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CLINICAL DIAGNOSIS AND MOLECULAR CHARACTERIZATIONS OF SHEEP PASTEURELLOSIS

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ABSTRACT:

Pasteurella multocida and Mannheimia haemolytica are the known bacterial pathogens, which cause severe respiratory diseases of sheep. So, this work intended to detect Pasteurella pathogens involved in sheep respiratory infections using culturing method, biochemical tests and PCR assay. To realize this, 75 samples of nasal swabs collected from clinically diseased sheep at Sohag Governorate. These samples subjected to bacteriological examination and the result showed that, 17 (22.7%) samples of the 75examined samples were positive to pasteurella spp.(P. multocida positive samples were15 (88.2%) and P.haemolytica positive samples were 2 (11.7%) on blood agar and biochemical tests. PCR using KMT1 gene (P. multocida) and ssa gene (P. haemolytica) specific primers, was carried out on these 17 positive samples. The results of PCR proved that only 3 (17.6%) isolates were positive for P. multocid and 2 (11.8%) isolates were positive for P. heamolytica. Considering of this fact this study suggest screening and detecting Pasteurella.Spp. By using of traditional methods and PCR technique in pneumonic sheep.

Key ***pasteurella spp., M. mannheimia spp., sheep.***

INTRODUCTION:

Sheep are important agricultural animals in all countries, which can compensate the shortage in cattle and buffaloes meat production besides wool Hakim et al. (2014).

Respiratory diseases are major cause of deaths in lambs and one of the most common associated problem of lower respiratory tract is that which caused by pasteurella species. Pasteurella causes two main diseases in sheep, pneumonic pasteurellosis and systemic pasteurellosis. The bacterium Mannheimia haemolytica (formerly Pasteurella haemolytica) is the most common cause of acute pneumonia in sheep Donachie (2000).

Both Mannheimia and Pasteurella species are commensally resident in the respiratory tract of healthy ruminants and are capable of causing infection in animals with compromised pulmonary defense system. Hence, the disease essentially triggered by physical or physiological stress created by adverse environmental and climatic conditions such as extremely bad weather, poor management, overcrowding, transportation or previous infection with respiratory viruses, mycoplasma or some

other pathogenic organisms Mohammed and Abd-elsalam (2008).

Routine identification of pasteurella spp. usually carried out by using of traditional methods was discomfort and time consuming. In recent years, genotypic methods especially nucleic-acid based assays, allow the detection of microorganisms by dramatically improving the sensitivity and decreasing the time required for bacterial identification McPherson and Moller (2000).

MATERIALS AND METHODS:

Animals and samples:

75sample of nasal swabs collected aseptically from sheep age ranged from 3 months to 5 years of different sex and different areas of Sohag Governorate. Diseased sheep showed respiratory signs such as cough, nasal discharges and fever according to Radiostitis et al., (2000).

The broth containing swabs kept in an ice box and transferred to the laboratory for bacteriological examination according to Sisay and Zerihun, (2003).

Nasal swabs inoculated on blood agar according to Cowan, (1974) then primary differentiation of the pathogen by inoculation on MacConkey agar and gram stain.

Biochemical identification carried out according to MacFaddin, (2000) for secondary identification included catalase, oxidase and indol. 17 isolates submitted for mPCR by using Kmt1 and ssa primers according to OIE, 2012 and Hawari et al., (2008) as showed in table 1.

Table 1: Oligonucleotide primers sequences, Source: Metabion (Germany).

Target agent	Target gene	Primers sequences	Amplified segment (bp)	Reference
P. multocida	Kmt1	ATC-CGC-TAT-TTA-CCC-AGT-GG	460 bp	OIE (2012)
		GCT-GTA-AAC-GAA-CTC-GCC-AC		
M. haemolytica	Ssa	TTCACATCTTCATCCTC	325 bp	Hawari et al., (2008)
		TTTTCATCCTCTTCGTC		

RESULTS

The studied animals showed respiratory signs including fever, respiratory distress and an irregular breathing pattern, serous to mucopurulent nasal discharges, lacrimation, inappetence and cough in some cases abdominal cough.

Bacteriological examination and biochemical identification revealed that number of P. multocida was 15 isolates by percentage (88.2%) and number of M. haemolytica isolates was 2 by percentage (11.7%) as showed in table 2 and figure 1.

PCR by using specific primers for (Kmt1 and ssa genes), confirmed the presence of P. spp. DNA in 5 isolates out of 17 isolates (3 P. multocida and 2 P. haemolytica) as The result of mPCR showed in table 3 and figure 2 and 3.

Table 2: number of P. spp. Isolates and percentages by using of blood agar and biochemical tests:

P. spp. on blood agar and biochemical reaction.	*P. multocida*	*P. haemolytica*
Number of positive samples17 (22.7%)	15 (88.2%)	2 (11.7%)

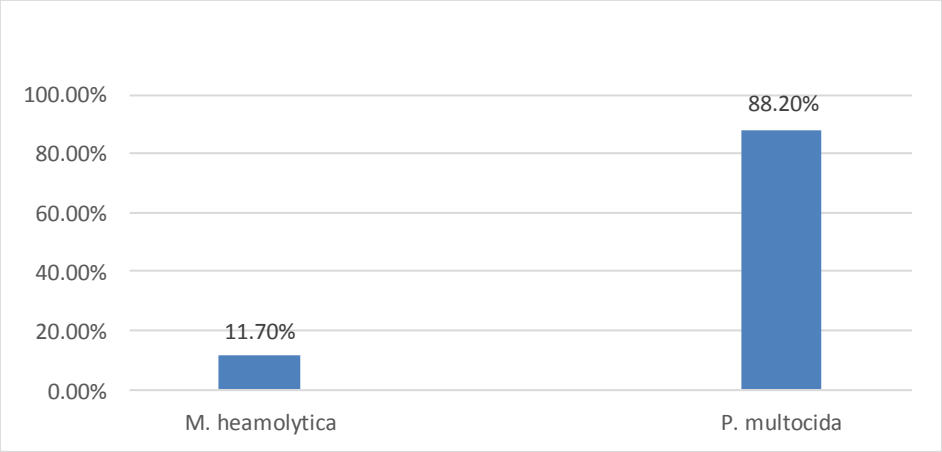

Figure 1: incidence of pasteurella spp. by using blood agar and biochemical tests.

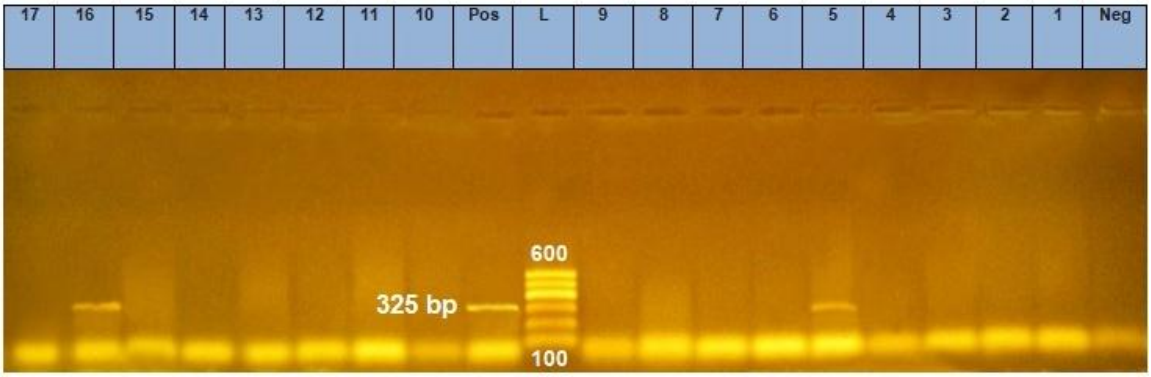

Figure 2: amplified profile of M. haemolytica DNA positive for ssa gene at 325bp lane 5 and 16 positive isolates.

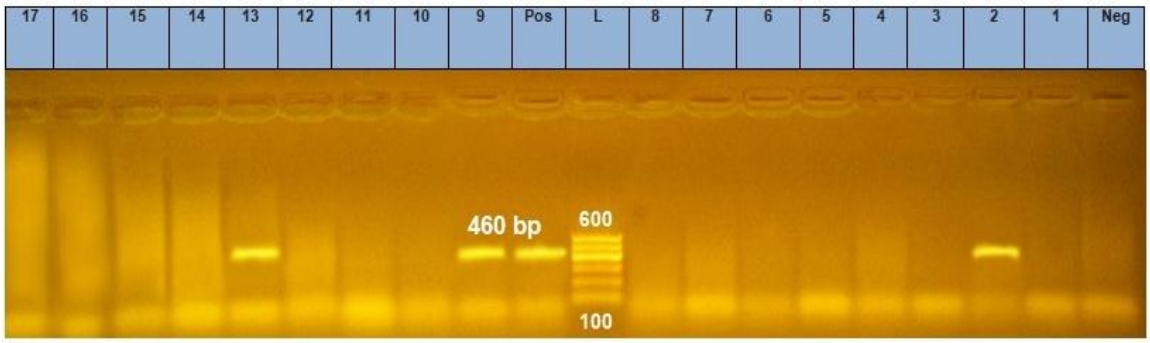

Figure 3: amplified profile of P. multocida DNA positive for Kmt1 gene at 460 bp lane 2, 9 and 13 positive isolates.

Table3: Results of PCR analysis for P. multocida and M. haemolytica in comparison with standard diagnostic methods:

Characteristics		Analyzed nasal swabs		
PCR		*P. multocida* 3 (17.6%)	*M. hemolytica* 2 (11.8%)	Negative (70)
Growth on sheep blood agar, 24 h	sheep blood agar, 24 h round and grayish with a musty or mushroom sort of smelly colonies (some variants were more opaque), non-hemolytic.	(15) +ve	-ve	-
	Circular, glistening colonies, narrow double-zone hemolysis.	-ve	(2) +ve	-
Growth on MacConkey agar, 24 h	None	+ve	-ve	-
	pink to red colonies	-ve	+ve	-
Gram stain	Gram negative coccobaccili numerous, bipolar stain marked or evenly stained rods.	+ve	+ve	-
Indole test		+ve	-ve	-
Catalase test		+ve	+ve	-
Oxidase test		+ve	+ve	-

DISCUSSION:

The studied animals showed respiratory signs including fever, respiratory distress and an irregular breathing pattern, serous to mucopurulent nasal discharges, lacrimation, inappetence and cough in some cases abdominal cough.

these results agreed with that reported by Bell, (2008); Hala et al., (2009); Jess et al., (2014) and Kumar et al., (2015).

Pasteurella isolates were recovered from 22.7% of nasal swabs samples on blood agar and biochemical tests (88.2% P. multocida more than 11.7% M.heamolytica) and this results agreed with (Kumar et al., 2000; Deressa et al., 2010; Tahamtan et al., 2014 and El Dokmak et al., 2015) and disagreed with (Hala et al., 2009; Marru et al., 2013 and Abera et al., 2014).

The variation in incidence percentage is likely to be caused by several factors including different isolation techniques, misidentification, stress factors, changes in management and immune status of infected animals and seasonal variation.

The PCR results indicated that only 3 (17.6%) of 17 isolates were positive for P. multocida while, 2 (11.8%) were positive for M. haemolytica and this results agreed with (Deressea et al., 2010). And this attributed to the PCR is more accurate than other techniques in detection of Pasteurella spp. Tabatabaei and Abdollahi (2018). Moreover, Beker et al., (2018) added that molecular techniques have a grand worth in detection and typing of the different strains belong to the family of Pasteurellaceae.

In the current study the percentage of pasteurella multocida is more than the percentage of haemolytica and this may attribute to that P.multocida is related to nasopharenx infection in acute cases more than P.haemolytica which related to lung infection and M.heamolytica infection also more common when the case is complicated by other microorganisms including respiratory viruses and mycoplasma infection also it may be attribute to type of the sample that was only from one origin.

CONCLUSION:

Phenotyping methods not reach to a high grade in specificity of pasteurella spp. identification while, PCR plays a confirmative role in and more accurate in detection of pasteurella spp. in diseased and apparent healthy animals. We suggest more sanitary conditions in rearing of sheep to avoid predisposing factors leads to occurrence of pneumonia.

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التشخيص الأكلينيكي والخصائص الجزيئية لباستيريلا الأغنام

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الملخص العربي:

تعتبر الباستيريلا مالتوسيدا والباستيريلاهيمولتيكا من العترات البكتيرية التى تسبب أعراض تنفسية شديدة فى الأغنام.

تم اجراء هذه الدراسة على الأغنام المصابة بأعراض تنفسية لتشمل عدد ٧٥ عينة (من المسحات الأنفية) تم جمعها من محافظة سوهاج.

وقد خضعت جميع العينات للفحص البكتريولوجي. باستخدام الطرق التقليدية في العزل و تم الحصول علي العزل المبدئي في الأغنام المصابة وكانت النتيجة (١٥) معزولات بكتيرية من الباستيريلا ملتوسيدا (٢٠%) و (٢) معزولات بكتيرية من الباستيريلا هيموليتيكا (٢.٧%) وبذلك فإن العدد الكلي للعزل كان (١٧) معزولة (٢٢.٧%) من المسحات الأنفية باستخدام الفحوصات التقليدية الظاهرية والاختبارات البيو كيميائيه التقليدية .

وقد تم إجراء الـــطريقة التقليدية لل ١٧ عتره المعزولة باستخدام تفاعل البلمره المتسلسل لتحديد الجين الخاص بجنس الباستيريلا ملتوسيدا باستخدام بادىء الجين ونسبة الباستيريلا هيموليتيكا KMT1 وكانت نسبة تواجد الجين (١٧.٦%) و(١١.٨%) على التوالى.ssa