

Research article

Clinical and immunological effects of experimental infection with *Klebsiella pneumoniae* in lambs in Iraq

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Abstract

The study was conducted on ten Awassi male sheep to study experimentally pathological changes of *Klebsiella pneumoniae* infection during (3 months). Experimental animals were divided randomly into equal groups (each group included 5 animals), induction of pneumonia in one of these groups in sheep by intratracheal route with the infective dose (3 ml) of bacterial suspension in concentration (10^5 CFU/ml) & the second represent as control and gave a sterile D.W. in the same dose & route of infection) and clinically exam daily with weekly complete blood indices, humoral and cellular immunity were examined by passive hemagglutination test and phagocytic activity as well as histopathological examination of lungs from two scarified sheep in the first group. Nasal discharge appeared as serous then turned to mucopurulent, intermittent cough, mild fever, increase in pulse and respiratory rates (42.4 ± 1.39 and 35.2 ± 1.77 respectively with significant differences between the two groups. WBC count increased significantly in the infected group as compared with the control group. GRN% showed statistically a significant increase in the values reached to (8.88 ± 0.86) during that period of infection compared with control and Mon% showed a statistically significant increase in values during that period in the infected group, and the Abs titration was the higher in the infected group significantly than control and the cellular response in the infected group revealed increment in phagocytic index of neutrophils than in the control group.

Keywords: *Klebsiella*, pathological, pneumonia, sheep, experiment.

Introduction

Respiratory diseases are due to complex factors that often interact to produce disease, various conditions such as inclement weather, weaning, transportation, poorly ventilated housing and nutritional deficiencies known to play a predisposing role in animals' immunity weakness sequel to the stressful conditions. In such conditions, flare up of the normal flora of upper respiratory tract and subsequent infection of the lungs is all documented (1). Pneumonia is an infection of lung tissue with multiple causes. It is an important medical problem of sheep and goats of all ages. In younger animals various bacteria, viruses and parasites of the upper and lower respiratory

tract are often involved in the development of pneumonia. Ovine pathogens, such as *Pasteurella multocida*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Corynebacterium pseudotuberculosis* and *Actinomyces pyogenes* (2, 3). *Klebsiella* were isolated from respiratory diseases from various domestics including sheep (4), also found highest prevalent of *Klebsiella pneumoniae* (24.2%). *Kl. pneumoniae* is an opportunistic pathogenic bacterium, present in water, soil, plants and as a saprophyte over mucus and intestine of mammals (5). In addition to pneumonia, *Klebsiella* can also cause infections in urinary tract, lower biliary tract and surgical wound sites. The range of

clinical discuses include pneumonia, thrombophlebitis, urinary tract infection (UTI), cholecystitis, diarrhea, upper respiratory tract infection, wound infection, osteomyelitis, meningitis and bacteremia and septicemia(6).The pathogenicity of *Klebsiella* attributed to its production of a heat-stable enterotoxin further to virulence factors include capsular polysaccharides (CPS) and lipopolysaccharides, adhesions and iron acquisition systems(7).

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: 408

Animals

Ten male Awassi lambs with ages from (5.5 -6) months were used in this study (in the period extending from 20th April 2014 to 29th June 2014) in the College of Veterinary Medicine, University of Al-Qadisiyah. All lambs remained for (45) days before beginning of experiment for adaptation and received appropriate prophylactic program by used of anthelmintic drugs and vaccination with enterotoxaemia and FMD vaccines. Feces samples were collected from all animals and examined by flotation and sedimentation tests and by Bergmann technique to ensure absence of endo-parasites and lungworms.

Experimental design:

Induction of pneumonia in sheep by intratracheal route with the infective dose of the selective bacteria & daily clinical examination for signs of pneumonia such as nasal discharge, coughing, rales, respiratory rate, heart rate, temperature, with weekly tests of CBC, PHAT for antibodies & phagocytic activity.

Preparing of bacterial dose

A stock culture of *Klebsiella* was transferred into blood agar then single colony was taken and cultured into brain heart infusion broth, incubated overnight at 37^oC.

The bacteria in this broth culture were precipitated by centrifuging at (3000) rpm for (10) minute and were then suspended in PBS, pH: 7.2. This suspension was washed by centrifuging again three times then sediment bacteria were suspended in appropriate amounts of PBS (8).

Preparation of bacterial suspension:

Experimental induction of pneumonia was performed using *Klebsiella pneumoniae* isolated from microbiological lab. College of veterinary medicine, University of Al-Qadisiyah. In this study from pneumonic lungs of sheep. Bacteria were grown on blood agar plates for (18) hour then harvested and suspended in sterile saline solution. Then several dilution made then bacterial suspension adjusted with Denis-check to a final concentration of (10⁵ CFU/ml) (8).

Induction of *Klebsiella pneumoniae* infection in sheep

The experimental induction of pneumonia in one group (infected A group) was performed by injection of (3ml) of bacterial suspension which contain (10⁵ CFU/ml) between two ring of mid trachea after clipping and shaving and sterilizing the region with alcohol (70%) then injected toward the bottom of trachea and noticed the cough reflex on animal after injection.

Hematological parameters:

In addition to total WBC cell/ml and differential leukocyte counts, include granulocytes (GRN) %, lymphocytes (LYM) % and monocytes (MON) %.

Clinical examination

Clinical signs of pneumonia infection with *Klebsiella*, which included temperature, pulse and respiratory rates and auscultation of the chest, post mortem examination to internal organs included (Lung). Passive hemagglutination test (PHA) (9) and Phagocytosis (by nitro blue tetrazolium dye NBT test) (10)

Statistical analysis:

To determine statistical differences between the different two groups in this

Results**Clinical examination:**

Nasal discharge appeared as serous then turned to mucopurulent and coughing (intermittent cough) which appeared firstly at group A. Mild fever, increase in pulse and respiratory rates (85.2 ± 1.77 and 35.2 ± 1.77 respectively Table (1) and significant differences between A with C groups at ($P \leq 0.05$).

Table (1). The clinical examination of the experimental animals during pneumonia

Mean +SE	Temperature	Respiration	Pulse
Date	21/6	21/6	21/6
Group	Mean +SE		
A	40.02 ± 0.03 Ab	35.2 ± 1.77 B b	85.2 ± 1.77 Ab
C	39.16 ± 0.21 Ba	27.4 ± 0.87 C a	77.2 ± 0.86 Ba

The different letters referred to significant differences at level ($P \leq 0.05$) between groups
Similarity letters represent no significant differences at level ($P \leq 0.05$) between groups
Capital letters referred to vertical compression, small letters referred to horizontal compression.

Auscultation of the lung revealed presence of abnormal sound over the chest regions (crackles) in A group.

Blood parameters during period of pneumonia induction:

A significant increase in WBC count through the infection period in the (A) group as compared with group C with presence of statistical significant differences Table (2). The percentage of granulocytes (GRN %) showed statistically significant increase in the values reached to (8.88 ± 0.86) during that period of infection compared with group C Table (3). There were restricted period of infection which occurred statistical significant decreased in LYM % in the group A Table (4). Mon % showed statistical significant increase in values during that period in the (A) group with the latter group was more than the group compared with group C Table (5), at $P \leq 0.05$.

experimental study by using two ways ANOVA in the SPSS windows program Statistical Package for Social Science.

Table (2): WBC (cell/ml) level during pneumonia in the experimental animals

Date	3/6	17/6	29/6
Group	Mean +SE		
A	75.22 ± 7.18 Bb	77.4 ± 8.12 Bb	86.42 ± 23.1 Bc
C	48.76 ± 10.11 Aa	49.58 ± 3.85 Aa	49.64 ± 4.24 Aa

The different letters referred to significant differences at level ($P \leq 0.05$) between groups
Similarity letters represent no significant differences at level ($P \leq 0.05$) between groups
Capital letters referred to vertical compression, small letters referred to horizontal compression.

Table (3): GRN % level during pneumonia in the experimental animals

Date	3/6	17/6	29/6
Group	Mean +SE		
A	8.5 ± 0.12 Ab	8.64 ± 0.92 Ab	8.88 ± 0.86 Ab
C	6.9 ± 1.22 Ba	6.85 ± 1.83 Ba	6.81 ± 2.34 Ba

The different letters referred to significant differences at level ($P \leq 0.05$) between groups
Similarity letters represent no significant differences at level ($P \leq 0.05$) between groups
Capital letters referred to vertical compression, small letters referred to horizontal compression.

Table (4): LYM % level during pneumonia in the experimental animals

Date	3/6	17/6	29/6
Group	Mean +SE		
A	90.18 ± 1.19 Ba	88.32 ± 2.18 Aa	84.94 ± 3.39 Ab
C	87.58 ± 1.41 Ba	85.46 ± 1.159 Aa	85.66 ± 2.3 Aa

The different letters referred to significant differences at level ($P \leq 0.05$) between groups
Similarity letters represent no significant differences at level ($P \leq 0.05$) between groups
Capital letters referred to vertical compression, small letters referred to horizontal compression.

Table (5): MON % level during pneumonia in the experimental animals

Date	3/6	17/6	29/6
Group	Mean +SE		
A	11.46 ± 1.77 Ab	12.96 ± 1.76 Ab	12.02 ± 1.55 Ab
C	6.54 ± 0.58 Ba	6.74 ± 1.44 Ba	6.72 ± 1.55 Ba

The different letters referred to significant differences at level ($P \leq 0.05$) between groups
Similarity letters represent no significant differences at level ($P \leq 0.05$) between groups
Capital letters referred to vertical compression, small letters referred to horizontal compression.

Passive hemagglutination test

Statistical significant gradual increment of antibodies titers values in experimental animals after infection with *Kl. pneumoniae*, with presence of statistical differences between (A) groups, so the higher antibodies titer was recorded in-group A than group C at ($P \leq 0.05$), Table (6).

Table (6). Mean antibodies titer by passive hemagglutination test in the experimental animals:

Date Group	Antibodies titer Mean \pm SE		
	27/5	3\6	17\6
A	0	70.2 \pm 12.2 Ab	124.4 \pm 13.18 Ac
C	0	0 Ba	0 Ba

The different letters referred to significant differences at level ($P \leq 0.05$) between groups
Similarity letters represent no significant differences at level ($P \leq 0.05$) between groups
Capital letters referred to vertical compression, small letters referred to horizontal compression.

Phagocytosis of neutrophils

There was increase in phagocytic activity of neutrophils after infection in the group A compared with group C (52 ± 3.74 and 46 ± 5.09 respectively) Table (7).

Discussion

These results conformed to (1) and (11) as they mentioned these signs of chronic bacterial bronchopneumonia. The signs appeared after (21) days of induction of pneumonia. Less researchers studied the chronic infection of pneumonia in different animals (12; 13) in Rats and (14) in mice while (15) studied the chronic lung diseases of sheep.(12),(16) and (17) who noticed infiltration of inflammatory cells and macrophage in experimental pneumonia infection with *Kl. pneumoniae* in mice and rats. (18) Showed infiltration of inflammatory cells with desquamation beside edema and hemorrhage with emphysema in histopathological change of pneumonia lungs when they studied pathological lesion of pneumonic lung infected with *Kl. pneumoniae* in sheep. Neutrophil recruitment to the lungs is an important first line of defense against bacterial infection. Also with lung macrophages, lung epithelium and

Table (7): phagocytic index in animals during the experimental study:

Group	During pneumonia
A	52 \pm 3.74a
C	46 \pm 5.09b

Values represented Mean \pm SE

The different letters referred to significant differences at level ($P \leq 0.05$) between groups

Similarity letters represent no significant differences at level ($P \leq 0.05$) between groups

Nitro Blue Tetrazolium reduction assay gave lower results (neutrophils which stimulated by opsonized zymosan). Positive neutrophil in Figure (1).

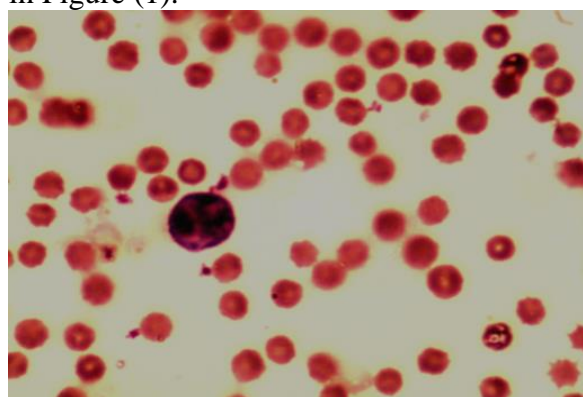


Figure (1) Neutrophil engulfed NBT dye (100X Oil immersion).

endothelium attributed to the initial recognition of bacteria, and production of inflammatory mediators and host defense (20). Interstitial pneumonia regarded as chronic inflammatory condition in which there is predominantly a proliferative response involving alveolar walls and supporting stroma (21). (18) Reveled significant increase in total WBC, eosinophil, neutrophil and monocyte count in pneumonia sheep, which suffered from infection by *Klebsiella pneumoniae*. Increase in total WBC count could be attributed to bacterial infection and product of tissue injury, which stimulate the release of growth factors, cytokines, and other mediators of inflammatory response, which related to increased. WBCs count and proliferation and maturation. Stress of respiratory disease leads to release of endogenous corticosteroids, which have major role in regulating circulating concentration of WBC

in moderate and severe pneumonia (22). The gradual increment of antibody titers was agreed with (23) who noticed alveolar macrophage activation after 4-14 days of treated with free and liposome entrapped

from of LPS of *Klebsiella pneumoniae*. In

Conclusion:

The effects of *Klebsiella pneumoniae* in experimental pneumonic sheep were more harmful.

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