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Characterization of *Pasteurella multocida* associated with epizootic pneumonia in fattening sheep in Egypt

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Pneumonia has frequently been identified as an important mortality factor in fattening sheep populations in Egypt. Resulting poor sheep survival and low selection rates and fatalities in adults may insistently affect population designs. *Pasteurella* species are the most frequently isolated pathogens. In spite of *P. multocida* is not customarily connected with ailment in fattening sheep, high recovery of *P. multocida* was detected during the winter of 2016–2017. The overall recovery rate of *P. multocida* was (13%) of examined samples, with isolation percent from nasal cavities of apparently healthy animals (9.3%), while diseased animals were (12.0%) and (15.3%) from pneumonic lungs of emergency slaughtered animals. Two non-culturable methods were applied for direct detection of *P. multocida*. The highest detection rate (33.3%) of *P. multocida* was explored by direct PCR on preselective broth in comparison to other two methods. The most prevalent capsule biosynthesis gene among clinical samples examined was capA (57.0%) followed by capB (26.0%) and both types capA & capB (17.0%) In conclusion, *P. multocida* serogroup A represent one of major cause associated with epizootic pneumonia in fattening sheep in Egypt.

Keywords: Capsular type*, Pasteurella multocida,* pneumonia epizootic, polymerase chain reaction, Sheep.

INTRODUCTION

Pneumonia is incredibly ordinary in sheep and can be accountable for colossal budgetary setbacks around the globe. The condition regularly shows up when sheep exposed to combinations of predisposing factors, for instance, hostile physical condition, and physiological uneasiness, bacterial and viral sicknesses (Marru et al. 2013). Although many predisposing factors have been identified, none other than "stress" as a general phenomenon have exhibited dependable shared elements. Sever bacterial fibropurulen bronchopneumonias are usually the ultimate cause of death whether, accompanied with viral, nutritional or transportion stress (Quinn et al. 1994, Brogden et al. 1998) or immune suppression are the initiating factors (Garedew et al. 2010) *Pasteurella multocida* is known to be a widespread veterinary pathogen and also has the potential to cause zoonotic infections in humans.. it is associated with pneumonia in swine and ruminants, with respiratory tract diseases in rodents, and with sporadic human infections (Magyar & Lax, 2010).Sheep are especially vulnerable to bacterial pneumonias, particularly domestic and bighorn sheep. They act as normal carriers of more pathogenic strains of bacteria, particularly Pasteurella species (Foreyt et al. 1996

; George et al. 2008)

This study was conducted to determine morbidity & morbidity rate of sheep pneumonia which were associated with *P. multocida*. The samples were collected to determine the prevalence of *P. multocida* associated with pneumonia infection in fattening sheep. In addition to phenotypic & genotypic characterizations of the detected *P. multocida* strains.

MATERIALS AND METHODS

Animals:

The animals used in this study were sheep emergency slaughtered at Bastten abattoir, the lungs of the slaughtered animals were visualized, palpated and inspected thoroughly and altogether lastly the pneumonic lungs were considered. The lungs of the emergency slaughtered animal were visualized and palpated for hemorrhage, edema and pneumonia. The lungs with consolidated inflamed area, deep red and sharply demarcated lesion were considered as pneumonic lungs (Herenda et al. 2000). A total of 150 pneumonic lungs were collected from fattening sheep at slaughter house. All lung samples were collected under aseptic conditions and transported on cold ice to Microbiology Department, Faculty of Veterinary Medicine, Cairo University.

Bacteriological Examination:

Pneumonic Lung samples from each animal were inoculated onto Columbia blood (5% ovine) agar (CBA) and MacConkey agar. The streaking was further spread with inoculating loop to aid colony isolation. The plates were labeled and incubated aerobically at 37°C for 24-48 h. Mixed colonies and Gram negative bacteria were subcultured on both blood and MacConkey agars and further incubated aerobically for 24 h. Pure culture of single colony type from both blood agar were transferred onto nutrient agar slants for a series of biochemical tests. The biochemical utilization tests were performed to confirm identification and differentiate isolates into subspecies and biotypes (Quinn et al. 1994).

Preselective Enrichment Broth:

All samples (Nasal swabs & lung tissues) were inoculated onto preselective broth for 34 hours according to Dyer et al. 2001 with slight modification as the following brain heart broth with antibiotics (5 µg of vancomycin, 5 µg of neomycin,100 u of nystatin, and 100 µg of cycloheximide [Sigma Chemical Co., St. Louis, Mo.] plus 50 µg of sodium azide and 1 µg of thiamine monophosphate [Sigma] per ml) to provide selectivity for Pasteurella spp. which were incubated for 24 hr at 37ºC. The broth was centrifuged for pelleting the grown bacterial cells. The bacterial pellets were washed twice by sterile PBS and transferred for genomic DNA isolation.

Genomic DNA isolation

Genomic DNA was isolated using the PureLink® Genomic DNA Mini Kit (Invitrogen) following manufacture instructions.

Direct PCR on Nasal swabs & Lung Tissue

Direct PCR on nasal swabs and Lung was performed using (**Phire Animal Tissue direct PCR Kit**) as per manufacturer instruction. Briefly puncher was used to lung tissue to obtain uniform and small (0.4mm) sample. Then samples were place directly into the PCR reaction (50 µL of volume). **(**Hossam et al. 2016)

Molecular characterization

P. multocida isolates identification was confirmed by the *P. multocida*- specific primer set (KMT1T7/KMT1SP6) in uniplex PCR reaction. The capsular typing was applied by multiplex PCR analysis to investigate the presence of cap genes. Besides the capsule-specific primers (CAPA, CAPB, CAPD, CAPE and CAPF), (Townsend et al. 2001). Each 50 µl reaction contained 10ng of DNA, in DreamTaq PCR master mix (Thermo). Forty cycles, with each cycle consisting of denaturing at 94 C, annealing at 55 C and extension at 72 C for 30 sec each, followed by a final extension at 72 C for 5 min were performed in a thermal cycler (Esco-swift minipro

RESULTS

The consequence of *P. multocida* in this review affirms that Pasteurella species cannot be ignored when the research concerning ovine pasteurellosis are made, especially fattening sheep. From 300 samples (150 nasal swabs and 150 lung swab) collected and cultured, Pasteurella was isolated successfully in 39 (13%) of examined samples. *P. multocida* identification was done molecularly by KMT1T7/KMT1SP6 primers (Fig.2). The recovery rate from nasal cavities of apparently healthy animals were 7 (9.3%), while diseased animals were 9 (12.0%) and 23 (15.3%) from pneumonic lungs of emergency slaughtered animals (Table 1). Two non-culturable methods were applied for direct detection of *P. multocida* without conventional culturing, depending on direct molecular detection

on either examined sample or 24 hours preselective broth. The direct PCR on samples detection percent were 16.0 % , 13.3 % & 22.0% from nasal swabs of healthy , diseased and pneumonic lung tissues, respectively (Table 1).

Table 1: Recovery and detection rate of P. multocida from different clinical samples

Sample source / Animal status	Sample Number	Recovery rate of *P. multocida*					
		Bacteriological isolation		Direct PCR on samples		PCR on 24 incubation Pre-selective broth	
		No.	%	No.	%	No.	%
Nasal Swabs/ Apparently Healthy sheep	75	7	9.3	12	16.0	9	12.0
Nasal swab/ Diseased sheep	75	9	12.0	10	13.3	18	24
Pneumonic Lung / Emergency slaughter sheep	150	23	15.3	33	22.0	73	48.6
Total	300	39	13.0	55	18.3	100	33.3

Table 2. Distribution of *P. multocida* subspecies detection by direct PCR on 24 hours pre-selective broth

P.multocids subtype / subspecies	Direct PCR on pre-selective broth						Total	
	Nasal swabs of Apparently Healthy sheep n = 9		Nasal swabs of Diseased sheep n = 18		Lung tissues of Emergency slaughter sheep n = 73			
	No.	%	No.	%	No.	%	No.	%
Serogroup A	6	66.6	11	61.1	40	54.7	57	57.0
Serogroup B	-	-	3	16.6	23	31.5	26	26.0
Serogroup A & B	3	33.3	4	22.2	10	13.6	17	17.0
Serogroup D	-	-	-		-		-	-
Serogroup E	-	-	-		-		-	-
Serogroup F	-	-	-		-		-	-

Fig. 1: Electropherotic profile of positive *P. multocida* isolates producing 620 bp amplicons by KMT1T7/KMT1SP6 primer, M.: DNA ladder 100 bp (Jena Bioscience, Germany

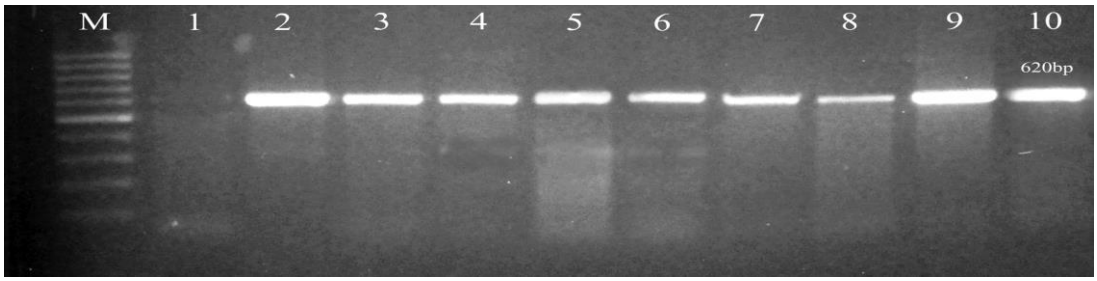
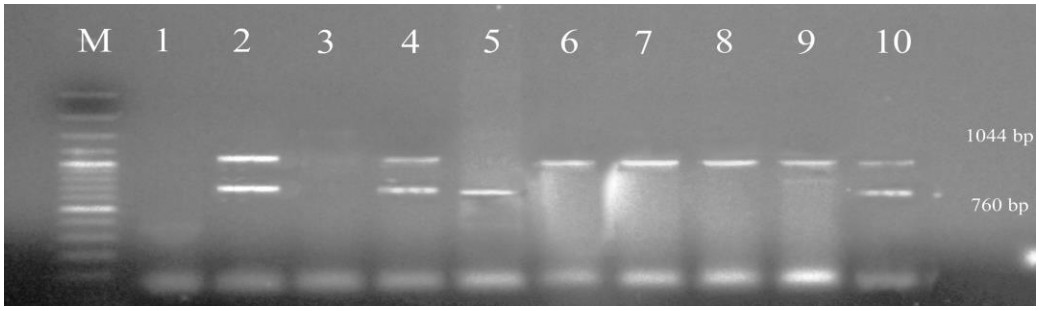


Fig. 2: Electropherotic profile of capsular typing profile of positive *P. multocida* DNA producing 1044 bp amplicons for capA type and 760 bp for capB type, M.: DNA ladder 100 bp (GeneRuler, Thermo)



The highest detection rate (33.3%) of *P. multocida* was explored by direct PCR on preselective broth in comparison to other two methods. Out of examined nasal sample of apparently healthy 9 (12.0%), diseased animals 18 (24.0%) and emergency slaughtered sheep 73 (48.6%). Molecular capsular typing were done in multiplex PCR producing amplicons of average size 1044 bp for capA type , while capB was 760 bp (Fig. 2), and the other capsular types were not produce any amplicons. The most prevalent capsule biosynthesis gene among the 100 clinical samples examined was capA (57.0%) followed by capB (26.0%) and both types capA & capB (17.0%) (Table 2). On the basis of these results *P. multocida* was associated with the most common cause of pneumonic pasteurellosis in sheep. *P. multocida* was isolated in all tested cases healthy, clinical and pneumonic.

DISCUSSION

Pasteurella multocida is a natural inhabitant of the oropharyngeal and gastrointestinal floras of a wide range of domestic animals (Yates, 1982). It can survive in the upper part of the respiratory tract of apparently healthy animals, and these carriers are believed to serve as the main spreading sources of the bacteria (Magyar and Lax, 2014). It is commonly the association of Pasteurellaceae with disease when favored by adverse combinations of host, agent, and environmental factors.

In the present study, *P. multocida* detection from samples of healthy individual animals by direct PCR either on samples (16.0 %) and on 24 hours preselective broth (12.0 %), whereas the bacteriological isolation from healthy sheep revealed (9.0%), as Pasteurella species can be isolated from most clinically healthy bighorn sheep when samples are appropriately collected and preserved prior to culture (Wild and Miller, 1991).Moreover, *P. multocida*, have been isolated from both healthy & ill bighorns sheep (Kelley et al. 2007).

High prevalence of *P. multocida* in association with pneumonic cases of emergency slathered fattening sheep due to respiratory disorders is recorded with recovery rate 15.3 % for bacteriological isolation, (22.0%) by direct PCR on samples , while direct PCR on preselective broth were (48.6%) (Table 1). The respiratory disease in fattening sheep populations is no doubt the zenith of a few biotic and abiotic elements involved in pathogenesis (Rimler, 2000),

Molecular capsular typing was conducted on genomic DNA of 24 incubation Pre-selective broth. Serogroup A, predominant in the present study, was particularly (66.6%) for healthy animals, (61.1%) for diseased, (54.4%) for emergency slaughtered animals. The highest rate of serogroup A in healthy animals confirms that Pasteurella species can cause pneumonic and septicemic pasteurellosis in fattening sheep, even the causative Pasteurella spp. strains are ‘‘normal’’ flora in sheep populations (Miller, 2001; Jenkins et al. 2007). Some cases were detected the both serogroup A & B with detection rate 13.6% for pneumonic cases, 22.2% for diseased individuals and 33.3% for the healthy individuals. The presence of *P. multocida* strains in fattening sheep population and the possible deadly consequences of unchecked transmission of pneumonic pasteurellosis within and between sheep populations. Biggest problems in this subject are; fattening sheep in the region does not take precautions or treat their animals unless there are large number of deaths and incompleted treatments or random medicine use makes disease asymptomatic or chronic. Results of the present studies clarify the complete association

between *P. multocida* and pneumonia in fattening sheep populations in Egypt. Moreover, the investigation of ovine pneumonia causes and source tracking, which can be useful in outbreak investigations and sheep population health surveillance.

CONCLUSION

Direct molecular non-culturable methods of *P. multocida* are more valuable for diagnosis of Pasteurella infection. *P. multocida* serogroup A represent one of major cause associated with epizootic pneumonia in fattening sheep in Egypt.

CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

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