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CORYNEBACTERIUM PSEUDOTUBERCULOSIS IN CATTLE SKIN OEDEMATOUS DISEASE IN ASSIUT GOVERNORATE

(With 3 Tables)

By

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الكوريني باكتريم سودوتيوبركولوزيس والتهاب الجلد الاوديمي
في الماشية بمحافظة أسيوط

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أجريت هذه الدراسة لمعرفة مدى تواجد الدور الذي يلعبه ميكروب الكوريني باكتريم سودوتيوبركولوزيس في حالات التهاب الجلد الاوديمي والمنتشر بين الأبقار والجاموس بمحافظة أسيوط. تم تجميع ٥٤ حالة تعاني من هذا المرض من ٤ قرى مختلفة بالمحافظة. أوضحت الدراسة الإكلينيكية عن وجود شكلين للمرض وهما النوع الاوديمي الصديدي المغلق (٣٠ حالة) والنوع الصديدي المفتوح (٢٤ حالة) وتم تسجيل أهم الأعراض الإكلينيكية. ولقد أسفرت نتائج الفحص البكتريولوجي عن عزل وتصنيف ميكروب الكوريني باكتريم سودوتيوبركولوزيس من ٧٤% (٥٤/٤٠) من الحالات المصابة بصفة عامة. وكانت نسبة عزله من النوع الاوديمي المغلق (٨٦.٦%) أعلى من مثيلتها في حالة النوع الصديدي المفتوح (٥٨.٣%). وقد تم عزل ميكروب الكوريني باكتريم سودوتيوبركولوزيس منفردا في ٣١ حالة (٢٣ حالة من النوع الاوديمي المغلق، ٨ حالات من النوع الصديدي المفتوح) ومصاحبا للميكروب العقودي الذهبي في ٤ حالات ولميكروب الانثراكويد في ٣ حالات ولكل من الميكروب السبحي الصديدي والميكروب القولوني في حالة واحدة فقط. وبأجراء اختبار حساسية ميكروب الكوريني باكتريم سودوتيوبركولوزيس المعزول للمضادات الحيوية المختلفة تبين أن معظم العترات المعزولة كانت شديدة الحساسية للجاراميسين والاسبكتراما والسيفالوسين والتتراسيكلين والاميسلين والاريثروميسين. وقد تمت مناقشة نتائج البحث والتوصيات اللازمة للإقلال من تكرار مثل هذه الإصابات.

SUMMARY

This study was performed to determine the role and the incidence of *Corynebacterium pseudotuberculosis* in case of oedematous skin disease

in cattle in Assiut Governorate. A total number of 54 samples were collected from cows and buffaloes showing clinical signs of skin infection from four villages belonging to Assiut Governorate. Two forms of the disease were recorded: a closed form (30 cases) and an opened suppurative oedematous form (24 cases). The main clinical signs of the infected animals had been described. The collected samples were submitted to bacteriological examination. Isolated bacteria were subjected for identification, biochemical characterization and biotyping. The results obtained revealed the isolation and identification of *C.pseudotuberculosis* from 74% (40/50) of the total infected cases. It was isolated from closed lesions in a higher rate than that from the opened lesion with a percentage of 86.6% and 58.3% respectively. *C.pseudotuberculosis* was isolated in pure form in 31 cases (77.5%) (23 from closed lesions and 8 from the opened lesions) and associated with *Staph. aureus*, *Anthraxoid*, *Strept. pyogenes* and *E. coli* from 4, 3, 1 and 1 cases respectively. Results of Antibigram indicated that most of the *C.pseudotuberculosis* strains were highly sensitive to garamycin, spectram, cephalothin, tetracycline, ampicillin and erythromycin. The results and measures for handling the disease were discussed.

Keywords: *Corynebacterium pseudotuberculosis*, Skin oedematous diseases, Cattle, Assiut, Egypt.

INTRODUCTION

Corynebacterium pseudotuberculosis is a Gram-positive rod shaped organism that produces a phospholipase -D exotoxin (Brown and Olander, 1987); it is an intracellular bacterium which is capable of surviving within the differentiated phagocytic cells (Tashjian and Campbell, 1983). The survival of *C.pseudotuberculosis* inside the phagolysosome, which is thought to be due to the lipid layer present on the cell bacterial surface lead to the development of typical pyogranulomas in lymph nodes and in internal organs, particularly in the lungs (Brown and Olander, 1987). *C.pseudotuberculosis* causes a chronic infectious disease in cattle, buffaloes, sheep, goats and horses (Shpigel et al. 1993, and Yeruham, et al. 1997).

Oedematous skin disease (Ulcerative lymphangitis) is an infectious disease which spread over large areas. The condition is most

commonly associated with poor hygiene and management and through insect transmission (Abou-Zaid and Hammam, 1994).

Cattle are known to be susceptible to *C.pseudotuberculosis* infection as recorded by Riising and Hesselholt (1973) in Denmark, Addo and Dennis (1977) in Northern Nigeria and Kariuki and Poulton (1982) in Kenya.

In Egypt, Carpano (1934) was the first who described ulcerative dermatitis as a particular disease mainly affecting skin of cattle, buffaloes and sheep caused by a diphtheria-like organism. Soliman *et al.* (1963) reported two outbreaks of ulcerative lymphangitis (oedematous skin disease) in local breed cattle. The infected animal had one or more swelling in the dewlap, fore and hind limbs, shoulder, abdomen and side of head and neck. The swelling varied in size from an egg of a hen to the size of watermelon. Sometimes the nodules appeared in the form of chain along the lymphatic vessels, accompanied with enlargement of regional lymph nodes (Soliman *et al.* 1963). Further investigations of oedematous skin disease in bovine in Egypt were done by Mostafa (1984), Hassan (1988), Abou-Zaid and Hammam (1994), Zaghawa and El-Gharib (1996) and Zaki, (1999).

The aim of this study was of two aspects: (a) isolation and identification of causative bacterial agent (s) of oedematous skin disease in cattle and to explore the incidence and the role of *C.pseudotuberculosis* in this disease in Assiut Governorate, (b) AntibioGram for the isolated *C.pseudotuberculosis* strains to reach a valuable and specific treatment.

MATERIAL and METHODS

Animals:

A total number of 32 buffaloes and 22 cattle of both sexes and different ages (mainly over 2 years) was investigated. The animals were admitted to the clinics- came from 4 villages belonging to Assiut Governorate- with their different disease histories. Bad hygiene and spread of insects especially house fly (*Musca domestica*) were noticed. All clinical cases were recorded as regards the location, size and appearance of any skin lesions.

Samples:

Two groups of samples were collected:- The first group consisted of 30 samples collected from intact unopened lesions by aspirating the

content of swollen lymph node and/or from swollen subcutaneous nodule by using a large, 16-gauge needle. The second group consisted of 24 samples, which were collected from open lesions by sterilized cotton swabs. All samples were transported as rapidly as possible in ice bag container to the laboratory where isolation and identification of the organisms were performed.

Bacteriological examination:

The samples were inoculated into nutrient broth media which were aerobically incubated at 37°C for 24h, then subcultures were made by streaking on 10% sheep blood agar, MacConkey's agar and nutrient agar. After incubating the plates for 48h. at 37°C suspected colonies were identified morphologically by Gram's stain and biochemically according to Quinn *et al.* (1994): catalase, oxidase, motility test, aesculin, gelatin and hippurate hydrolysis, urea production, indole test, nitrate reduction and H₂S production, triple sugar iron, and fermentation of glucose, ribose, xylose, lactose and galactose.

***In vitro* antibiotic sensitivity test:**

Antibiogram sensitivity was performed for isolated strains of *C.pseudotuberculosis* by the agar diffusion technique (Quinn *et al.* 1994). The used antibiotic discs were cephalothin (30 µg), nalidixic acid (30 µg), spectramin (10 µg), streptomycin (10 µg), garamycin (30 µg), tetracycline (30 µg), erythromycin (10 µg), amikacin (30 µg), duracef (30 µg), claforan (30 µg), ampicillin (10 µg), and netilmicin (30 µg). The results were interpreted according to Konemen *et al.* (1992).

RESULTS

The main clinical signs in cattle and buffaloes included acute oedematous swellings accompanied with single or multiple abscesses or ulcerative areas of 5 to 25 cm diameter. They were present on the flanks, shoulders, neck, one or more limbs, dewlap, head and side of abdomen. Palpation of the abscess revealed doughy or soft contents surrounded by a firm wall. The abscesses ulcerated and developed draining tract. The ruptured abscesses or ulcerated areas had raised necrotic margin, they contained either a sero-sanguineous exudate or a blood-stained yellowish or greenish foul-smelling pus. The regional lymph nodes were involved

in some cases but generalized lymphadenitis and lymphangitis was not observed in the majority of the animals examined.

After 24 hours incubation the subcultures revealed non haemolytic, pinpoint sized colonies appeared on most plates of blood and nutrient agar. After further incubation for 24 hours the colonies enlarged in size, they were opalescent white and were encompassed by a narrow zone of haemolysis. There was no growth on MacConkey agar plates. The microorganisms which was Gram positive, coryneform bacilli were identified as *C.pseudotuberculosis* on the basis of positive reactions for catalase and nitrate reduction, glucose fermentation, urease production as well as negative reactions for aesculin hydrolysis, gelatin liquefaction and lactose fermentation and H₂S production.

Plates that showed bacterial growth, other than *C.pseudotuberculosis* were also subjected for bacteriological studies. Separate colonies were subcultured, characterized and identified biochemically.

The results of incidence and percentage of *C.pseudotuberculosis* isolates from closed and opened lesions, either in pure or in mixed forms are illustrated in tables 1&2. The antibiogram assay of 40 strains of *C.pseudotuberculosis* is shown in Table 3.

DISCUSSION

Oedematous skin disease (Bovine ulcerative lymphangitis) has become prevalent in many provinces in Egypt over the past few years. It causes severe losses in animal production in cattle and buffaloes. These economic losses are mainly attributed to decrease milk and meat production, low quality of skin as well as the cost of long time treatment of infected animals. The disease appeared in two epidemiological forms, an epidemic form and sporadic form (Abou-Zaid and Hammam, 1994; Zagahawa and El-Gharib, 1996 and Zaki, 1999).

During summer 2000, sporadic cases of oedematous skin diseases were observed in 4 villages belonging to Assiut Governorate. The clinical signs recorded in cattle and buffaloes were either closed or opened suppurative oedematous form. The affected animals were mainly over two years old and most of them had one or two cutaneous lesions in various sites of the body. These findings are quite similar to that described by Kariuki and Poulton (1982); Ibrahim *et al.* (1983); Esmat,

(1984) and Hassan (1988). The increased susceptibility of older cows had also previously been described by Kariuki and Poulton (1982).

The results of the bacteriological examination revealed the isolation of *C.pseudotuberculosis* from 74% (40/54) of the total collected samples (Table 1). These results confirm that *C.pseudotuberculosis* is the main biological cause of oedematous skin disease in cattle and is supported by the previous findings of Soliman *et al.* (1963); Riising and Hesselholt (1973); Addo and Dennis (1977) and Abou-Zaid and Hammam (1994). A lower percentage 41.5% was stated by Zaki (1999), whereas higher percentage 87% was recorded by Yeruham *et al.* (1997). *C.pseudotuberculosis* was isolated from closed lesions in a higher rate than that from opened ones, namely 86.6% and 58.3% respectively (Table 1&2). Such difference was noticed by Zaghawa and El- Gharib (1996) and Zaki (1999).

C.pseudotuberculosis was isolated in pure culture from 23/26 of the closed lesions and only from 8/14 of opened lesions. The other lesions showed mixed infection with *Staphylococcus aureus* (4 cases), Anthracoid (3 cases), *Streptococcus pyogenes* (1 case) or *E.coli* (1 case) (Table 2). This result is nearly similar to that reported by Abou-Zaid and Hammam (1994). Zaki (1999) found mixed culture of *C.pseudotuberculosis* with *Staphylococcus aureus*, *Pseudomonas aeruginosa* or *Streptococcus haemolyticus*.

The means of transmission of the infection are uncertain. It may spread either by direct contact between infected and uninfected animals or by mechanical transmission by house fly (Yeruham *et al.* 1997). In the present study it observed that the house fly might become important in the transmission and epidemiology of the disease as it spread between very large numbers of infected animals. This observation was supported by Addo (1983) who can isolate *C.pseudotuberculosis* from feet, body surface, mouth parts and the gastrointestinal tract of the house fly fed on oozing lesions of ulcerative lymphangitis in horses.

Khater *et al.* (1983) could reproduce the disease by intradermal inoculation of a local isolated strain of *C.pseudotuberculosis* in buffaloes. Torkey *et al.* (1982) pointed out that *C.pseudotuberculosis* produced a potent phospholipase-D exotoxin after infection or experimental injection resulting in a local area of oedema. Macrophages migrate to the invasion site and engulf the organism in a phagosome. Fusion of the phagosomes and lysosomes does not bring about the destruction of the organism owing to their ability to resist the action of

the lysosomal enzymes. This property is attributed to the high levels of specific cell wall lipids. The surviving intracellular organisms multiply rapidly and the infected macrophages degenerate and die, releasing the organism to be engulfed by secondary macrophages. This process leads to the formation of the pyogranulomas observed in the skin of infected animals (Shpigel *et al.* 1993).

Biotyping of the isolated strains of *C.pseudotuberculosis* showed positive nitrate reduction test, a result which came in accordance with that mentioned by Zaki (1999). Barakat *et al.* (1984) recorded that most cattle and horse *C.pseudotuberculosis* strains reduced nitrate (nitrate positive) whereas most sheep and goat strains were nitrate negative. On the other hand Shpigel *et al.* (1993) and Yeruham *et al.* (1997) found that all isolates of *C.pseudotuberculosis* affecting cow failed to reduce nitrate.

The results of antibiogram revealed that the majority of *C.pseudotuberculosis* isolates were sensitive to garamycin (90%), spectram (90%), cephalothin (80%), tetracycline (75%), erythromycin (72.5%) and ampicillin (65%) (Table 3). Similar results were reported by Abd-El-Galil *et al.* (1986) and Abou-Zaid and Hammam (1994). However, variable observations were reported by Soliman *et al.* (1963); Fouad *et al.* (1972) and Esmat (1984). As *C.pseudotuberculosis* is a facultative, intracellular parasite, the selection of antimicrobial drugs should have considerable diffusibility, targeting and activating in the phagolysosomal macrophage compartment (Shpigel *et al.* 1993). Surgical treatment of skin lesions accompanied with the effective local and systematic antibiotics for 10 days gave good improvement (Ibrahim *et al.*, 1983 and Abou Zaid and Hammam, 1994).

Therefore to prevent this disease it is recommended that hygienic measures must be taken in consideration in rearing animals in an area free from infected animals together with rapid efficient treatment of wound and control of insect vectors.

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Table (1): The incidence of *C.pseudotuberculosis* collected from cattle and buffaloes showing oedematous skin disease.

Total numbers of samples	Total isolates		Isolates collected from			
			Closed lesions (30)		Opened lesions (24)	
	No.	%	No.	%	No.	%
54	40	74	26	86.6	14	58.3

Table (2): Incidence of single and mixed infections in closed and opened lesions

Types of bacterial isolates	Closed lesions	opened lesions	Total isolates	
			No.	%
<i>C.pseudotuberculosis</i>	23	8	31	77.5
<i>C.pseudotuberculosis</i> + <i>Staph. aureus</i>	2	2	4	10
<i>C.pseudotuberculosis</i> + <i>Anthraxoid</i>	1	2	3	7.5
<i>C.pseudotuberculosis</i> + <i>Strept. pyogenes</i>	0	1	1	2.5
<i>C.pseudotuberculosis</i> + <i>E. coli</i>	0	1	1	2.5
Total	26	14	40	100

Table (3): Antibigram of 40 *C.pseudotuberculosis* isolates.

Antibiotics	sensitive isolates		Antibiotics	sensitive isolates	
	No.	%		No.	%
Garamycin (30 µg)	36	90	Duracef (30 µg)	18	45
Spectrama (10 µg)	36	90	Claforan (30 µg)	15	37.5
Cephalothin (30 µg)	32	80	Nalidixic acid (30 µg)	12	30
Tetraacycline (30 µg)	30	75	Amikan (30 µg)	10	25
Erythromycin (10 µg)	29	72.5	Netlimycin (30 µg)	6	15
Ampicillin (10 µg)	26	65	Streptomycin (10 µg)	3	7.5