

## **BACTERIOLOGICAL STUDIES ON PASTEURELLA INFECTION IN SHEEP**

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### **ABSTRACT**

*A total of 51 samples (43 nasopharyngeal swabs and 8 lung tissues) were taken from 43 animals of private sheep flock at Dakahlia province some of which were showing respiratory manifestations, the examined include 30 lambs (8 emergency slaughtered, 14 diseased and 8 apparently healthy) and 13 adult sheep (9 diseased and 4 apparently healthy). The bacteriological examinations revealed that 58.14% of examined animals were positive for pasteurella species, Mannheimia hemolytica (M.hemolytica) represent the most common isolates as 41.86% while 23.26% were infected with pasteurella multocida (P.multocida) . Mixed infection with the isolated species was found as 6.98%. Meanwhile the isolated species were pathogenic for mice. On the other hand, the sensitivity test in vitro for pasteurella specis proved that all examined isolates were sensitive for norfloxacin and enrofloxacin while 94.12% and 88.24% were sensitive for oxytetracycline and ciprofloxacin respectively, on the other hand all isolates were resistant for amoxicillin and gentamicin but 76.47% of the isolates were resistant for streptomycin and lincomycin.*

## INTRODUCTION

Pasteurella organisms has a wide host spectrum and cause epidemic and septicemic diseases of both animals and birds. It has been incriminated as a primary or secondary cause of pneumonia (enzootic pneumonia) (*Gilmour and Angus, 1983*). Concerning sheep, there is no doubt that pasteurellosis is one of the most important infectious diseases which causes economic loss not only due to high mortalities, but also due to loss in body weight of pasteurellosis recovered sheep (*Winkler,1982*). *Jubb et al.,(1985)* mentioned that the pasteurella species were a part of normal flora of the nasopharynx of various animals, and stress factors such as shipping and climatic changes have been incriminated as an important predisposing for the proliferation of these organisms in the nasal cavity before the lung lesions were produced. Other stress factors were transportation, overcrowding, poor ventilation, heat and gathering handling (*Brogden et al., 1998*) .Pasteurella organisms transmitted from carrier sheep through contaminated air, water, feed and equipments into susceptible sheep by inhalation and by ingestion (*Jensen and Swift, 1982*). The ovine pneumonic pasteurellosis affecting all ages with high prevalence in nursing and feedlot lambs (*Jensen and Swift, 1988*). The pasteurellosis may have an acute, subacute and chronic course (*Nesbit et al.,1994*).

Investigation of pasteurella microorganisms that affect the respiratory system of sheep is very important as it enable us to evaluate the role played by these organisms ,therefore the present work was designed to enlighten the following aspects:

## **Bacteriological Studies On Pasteurella Infection In Sheep.**

- 1- Prevalence of pasteurella species in sheep suffering from respiratory disorders at Dakahlia province.
- 2- Identification of pasteurella microorganisms by their morphology and biochemical activities.
- 3- Determination of the pathogenicity of pasteurella isolates.
- 4- Testing the susceptibility of the isolates to some antimicrobial agents.

### **MATERIAL AND METHODS**

The present investigation was done on 43 adult sheep and lambs from private sheep flock suffering from respiratory manifestations at Dakahlia Province as showed in Table (1).

**Table (1):** Number of examined animals.

<b>Animal</b>	<b>Emergency slaughtered</b>	<b>Diseased</b>	<b>Apparently healthy</b>	<b>Total</b>
Lamb	8	14	8	30
Adult sheep	-	9	4	13
<b>Total</b>	<b>8</b>	<b>23</b>	<b>12</b>	<b>43</b>

#### **Samplings:**

A total of 51 samples (43 nasopharyngeal swabs from all examined sheep and 8 lung samples from emergency slaughtered lambs) were collected. The samples were collected under aseptic conditions in sterile swabs and plastic bags, and transported in ice box to the laboratory without delay.

## **Bacteriological examination:**

### **Culturing:**

Nasopharyngeal swabs were inoculated into sterile nutrient broth and incubated at 37°C for 24 hours , then subcultured onto DAS medium (Crystal Violet Cobalt Agar) which used as a selective medium for isolation of *Pasteurella* species and incubated at 37°C for 24 -48 hours.

Surface of lung tissue samples were sterilized with a hot spatula then incised with sterile scalpel and the specimen were taken by sterile platenium loop and inoculated into sterile DAS medium, incubated at 37°C for 24- 48 hours ( *Cruickshank et al., 1975*)

### **Identification:**

The resultant colonies were identified by their morphology and biochemical activities according to *Quinn et.al., (2002)*.

Pathogenicity of isolated *Pasteurella* species to mice (*Wessman (1964)* ).

Bacterial suspension for each 17 *Pasteurella* isolate (11 *M.hemolytica* and 6 *P.multocida* ) were prepared by plate washing technique. 0.1 ml of bacterial suspension of isolate ( $1.5 \times 10^8$  CFU) was injected intraperitoneally for Swiss albino mice 16-20 grams weight (2 mice used for each isolate), 5 mice used as control. The dead mice were subjected to postmortem examination, blood smears were prepared from heart blood of dead mice, stained with Leshiman's stain to notice the characteristic bipolarity of *Pasteurella* and reisolation of inoculated *Pasteurella* strains from spleen and heart blood of dead mice were carried out.

**Antimicrobial sensitivity test:**

The sensitivity of the recovered isolates of pasteurella species to antibacterial agents were done by disc diffusion method according to *Cruickshank et al., (1975)* and *koneman et al., (1992)* (10)discs of antimicrobial agents were used in the test, which produced by oxoid .

**RESULTS**

**Table (2):** Prevalence of Pasteurella species in examined sheep .

Examined animals	No. of samples	<i>Pasteurella species</i>		<i>Mannheimia hemolytica</i>		<i>Pasteurella multocida</i>	
		Positive samples	%	Positive samples	%	Positive samples	%
Emergency Slaughtered lambs	8	6	75	5	62.5	2	25
Diseased lambs	14	9	64.29	7	50	3	21.43
Apparently healthy lambs	8	2	25	-	-	2	25
Diseased adult sheep	9	5	55.56	5	55.56	1	11.11
Apparently healthy adult sheep	4	3	75	1	25	2	50
<b>Total</b>	<b>43</b>	<b>25</b>	<b>58.14</b>	<b>18</b>	<b>41.86</b>	<b>10</b>	<b>23.26</b>

N.B. 3 animals(6.98%) showed mixed infection with *Mannheimia hemolytica* and *Pasteurella multocida*

**Table (3):** Prevalence of *Pasteurella* species in examined samples

Type of sample	No. of samples	<i>Manheimia hemolytica</i>		<i>Pasteurella multocida</i>	
		Positive samples	%	Positive Samples	%
Lung of slaughtered of lambs	8	5	62.5	2	25
Nasopharyngeal swabs of slaughtered lambs	8	4	50	2	25
Nasopharyngeal swabs of diseased lambs	14	7	50	3	21.43
Nasopharyngeal swabs of apparently healthy lambs	8	-	-	2	25
Nasopharyngeal swabs of diseased adult sheep	9	5	55.56	1	11.11
Nasopharyngeal swabs of apparently healthy adult sheep	4	1	25	2	50
<b>Total</b>	<b>51</b>	<b>22</b>	<b>43.14</b>	<b>12</b>	<b>23.53</b>

**Table (4):** Sensitivity of *Pasteurella* species isolates from sheep samples (n=34) to some antimicrobial agents.

Chemotherapeutic	Disc potency	Sensitive isolates		Resistant isolates	
		No.	%	No.	%
Oxytetracycline	30 ug	32	94.12	2	5.88
Ciprofloxacin	30 ug	30	88.24	4	11.76
Norfloxacin	10 ug	34	100	-	-
Enrofloxacin	5 ug	34	100	-	-
Streptomycin	10 ug	8	23.53	26	76.47
Sulfamethaxole	23.75 ug	10	29.41	24	70.59
Trimethprim	5 ug	12	35.29	22	64.71
Gentamycin	10 ug	-	-	34	100
Lincomycin	15 ug	8	23.53	26	76.47
Amoxicillin	25 ug	-	-	34	100

## DISCUSSION

Pneumonic pasteurellosis is one of the most important diseases of sheep throughout the world . Both *Manheimia hemolytica* and *Pasteurella multocida* have been associated with the disease (**Oden Daal and Henton 1995**).

The overall prevalence of Pasteurella species in examined sheep of this study was 58.14%. which was higher than that recorded by **Andrawis (2001)** but lower than that found by **Black (1997)**. The high incidence of Pasteurella species may be attributed to the nature of breeding which mainly grazing of the examined sheep flock and also the transportation from place to another which increase the propagation of infection beside its act as predisposing factor for Pasteurellosis.

Also the obtained results proved that the lambs were more susceptible to the infection than adults and this was agreed with **Gilmour and Gilmour (1989)** and **Abd El Rahiem (2002)** who recorded the same result in Menofia, Damietta and Kafr El Sheikh Provinces, Egypt. On other hand **Frank (1982)**, **Baysal and Guler (1992)** and **El Beskawy (2005)** reported that adult sheep were higher in rate of isolation than lambs and they attributed this to that, the carrier state increased in older age than young.

The results reported in (Table 3) showed that the *Manheimia hemolytica* was isolated as 25 % from nasopharyngeal swabs of apparently healthy adult sheep and not isolated from apparently healthy lambs. These results were in agreement with that reported by **Gilmour and Angus (1983)** and **Davies (1985)** who stated that *M. hemolytica* is a part of normal nasal microflora of healthy sheep, but the obtained results were

higher than that recorded by *Hussein et.al., (2000) and El Beskawy (2005)*. Meanwhile *Pasteurella multocida* was observed as 50 % and 25 % in nasal swabs of apparently healthy adult sheep and lambs respectively which agreed with *Chandrasekaran et.al., (1991)* who recorded that *P. multocida* was found as a respiratory common pathogen in sheep in temperate areas but may be of greater importance in tropical areas. On the other hand the detected results were higher than reported by *Hamdy et .al., (1989) and Elyas (1993)*. From nasopharyngeal swabs of diseased adult sheep *M. hemolytica* was isolated as 55.56 % while it was 50 % in diseased lambs. *Pasteurella multocida* was recorded in nasal swabs of diseased adult sheep as 11.11 % while diseased lambs recorded 21.43 % .The obtained results were higher than that recorded by *Kaya and Kirkan (1999) and Abd El Rahiem ( 2002 )* .On the other hand *Abd El- Latif and El Dessouky (2006) stated that Pasteurella multocida and M. hemolytica* were found in clinically diseased lambs in Dakahlia province as 17.14 and 5.71 % respectively.

Concerning lung samples of slaughtered lambs, this study declared that the *Pasteurella* species were isolated as 87.5 % ( *M. hemolytica* 62.5 % and *Pasteurella*

*multocida* 25 %), the obtained results were lower than that recorded by *El Beskawy (2005)* who declared that *Pasteurella* species isolated as 99.99% (*M. hemolytica* 80.88 % and *Pasteurella multocida* 19.11 %), also *Hatem et al., (2003)* isolated *Pasteurella multocida* as 30% and *El Sukhon (1995)* isolated *Pasteurella hemolytica* in Jordon as 36 % .

The present study revealed that, all tested isolates of *Pasteurella* speices were pathogenic to injected mice (the death have been occurred within 72 hours post inoculation).

Regarding to the sensitivity test, Table 4 showed that both tested isolates of *Pasteurella hemolytica* and *pasteurella multocida* were sensitive to n.orfloxacin and enrofloxacin (100 %), oxytetracycline (94.12%) and ciprofloxacin(88.24%). The isolates were resistant to gentamicin and amoxicillin with percentage 100%, streptomycin and lincomycin (76.47 %) ,sulfamethaxole (70.59 %) and trimethprim (64.71%). These results were some what differ from **Mackie et.al.,(1995)** who found that *M. hemolytica* where sensitive to oxytetracycline ,pencillin, amoxicillin, cephalothin, erythromycin and resistant to Sulfamethoxole, trimethprim, lincomycin, streptomycin, neomycin, gentamicin and kanamycin, While **Lin et al., (2001)** showed that both *M. hemolytica* and *P. multocida* isolated from chicken were sensitive to ceftiofur , amoxicillin , lincomycin and spectinomycin, moderately sensitive to tetracyclin and slightly sensitive to chloramphenicol. Also **Talab (2002)** found that *Pasteurella hemolytica* were sensitive to ciprofloxacin (92.5 %) and gentamicin (20 %) and in the same time resistant to neomycin. So sensitivity test for drug choice to treatment of Pasteurella infection must be done.

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## دراسات بكتريولوجية للإصابة بالباستريلا في الأغنام

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معهد بحوث صحة الحيوان - فرع المنصورة

أجريت الدراسة على 51 عينة أخذت من 43 من الأغنام والحملان في قطيع من الأغنام في محافظة الدقهلية مصابة بأعراض تنفسية وتشمل 30 حمل (8 ذبحت اضطراريا و14 مصابة و8 سليمة ظاهريا) وكذلك 13 من الأغنام (9 مصابة و4 سليمة ظاهريا) وقد أظهر الفحص البكتريولوجي ان 58.14% من الحيوانات المفحوصة ايجابية لميكروب الباستريلا. حيث وجد أن نسبة كبيرة (41.86%) من تلك الحيوانات مصابة بميكروب المانهيميا هيملتكا بينما 23.26% مصابة بميكروب الباستريلا مالتوسيدا وفي الوقت نفسه وجد أن 6.98% من هذه الحيوانات مصابة بكل منهما ومن ناحية أخرى وجد أن تلك المعزولات مميّنة للفئران الالبنو وبإجراء اختبار الحساسية لتلك الميكروبات المعزولة وجد أن جميع المعزولات المختبرة حساسة لنورفلوكساسين والانروفلوكساسين، بينما 94.12%، 88.24% من المعزولات حساسة للأوكسى تتراسيكلين والسبروفلوكساسين على التوالي وعلى النقيض من ذلك وجد أن جميع المعزولات مقاومة للأموكساسلين والجينتاميسين ولكن 76.47% من المعزولات المختبره مقاومة للإستربتومايسن والنكوممايسن.