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Research article

Clinical and diagnostic study of sheep Pneumonic pasteurellosis in Basrah, Iraq

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Abstract

The study included clinical investigation on pneumonia that caused by Mannheimia haemolytica (M. haemolytica) in sheep of Basrah Province, also isolation and identification were done and confirmed the diagnosis by PCR technology. The blood samples and nasal swabs were collected from 410 local sheep breeds of both sexes, and different ages. The results showed that from 410 sheep there were 25 healthy against clinical and cultural tests, which concerned as control group. The rest 385 sheep were revealed clinical pneumonia. The most important pneumonic signs included coughing, fever, abnormal lung sounds, dyspnoea, depression, mucopurulent nasal discharge as well as loss of appetite and separated from the herd. The laboratory bacterial culture and biochemical tests for samples from 385 pneumonic sheep appeared M. haemolytica in 81 (21 %) cases, which characterised by moist, round, white or grey colony with β-type haemolysis on blood agar. On MacConkey agar showed pink–red pinpoint colonies. While when stained by gram stain appeared as pink, short rods or coccobacilli and bipolar in methylene blue stain. The biochemical reactions included negative indole, urease and citrate whereas positive for oxidase and catalase tests. The PCR technique indicated that from 81 isolates there were 48 59, 2% cases had evidence by Rpt2 gen as M. haemolytica in local sheep of Basrah Province.

Keywords: Basrah, Pneumonic pasteurellosis, sheep

Introduction

Respiratory diseases of sheep particularly pneumonia continues to be a major problem commonly encountered in sheep flocks, affecting groups or individuals of all ages and types (1). Pneumonia was refer to the inflammation of the pulmonary parenchyma as well as associated with inflammation of bronchioles and pleurisy; otherwise it may characterized by respiratory embarrassment or sometimes toxemia (2). Pneumonia is regarded as significant cause of loss to the sheep industry (3, 4, 5). There are multiple agents causing pneumonia in sheep, such as bacterial agents, which drawn attention due to variable clinical manifestations, severity of diseases, and re-emergence of strains resistant to a number of chemotherapeutic agents (6). The most common causes of bacterial pneumonia in sheep were *Mannheimia haemolytica* (7). This organism is an opportunistic pathogen, which has been recovered from the mucous membranes of the nasopharyngeal and oral regions of clinically healthy sheep (8). For clinical economic importance of the pneumonia caused by *Mannheimia haemolytica* in sheep, the present study aimed for Clinical investigation of pneumonia caused by *Mannheimia haemolytica* in sheep of Basrah Province. Then confirm the isolated *Mannheimia haemolytica* by using of PCR.

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: 401

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1. Animals: four hundred and ten of sheep were used for investigation of pneumonia caused by *M. haemolytica*, which from Basra regions: North (Qurna, Deer and Hartha) East: (Shat Al-Arab) South: (Abo Al-Qasseb), West: (Al-Zubair and Sofwan). The clinical findings were recorded for all those sheep. EDTA tubes for bacterial culture (9). Nasal swabs were taken gently from entire nasal cavity and preserve in sterile tube with brain heart infusion broth for bacterial culture (10). The bacterial culture was done in clinical pathology laboratory, college of veterinary medicine, University of Basrah. The bacterial isolation and biochemical identification methods were conducted from (7). 2. DNA extraction: The DNA extraction of identified *M. haemolytica* was adapted from (11). The gens used for detection of *M. haemolytica* were adapted from (12) as in table (1) and provided with mastermix Table (2) by Bioneer –Korea.

Table (1): Oligonucleotide primers sequences for PCR amplification of *Mannheimia haemolytica*

Primer	Sequence	Length of primer
Rpt2 **Rpt2rev**	GTTTGTAAGATATCCCATTT CGTTTTCCACTTGCGTGA	1022bp

Table (2): Mastermix for amplification of *Mannheimia haemolytica*

No.	Component	20 µl reaction
1	Top DNA polymerase	1 U
2	Each dNTP (dATP, dCTP, dGTP, dTTP	250 µM
3	Tris-HCl (pH 9.0)	10 mM
4	KCl	30 mM
5	$MgCl_2$	1.5 mM
6	**Stabilizer and tracking dye**	

The PCR cycling protocol was done as in (12) which described in Table (3) and use Thermocycler apparatus (Techne, UK).The significance of variations between infected and healthy sheep were statistically analyzed by using SPSS student t-test (13).

Table (3): PCR cycling protocol for *Mannheimia haemolytica*

PCR cycle		Temp.	Time
1cycle	Initial Denaturation	95ºC	3 min.
35 cycles	Denaturation	95ºC	1 min.
	Annealing	48ºC	1 min.
	Extension	72ºC	30 sec.
1cycle	Final Extension	72ºC	5min.

Results

The clinical examination of 410 sheep from different regions in Basrah revealed that 25 sheep were clinically healthy, that enabled them to represent the control group. Other 385 sheep had clinical signs of pneumonia, which manifested by: Coughing (93.5%), Fever (91.4%), abnormal lung sounds (86.2%), Polypnea and Dyspnea (85.5%), depression (83.4%), mucopurulent nasal discharge (81.8%), loss of appetite (75.8%), isolation from flock (70.4%), Pale mucus membrane (65.2%), crusting around nostrils (64.2%), lacrimation (43.4%) and decreased milk production(26.5%) as in Table (4).The vital signs such as body temperature ,respiratory and heart rate of diseased sheep described in Table (5) which ware statistically significant increase ($p<0.05$) in compared with control group. Cultural characters of *Mannheimia haemolytica* isolates appeared as moist, round, white or grey and colourless colony on blood agar with small area of β-type haemolysis around the colony. On MacConkey agar showed pink–red pinpoint colonies**.** While when stained by gram stain appeared as pink, short rods or coccobacilli more over tendency for bipolar staining when using methylene blue stain. The biochemical data relating to *M. haemolytica* was described in (Table 6), and out of total 385 nasal swab and blood samples, *M.haemolytica* isolates were 81(21

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%), divided into 23(5.97%) from the nasal swabs and 58 (15.03%) from the blood according to cultural and biochemical identifications.

Table (4): Clinical signs in sheep with bacterial pneumonia.

Clinical signs	Affected sheep n=385	%
Coughing (moist, dry)	360	93.5
Fever	352	91.4
Abnormal lung sounds	332	86.2
Polypnea and dyspnea	329	85.5
Depression	321	83.4
Mucopurulent nasal discharge	315	81.8
Partial or complete loss of appetite	292	75.8
Pale mucus membrane	251	65.2
Isolation from flock	271	70.4
Crusting around nostrils	247	64.2
Lacrimation	167	43.4
Decreased milk production	102	26.5

Table (5): Body temperature, respiratory and heart rate, of pneumonic sheep and control group

Parameters	Controls n=25	Diseased sheep n=385
Body temperature C°	39.2 ± 0.086	41.45± 0.1*
Respiratory rate/ mint	24.4±0.520242	44.15± 0.3*
Heart rate/ mint	73.8± 0.59	89.78± 2.2*

Values are mean ± standard error of mean. * (P<0.05).

Table (6): Biochemical tests of *Mannheimia haemolytica*

Indole	Urease	Citrate	Oxidase	Catalase	Haemolysis
-ve	-ve	-ve	+ve	+ve	+ve

Table (7) PCR amplification of Rpt2 primer specific for *M. haemolytica*

Type of sample	No. of samples	Positive isolate for Rpt2 primer No	percentage
Nasal swab	23	13	16.0%
Blood sample	58	35	43.2%
Total	81	48	59.2%

The PCR resulted from 81 isolate according to culture and biochemical identification which aimed by using Rpt2 primer specific for *M. haemolytica*, and then showed that 48 (59.2%) had been successfully amplified, which included 13 from nasal cavities and 35 from the blood (Table - 7 and figure -1). Appeared PCR amplification (1022 base pair) primer for *Mannheimia haemolytica* extracted DNA. Lane (1): Molecular Weight Marker (10000 base pair). Lane (2, 3, 4, 5, 6, 7, 8) Note: Positive samples (1022bp).

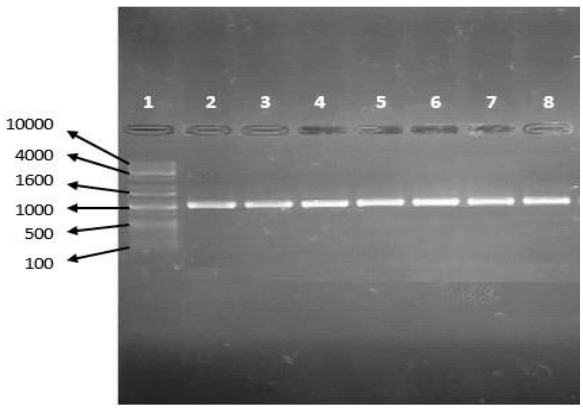

Figure (1): Gel electrophoresis with ethidium bromide

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Discussion

The clinical investigation for bacterial pneumonia that caused by *Mannheimia haemolytica* in sheep from Basra governorate by examination of 410 sheep showed that 25 heads had no clinical signs of pneumonia as well as negative for bacteria culture, so they concerned as control (14,15). The other 385 heads of sheep appeared signs of pneumonia, such as: fever, coughing, polypnea, dyspnoea, mucopurulent nasal discharge, lacrimation, partial or complete loss of appetite, paler of mucus membranes, weakness, separation from the herd, decrease milk and abnormal lung sounds, which also recorded by many authors in relation to bacterial pneumonia and *M. haemolytica* infections in sheep (16,17). The most severe signs were coughing (93.5%), Fever (91.4%), abnormal lung sounds (86.2%), Polypnea and Dyspnea (85.5%), depression (83.4%), mucopurulent nasal discharge (81.8%) whereas *M. haemolytica* mostly associated with severe illness than other bacterial causes of pneumonia (14, 18). However field researchers added that the severity perhaps depend on the severity of pathogenic agent, stage of the disease, type of the lesion and generalized of infection (15). Abnormal lung sounds were heard over the auscultation area, include crackles, wheezing and pleural frictional rub. Crackles or moist rales are initiated if presence of fluids or exudate in the small bronchi, bronchioles and alveoli, while wheezing sound or dry rales occurs when the bronchial tubes inflamed and narrowed (19). Moreover, frictional sound heard due to inflammation in the pleural cavity and the rubbing together of vesicular and parietal pleura (2). Lungs sound may clearly heard following dyspnea (19). According to the bacteriological characters, *M. haemolytica* in present study discovered on blood agar as smooth round, white to grey colonies and small area of β-type haemolysis which agreed with (20), while on MacConkey agar appeared in small pink and pin point colonies as results that showed by (21,22). Otherwise isolated bacteria taken pink on gram stain, also short rods or coccobacilli, as well as appeared bipolar staining when using methylene blue stain that have been approved in (7). The biochemical reactions for isolated *M. haemolytica* manifested by negative indole, urease and citrate while positive for oxidase and catalase in agreement with (7, 21).The conventional culture and biochemical identification revealed that from 385 pneumonic sheep there were 81 (21 %) sheep infected with *M.haemolytica* (7,14). Polymerase Chain Reaction was aimed on those 81 isolate and disclosed that 48 (59.2%) samples had been successfully amplified by Rpt2 primer used as based specific gen for *M. haemolytica* as recorded in (12), also the differences between the conventional bacterial and biochemical identification and the PCR tool for the diagnosis of *M. haemolytica* may related to several serotypes of this bacterium (2,7,23), more over PCR system was designed for the detection of many virulence genes and are often the most sensitive, rapid and specific technology for detecting *M. haemolytica* (20). PCR as a test can detect the organism even in very small amount, and included in many studies that reported the use of PCR technology to identify the *Mannheimia haemolytica* (12, 20, 23, 24).The present result evidenced higher infection with *M. haemolytica* in sheep than founded by (25) in Iraq, that in fact of increased virulent of pneumonia caused by *M. haemolytica* was related to many predisposing factors included environmental factors and previous infection (6, 26).

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