji and Wajid (1990) found 83 cases of interstitial pneumonia in Indian Dromedary. Rana *et.al.* (1993) had isolated *E. coli* from pneumonic lungs of three dromedaries in Pakistan. Al-Doughaym *et.al.* (1999) identified

460 isolates from 196 pneumonic camels in KSA constituted 9 genera of bacteria; 7% of them were *E coli*. In northern Jordan, interstitial pneumonia was diagnosed due to *E. coli* 58.6% prevalence with higher incidence in young camels 6 months to 4 years (9). Amer *et.al.* (2002) found *E. coli* in condemned lungs of slaughtered calves in Egypt. Seddek (2002) reported 9 cases with pulmonary infection in Egypt infected with *E. coli*. The isolated *E. coli* strains were identified serologically from 15 camels with respiratory infection in Egypt (12). Tarek & Wafaa (2012) isolate *E. coli* from pneumonic lungs in Egypt.

Transtracheal washes collected using the percutaneous transtracheal technique is preferred for bacterial culture because these are not contaminated by oropharyngeal organisms. (14)

The VITEK 2 is an automated microbial identification system that provides highly accurate and reproducible results as shown in multiple independent studies. With its colorimetric reagent cards, and associated hardware and software advances, the VITEK 2 offers a state-of-the-art technology platform for phenotypic identification methods (15).

E. coli is a gram-negative, facultative aerobic, motile, non-hemolytic on blood agar and non–spore-forming bacilli. It's responsible for a wide variety of diseases of animals, including diarrhea, septicemia, hemorrhagic enteritis, respiratory diseases, ear infections, urinary tract infections, and mastitis (16). Strains of the E coli, which were reclassified, based on 16S rRNA analyses, contain a specific gene with 537bp (17). The complete nucleotide sequence of the 16S RNA gene from *E. coli* has been determined by using three rapid DNA sequencing methods (18). Definition of *E. coli* partial 16S rRNA gene, strain ATCC 35218 was done by (19).

Materials & Methods

Animals: One hundred fifty camels in abattoirs of Al-Najaf & Al-Qadissiya provinces were

suspected to have pneumonia from which twenty two camels were diagnosed with interstitial bronchpneumonia.

Methods:

1- Physical examination:

In regard to respiratory system, an examination has been done on suspected animals; those suffering irregular respiration, depression & extended neck; include body condition, hydration, nares, body temperature, heart rate, breathing rate & rhythm , mucus membrane, lung sounds & palpable lymph nodes after taking the possible case history (20).

2- Sampling:

A- **Transtracheal washes**:- Animals were adequately restrained with intramuscular injection of 0.25 ml/100kg xylazine which was useful as a sedative while the local anesthetic drug was 2% lidocaine (21). The skin over the selected site (about 10 cm^2) at the mid ventral aspect of the neck, where trachea can be grasped & the rings easily palpated, clipped and surgically prepared. Skin was penetrated with a stab blade & a trocar & cannula of suitable size (5 mm) was pushed firmly between two tracheal rings vertical to the long axis of trachea. The trocar is withdrawn to push the cannula down the tracheal lumen & imbed the catheter distally to the thoracic inlet. A 50 ml syringe filled with sterile, antibiotic-free & pre-warmed normal saline to be injected 1-3 times & immediately aspirated carrying the respiratory secretions from the lowest point of the trachea to be stored in the EDTA tubes at 4c^o (14).

Total protein: Spectrophotometer (CT Chrome Tech) was used to find the total protein according to the protocol administrated by the kit (22). This method is fairly accurate & the assay depends on the presence of amino acids which absorb UV light (23).

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 & differential WBC count. (24)

TTW smear:

Small drop of well-mixed TTW placed on end of a clean, grease-free slide, using an applicator stick or capillary tube. Immediately after placing TTW on the slide, a second slide "spreader" placed in front of the drop of TTW at an angle of approximately 30 degree and it pulled back until it comes to contact with the drop of TTW, and pause until the TTW spreads along the edge of the spreader. The greater the angle the thicker and shorter the TTW smear, and the smaller the angle the thinner and longer the smear (25).

White Blood Cells count (WBCc): Hemocytometer was used for enumeration of total leukocytes according to (25).

Differential WBCc: Differential leukocytes are counted by TTW film. The TTW film should be made from fresh sample as possible after collection of the TTW; otherwise, best results are obtained if EDTA is used as the anticoagulants. (25)

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 (26).

B- **Postmortem exam**: The whole lung was taken after slaughter; hot spatula was used to sterilize the outer part that we were going to sample from. By sterile forceps, a piece of the lung; about a half cm^3 from the edge of the obvious lesion; was taken into the thioglycolate broth to promote the growth of aerobic & anaerobic bacteria. (27) Another piece of 2 cm^3 from the same site was persevered in 10% formalin for the histopathological dissection according to (28).

C- **Biochemical identification**: A reagent card of VITEK 2 Compact has 64 wells that contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalization, enzyme

hydrolysis, and growth in the presence of inhibitory substances. An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel that prevents contact with the organism-substrate admixtures. Each card has a pre-inserted transfer tube used for inoculation. Cards have bar codes that contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the sample either before or after loading the card onto the system. (15)

D- **Conventional polymerase chain reaction**: A specific primer of *E coli* partial 16S rRNA gene, strain ATCC 35218 GenBank: AM980865.1, was designed according to NCBI Gene-Bank and Primer3 plus program online and provided by (Bioneer Company, Korea) as following in table (1):(16, 17, 18, 19, 29). Deoxyribonucleic acid (DNA) was extracted from the bacteria using Genomic DNA Mini Kit, according to (17).

Table (1): specific primer of *E. coli*; F (Forward) & R (reverse).

Primer	Sequence		Amplicon
E. coli	CGCGAAGAACCTTACCTGGT	F	537bp
	CCTTGTTACGACCTCACCCC	R	

Results & discussion

The **physical examination** of twenty two camels indicated that camels have extended neck to inhale a lot of air due to lung insufficiency, no cough, rapid shallow respiratory rate (24±0.46) because of decreased lung volume, an elevated heart rate (44±0.75) to compensate the blood supply of lungs, fever (39.8±0.12) (because it was recorded in winter) and congested mucus membrane, as a result of the inflammation, and emaciation. These finds seem acceptable in regard to (1).

Moist crackles heard in auscultation; which indicates the

presence of acute inflammation and no enlargement in palpable lymph nodes. These findings agreed with (30), (31), (32), (33) and disagree with (34) who supposed that bacterial bronchopneumonia is usually accompanied by a moist and painful cough in other ruminants.

Cytological analysis referred to an increase in TP (308±3.6) mg/dl as compared with the normal value (70±0.02) mg/dl; increased Leukocyte count (1318±12.5) c/µl as compared with normal value (620) c/µl with obvious neutrophilia (51%) unlike healthy camels that have (60%) lymphocytes in their TTW. It seems to be an acute infection; as an agreement with (35), (36) and (37).

Culture: culture formed on blood agar from the TTW indicated sticky and greyish white non-hemolytic pure colonies after 24-36 hr at 37.5.Cº as in figure (1) which resembles (16). 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 *Escherichia coli* as in figure (3). These results resemble those found in (38). In other hand, growth in thioglycolate broth revealed aerobic colonies from which three colonies were seen on blood agar soon after. After gram staining; a gram negative rods indicate the presence of *E. coli* with two gram positive cocci *Staphylococcus lentus & St. vitulinus* which all were identified biochemically. It's clear now that *E. coli,* likewise (3,4,5,6,7,8,9,10,11,12 &13), was the causative agent of pneumonia and the others were postmortem contaminants.

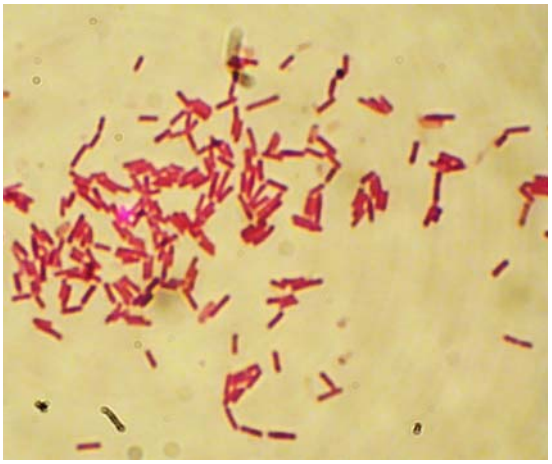

Fig. (2): Gram negative rods of *Escherichia coli***.** X (3000)

Fig. (1): Sticky, Greyish, White, and Non-hemolytic Colonies on Blood Agar.

Postmortem examination, Similar to (35), discloses lungs with elastic texture, parts of consolidations & meaty cut-surface as in figure (4). Histopathological dissection discovers the thick alveolar walls due to increased cellularity of the interstitium leading to lose air spaces as in figure (5). Besides, bronchopneumonia was obvious in figure (6) in which degeneration of epithelial cells lining the bronchus & desquamation of these cells in the lumen with infiltration of inflammatory cells in the interstitial tissue with presence of pulmonary emphysema. These results came true with the classification of pneumonia according to (2 & 34).

College of Veterinary Medicine

bioMerieux Customer:
System #:

Laboratory Report

Printed Feb14 , 2014 11:25 CDT
Printed by: labadmin

Patient Name: 22
Isolate Group:

Patient ID:

Bionumber: 2405610550526611
Selected Organism: Escherichia coli

Comments:	

Identification Information	Card:	GN	Lot Number:	241276840	Expires:	Jul 13, 2014 13:00 CDT
	Completed:	Feb 13, 2014 18:28 CST	Status:	Final	Analysis Time:	5.00 hours
Selected Organism	99% Probability		Escherichia coli			
	Bionumber:	2405610550526611			Confidence:	Excellent identification
SRF Organism						
Analysis Organisms and Tests to Separate:						
Analysis Messages:						
Contraindicating Typical Biopattern(s)						

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

Installed VITEK 2 Systems Version: 05.04
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Page 1 of 1

Fig. (3): Laboratory Report of VITEK 2 System which indicate the identification of *E. coli*

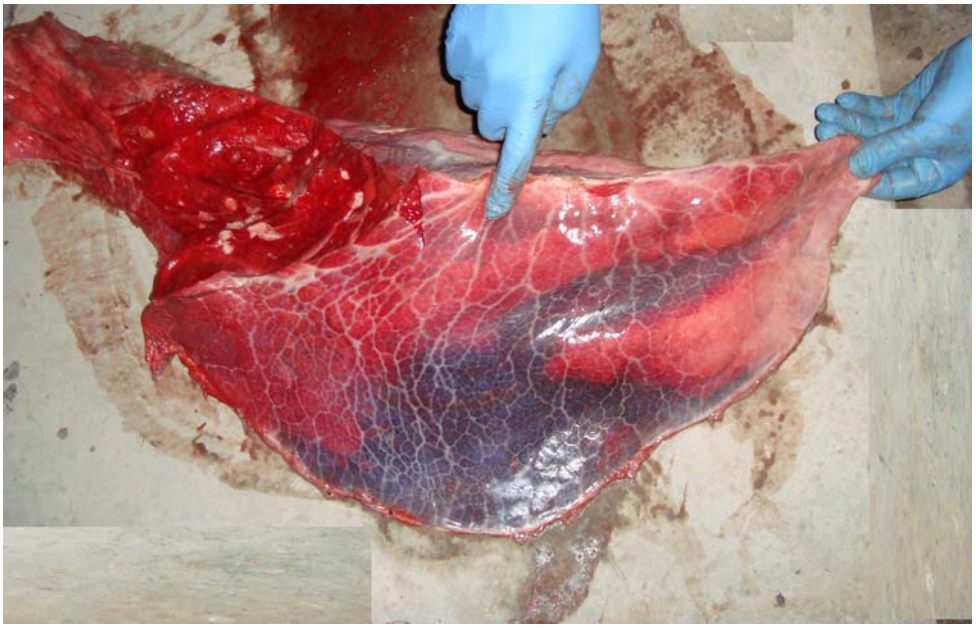

Fig. (4): Right Lung of Camel with Interstitial Bronchopneumonia; congestion, meaty cut-surface and a large consolidation

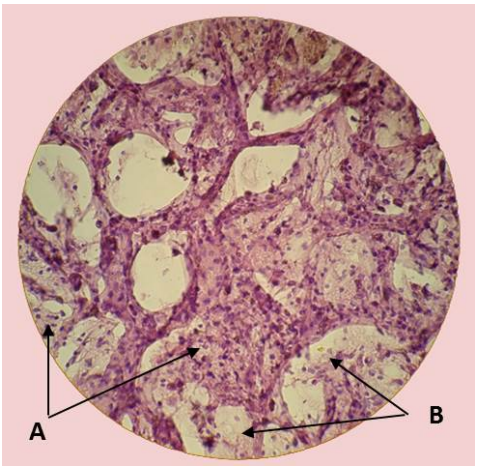

Fig. (5): Interstitial Pneumonia of Camel Lung: A: infiltration of inflammatory cells, B: damaged alveolar walls (Histopathological Examination).H&E,X(800)

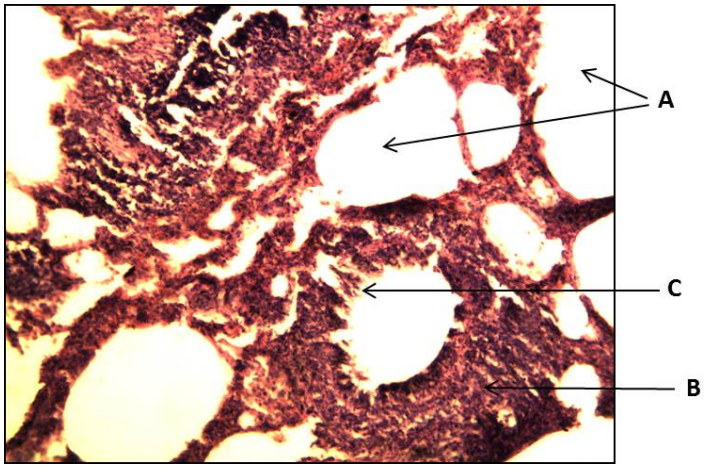

Fig. (6): Interstitial Bronchopneumonia of Camel Lung. A: Pulmonary Emphysema, B: Infiltration of Inflammatory cells, C:Degeneration and Desquamation of Epithelial Cells, , (Histopathological Examination) H&E, X(400)

All samples of the TTW; after DNA extraction; showed positive response in the conventional PCR containing the specific gene with an agreement with (20,21,22 & 23) as in figure (7), in which they present the molecular weight of 537bp formerly administered with the imported primers affirming the diagnosis of the *E. coli* by the VITEK 2 Compact technique.

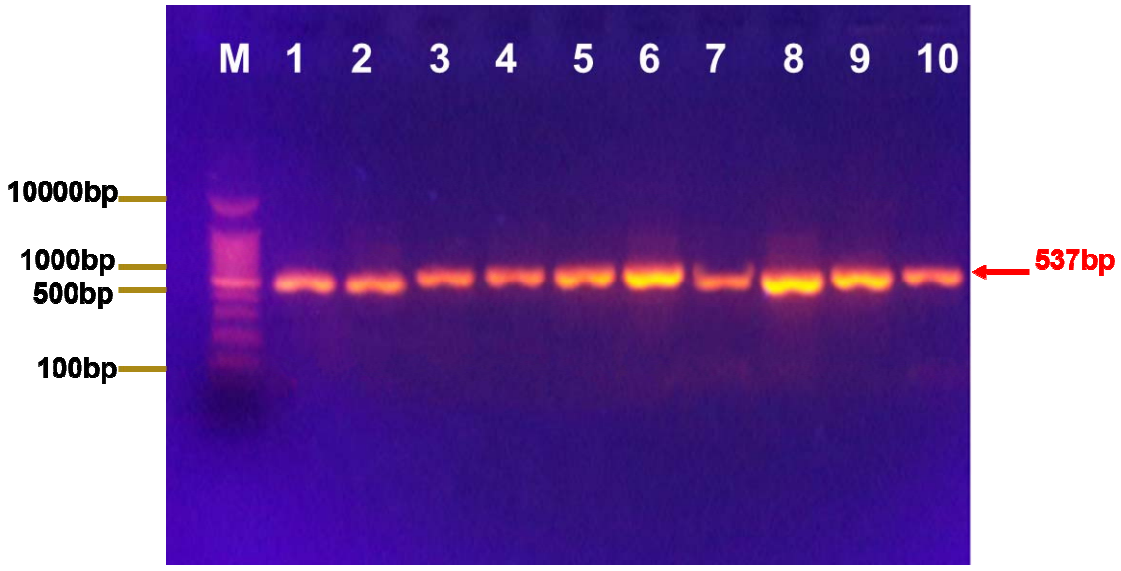

Fig. (7): Agarose gel electrophoresis of the 16S rRNA gene of *E. coli* from pure culture isolates. M: marker (100bp), lane (1-10) positive samples at 537bp PCR product.

There was no statistical significance ($p>0.05$) of gender with this infection while it was, unlike (9), 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 (39) 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 (40).

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