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**STUDIES ON SOME BACTERIAL CAUSES
ASSOCIATED WITH OEDEMATOUS SKIN DISEASE
IN BUFFALOES IN DAKAHLIA GOVERNORATE**
(With 5 Tables)

By

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دراسات علي بعض الأسباب البكتيرية المصاحبة للالتهاب الجلدي الأوديومي
في الجاموس في محافظة الدقهلية
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أجريت هذه الدراسة علي عدد ٨٦ جاموسة من المزارع الخاصة بمحافظة الدقهلية لمعرفة مدى تواجد البكتريا المصاحبة للالتهاب الجلدي الأوديومي حيث أظهر الفحص البكتريولوجي للحالات عن وجود إصابة بالمرض في ٣٦ حالة (٤١,٩%). ٢٣ حالة (٦٣,٩%) كانت في صورة النوع الأوديومي الصديدي المغلق والنوع الصديدي المفتوح ١٣ حالة (٣٦,١%). وتم تسجيل أهم الأعراض الإكلينيكية. وقد أظهرت نتائج الفحص المورفولوجي والبيوكيميائي للمعزولات البكتيرية عن عزل وتصنيف الميكروب الكوريني باكتريام سودوتيبيركلوزس بصورة منفردة في ثلاثين حالة (٨٣,٣%) وفي صورة مشتركة مع كل من الميكروب العنقودي الذهبي في أربعة حالات (١١,١%) ومع الميكروب السبحي الصديدي في حالتين (٥,٦%). وبإجراء اختبار العدوى الصناعية لميكروب الكوريني باكتريام سودوتيبيركلوزس في الأرنب الغيني كانت كل المعزولات من النوع الممرض. وأظهرت نتائج اختبار الحساسية للمضادات الحيوية أن جميع العترات المعزولة كانت حساسة لكل من الجنتاميسين والبنسيللين والسبروفلوكساسين والاستريptomاميسين وبالعلاج باستخدام المضادات الحيوية طبقاً لنتائج اختبار الحساسية مع مضادات الهستامين وكذلك مكافحة الحشرات الطائرة أمكن الوصول إلي نسبة شفاء عالية من المرض.

SUMMARY

This study was carried out on 86 buffaloes from a private farms at Dakahlia Governorate to investigate the bacterial causes associated with oedematous skin disease. Bacteriological examination of the samples revealed that 36 cases (41.9%) were positive. 23 cases (63.9%) were in form of the closed lesions and 13 cases (36.1%) were open lesions. The main clinical signs of the infected buffaloes had been described. Isolated bacteria were subjected for morphological and biochemical identification. The results obtained

revealed that 30 isolates (83.3%) were *Corynebacterium pseudotuberculosis* in single infection and mixed infection with both *Staph. aureus* 4 isolates (11.1%) and *Strept. pyogenes* 2 isolates (5.6%). Pathogenicity test for *C. pseudotuberculosis* in guinea pigs indicated that all isolates were pathogenic and cause the death of inoculated animals. Results of antibiogram indicated that most of the isolates were highly sensitive to Gentamycin, Pencillin, Ciprofloxacin and Streptomycin. Treatment by effective antibiotics, antihistaminic and eradication of the flies achieved the recovery from the disease.

Key words: *Oedematous skin disease, Bacterial causes, buffaloes, antibiogram.*

INTRODUCTION

Buffaloes may be considered the principles sector of livestock in Egypt. They represent the main source for meat and milk production. This sector had been exposed to different infectious diseases with great economic impact. One of the most serious and specific infectious diseases is the so called oedematous skin disease (OSD) which becomes an endemic disease in Egypt (Selim, 2001). The disease was characterized by low mortality and high morbidity (Khalil *et al.*, 1995) and clinically by hot painful inflammatory swelling that appeared at different areas of the skin and the lymph vessels draining the inflamed area appeared as cord, also swollen local lymph node. The condition of diseased animals was fair with little change in appetite, decreased milk yield and slight rise in body temperature (Al-Gaabary and Ammar, 1999; Pandey *et al.*, 2007; Fontaire and Baird, 2008). The disease has a prolonged course and is highly expensive for treatment (Abd El-Hakeim 2005; Sayed *et al.*, 2007). Several investigations in different Governorate which established that the etiological agent was *Corynbacterium pseudotuberculosis* as alone or in mixed infection with other pathogen as *Staphylococcus spp.* and *Streptococcus spp.* (Barakat *et al.*, 1984; Mostafa, 1984; Ali and Zaitoun 1999; Ghoneim *et al.*, 2001; Sayed *et al.*, 2007; Abd El-Ghafar, 2009). *C.pseudotuberculosis* serotype II is the main cause of OSD and exotoxin phospholipase D and its lipid contents of the cell wall are the major cause of pathogenesis (Selim, 2001). Several authors suggested that the route of transmission of OSD is through the mechanical way only either by contamination of external environment (soil and water) with *C.pseudotuberculosis* or by external parasites (*Hippobosca equina*). (Barakat *et al.*, 1984; Khalil *et al.*, 1995; Sayed *et al.*, 2007).

The aim of this study was planned to investigate the prevalence of oedematous skin disease in buffaloes beside identification of the causative

pathogens. In addition to detect the pathogenicity and antibiogram for the most isolated bacteria.

MATERIALS and METHODS

Animals:

A total number of 86 buffaloes aged from 6 months up to 7 years from a private farms in Dakahlia Governorate during summer season, were investigated in this study. Diseased buffaloes were suffering from skin lesions either closed or opened in the form of diffuse oedematous swelling in different parts of the skin, ulceration and nodular lesions, were also present.

Bacteriological samples:

A total number of 86 samples were collected from diseased buffaloes by aspiration from closed lesions by sterile syringes after disinfecting the surface using 5% tincture iodine. A sterile disposable syringe filled with 5 ml sterile saline solution was injected and the contents of the lesion were aspirated with the same syringe. Samples from open lesions were taken by sterile cotton swabs. All samples were taken under complete aseptic conditions and transported as rapidly as possible in ice bag container to the laboratory where isolation and identification of the organisms were performed. Both aspirated exudates and cotton swabs were inoculated into Nutrient broth media overnight and incubated aerobically at 37°C, then streaked onto 10% sheep blood agar, Nutrient agar and MacConkey's agar plates and incubated at 37°C for 48 hours aerobically. Growing colonies were purified and identified morphologically by Gram's stain (Bailey and Scott's, 1990) and biochemically for glucose, sucrose and maltose fermentation, catalase activity, gelatine liquefaction, urea production, methyl red and nitrate reduction were adopted according to Koneman *et al.* (1992)

Biotyping of *C. pseudotuberculosis*:

C. pseudotuberculosis was biotyped according to the presence or absence of nitrate reductase enzyme. Biotype I did not express the enzyme (nitrate reduction test negative) whereas biotype II was capable of producing the enzyme (nitrate reduction test positive) (Quinn *et al.*, 2002).

Pathogenicity test :

Eight guinea pigs of about 250- 350 gm body weight were used in the reisolation of *C. pseudotuberculosis* (The most causative agent of OSD) as well as determination of its pathogenicity. Six guinea pigs were inoculated subcutaneously with isolated *C.pseudotuberculosis* at dose of 0.25ml (1ml containing 2×10^6 CFU) according to Ibrahim *et al.* (2007). At

the same time two guinea pigs (as control) were inoculated with sterile broth by using the same dose and route of inoculation. From dead guinea pigs showed post mortem changes and reisolation of inoculated isolates according to El- Far (1976) and Rafequ and Mahmoud (2007).

In-vitro antimicrobial susceptibility testing:

Antibiogram sensitivity was performed for isolated strains by the agar diffusion technique Quinn *et al.* (2002). The used chemotherapeutic discs were Pencillin (10 IU), Streptomycin (10 µg), Gentamycin (10 µg), Oxytetracycline (30 µg), Ampicillin (10 µg), Amoxycillin (25 µg), Ciprofloxacin (20µg) and Enrofloxacin (5 µg). The degree of sensitivity was determined and interpreted according to Koneman *et al.* (1992).

RESULTS

A- Clinical signs :

The main clinical signs in buffaloes included acute oedematous swellings accompanied with single or multiple abscesses or ulcerative lesions. The lesions were present on the flanks, shoulders, neck, one or more limbs, dewlap and contained either a sero-sanguineous exudates or blood stained, yellowish or greenish pus.

B- Results of bacteriological examination :

The obtained bacteriological results were tabulated in tables (1 -5).

Table 1: Bacteriological examination of the affected buffaloes.

Case of animal	Number of examined buffaloes	Positive samples		Negative samples	
		No.	%	No.	%
Affected buffaloes with closed and open lesions.	86	36	41.9	50	58.1

Table 2: Biochemical activities of *C. pseudotuberculosis* strains isolated from OSD buffaloes.

Organism	Biochemical activities							
	Nitrate reduction	Catalase	Urease	Glucose	Maltose	Sucrose	Gelatin liquification	Methyl red
<i>C.pseudotuberculosis</i>	+	+	+	+	+	-	-	+

Table 3: Bacterial species isolated from skin lesions of OSD.

Bacterial species	Forms of the positive OSD buffaloes (36)				Total	
	Closed Lesions (23)		Open lesions (13)			
	No.	%	No.	%	No.	%
<i>C. pseudotuberculosis</i>	23	100.0	7	53.8	30	83.3
<i>C. pseudotuberculosis</i> + <i>Staph. aureus</i>	0	0.0	4	30.8	4	11.1
<i>C. pseudotuberculosis</i> + <i>Strept. Pyogenes</i>	0	0.0	2	15.4	2	5.6

Table 4: Pathogenicity of *C. pseudotuberculosis* isolated from OSD infected buffaloes:

Inoculated isolate	No. of dead guinea pigs/day							Total	
	1	2	3	4	5	6	7	No.	%*
<i>C. pseudotuberculosis</i>	0	1	2	2	1	0	0	6/6	100%
Control guinea pigs	6	6	6	6	6	6	6	6	100%

No. : Number of dead guinea pigs

* : The percent was calculated according to the total number of inoculate guinea pigs

Table 5: Antimicrobial susceptibility testing of bacterial isolates recovered from OSD infected buffaloes

Organisms		<i>C.pseudotu be-rculosis</i> (36)	<i>Staph. aureus</i> (4)	<i>Strept. pyogenes</i> (2)
Anti microbial agent and its potency				
<i>Gentamycin</i>	10 µg	+++	+++	+++
<i>Streptomycin</i>	10 µg	+++	++	++
<i>Ciprofloxacin</i>	20 µg	+++	+++	++
<i>Penicillin</i>	10 µg	+++	++	R
<i>Amoxycillin</i>	25 µg	++	R	R
<i>Ampicillin</i>	10 µg	R	R	R
<i>Oxytetracycline</i>	30 µg	++	++	++
<i>Enrofloxacin</i>	5 µg	++	+++	+++

+++ = Highly sensitive

++ = Moderately sensitive

R = Resistant

DISCUSSION

Oedematous skin disease (OSD) is an endemic infectious disease that appear mainly among buffaloes and occasionally cows in Egypt (Rafequ and Mahmoud, 2007).

The main cause of OSD in buffaloes is *C. pseudotuberculosis* which is a Gram-positive bacilli, small pleomorphic (straight to slightly curved), appears as short chain or clumps resembling Chinese letters, aerobic or facultative anaerobic, non motile, non spore forming and grow slowly on enriched media and produce a toxic phospholipase D (Blarksdale *et al.*, 1981); Brown and Olander, 1987). The organism is capable of surviving within the phagocytes due to its high lipid content on the cell surface. (Tashjian and Campbell 1983; Pointkowski and Shivers 1998). Oedematous skin disease is an acute and seasonal disease appear often in summer and cause redness and swelling of skin in hairless areas, also cause sever economic losses through low quality of skin, decrease in meat production as well as long course of treatment (Ghoneim *et al.*, 2001; Zaki, 2004; Syame, 2006).

Out of the examined 86 buffaloes 36 showed clinical signs of OSD (Closed and open lesions) representing a morbidity rate of 41.9% whereas no mortalities were recorded (Table 1). These results are relatively near to that mentioned by Zaghawa and El-Gharib (1996) who found that OSD in buffaloes were 44.3%. Higher incidence of OSD was recorded by Sayed *et al.* (2007) who mentioned that morbidity of OSD was 95.5% between the buffaloes, while the lower incidence was recorded by Barakat *et al.* (1985); Abou-zaid (2001); Rafequ and Mahmoud (2007) with percent of 17.2%, 9.7% and 26.1% respectively.

Bacteriological results revealed that the isolation of *C. pseudotuberculosis* from closed lesions was 23(100%) from 23 positive cases and open lesions 7(53.8%) from 13 positive cases. This results are nearly similar to those recorded by Sayed (2001) who isolated *C.pseudotuberculosis* from closed lesions in a higher rate than from open lesions with percent of (86.6%) and (58.3%) respectively. On the other hand El-Sayed (2006) isolated *C.pseudotuberculosis* (15%) from closed lesions. *C.pseudotuberculosis* was isolated in pure culture 30 (83.3%) from both closed and open lesions (Table 3). These results go hand to hand with those recorded by Khalil *et al.* (1995) who recorded that *C.pseudotuberculosis* was isolated from OSD infected animals with an incidence of (83.3%). Zaghawa and El-Gharib (1996); Ali and Zaitoun (1999); Sayed (2001); Zaki (2004) and Sayed *et al.* (2007) detected *C. pseudotuberculosis*, in 88.2%, 94.5%, 77.5%, 12.8% and 80.0% of the

OSD infected buffaloes, respectively.

In the present work *C. pseudotuberculosis* was isolated in mixed culture from open lesions only with *Staphylococcus aureus* 4 (30.8%) and *Streptococcus pyogenes* 2 (15.4%) (Table 3). On the other hand, Sayed (2001) isolated *C. pseudotuberculosis*, with *Staphylococcus aureus* (4 cases), *anthracoid* (3 cases), *Streptococcus pyogenes* (1 case) and *E.coli* (1 case). Mostafa (1984); Ali and Zaitoun (1999) and Sayed *et al.* (2007) found that mixed culture of *C. pseudotuberculosis*, with *Staphylococcus spp.* and *Streptococcus spp.* with percent of 10%, with *Staphylococcus spp.* 15.3% and with *streptococcus spp.* 7.5% respectively. Biotyping of the isolated strains of *C.pseudotuberculosis* showed positive nitrate reduction test (Table 2). A result which come in accordance with that mentioned by Sayed (2001); Yeruham *et al.* (2003); Zaki (2004) and Abd El Ghafar (2009) recorded that the most of *C.pseudotuberculosis* which isolated from buffaloes were nitrate positive.

The variation in the disease frequency between the different studies may be attributed to the endemic nature of the disease which leads to variation in animal immunity and the variation in the presence of a few or large numbers of susceptible animals.

Concerning guinea pigs inoculation for studying both pathogenesis and re-isolation, all the inoculated isolates of *C.pseudotuberculosis* killed guinea pigs within 2 -5 days post injection (Table 4) where the dead guinea pigs showed congestion and maceration of muscles at site of injection in addition to congestion of the internal organs. This come in agreement with the findings of Barakat (1984); Khater *et al.* (1984); Galila (1998); El-Sawah (2002); Rafequ and Mahmoud (2007). Also Zaki (2004) reported that all isolates of *C.pseudotuberculosis* produced an exotoxin which was lethal for experimental animals usually within 48 hours of inoculation. The postmortem examination revealed congestion of internal organs. Table (5) revealed that the most isolates of *C.pseudotuberculosis* (main cause of OSD) were sensitive to Gentamycin, Penicillin, Ciprofloxacin and Streptomycin. Similar results were obtained by Hassan (1988) who said that combination of Penicillin with Sterptomycin showed good results in treatment of OSD. High sensitivity of Gentamycin was recorded by Khalil *et al.* (1995); Sayed (2001) and Sayed *et.al.* (2007). On other hand, Abou-Zaid (2001) found that *C.pseudotuberculosis* strains were sensitive to Doxycycline, Erythromycin, Gentamycin and Cephalocin.

Finally, it could be concluded that, the oedematous skin disease in buffaloes is acute disease occurs in different localities in Egypt and mainly caused by *C.pseudotuberculosis* in single infection or in mixed infection with other bacteria. Therefore to prevent this disease it is recommended

that hygienic measures must be taken in consideration in an area free from infected animals together with treatment with effective antibiotic as Gentamycin, Penicillin and Streptomycin in addition to control of insect vectors.

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