Prevalence of Caseous Lymphadenitis in sheep

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Abstract
Out of the 168 living sheep a total of 67 isolates of C*orynebacterium pusedotuberclosis* were obtained with an incidence of 39.9%. On the other hand for the samples collected from the slaughtered animals 17 samples were positive with an incidence of 20%.Adults were more affected than young Clinically the lesion appeared at different regions. The head and neck accounted for the largest number of lesions. For the internal abscesses body distribution and frequency of the 17 abscesses for sheep were recorded. The result also revealed high incidence of abscess after shearing (34.5%), while before shearing was 5.3 %. The result of serological microagglutination test for detection of *Corynebacterium pusedotuberclosis* for the 67 sheep with abscesses revealed that 65 (97%) with abscesses had positive titres and 2 (3%) showing abscesses had negative titre. 36 out of 101 sheep samples without abscesses were seropositive and 65 (64.4%) samples without abscesses were seronegative**.** The in vitro antibiogram studies for the isolated *C. pseudotuberculosis* strains ,which were chosen randomly revealed that most of isolates were highly sensitive to (ampicillin (100%), gentamycine (100%).While most isolates were resistance to streptomycin and Neomycin to each sensitivity each 5%) .Treatment was performed for 10 cases showed external abscesses and give good result.

Key words: C*orynebacterium pusedotuberclosis, microagglutination test, antibiogram* .

Introduction

Caseous lymphadenitis (CL), caused by *Corynebacterium pseudotuberculosis* is one of the most important diseases of sheep, causing considerable losses for herd owners, due to the chronic and generally subclinical nature of infection. Control is difficult and prevalence in animals and herds is high **(Alessandro *et al*., 2011).**

The bacteria produce an exotoxin called phospholypase D, a glycoprotein which dissociates the sphingomyelin of cell membranes. The disease has a long incubation period of up to 4 months. The bacteria can survive for prolonged periods in soil (8 months), straw and hay (2 months) and dip (24 hours**) (Baird, 1997** and **Brown and Olender, 1987).**

There are two forms of the disease, superficial and visceral. In the superficial form there is enlargement of the animal's lymph nodes due to abscessation, which are visible as swellings. In recent outbreaks the lumps have been around the head and neck but they may also appear on the shoulder, groin or legs where the superficial lymph nodes are located. The visceral form of the disease is endemic, insidious and sometimes subclinical. It is commonly, however, manifested by wasting and in some cases, death **(Gilmour, 1991 and Lloyd, 1994).**

The disease occurs worldwide and causes significant economical losses particularly in the sheep industry due to wasting, poor wool growth, decreased milk production reproductive disorders, premature disorders, premature culling, carcass condemnation and rarely death (**Paton *et al.,* 1994).**

It is considered to be one of the most economically important diseases of sheep and goats, the actual prevalence of CL in small ruminants is usually underestimated because it is not a notifiable disease in many countries and since animal owners are not aware of its economical impacts and do not usually applies for veterinary advice for superficial abscesses.

C .pseudotuberculosis is found in milk and the

ingestion of raw milk from CL-affected animals may therefore pose a risk for humans. It is also involved in occasional infections of cattle, horses and man (**Peel *et al.,* 1997)**Infection in humans as recorded particularly in those occupationally exposed to farm animals (farmers and abattoir workers, etc.)**.**

There is no satisfactory means to diagnose and control *C. pseudotuberculosis* infection in flocks by palpation of external lymph nodes. It is unreliable to detect carriers, early cases, or animals with internal abscesess consequently, numerous serodiagnostic tests have been proposed (**Brown and Olender, 1987,** and **Zaki and Abdel-Hamid, 1974).**

The objective of the present work to study the prevalence of this disease in sheep, isolation and identification of *Corynebacterium pseudotuberculosis,* use of microagglutination test as a method for diagnosis of the disease by antibody titrs, antibiotic sensitivity testing and finally trial for treatment of affected cases**.**

Material and methods

1. Material

Animals

A total of 168 living sheep at wide range of ages (1-4 years) were examined before and after shearing for detection the presence of abscesses in superficial lymph nodes at different farms at El Kaliobia and Giza governorates. Also 85 slaughtered cases from slaughter house were examined.

Samples were collected from 168 living sheep and 85 from slaughtered cases. These samples were purulent material from lymph node of living cases, while samples from slaughtered ones were lung, liver, kidney, intestine and lymph node from each animal**.**

Collection of samples:

Purulent material from abscessed lymph nodes was collected by using aspirating puncture with a fine needle by after careful antiseptic cleaning of the skin **Collett *et al.,* (1994).** For lanced abscesses the material was collected in a sterile container or with a sterilized bacteriological swab**.**

At abattoir:

All animals were inspected briefly antemortem for the presence of any enlarged superficial lymph nodes or obvious signs of the disease. Blood for serology was collected at the time of slaughtering. Superficial lymph nodes were palpated prior to skinning and examined again when the hide was removed. Viscera were inspected, with special attention to spleen and the mesenteric, tracheo- bronchial, and mediastinal lymph nodes. Lumbar nodes were checked insitu within the carcass. Any enlarged node was cut open with the excepation of superficial abscesses that were already open to the skin surface, purulent material was collected from all abscesses for bacteriological examination **(Nozaki *et al.*, 2000).**

Direct microscopically examination:

Material aspired from lymph node were stained by Gram and Giemsa stains. The bluish colour taken on by *C .pseudotuberculosis*, in contrast to the reddish colour of the cellular and inflammatory material helps in the identification of the infectious agent **(Radostits *et al*., 2002).**

2. Culture and biochemical identification

Purulent material collected from abscessed lymph nodes of living sheep and the internal organs from the slaughtered house were inoculated onto blood plates. Duplicated plates were inoculated and incubated for 48 hours at 37°C aerobically and anerobically for each plates. After incubation of plates small, white, dry and crumbly colonies were isolated, for further identification pure cultures were prepared from the colonies that were Gram-positive and showed small curved rod-shaped in the microscopic examination. The routine biochemical tests, such as catalase, urease, trehalose, maltotriose and synergistic haemolysis with *Rhodococcus equi* and antagonistic haemolysis with *Staphylococcus aureus* were carried out to identify the isolates. **(Jones and Collins, 1986 and Quinn *et al.,* 2005).**

Microagglutination:

Antigen preparation:

C. pseudotuberculosis was grown overnight at 37^0C with gentle rotation in brain heart infusion broth containing 0. 1% Tween 80 .Then was washed three times in phosphate buffered

saline solution pH 7.5, containing 1.0% Tween 80 and0.3% formalin (PBS-TF)**.**

Blood samples were collected from sheep by jugular venepuncture into 10 ml vacutainer tubes without anticoagulant. Serum was separated from clotted blood in vacutainer by centrifugation and decanting**.**

A known positive control serum from a sheep naturally infected with C. *pseudotuberculosis* and a negative control serum from a rabbit were included as controls. Atwofold dilution of serum samples was performed. Micro-agglutination test was applied according to **Paual *et al.,* (1989).**

A 0.05 mL volume of this formalized bacterial antigen suspension prepared in PBS-TF containing 0.005% safranin dye was agglutinated by 0.05 ml of twofold serum dilutions in "V" bottom microtiter plates.

Plates were covered and held overnight at room temperature, and of the last well in which visible agglutination had occurred was recorded as the direct agglutination titer**.** The minimum value for a positive titer in the microagglutination assay was set at 1:40**.**

Antimicrobial susceptibility testing:

Disc diffusion susceptibility test was used to analyze the susceptibility of *C. pseudotuberculos* isolates to nine different antimicrobials, ampicillin (25 mcg) chloramphenicol (30 mcg),Lincomicin (10 mcg), Sulfamethoxazole-trimethoprim (30 mcg), penicillin G(10 µcg), neomicin (30 mcg), gentamycin (30 mcg)and oxytetracycline (30 mcg)and streptomycin(30 mcg)The interpretation was made according to **NCCLS (1999**) depending on the diameter of zone of inhibition of bacterial growth.

Treatment

A total of 10 cases with an external abscess were collected and separated in a place with concrete floor to be disinfected easily to avoid spreading the microorganism. Both betadine solution (El Nile Company) and Hydrogen peroxide 20% were used for the treatment.

Results

Direct microscopically examination:

In stained smears, the isolates appear as rods and have pleomorphic forms, from coccoids to filamentous rods, grouped in parallel cells or in a format similar to Chinese letters.

Prevalence of *Corynebacterium pseudotuberculosis:*

Results revealed that the disease was present in all farms visited with different frequency. Of the 168 living sheep a total of 67 isolates of *Corynebacterium pseudotuberculosis* were obtained with an incidence of 39.9%. On the other hand the samples collected from the slaughtered sheep 17 isolates of *Corynebacterium pseudotuberculosis* isolated with an incidence of 20% (Table1).

As regarding to age, the result revealed that adults sheep were more affected than young with percentage of 23.2%for adult more than one year and 16.7% for less than one year(Table 2)) also the result revealed high incidence of abscess were recorded after sheering, 34.5% while before shearing was (5.3%) (Table 3). Clinically, the lesion appear at different region, the head and neck accounted for the largest number of lesions observed in living sheep lymph node with a percentage of 0.6% ,20.2%, 4.2%, ,9.5%,2% 3 % for cranial,parotid, submandibular ,prescapular ,prefemoral, superamammary and cervical respectively (Table 4).

For the slaughtered sheep the body distribution and frequency of *Corynebacterium pseudotuberculosis* revealed a total of 17 samples with percentage of 20% were isolated from tracheobronchial l.n (3.5%), lung (3.5%), liver (2.3%), lumbar l.n(2.3%), parotied l.n (3.5%), mandibular l.n (3.5%), prescapularr l.n(1.2%) (Table 5).

The result of serological microagglutination test for detection of *Corynebacterium pseudotuberculosis*(Table 6) revealed that out of the 67 sheep with abscesses, 65 (97%) had positive abscesses had positive titres greater than 1:80 and 2(3%) had positive abscesses had negative titre. For 101 sheep samples without abscesses 36 samples (35.6%) with abscess had positive titre 1:40 and 65 samples (64.4%). without abscess had negative titre.

Antimicrobial susceptibility Testing:

The in vitro antibiogram studies were conduct-

ed on 20 out of 84 *C. pseudotuberculosis* strains whichwere chosen randomly.Table 7 illustrated that most of isolates were highly sensitive to ampicillin (100%),gentamicin (100%) followed by chloramphenecol (95%), lincomycin (95%) pencillin G (95%) Oxytetracycline (90%) and Sulfamethoxazole-trimethoprim (93.2%) isolates were resistant to Streptomycin and neomycin (95%) and 95% respectively.

Table (1). Results of testing sheep samples for *C. pusedotuberclosis*

Sample origin	Number	Positive sample	Percentage (%)
Living sheep	168	67	39.9%
Slaughtered sheep	85	17	20

Table (2). Prevalence of *C.pusedotuberclosis* with sheep

Total NO. tested	Age group	Positive cases	
		No.	%
(168) Sheep	≤year	28	16. 7%
	>1 year	39	23. 2%

Prevalence calculated acc. to total number of examined animals

Table (3). Prevalence of *C.pusedotubercclosis* with shearing time

Species	Shearing time	Positive cases	
		No.	%
Sheep (168)	Before shearing	9	5.4%
	After shearing	58	34.5%

Table (4). Distribution of abscesses due to *C.pseudotuberculosis* in living sheep.

Types of L.N	Number	Frequency in sheep
Cranial	1	0.6%
Parotid	34	20.2%
Submandibular	7	4.2%
prescpular	16	9.5%
Prefemoral	2	1.2
superamammary	2	1.2
Cervical	5	3%
Total	67	39.9%

Prevalence was calculated acc. to total number of examined animals

Table (5). Body Location and frequency of abscesses in slaughtered sheep

Sheep	Number	percentage
Tracheobronchial L.N	3	3.5%
Lung	3	3.5%
liver	2	2.3%
Lumbar L.N	2	2.3%
Parotied L.N	3	3.5%
Mandibular L.N	3	3.5%
Prescapularr L.N	1	1.2%
Total	17	20%

Prevalence was calculated in relation to total slaughtered case

Table (6). Serological response in relation to presence of abscesses in 168 sheep

Microagglutinaton test	Animals with abscesses 67				Animals with abscesses 101			
	Positive		Negative		Positive		Negative	
	No.	%	No.	%	No.	%	No.	%
Animals with positive microagglutination test	65	97%	2	3%	36	35.6	65	64.4

Table (7). Sensitivity test of 20 isolated *Corynebacterium pseudotuberculosis* isolates to different antibiotics.

Antibiotic disc	Sensitive isolates		Resistant isolates	
	No.	%	No.	%
Ampicillin	20	100	-	-
Chloramphenecol	19	95	1	5
lincomycin	19	95	1	5
penicillin G	19	95	1	5
Gentamycine	20	100	-	-
Oxytetracycline	18	90	2	10
Streptomycine	1	5	19	95
Neomycin	3	15	17	85
sulfamethoxazole-trimethoprim	17	85	3	15

Treatment

In this study, the treatment was performed for 10 cases showing external abscesses. Although the organism was susceptible to more than antibiotics as penicillin G and ampicillin cannot be used any of them because the formation of abscesses limits their penetration and effectiveness. The following steps were performed, firstly the animals were isolated from the herd and placed on a concrete floor to be disinfected easily to avoid spreading the causative bacteria .By use of a disposable scalpel we made a cross cut (+) was made to drain pus completely. The resulting abscess cavity was washed thoroughly with hydrogen peroxide, then flushed the with betadine solution 10 %, as an antiseptic solution. The lifting cavity was packed with gauze or other bandage material to minimize contamination of the farm environment with the infected material and the affected animal was kept isolated until the wound heals.

Discussion

Caseous lymphadenitis (CL) is a sheep and goat disease that occurs throughout the world. It decreases meat yield through carcass condemnation, hinders reproductive efficiency, causes damage to pelts via abscess scars and may lead to death in severely infected animal **(Jollty, 2013).** Several authors claim that the ovine abscess is a disease found in all farms **(Malone and Fee, 2006 and Figuerora and Maier, 2007).** The prevalence of infection in small ruminants depends on the animal environment **(De La Fuente *et al*., 1997).**

The results observed in the present study proved that the disease was present in all farms visited with different frequency and these corroborate with those of **Musa (1998)** and **Malone and Fee (2006).** The frequency of the disease in living sheep was recorded to be 39.9%, which is higher than that obtained by **Paton *et al.(* 2003)** who recorded frequency of 26%this may be attributed to difference in location, season, and type of housing.

As regarding to the prevalence of CL differed among different age groups; the higher prevalence was recorded in animals of the age group over one year 23.2% than in those less than one years (16.7%)**.**This also was reported by **Alabam and Aubur (2012)** and **Al-Gaabary *et al*., (2010)** who saied that CL abscesses are more frequently found in older animals.

The present result the lesions appeared at different regions, the head and neck accounted for the largest number of lesion observed in sheep lymph node with percentage of 16.7% **,** 9.5% and 4.2 % for parotid, prescapular and mandibular respectively and this agree with that recorded by (**Carillo *et al*.,(2005) and El Sanousi *et al,*(1989).**This may be attributed to animals managed under farm conditions which initially will develop abscesses around the head and neck following infection from contaminated feed, feeders and panelling while that managed under range conditions, abscesses generally will be limited to the shoulders and neck because of limited contact with feeding facilities and other animals**(Justin and Charlie 2008).**

The results of the present investigation revealed high incidence of abscess after sheering (34.5%) while before shearing was 5.3% **.**This also coincide with that reported by (**Sayed *et al.,* 1995)** who concluded that in sheep, abscesses are usually not found until shearing during which the shearer may invader- tently nick the wall of an abscess or due to external parasites (ticks and scabies). Injuries of the skin of animals during the shearing or by foreign bodies (wire, barbed) play a role in the emergence of these abscesses with unsterilized shearing equipment, combs and other tack. Milking equipment between animals may play a role in transmission of disease**.**

As regard to the result of the sample collected from slaughter animals according to the anatomical localization revealed a total of 17 isolates of *corynebacterium pusedotuberclosis* with an incidence of (20%) isolated from tracheobronchial L.N(3.5%,) lung (3.5%), liver (2.3%), lumbar L.N(2.3%), Parotied l.n (9.5%) Mandibular L.N. (5%), Prescapularr L.N (1.1%), respectively**.** The mediastinal and bronchial lymph nodes were the most frequently affected thoracic lymph nodes in the internal form **Reshawh *et al.,* 1979** this also agreed with that of **Akpavie and Emikpe, (2000) Frazer *et al.,* (1991) and Mohamed *et al.,* (2011).**

As regard to microagglutination test revealed that out of the 67 sheep with abscesses, 65 (97%) had positive abscesses had positive titres that and 2 (3%) had positive abscesses had negative titre, 35 of 101 sheep samples without abscesses were seropositive and 65 (64.4%) samples without abscesses were seronegative, this may attributed to antibody levels remained high even after complete healing of the superficial lesion. In which caseous lymphadenitis is noted for its chronic recurring nature and it may be that animals once infected continue to harbor the organism and so retain seropositivity. The technique of the microagglutination assay for detection of antibodies to *C. pseudotuberculosis* was rapid and easy to use, and can be used as method for detection of *corynebacterium pusedotuberclosis.*

The susceptibility pattern of *C. pseudotuberculosis* to antimicrobial agents varies among isolates obtained. Table (8) illustrated that most of isolates were highly sensitive to Ampicillin (100%) and Gentamycine(100%) followed by Chloramphenecl (95%)**,** lincomycin (95% **)** penicillin G (95%) Oxytetracycline (90%) and-Sulfamethoxazole-trimethoprim(93.2%). Isolates were resistance to Streptomycin (5%) Neomycine (5 %.). These results agree with **Garg and Chandiramani, (1985)** and **Foley *et al.,* (2004).**

As regarding to antimicrobial sensitivity although most of them highly sensitive, it is not used in the treatment because the formation of abscesses limits their penetration and effectiveness, and this reported by **Michael (2009)** and **Jeremy Powell (1998).**

Conclusions

Caseous lymphadenitis is of great economic importance because it can decrease profitability of the herds. It is imperative that governmental agencies, researchers and laboratories work together to develop ways to eradicate Caseous lymphadenitis.

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