Prevalence of some respiratory diseases among sheep and goats in Shalateen , Halaieb and Abu-Ramad Areas

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Nasopharyngeal swabs and pnuemonic lung autopsies collected from diseased or slaughtered sheep and goats suffering from respiratory manifestation were subjected to microbiological sreening. In addition, serum samples were collected from all animals were investigated. *P. hemolytica* was the most prevalent recovered bacterial isolates followed by *S. aureus and E. coli*. On the other hand, *Aspergillus fumigatus* was the most prevalent fungus. *Aspergillus* species and *Candida albicans* were also isolated. Most of the isolated bacterial strains were found sensitive to spectrama and chloramphenicol. Serodiagnosis of *P. hemolytica* by ELISA using the whole cell antigen gave positive results in 18.3 and 22.5% of diseased sheep and goats respectively and 52% and 42.1% of slaughtered pneumonic sheep and goats respectively. Also serodiagnosis of *Aspergillus fumigatus* by indirect haemagg-lutination test revealed positive results in18.3and 17.5% of diseased sheep and goats respectively and 24% and 21% in sera of slaughtered pneumonic sheep and goats respectively. Histopathological changes due to *P. haemolytica* and *Aspergillus fumi-gatus* were recorded.

Respiratory disorders represent one of the most important causes of great economic losses among animals. Lung affections in farm animals constitute serious problem that hinders animal production and may result in great losses in animal husbandry. The primary causes of sheep and goat pneumonia are bacteria, viruses and fungi whereas pour hygienic measures and climate disorders are the most predisposing factors to infection (Hafez *et al,* 1991 and Elyas *et al,* (1984).

Pasteurella speices *(P. multocida, P. haemolytica*), *Pseudomonas* sp. and *Klebsiella pneuomniae* are the important bacterial causes of sheep pneumonia (Quinn *et al.*, 1994) while *aspergillus* species is the main cause of fungal pneumonia in sheep (Kamil and Parihar, 1991).

Kaya and Erganis (1991) isolated *S. aureus* alone or in combination with other organisms from sheep.

Mishra (1988) isolated Aerobacter, Coryne-bacterium, *Esherichia coli*, Klebsiella, Micro-coccus, Pasteurella , Pseudomonas , Staphyloco-ccus and Streptococcus species from pneumonic lungs of goats.

Elyas (1995) isolated *E. coli, S. aureus*, S. *epidermidis,, A. pyogenes , C. ovis , S. pneumoniae , P. aeruginosa , K. pueumouiae , P. multocida*, *As. flavus* and *As. terreus* from pneumonic lung sheep. *P. haemolytica* is the most important cause of bacterial respiratory mortality in cattle and sheep. It is also, identified as the major cause of systemic deaths in sheep. Rowe *et al*. (2001)

Antigens that may serve as potential immunogens have been characterized as whole cell and leukotoxin antigen (Derek *et al*, 1989).

The aim of this study was to isolate and identify the possible causative bacterial and fungal pathogens of such respiratory disease conditions as direct methods of their diagnosis and antibiogram of the isolated bacteria and fungi in was also to reach an available and specific treatment.

Material and methods

Animals. Sixty and 40 of clinically diseased sheep and goats respectively suffering from respiratory manifestation were examined and 25 and 19 of pneumonic lungs of (Slaughtered) sheep and goats respectively were employed in this study.

Samples. Nasopharyngeal swabs of 60 sheep and 40 goats suffering from respiratory disorders. From 25 and 19 slaughtered sheep and goats respectively. The samples were taken under aseptic conditions on transport media for bacteriological and mycological examination. Serum samples were collected from all examined animals.

Clinical examination. Animals were subjected

to clinical examination, this include recording the clinical signs, body temperature, pulse and respiratory rate.

Bacteriological and Mycological examination. Nasopharyngeal swabs were inoculated into nutrient broth and incubated at 37°C for 24 hr. Subcultured was performed onto the following media:

nutrient agar, 5% sheep blood agar, MacConkey agar and S.S agar at 37°C for 24-48 hr. as well as Sabouraud's dextrose agar at 25°C and 37°C for 5 days.

The surface of the lung specimens was sterilized with a hot spatula, then with sterile platinum loop to be inoculated in the previously mentioned media. Colonies were identified mycologically according to (Baily and Scott 1974; Conan *et al*., 1971 and Ellis, 1976).

Antibiogram sensitivity test for bacterial isolates was done by disc diffusion method using the following antibiotic discs, ampicillin (10 mg), kanamycin (30 mg), chloramphenicol (30 mg), Erythromycin (15 mg) oxytetracyclin (30 mg), spectrama (10 mg) and lincomycin (10 mg) according to the method described by (Cruickshank *et al*., 1975).

ELISA. serological diagnosis of pasteurellosis in sheep and goats by ELISA according to Derek *et a*l. (1989). Whole cell *P. haemolytica* antigen was prepared from fresh 18 hours cultures of *P. haemolytica* suspended to concentration of 10^9 cfu in phosphate buffer saline (pH7.4) according to Derek *et al.* (1989).

Indirect haemagglutination (IHA). Serological diagnosis of Aspergillosis in sheep and goats was performed using (Fumoze Diagnostics, France) according to the method of (Senet and Brisset, 1973).

Histopathological studies. Histopathological sections were prepared from pneumonic lungs. Tissue sections were stained by H&E and GMS (Clayden, 1971)

Results and discussion

In Table (1) the main clinical signs were rise of body temperature (39.5-40°C), depression, eye and nasal discharges, loss of appetite and acceleration of respiration. By auscultation, exaggerated vesicular sounds in some cases, moist ralles with frictional sounds. The post mortem findings included congestion of the lung accompanied with heavy fibrinous, grayish or yellowish exudates within the bronchi. In some cases grayish white abscesses with offensive odors and sometimes pulmonary edema were recorded. The most important bacterial and mycotic isolates of the nasopharyngeal swabs and pneumonic lungs of sheep and goats, the antibiogram of the bacterial isolates and serodiagnosis against *P. haemolytica*, *A. fumigatus* in sheep and goats are demonstrated in (Tables 1- 5).

Respiratory diseases in sheep and goats and in particular pneumonia are the result of interaction of many infectious agents under the influence of physical stresses (Rahman and Lyer, 1979 and Martin, 1983).

The results recorded in (Tables 2 and 3) revealed that *P. haemolytica* was the main bacterial cause of sheep and goats pneumonia as its percentage was 16.6% and 20% in nasopharyngeal swabs of sheep and goats respectively and 32% and 31.6% in the pneumonic lungs of sheep and goats. These results were agreed with Mishra (1988), Hafez *et al.* (1991); Mackie *et al,* (1995); Martrenchar *et al* (1995); Black and Duganzich (1995); Fodoral *et al*. (1999) and Mohamed and Shaker (2002) who isolated *P. haemolytica* from apparently healthy (56 %) and diseased sheep (75%) while 66 % and 90 % from emergency slaughtered and dead animals respectively.

In the present study, *S. aureus*, *E. coli,* Streptococci, *K. preumoniae*, *P. multocida* and *P. aeruginosa* were also isolated from both nasopharyngeal swabs and pneumonic lungs of sheep and goats. Similar findings were reported by Biberstein *et al.* (1967) and Sayed (1996).

Qunin *et al.* (1994), recorded that *K. pneumonae* and *P. aeruginosa* cause pneumonia and lung abscesses in sheep.

Isolation of Salmonella species from nasopharyngeal swabs of diseased sheep and from pneumonic lungs was in agreement with Thabet (1993).

The isolation of fungi concurrently mixed with one or two bacterial isolates are in agreement with Thabet (1993) and Sayed (1996) who reported that different bacterial strains could be isolated in combination with fungi from respiratory tract of camels and sheep. These results may be due to several factors as hygienic measures, environmental conditions, nutritional deficiencies and immune status of the animal (Darwish *et al.*, 2001)

Mycotic pneumonia in some cases is very dangerous due to lack of quick laboratory diagnosis and usually pneumonia is produced as a result of mixed infection with bacteria In this study the reported data proved that the overall incidence of aspergillosis infection in sheep and

Table (1) Clinical signs and post mortem findings of sheep and goats.

Species	No. and status	Samples	Clinical	Post mortem
Sheep	60 diseased	60 nasopharyngeal swabs	60 and 40 diseased sheep and goats showed rise of body temperature (39.5- 40ºC), depression, Increased eye and nasal discharge, loss of appetite and acceleration of respiration.	25 and 19 pneumonic lung of sheep and goats showed congestion of the lung accompanied with heavy fibrinous , grayish or yellowish exudates within bronchi , in some cases greyish white abscesses with offensive odours and sometimes pulmonary odema.
	25 pneumonic (Slaughtered animals).	25 samples of pneumonic lung		
Total	85			
Goats	40 diseased	40 nasopharyngeal swabs	Auscultation revealed exaggerated vesicular sounds, moist ralles with frictional sounds.	
	19 pneumonic (Slaughtered animals).	19 samples of pneumonic lung		
Total	59			

Table (2) Types and number of microorganisms isolated from infected sheep.

Microorganisms	Nasopharyngeal swabs (60)		Pneumomic lungs (25)	
	No	%	No	%
P. haemolytica	10	16.6	8	32
S. aureus	9	15	3	12
E. coli	7	11.7	2	8
K. pnoumoniae	5	8.3	2	8
S. Pyogenes	5	8.3	-	-
S. pneumoniae	4	6.7	1	4
P. multocida	3	5	2	8
P. aeruginosa	3	5	1	4
Salmonella species	2	3.3	1	4
P. multocida + E. coli + A. fumigatus	2	3.3	-	-
K. pneumoniae + S. aureus + A. fumigatus	2	3.3	-	-
S. aurens + C. albicans	2	3.3	2	8
S. aureus + E .coli + A. niger	1	1.7	-	-
A. fumigatus	5	8.3	3	12
Total	60	100%	25	100%

Table (3): Types and number of microorganisms isolated from infected goats.

Microorganisms	Nasopharyngeal swabs (60)		Pneumonic lungs (25)	
	No	%	No	%
P. haemolytica	8	20	6	31.6
S. aureus	7	17.5	4	21
E. coli	5	12.5	3	15.8
S. pyogenes	4	10	1	5.3
K. pnoumoniae	3	7.5	1	5.3
P. aeruginosa	3	7.5	1	5.3
S. pneumoniae	4	10	1	5.3
S. aureus + E. coli + A .niger	3	7.5	-	-
E. coli + C. albicans	1	2.5	-	-
S. pneumoiae + A. flavus	1	2.5	-	-
K. pnoumoniae + A .fumigatus	-	-	2	10.5
A. fumigatus + A. niger	1	2.5	-	-
	40	100%	19	100%

Table (4): Antibiogram of isolated bacteria from nasopharyngeal swabs and pneumonic lungs of sheep and goats.

Microorganisms	Ampicilin	Erythro-micin	Linco-mycin	Spectr-ama	Chloram-phnicol	Kana-mycin	Oxytetra-cyclin
P. haemolytica	+	+	-	+++	++	+	+
S. aureus	+++	+	-	+++	+++	++	+
E. coli	+	+	+	+++	++	++	+
K. pnoumoniae	-	-	-	+++	++	++	-
S. pyogenes	+	++	-	+++	-	++	-
S. pneumoniae	+	++	-	+++	+	++	+
P. multocida	+	+	-	+++	++	+	-
P, aeruginosa	+	+	-	+++	+	++	-
Salomonella spp.	+	+	-	++	+++	-	++

Table (5): Antibody response of sheep and goats to *P. haemolytica* whole cell antigen using ELISA.

Species	Sheep				Goats			
Status & number	Diseased (60)		Slaughtered with pneumonia (25)		Diseased (40)		Slaughter with pneumonia (19)	
	No	%	No	%	No	%	No	%
+ve culture & +ve ELISA	7	11.7	8	32	6	15	6	31.6
+ ve culture & -ve ELISA	3	5	-	-	2	5	-	-
-ve culture & +ve ELISA	4	6.7	5	20	3	7.5	2	10.5
- ve culture & - ve ELISA	46	77	12	48	29	72.5	11	57
Total + ve culture	10	16.7	8	32	8	20	6	31.6
Total +ve ELISA	11	18.3	13	52	9	22.5	8	42.1

Cut off sheep = 0.6
Goats = 0.5

Table (6): Antibody response of sheep and goats to *Aspergillus fumigatus* using IHA.

Species, status and No.	> 1/80	1/80	1/160	1/320	1/460	1/1280	1/2560	Positive culture		Positive IHA	
								No.	%	No	%
Sheep, diseased (60)	30	15	4	2	4*	3*	2*	9	15%	11	18.3
Slaughtered sheep, with pneumonia (25)	11	5	3	3	1*	1*	1*	3	12	6	24
Diseased goats (40)	17	8	5	3	1*	1*	2*	4	10	7	17.5
Slaughtered goats with pneumonia (19)	6	6	3	2	1*	1*	-	2	10.5	4	21

+ve IHA > 1/320
*= Positive culture
IHA = Indirect Haemagglutination

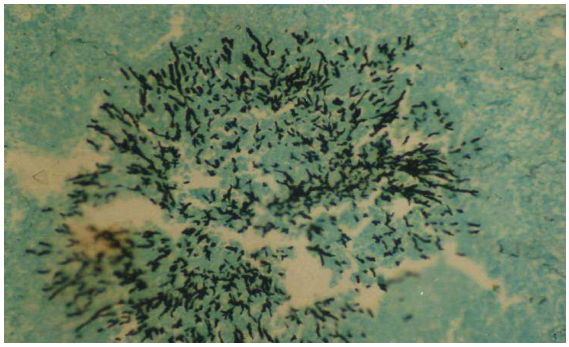

Fig. (1): Lung of sheep showing mononuclear cells infilteration with intra alveolar edema. (H&E. x100)

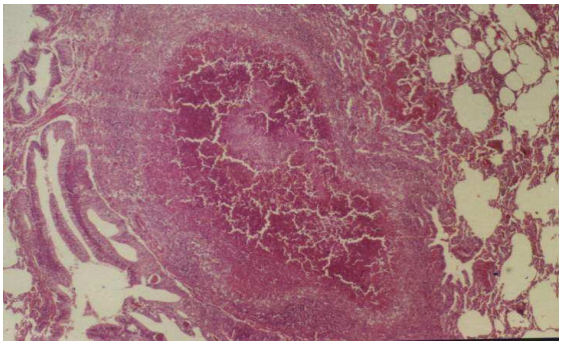

Fig. (2): lung of sheep showing large mycotic granuloma. (PAS, x100)

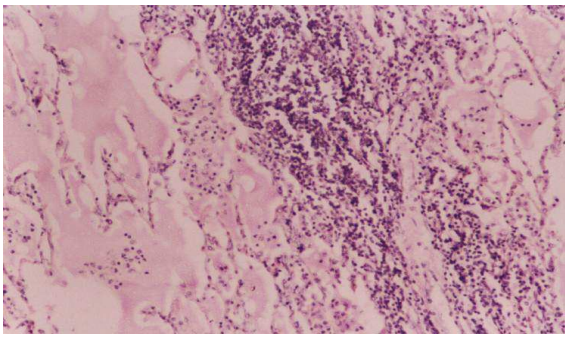

Fig. (3): Chronic mycotic granuloma in lung tissue stained by GM.s. showing central small nodule of A. fumigatus. (x220)

Nicolas *et al*. (1985) who reported that 12% of respiratory diseases in sheep were attributable to aspergillus infection lower value was reported by Singh *et al*. (1995), the incidence of aspergillosis was 4.9 % in both sheep and goats. In the present study *A. fumigatus* was the most common isolate among Aspergillus species constituting 15 and 10 % in diseased sheep and goats and 12 and 10% in pneumonic lungs of sheep and goats respectively proving that it was the major cause of pulmonary aspergllosis. These results simulated that reported by Austwick *et al.* (1960) and Austwick (1962) and Gonzalez *et al.* (1993) who reported that bovine and ovine pulmonary aspergillosis were mostly attributed to *A. fumigatus* infection.

Antibiogram of the isolated bacterial species yields the antibiotic of choice for proper treatment. In this investigation, most of bacterial isolates were highly sensitive to spectrama and chloramphenicol and moderately sensitive to kanamycin, erythromycin and ampicillin and less sensitive to lincomycin and oxytetracycline.

Table (5) showed, the results of antibody response of sheep and goats to *P. haemolytica* whole cell antigen using ELISA. Aquilar *et al*. (1994) suggested that whole cell antigen stimulate somatic antibody response.

The results of ELISA as presented in (Table 5), showed significant increase in antibody titer to whole cell antigen in 18.3% and 22.5% of examined diseased sheep and goats. 52% and 42.1% in sera of slaughtered sheep and goats respectively. These results go in hand with Sahr and Maysa (2002) who diagnosed *P. haemolytica* in sheep by isolation from lungs and serologically by ELISA.

Concerning the results of serodiagnosis against *A. fumigatus*, (Table 6), by using indirect heamagglutination (IHA) test, it was noticed that 18.3% and 17.5% of diseased sheep and goats respectively and 24% and 21% of slaughtered sheep and goats respectively had antibodies against *A. fumigatus* with significant titer $\geq$ 1/320. These results agree with Osman (2000) who used IHA for detection of antibodies against *A. fumigatus* in sera of sheep and goats, with approximately equal results El-Shayeb (1996) detected antibodies in serum of cattle with incidence of 46.6%. On the other hand Iwata *et al*. (1990) proved that 2.9 % of examined serum samples of cattle were positive. These results agreed with the findings recorded by Kate

(1981) who recorded that IHA test was a reliable and rapid method for detecting antibodies against aspergillosis in serum samples. Histopathological examination of the lung tissue of infected sheep and goats revealed mycotic pneumonia characterized by the presence of multiple focal mycotic granuloma, septated hyphae of *A. fumigatus* scattered in the center of granuloma surrounded by polymorphonuclear cells, lymphocytes and macrophages with proliferation of fibrous connective tissue. These pathological findings are in agreement with that recorded by Chattopathyay *et al*. (1987) and Singh *et al*. (1995) in sheep and goats.

In the present work, *P. haemolytica* was responsible for the severe pathological changes developed in the lung of sheep and goats, which were interstitial pneumonia and pleuritis. These results were inagreement with Mohamed and Shaker (2002).

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