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Microbiological and Epidemiological Studies on Sheep and Goat Deaths in New Valley Governorates, Egypt

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ABSTRACT

This study was carried out on four flocks of sheep and goats in New Valley governorate in July and August, 2015 in which 154 sheep and goats were dead in these two months after a short course of respiratory signs. It was noticed that the rate of deaths was higher in sheep (71.43%) than in goats (28.57%) and Flock (3) located in Shrq Aleuaynat district showed the highest rate of deaths by 15%. In addition, the highest rate of deaths occurred in age group (8 month) (38.31%) followed by age group (2 month) (25.975%) then the age group (6 month) (24.68%). Postmortem examination (PM) showed that the lesions were restricted to the thoracic cavity where lungs were firm, congested and lobulated with presence of straw fluid in the thoracic cavity with congestion in heart muscles and liver. Bacteriological examination of samples obtained from 13 freshly dead sheep and goats revealed the isolation of 10 isolates of Staphylococcus aureus, 3 isolates of Escherichia coli and 6 isolates of Klebseilla pneumoniae from samples of lung and liver tissues while no isolation was recorded from thoracic fluid, heart tissue or heart blood. PCR technique succeeded in detection of *M. ovipneumoniae* in two lung samples out of 9 samples at percentage of 22.22%. On contrary, Pasteurella Multocida could not be detected by either traditional culture or PCR technique in the examined samples. Antibiotic sensitivity test revealed that the recovered bacterial isolates were highly sensitive to tulathromycin followed by ciprofloxacin (CIP) while they were less sensitive to tetracycline (TE). Finally, it was concluded that M. Ovipneumoniae may be accused in increased death rates in sheep and goats flocks in New Valley governorate with other bacteria. In addition, it was the first recording of M. Ovipneumoniae in New Valley governorate and further epidemiological studies needed to determine the risk factors associated with occurrence of infection. It was suggested that Tulathromycin should be given to treat respiratory manifestations in sheep.

1. INTRODUCTION

Respiratory system disease is the main cause of death in the lambs and kids and leads to reduction in the productivity of farm animals Gamal et al. (2016). Althugh the predominant cause of pneumonia in sheep, is pasteurellosis or mannheimiosis, there are many pathogens also responsible for inducing pneumonia in sheep such as parasites, chlamydia, viruses and mycoplasmas but there is no great attention paid to mycoplasmal pneumonia. Other bacteria occasionally associated with respiratory disease in small ruminants are *Staphylococcus spp., Streptococcus spp., Haemophilus spp., Arcanobacterium pyogenes and Klebsiella pneumonia* (Martin 1996 and Nicholas et al 2008). Small ruminant's mycoplasma infection recorded in the entire world but it is rigorous in the Mediterranean region Zendulkova et al., (2007).

Mycoplasma ovipneumoniae (M. ovipneumoniae) is one of the mycoplasma classes, affect primarily ovine and produces lethal pneumonia in both sheep and goats (Besser et al., 2013; Dassanayake et al., 2010; Lin et al., 2008). In sheep and goats *M. ovipneumoniae* mainly causes non progressive pneumonia (Parham *et al*.2006; Besser *et al.* 2013), moreover the infected animals becomes more susceptible to other dangerous respiratory pathogens such as parainfluenza 3 virus *Pasteurella* and *M.haemolytica*, (Jones, G. E.et al., 1982; Dassanayake,.et al., 2010.).

Pasteurella (P) multocida, from the gram-negative bacteria makes respiratory manifestation in almost all animal species. On the other hand, it acts as a main cause of pneumonia or as an opportunistic microorganism to other upper respiratory tract pathogens. (Valadan et al; 2014, Odugbo 2006).

It is dramatically important to detect the real pathogens responsible for inducing the diseases to over com time losses and making suitable programs for controlling those diseases Settypalli et al. (2016). Mycoplasmas are highly fastidious bacteria difficult to culture and the best method for identification is PCR assay then denaturing gradient gel electrophoresis (Nicholas, 2002 and McAuliffe et al. 2003). Using PCR technique by amplifying the DNA fragment within *KMT1* gene is more suitable for specific detection of *P. multocida* compared with traditional bacteriological methods. Gamal (2016)

This study aimed to inspect the role of M. ovipneumoniae as a main cause of death in lambs and kids in flocks with respiratory manifestations by detection of M. ovipneumoniae in lung tissue of recently dead lambs and kids using PCR technique. In addition, isolation of the most important bacteria incriminated in causing pneumonia of sheep was attempted. Finally, the role of P. multocida in inducing high percentage of deaths particularly when there was no previous vaccination program.

2. MATERIAL AND METHODS:

2.1. Study area:

This study was conducted in months July and August in 2015 on four private sheep flocks in Shrq Aleuaynat and El-kharga city in New Valley Governorates, in history of high deaths in sheep and goats after short course of respiratory manifestation in these two months; however, treatment using tetracycline and nuflour by therapeutic doses not effect on percent of deaths. The data of dead animals were recorded. And history revealed there's no regular vaccination program for the flocks.

2.2. PM examination

Postmortem examination carried out on 13 freshly dead sheep and goat from these flocks.

2.3. Samples

From 13 dead carcasses, we take parts of pneumonic lung, liver, heart, thoracic fluid, heart blood.

2.4. DNA extraction and PCR amplification

The QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was used for DNA extraction from samples with performing some modifications from the manufacturer's recommendations. Simply, the tissue sample (25 mg) was incubated with (20 µl) Qiagen protease and ATL buffer (180 µl) at 56˚C. The buffer (200 µl) was added to the sample, pulse vortexing for 15 seconds was done for mixing. Then the mixture was incubated for 10 min at 72˚C. After incubation, 100% ethanol (200 µl) was added to the lysate. Washing and centrifugation of the sample was done according to the manufacturer's recommendations. Elution buffer provided in the kit (100 µl) was used for nucleic acid elution, the amplification of the PCR product occurred using primers from Metabion (Germany, listed in table (2).the primers were utilized in (25- µl) reaction containing (12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), for each primer (1 µl) of 20 pmol concentrations, water (4.5 µl), and DNA template (6 µl). T3 Biometra thermal cycler was used for performing the reaction.

Table (1): Types of samples collected from freshly dead carcasses

Flock No.	Lungs	Liver	Heart	Thoracic fluid	Heart blood
1	**2**	**2**	**0**	**0**	**0**
2	**3**	**2**	**0**	**0**	**0**
3	**6**	**6**	**3**	**3**	**5**
4	**2**	**2**	**2**	**0**	**0**
Total	**13**	**12**	**5**	**3**	**5**

2.5. Analysis of the PCR Products.

Agarose gel 1.5% (Applichem, Germany, GmbH) was used for PCR product separation by electrophoresis in 1x TBE buffer at room temperature using gradients of 5V/cm. the products (15 µl) was loaded in each gel slot, for gel analysis. The fragment sizes were determined by using a Gene ruler 100 bp DNA ladder (Fermentas). Photographing of the gel was done by the gel documentation system (Alpha Innotech, Biometra) and finally the data was analyzed by computer software.

2.6.1. Bacteriological examination:

The samples were thawed at room temperature and a bacteriological loop full of lung, liver, thoracic fluid and heart blood was streaked on 5% blood agar and MacConkey agar Barrow *et al*, (1993). The cultured plates were incubated aerobically at 37°C for 18-24 hours. Pure cultures were obtained by sub-culturing part of typical and well isolated colony on a corresponding medium. This method was repeated at least twice. The resulting growth was checked for purity by staining smear samples with Gram's stain. The pure isolates of bacteria were identified by using standard biochemical tests. Cowan and Steel, (1985).

2.6.2.Antimicrobial agents and media: The sensitivity of the organisms to different antimicrobial agents was done using Oxoid discs including, cefotaxime (30µg CTX), tetracycline (30µg TE), trimethoprim-sulfamethoxazole (1.25/23.75µg SXT), gentamycin (10µg GN), cephalexine (30µg CFX),ciprofloxacin (5µg CIP) and Tulathromycin (30µg TUL). The media used was Muller Hinton medium.

2.6.3. Test procedure

The method used was Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2011).

Table (2): Sequences of the used primers, target genes, amplicon sizes and cycling conditions.

Target agent	Target gene	Primers sequences	(bp) of Amplif	Frist den.	Amplification (35 cycles)				Reference
					Sec. den.	Ann.	Ext.	Final ext.	
M. ovipneu.	16S–23S inter	GGAACACCTCCTTTCTACGG	**492**	94˚C 5 min.	94˚C 30 sec.	58˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	Besser et al., 2012
		CCAAGGCATCCACCAAATAC							
P. multocid	*Kmt1*	ATC-CGC-TAT-TTA-CCC-AGT-GG	**460**		94˚C 1 min.	55˚C 1 min.	72˚C 1 min.		OIE (2012)
		GCT-GTA-AAC-GAA-CTC-GCC-AC							

Table (3): Standard inhibition zone diameter interpretive chart (CLSI, 2011)

Antibiotic		Zone of inhibition (mm)		
	Disk contents	Res. ≤	Inter.	Sens. ≥
CFX	30 µg	14	15-17	18
CIP	5 µg	15	16-20	21
CTX	30 µg	14	15-22	23
SXT	25 µg	11	12-16	17
GN	10 µg	12	13-14	15
TE	30µg	14	15-18	19
TUL	30µg	14	15-17	18

Res. = resistant; Inter. = intermediate resistance; Sens. = sensitive.

3. RESULTS

The percent of the totally dead sheep and goat in four flocks reached 4.38% in which 154 dead sheep and goat out of 3516 sheep and goats during the two months July and August 2015, the high percent of deaths recorded in flock No. 3 as it reach 15% and the low percent were in flock No. 2 as it reach 0.94%, as show in table (4) and Fig (1).

3.1.Postmortem examination revealed:

Postmortem examination for the freshly dead carcasses revealed that, pleuritis, fibrinous pneumonia associated with severe congestion of lung, patchy area of whitish grey color, perihepatitis with fibrin deposition in liver and hypertrophy of the heart and congestion of coronary artery in Heart.

The age of the dead animals explain that the high number of the deaths occurred in the age group 8 months 59 (38.31%) animals followed by age 2 month and age 6month 40 (25.97), 38 (24.68%) animals for each respectively (Table 5).

Table (4): Percent of deaths in flocks

Flock No.	Total	NO. of dead animals		Total	%
		Goat	Sheep		
1	1517	-	15	15	0.99%
2	1060	-	10	10	0.94%
3	800	36	84	120	15%
4	139	8	1	9	6.47%
Total	3516	44	110	154	4.38%

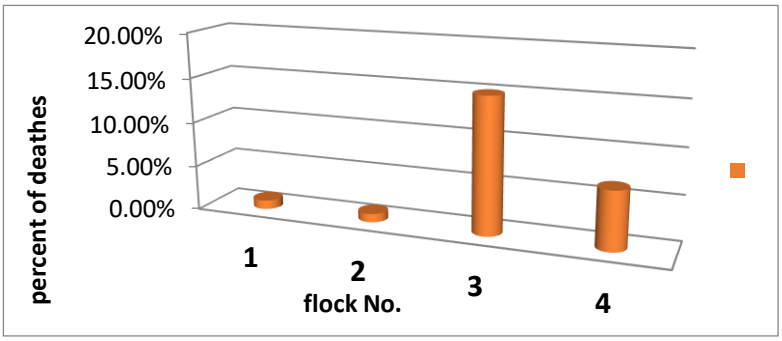

Fig (1): Percent of deaths among sheep and goats flocks

Fig: (2): Lesions from dead carcasses show: Lung, pleuritis, fibrinous pneumonia associated with severe congestion of lung. Liver, patchy area of whitish grey color---perihepatitis with fibrin deposition. Heart, hypertrophy of the heart and congestion of coronary artery.

Table (5): Ages of the dead sheep and goats

Flock	2 month	4 month	6 month	8 month	1 year	2 year	Total
1	3 (20%)	2 (13.3%)	3 (20%)	7 (46.7%)	0	0	15
2	3 (30%)	4 (40%)	0	0	0	3 (30%)	10
3	30 (25%)	7 (5.8)	33 (27.5%)	50 (41.7%)	0	0	120
4	4 (44.4%)	1 (11.1%)	2 (22.2%)	2 (22.2%)	0	0	9
Total	40 (25.97)	14 (9.09%)	38 (24.68%)	59 (38.31%)	0	3 (1.95%)	154

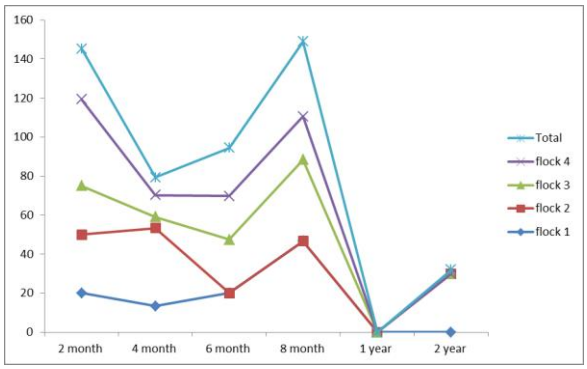

Fig (3): Ages of the dead sheep and goats.

Table (6): PCR Detection of mycoplasma ovipneumoniae and Bacterial Isolation from postmortem lesions.

Bacterial spp	Total number of isolates	Liver	Lung	PCR result
S. aureus	10	2	8	-
E – coli	3	1	2	-
K. pneumoniae	6	2	4	-
P. multocida	0	0	0	0
M. ovipneumoniae	0	0	0	2 (lung)
Total	19	5	14	2

In Table (6). PCR technique was used to detect *M. ovipneumonia*e from lung samples which revealed that the *M. ovipneumonia*e were detected in 2 lung samples out of 9 samples at percentage of (22.22%) in Fig (4).Bacterial isolation from 13 lung samples and 12 liver samples revealed that there were (10) isolates for *S. aureus*, (3) isolates for *E-coli* spp. and (6) isolates for *K. pneumoniae* while there is no isolation from thoracic fluid ,heart tissue and heart blood, and no Pasteurella spp. Were isolated from the lesions. The antibiotic sensitivity test revealed that the isolated bacteria spp. were highly sensitive to tulathromycin by 100% followed by ciprofloxacin (CIP) and low sensitive to tetracycline (TE) (Table 7).

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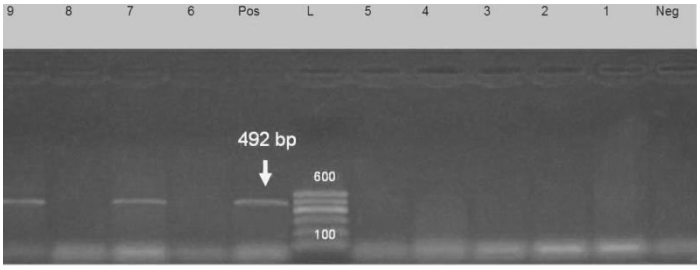

Fig (4): Gel electrophoresis of PCR products of *M. ovipneumoniae,* Lane 7&9: The amplified products prepared from positive lung samples, lanes 1-5 and 6&8 negative samples. While in fig (5) there is no detection *P. multocida*.

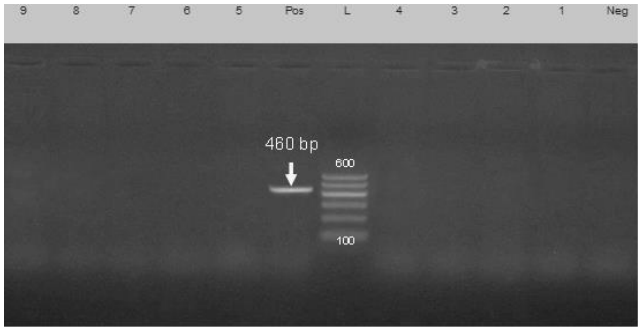

Fig (5). Gel electrophoresis of PCR product of P. multocida .there is no detection to P. multocida

Table (7): Antimicrobial Sensitivity test on the isolated bacteria.

Bacterial Spp.	Total No.	CTX		TE		SXT		CIP		GN		CFX		TUL30	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
S. aureus	10	6	60	4	40	6	60	8	80	5	50	4	40	10	100
E-coli	3	1	33.33	1	33.33	2	66.67	2	66.67	2	66.67	1	33.33	2	66.67
K. pneumoniae	6	4	83.33	1	16.6	5	83.33	6	100	4	66.67	3	50	6	100

4. DISCUSSION

Mycoplasmas have a worldwide distribution. Infection principally occurs in housed or closely stocked store lambs, particularly following two months of housing. Outbreaks can take place following the mixing Suzanna Bell (2008)

M. ovipneumoniae is serious and highly infectious pathogen causing lethal pneumonia in sheep and goat which appear in all ages especially in lambs Besser et al., (2013), Eisele and Anderson, (2011). In this study, from 13 freshly dead sheep and goats in 4 flocks in New valley *M. ovipneumoniae* were detected in 2 lung samples out of 9 samples at percentage of (22.22%) by using PCR Fig (4) this result agree with Besser et al (2013), in recent years *M.ovipneumoniae* was detected in all bighorn sheep pneumonia outbreak particularly in the presence of reliable diagnostic tools.

Bacterial cultivation revealed that presence of *S.aureus*, *E.coli* and *K.pneumonea* from lung and liver samples while there is no isolation from thoracic fluid, heart tissue and heart blood Table (6). *Harvey et al. (2007) stated that* infected sheep with *p*opulations of *M. ovipneumoniae* always found to have varying strains of the bacterium which differ in virulence. The bacterium can be found within the lungs, trachea, and nasal cavity of small ruminants. also Mebratu Asaye *et al* (2015) isolated many species of bacteria from pneumonic sheep lung but *Staphylococcus spp*.was the predominant one by percentage of 42.4%on the other hand in this study no detection to *P.multocida* that is differ from others Rosário Gonçalves (2010) recorded the high mortality rate related to combination of *M.ovipneumoniea* with *P.multocida* and *Mannheimia haemolytica* (the main cause of pneumonia in sheep and goat),which my explained by Besser et al (2008) mentioned that *M.ovipneumoniae* may act as the only pneumonia pathogen in lamb as it was the predominant one or the primary pathogen which enhances the secondary bacterial infection

Post mortem examination, the lesions in the dead animals appear to be the same with Rosário Gonçalves (2010) in epidemiological point of view the deaths were in the summer months (July and August) (Table 2, Fig 1), the total number of the dead animals are 154 sheep and goats the high percent of deaths occurred in flock 3 in Shrq Aleuaynat by 15% , the high deaths occurred in sheep species by 71.43% while goat species 28.57% the result are agree with Besser et al (2008) who mentioned that *M.ovipneumoniae* linked to the deaths of bighorn sheep in the Western United States in July 2007.

The high percent of dead animals occurred in age group 8 months 59 (38.31%) animals followed by age 2 month and age 6 month 40 (25.97), 38 (24.68%) animals for each respectively. (Table 5 &

Fig 2), which agree with Beseer *et al* (2008) who recorded that the high mortality rate in lamb with *M.ovipneumoniae* occurred at age of 42 to 70 days, also Suzanna Bell (2008) mentioned that acute disease has been seen in young lambs, a chronic infection is often found in older lambs and adults.

Antibiotic sensitivity test on isolated bacteria in table (7) revealed that bacteria were highly sensitive to tulathromycin by 100%. The results are similar to that mentioned by Evans (2005) and Clothier *et al* (2012) tulathromycin is highly effective against goat respiratory pathogens which could make it a valuable medication in this species,

5. CONCLUSION.

From our study we concluded that *mycoplasma ovipneumoni* has a great role in respiratory manifestation in sheep and cause high mortality in new valley governorates.

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