

Mycotic and bacterial studies on pneumonia among sheep flocks in matrouh governorate

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Abstract

The study was included 424 animals of sheep flocks of different ages and sexes belonged to 3 localities in Matrouh governorate, Egypt. Each flock was divided into two groups according to their ages (1day-3month) and (3-6 months). Clinical observations and examination revealed that 198 animals showed respiratory distress. 120 cases emergency slaughtered and 98 dead of the total diseased animals formed the materials of the work. Swabs from nose, trachea, larynx and trachea were taken under complete aseptic conditions for bacteriological examinations. Also p.m. sample from affected lungs has been taken under complete aseptic conditions for bacterial and mycotic examinations. Isolated microbes obtained were identified morphologically, culturally and biochemically according to Cruickshank (1975). In mycotic examination the samples were cultured on modified Sabauraud dextrose agar and the fungi was based on the observation, classification and description of Gilman (1957). Clinical examination of sheep flocks revealed a general trend of high prevalence of respiratory affections of newly born lambs as compared with older ones. The prevalence of respiratory distress reached 75% and 61.4% in some sheep flocks. Also, prevalence of death among newly born lambs reached 67.4% in some animal flocks. Various types of bacteria were isolated from both emergency slaughtered and dead cases. Total of 34 bacterial isolates were obtained. Among the total bacterial isolates Staph.aureus (11.11%) and E.coli (6.35%) constituted the highest incidences. The isolated Fungi from pneumonic cases were belonged to Aspergillus, Mucor, Rhizopus and Pencillium Species. The various types of Aspergillus spp. constitute the highest incidences 36.6% in relation to all cases and 41.48% in relation to all isolates.

Key words: Sheep diseases, Mycotic pneumonia, bacterial pneumonia, respiratory infection.

Introduction

Fungi are eukaryotic, unicellular or filamentous, multi cellular hyphal, mostly non flagellate, nutritionally absorptive organisms with sell walls containing chitin and beta- glucans that reproduce sexually or asexually (Ross bond 2008).

Mycosis is an infectious diseases caused by fungus. A systemic mycosis is a fungal infection involving one or more deep organs. (Ross bond 2008) Taxonomic studies of the fungi that cause pulmonary diseases have resolved the most urgent problems of classification and identification.

Knowledge regarding the ecology of these

fungi, i.e. the study of the relationship to their environment, is rather superficial and scanty (Libero Ajello 1967). Fungal pneumonia is an infection of the lungs by fungi. It can be caused by either endemic or opportunistic fungi or a combination of both. (Meersseman, et al., 2007).

Specific instances of fungal infections with pulmonary involvement include: Histoplasmosis, which has primary pulmonary lesions. Coccidioidomycosis that cause Valley Fever. Pulmonary bastomycosis, pneumocystis pneumonia, in immunocompromised cases and sporotrichosis can involve the lung. Cryptococ-

cosis occurred through inhalation of soil contaminated with the yeast, Aspergillosis, and rarely by candidiasis. (Dulpa, *et al.*, 2004).

Pneumonia in sheep is rarely simple, especially in a group of lambs. The actual infecting agent could be one of a number of bacteria, a mycoplasma or a virus or an interaction of viruses, fungi and bacteria. Many of these organisms are commonly found in the respiratory tract of healthy lambs where they cause no trouble that is until some predisposing factor stresses. Respiratory infections constitute the most costly and troublesome disease problem in sheep in Egypt. Sheep respiratory disease complex is an acute or sub acute inflammatory reaction within the nose, Para nasal sinuses, larynx, trachea, bronchi and lungs.

The scope of these studies is summarized as follows:

Age of affected animals, morbidity, mortality rate and the general therapeutic measures applied by the local veterinarians. Clinical examination of the animals with selection of those

with apparent respiratory manifestation. Post-mortem examination of dead and emergency slaughtered animals was aimed. Isolation and identification of pathogenic bacteria as well as the pathogenic fungi responsible for the present diseased condition.

Materials and methods

During the last year, there were varying degrees of respiratory affection among sheep flocks. The total number of animals was 424 of different ages, sexes and belonged to 3 localities in Matrouh governorate, Egypt. The work was done during years 2009 and 2010. Animals of each flock were divided into two groups according to their ages (one day-3 months) and (3-6 months). Clinical observations and examination revealed that 198 animals showed respiratory distress. 120 cases (22 emergency slaughtered and 98 dead) of the total diseased animals.

These animals were grouped as follow:

Table (1): Animal grouping according to their ages.

Age (months)	No. of lambs	Diseased	slaughtered	dead
1 day -3	260	141		85
3-6 months	164	57	22	13
Total no. of animals	424	198	22	98

Swabs from the nose, larynx and samples from the affected lungs tissues of each animal were collected in clean plastic bags. During collection it was noted that nasal and laryngeal swabs were taken under complete aseptic conditions. Lungs showing pathological changes were taken in plastic bags, placed in sterile jars. The surface of affected organs was seared with hot spatula then opened with sterile scalpel. The pus materials was taken with a sterile platinum loop and inoculated in the culture media for bacterial and fungal examination.

A- Bacteriological examination: Samples were collected immediately after evisceration, transferred to laboratory at the research institute, Matrouh province. These samples were subjected to:

1- Direct microscopic examination. Two blood samples were made from peripheral blood was taken into clean, dry and sterile glass slide and stained with Giemsa stain for direct microscopically examination to detected the presence of bipolar microorganisms.

2- Isolation and identification of microorganisms. The taken swabs inoculated into sterile broth culture media, and incubated for 24 hours at 37°C and inoculated into blood agar and

MacConkey agar plates. Isolates obtained were identified morphologically, culturally and biochemically according to (Cruickshank, 1975).

B- Mycotic examination: Collected materials were cultured on modified Sabouraud's dextrose agar medium with and without Actidions

(Moss, and MoQuown, 1969) Identification of respiratory fungi generally was based on the observation, classification, and description of (Gilman, 1957).

Results

The clinical signs include coughing, difficult breathing, and high temperature, some may die. Clinical examination of sheep flocks revealed a general trend of high prevalence of respiratory affection of newly born lambs as compared with older ones. The prevalence of respiratory distress reached 75% and 61.4% in some sheep flocks. Also, prevalence of deaths among newly born lambs reached 67.4% in some animal flocks. Various types of bacteria were isolated from both emergency slaughtered

and dead cases. (Table, 2) .Total of 34 bacterial isolates were obtained. Among the total bacterial isolates, *Staph. Aureus* and *E.coli* constituted the highest incidences. (Table, 3).On the other side, the isolated fungi from pneumonic cases were belonged to *Aspergillus*, *Mucor*, *Rhizopus* and *Pencillium species*. 92 isolated fungal strains were identified from slaughtered and dead cases. The various types of *Aspergillus spp.* constitute the highest incidences 36.6% and 41.48% in relation to all cases and to all isolates respectively. Other fungal spp., showed lower percentage. (3.9%) (table, 4). A few cases showed mixed infection of both bacterial and fungal infection (6 cases) (table, 5).

Table (2): Prevalence of respiratory affections among sheep flocks.

Age/region	Total no. of animals	diseased		Slaughtered		Dead	
		No.	prevalence	No.	%	No.	%
Region1							
1 day-3 m.	150	68	45.3	-----	-----	37	54.4%
3-6 m.	80	20	25	10	50%	6	30%
Region 2							
1 day-3m.	70	43	61.4	-----	-----	29	67.4%
3-6 m.	52	22	42.3	8	36.4%	4	18.2%
Region 3							
1 day-3m.	40	30	75	-----	-----	19	63.3%
3-6 m.	32	15	46.9	4	26.7%	3	20%
Total no.	424	198	46.7	22	11.1	98	49.5

Table (3): Pathogenic bacteria isolated from pneumonic cases collected from the examined sheep flocks.

Isolated bacteria	No. of +ve cases	% to all cases (120)	% to all isolates (126)
<i>Staph. aureus</i>	14	11.67	11.11
<i>E. coli</i>	8	6.67	6.35
<i>Strept- pyogenes</i>	5	4.17	3.97
<i>Coryne pyogenes</i>	7	5.8	5.56

Table (4): Fungi isolated from pneumonic cases collected from the examined sheep flocks.

Isolated Fungi	No. of isolates	% to all cases (120)	%to all isolates(126)
<i>Aspergillus spp.</i> <i>A. Fumigatus fres.</i>	11	9.1	8.7
<i>A. flavus (link) fres.</i>	9	7.5	7.1
<i>A. niger Vantiegh</i>	6	5	4.67
<i>A. paraqsiticous spear</i>	5	4.2	3.97
<i>A. terrus thom</i>	9	7.5	7.14
<i>A. ustus (bain.)thom&church</i>	10	8.3	8.3
<i>Mucor spp.</i> <i>Mucor hiemolis wehmer</i>	5	4.17	3.97
<i>Mucor fragilis bainier</i>	7	5.8	5.56
<i>Mucor circinelloides van tieghem</i>	8	6.67	6.35
<i>Rhizopus spp.</i> <i>Rhizopus oryzae vent+ prinsen geerlings</i>	6	5	4.76
<i>Penicillin spp</i> <i>Pencillium notatum westl.</i>	10	8.3	7.9
<i>Penicillium funiculosum thom</i>	6	5	4.76

Table (5): Mixed fungal and bacterial isolated from sheep flocks.

No of cases	Types of microbes
1	<i>Aspergillus flavus</i> + <i>E. coli</i>
1	<i>Aspergillus terrus</i> + <i>staph. aureus</i>
1	<i>Aspergillus fumigatus</i> + <i>E.coli</i>
1	<i>Aspergillus funiculosum</i> + <i>Staph. aureus</i>
1	<i>Mucor fragilis</i> + <i>Staph. aureus</i>
1	<i>Aspergillus flavus</i> + <i>Staph. aureus</i>

Post mortem examination:

Examination of the infected lungs of slaughtered and dead cases revealed presence of catarrhal exudation on the inner surface of the respiratory passages with the occurrence of dark red patches on the lungs surface which in

most cases extended deeply into the parenchyma with dark red to grayish patches which may be attributed to the occurring of red and grey hepatization and eventually colonization of bacteria in theses patches. (**Sadiek *et al.*, 1993**).

Discussion

Ovine pneumonia affecting all ages with high incidences in nursing (one day- 3 months) and feedlot lambs (3-6 months) as stated by **Cleon, (1988)**. This is caused by complex interaction of stress and microbial agents. The stress factors involved are sudden movement for grazing crowding into an inadequate ventilated shed (**Lehmkuhl and Cough 1997**). Furthermore high level of ammonia, high relative humidity and extreme temperature variations, deficiency of colostrums intake, sudden climatic changes and bad hygienic condition act as a predisposing causes for sever outbreaks of infectious respiratory diseases. On the other hand the disease may occur at any time through out the year but is more frequent during the warm and moist seasons of summer months and also during weather changes due to air drafts and dusty environments. Which is the characterized weather in desert nature countries like Matrouh.

Clinical signs showed rise of body temperature hurried respiration and reduced appetite, with dry cough and serous to seromucoid nasal discharges and dyspnoea were observed in some sheep it is clear that spreading of infection to other animals occurred due to air drafts and dusty weather. Dyspnoea with mouth breathing and extension of the head and neck was noticed specially in young ages, which may be due to air way obstruction by interstitial edema. Which lead to death of young lambs.

Laboratory findings: Revealed that most cases are infected by bacteria like *Staphylococcus aureus* which constitute the highest percent (11.11%). And *E.coli* (6.35%). And the other cases infected by fungi like that *Aspergillus Fumigatus* (8.7%). Fungal pneumonia can be diagnosed by many ways, the simplest method is culture the fungus but it is slow method which may increase mortality risk (**Morrell et al., 2005**), or by microscopic method which is also slow and imprecise. Other cases showed mixed infections from both bacterial and fungal infection constitute 6 cases only. From the mentioned data, it is noticed that the respira-

tory affection is a complex syndrome eventually affected by stress factors and by either bacteria or fungal infection or both of them. But bacteria are the main factors that cause the serious damages and troubles. But Fungi that cause pulmonary diseases are generally come to medical attention as secondary invader. Also in immunosuppression resulting from treatment with steroids and cytotoxins frequently is associated with opportunistic mycotic infections, pneumonia caused by *Aspergillus Fumigatus* occurred in calves treated with dexamethasone and inadvertently fed hay that contained large numbers of *A. fumigatus* spores (**Rippon, 1988**). Treatment with cortisone prior to aerosol challenge greatly enhanced pulmonary fungi lesions and mortality, Alveolar macrophages and neutrophils obtained from animals treated with cortisone are unable to kill ingested fungal spores (**William et al., 1992**). Stress factors Which could be a collection of a group of lambs from grass to dusty yard for shipping, poor ventilation in a barn, build up of ammonia from a manure pack in a building, high humidity, overcrowding or any combination of these (**Dr. S. John Martin, 1999**).

Conclusion

There are economic losses due to lambs with chronic pneumonia may appear normal have a slowed growth rate. These are after there have been deaths and expensive treatments have been used. The results confirm the role played by bacteria and fungi in causing deaths among sheep flocks. The disease seemed to be severe in newly born lambs. Proper hygienic measurements and the use of suitable medicaments are necessary to control the problem.

References

Cleon V. Kimberling (1988). "Jensen and Swifts, Diseases of sheep" 3rd Edition. DVM, MPH college of veterinary medicine and biochemical science Colorado State University Ft. Collins, Colorado.

- Cruichshank, R. (1975).** Medical Microbiology. A Guide to the Laboratory Diagnosis and Control of Infection. 11th Ed., Livingstone Limit., Edinburgh. pp2-115.
- Dulpa P., Dive A., and Silbille Y. (2004).** Invasive pulmonary Aspergillosis in patients with chronic obstructive pulmonary diseases. Eur Respir. J. Oct; 30 (4): 782-800.
- Dr. S. John Martin-Veterinary Scientist, Sheep, Goat and Swine / OMAFRA (1999).** Pneumonia in sheep 1 June 1999 reviewed 16 April 2010.
- Gilman, J.C. (1957).** A manual of soil fungi Iowa State Univ. Press, Ames-Iowa, U.S.A. (1957).
- Lehmkuhl, H.D. and Cough, P.M. (1997).** Investigation of causative agents of bovine respiratory tract disease in beef calves, calf herd with an early weaning program. A.J.V. Res. 38, 1717-1720.
- Libero Ajello (1967).** Comparative ecology of respiratory mycotic diseases agents National communicable disease center. U.S. Public Service, Atlanta, Georgia. Bacteriological Review, Mar., American society for mycosis.vol.31, No.1 p. 6-24
- Meersseman W, Lagrou K, Maertens J, and Van Wijngaerden E. (2007).** Invasive Aspergillosis in the intensive care unites. Clin. Infect. Dis. Jul 15; 54 (2): 205-16.
- Morrell M., Fraser V.J., and Kollef MH. (2005).** Delaying the empiric treatment of candida bloodstream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. Antimicrob Agents Cheother; 49:3640-5.
- Moss, E. and MoQuown, A. (1969).** Atlas of Medical Mycology. 3rd .Ed. Beltimore: The Williams & Wilkins company, Edinburgh and London: E.&S. Livingstone. Pp. ix and 366.
- Raper K., B., and Fennell, D.I. (1965).** The genus Aspergillus Williams and Wilkins, Baltimore, U.S.A. (1966).
- Rippon J., W., (1988).** The pathogenic fungi and the pathogenic Actinomycetes Medical mycology, 3rd ed. WB Saunders co., Philadelphia, PA. Chapter 15, p. 381
- Ross B., BVMS PhD DVD MRCVS Dip ECVD (2008).** Nomenclature in Fungal Diseases Department of Veterinary Clinical Sciences Royal Veterinary College, Hawkshead Lane North Mymms, Hatfield, and Herts AL97TA
- Sadiek, A.H.; Abdel-salam, M.N. and ZaitounA. M., (1993).** Field investigations on ovine pneumonic Pasteurellosis with some blood and blood serum analysis 2nd Sci. Cong. Egyptian Society for cattle diseases 5-7 Dec., Assiut Egypt. P.10-15.
- William U. Knudtson and Clyde A. Kirkbride (1992).** Fungi associated with bovine abortion in the northern plains states (USA) J Vet. Diagn Invest 4:181-185.

دراسات فطرية وبكتيرية على الالتهاب الرئوي في قطعان الاغنام في محافظة مطروح

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الملخص العربي

اشتملت الدراسة على 424 حيوان من قطعان الخراف من مختلف الاعمار والنوع من ثلاثة مناطق في محافظة مطروح كل قطيع تم تقسيمه الى مجموعتين طبقا للعمر الفحص الاكلينيكي بينت ان 198 حيوان اظهروا اعراض تنفسية 120 حالة تم ذبحها اضطراريا و98 نفقوا من مجموع الحيوانات المريضة المكونة للدراسة . تم اخذ عينان من الانف والقصبة الهوائية تحت تعقيم كامل لعمل الفحص البكتريولوجي واخذت عينات بعد الوفاة للفحص البكتريولوجي والفطري . البكتريا المنعزلة تم فحصه مورفولوجي وبيوكيميائي طبقا لكروكشانك 1975 . للفحص الفطري تم عزل الفطريات على سابروود اجار . الفحص الاكلينيكي اظهر حدوث اصابات تنفسية في الحملان الصغيرة بالمقارنة بالكبيرة معدل الاصابة بالاضطرابات التنفسية 75% و61.4% في بعض القطعان. وايضا معدل الحيوانات النافقة بين الحملان الصغيرة وصلت الى 67.4% في بعض القطعان تم عزل انواع مختلفة من البكتريا من الحيوانات النافقة والمذبوحة اضطراريا المجموع الكلي الحاصل عليه 34 من العترات من بينهم الستافيلوكوكس اوريوس والاي كولاي من اكثر المكونات للعترات المعزولة. الفطريات المعزولة من الاسبيرجيليس والميكور والريزويس. وكانت الانواع المختلفة للاسبيرجيليس تشكل اعلى اصابة 36.6% بالنسبة لكل الحالات المصابة و41.48% بالنسبة لكل العترات المعزولة. النتائج تؤكد على الدور الذي تقوم به البكتريا والفطريات في الاصابات والنفوق بين قطعان الاغنام . المرض يكون خطيرا في الحملان مما يؤدي الى زياده نسبة النفوق. وللتغلب على الاصابة يجب توفير البيئة الصحية الملائمة الخالية من التلوث وعند حدوث اصابات او عدوى يؤخذ الدواء الملائم للتغلب على المشكلة.