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Some studies on Corynebacterium pseudotuberculosis causing Oedematous Skin Disease in Egyptian buffaloes

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Out of 63 bacteriologically examined sanguineous fluids samples which were collected from Oedematous Skin Disease (O.S.D.) lesions, 37 isolates of C. pseudotuberculosis (C.p.) were recovered. The sensitivity tests revealed that isolates were highly sensitive to trimethoprim + sulphamethoxazole, amoxycillin, gentamicin and enrofloxacin. Diphtheria toxin (DT) produced by C. pseudotuberculosis of buffalo was determined by using the double immunodiffusion technique, it was applied on concentrated exotoxins which was prepared from C.p. isolates against Diphtheria toxin antiserum, its results were 15 (40.54%) positive and 22 (59.46) %) negative to presence of DT. Virulence of isolates having only phospholipase D (PLD) or both PLD and DT was assayed by S/C inoculation of exotoxines prepared from isolates in guinea pigs, 15(40.54%) guinea pigs died within 18 hours while 22 (59.46%) guinea pigs died during 48 hours. An important point in this investigation that there have been very rare previous reports describing the production of DT by local isolates of C. pseudotuberculosis of buffalo.

Corynebacterium pseudotuberculosis is the main cause of Oedematous Skin Disease (O.S.D.) of buffaloes in Egypt (Maarouf, 2003). O.S.D. is an endemic disease of buffaloes in Egypt characterized by oedematous swellings at the initial site of infection and usually involves the regional drainage lymph nodes. O.S.D. causes significant economic losses mainly, decrease in milk and meat production, low quality of hide, highly expensive medical treatment and decreased work efficiency (Selim, 2000). The aim of this study is directed to the following: Isolation and identification of C. pseudotuberculosis from buffaloes suffering from Oedematous Skin Disease (O.S.D.), determination of antibiogram of the isolated C. pseudotuberculosis strains, detection of diphtheria toxin producing *C. pseudotuberculosis* isolates by using the double immunodiffusion technique and detection of the lethality of *C. pseudotuberculosis* filtrate to guinea pigs.

Materials and methods

Samples. Sanguineous fluids were collected from oedematous swellings of 63 buffaloes with lesions suspected to be O.S.D., using sterile syringes and Mac-cartney bottles. All samples were sent to laboratory in an ice box with a minimum of delay. Isolation and identification of isolates were done according to (Koneman *et al.,* 1992; Quine *et al.,* 2002).

Media used for isolation of C. pseudotuberculosis. 10% sheep blood agar, brain heart agar and brain heart infusion broth with tween 80 were used.

Antibiogram of the isolated strains. The antibiotic sensitivity test was done according to Finegold and Martin, (1982) using the following discs: amoxycillin (10), cefadroxil (30), chloramphenicol (30), enrofloxacin (10), erythromycin (15), gentamicin (30), Oxytetracycline (30) and trimethoprim + sulphamethoxazole (1.25 + 23.75).

Detection of diphtheria toxin producing C. pseudotuberculosis isolates by using double immunodiffusion technique. The technique was done according to Arab et al., (1989). The central wells were filled with the Diphtheria toxin (D.T.) antiserum (it was obtained from Holding Company of Vaccine and Sera (VAC SERA) while the peripheral wells were filled with the concentrated toxins prepared according to Knight, (1978) from C. pseudotuberculosis isolates, a well was filled with the control positive DT (it was kindly provided from VAC SERA) and other well was filled with the control negative (Brain heart infusion broth). The plates were kept in a moist chamber at 37°C and examined after 24 h (Fig. 1).

Lethality of *C. pseudotuberculosis* filtrate to guinea pigs. It was done according to (Cameron and Buchan, 1966; Soheir 2006). 37 coryne-bacterium isolates were grown in cooked meat medium broth and incubated for 24 hours at 37°C, followed by inoculation of isolates to

brain heart infusion broth with 0.1% tween 80 and incubated at 37°C for 72 hours, the cultures were centrifuged at 5000 r.p.m. for 20 min., the supernatants were filtered through membrane filters with 0.22u Millipore, 0.5 ml of each original filtrate was inoculated subcutaneously (S/C) in one guinea pig-average weight 250-300 gm. At the same time 2 guinea pigs were inoculated with sterile saline solution using the same dose and route. The injected guinea pigs were observed and dead animals were examined to detect macroscopic changes in the internal viscera (Fig. 2).

Results and discussion

Results of lethality of *C. pseudotuberculosis* filtrate in guinea pigs. 22 (59.46%) guinea pigs were inoculated with *C. pseudotuberculosis* filtrate-negative to D.T by D.I.T.-died within 48 hours post-inoculation while 15 (40.54%) guinea pigs were inoculated with C.p. filtrate-positive to DT. by D.I.T. died within 18 hours and the control ones survived and were sacrificed after 96 hours.

Postmortem lesions were recorded as follows. 37 guinea pigs showed local oedema at the inoculation sites, congestion with different degrees in livers, spleens and prefemoral lymph nodes lungs were congested and hepatized. Heart and kidneys were pale in colour, Orchitis in 12 of them. The control ones were normal.

Oedematous Skin Disease (O.S.D.) is an acute disease of buffaloes in Egypt, which is caused by *Corynebacterium pseudotuberculosis* (C.p.) biotype 2. The disease causes severe economic losses which represented by reduced productivity, decreased work efficiency of the animals beside high costs of medical treatment. Two important components are included in pathogenesis of the etiological agent, the lipids of cell wall surface which is a toxic component of the bacterial cell that acts as pyogenic factor and the protoplasmic exotoxin phospholipase D which serves to promote the local spread of the organisms (Selim, 2000). Therefore, this investigation tries to spote light on an other C.p. exotoxin which enhance its virulence.

Table (1) showed the prevalence of C.p. in samples suspect to be O.S.D., out of 63 examined samples, 37 (58.73%) isolates of C.p. were recovered. While, Maarouf, (2003) found that the incidence of isolation of C.p. was 100% in buffaloes suffered from O.S.D. symptoms. The bacteriologically negative samples for C.p. were 26 (41.27), this may be resulted from that the animals were under therapeutic treatment.

Antibiogram of 8 chemotherapeutic agents on 37 isolates from buffaloes suffered from O.S.D. were presented in Table (2) all isolates were completely resistant to chloramphenicol. There were differences in isolates susceptibilities and zones of inhibition to different chemotherapeutic agents. All isolates were sensitive to trimethoprim + sulphamethoxazole, amoxycillin, gentamicin and enrofloxacine. It was clear that many isolates showed resistant to many antibiotics, this may be attributed to wrong dose, duration of drugs and route of administration or plasmid resistant. The obtained results coincided to large extent with that of (Selim *et al.,* 1998) who recognized that all Gram positive isolates were sensitive to penicillin, enrofloxacin, ampicillin, flumequine, erythromycin and trimethoprim + sulphame thoxazole. Also, nearly similar results were obtained by (Sayed, 2001). Results illustrated in Table (3) showed the detection of Diphtheria Toxin producing *C. pseudotuberculosis* isolates by using the double immunodiffusion technique. Out of 37 isolate, 15 (40.54%) were positive to Diphtheria toxin production. While 22 (59.46%) were negative to Diphtheria Toxin production. These results coincided with Ahmed, (2007) who could detect DT produced from 10 (55%) strain out of 17 isolates of *C. pseudotuberculosis* of buffalo origin. Moreover, Groman *et al.* (1984) who could detect DT produced from 2 strains isolated from Egyptian buffaloes. The results of lethality for C. pseudo-tuberculosis filtrate inoculation in guinea pigs revealed that 22 (59.46%) guinea pigs which were inoculated with *C. pseudo-tuberculosis* negative to DT Died within 48 hours, but 15 (40.54%) guinea pigs which were inoculated with C.p. filterate positive to DT, died within 18 h.

It is well known that phospholipase D (PLD) of *C. pseudotuberculosis* was identified as the major virulence factor of 31 KDa and it was suggested that PLD exotoxin facilitate the dissemination and infiltration of the bacteria in host tissues (Hodgson et al., 1992). The duration of lethality depends upon the number of exotoxins contained in the same isolate. The shortest duration of lethality 18 hours was produced by isolates contain PLD and DT while isolates contain PLD and not produce DT cause lethality of guinea pigs within 48 hours. In this concern Ahmed, (2007*)* reported that the duration of lethality for guinea pigs which were inoculated with exotoxins contained PLD and DT was 24-48 h, while exotoxin contained only PLD cause

Table (1): Prevalence of *C. pseudotuberculosis* in samples suspected to be O.S.D.

No. of examined samples	No. of + Ve	%	No. of – Ve	%
63	37	58.73	26	41.27

* The percent was calculated according to the number of examined samples.

Table (2): Antibiogram of the isolated strains.

Antibiotic disc used (mg/disc)			Sensitivity		
			Sensitive	Moderately sensitive	Resistant
Amoxycillin	(10)	No.	32	4	1
		%	86.49	10.81	2.70
Cefadroxil	(30)	No.	3	3	31
		%	8.10	8.10	83.78
Chloramphenicol	(30)	No.	0.0	0.0	37
		%	0.0	0.0	100
Enrofloxacin	(10)	No.	29	5	3
		%	78.38	13.51	8.10
Erythromycin	(15)	No.	1	7	29
		%	2.70	18.92	78.38
Gentamicin	(30)	No.	31	4	2
		%	83.78	10.81	5.41
Oxytetracycline	(30)	No.	5	11	21
		%	13.51	29.73	56.76
Trimethoprim + Sulphamethoxazole	(1.25 + 23.75)	No.	33	4	0
		%	89.19	10.81	0.0

* The percent was calculated according to the total number of isolates (37)

Detection of Diphtheria toxin producing *C. pseudotuberculosis*

Table (3): Isolates by using the double immunodiffuion technique (DIT).

Total number	Positive		Negative	
	No.	%	No.	%
37	15	40.54	22	59.46
Control +ve	+ ve			
Control - ve	- ve			

The percent was calculated according to the total number of isolates (37).

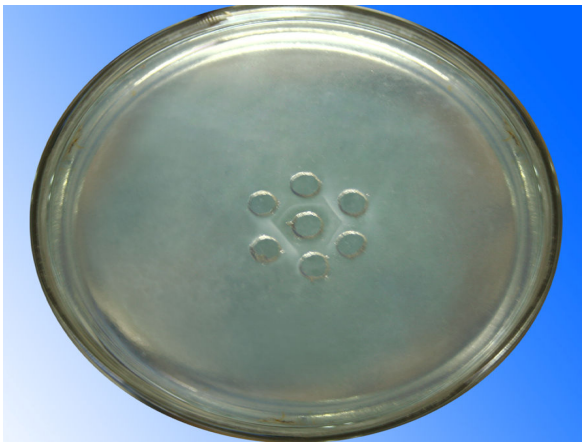

Fig. (1): The double immunodiffusion test.

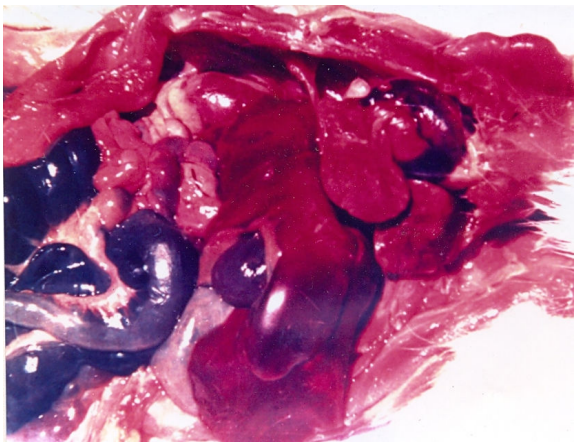

Fig. (2): Severe congestion of internal organs in guinea pigs inoculated with C.p. exotoxin.

death to guinea pigs within 48-72 hours. It could be concluded that, virulence of *C. pseudotuberculosis* depends upon 2 virulence factors which are PLD and DT., these factors have synergistic effect and the extent of virulence depends upon the number of factors possessed by the same strain. Thus the severity of O.S.D. in buffaloes under field conditions depends upon the presence of one or the two virulence factors (PLD. and DT.) we believe that, this micro-organism needs more investigations to identify on more virulence factors.

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بعض الدراسات على ميكروب كورينى السل الكاذب المسببة لمرض الجلد الأوديمى في الجاموس

أجريت هذه الدراسة على ٦٣ عينة سائل ارتشاحى من جاموس تبدو عليه أعراض إكلينيكية مشابهة لمرض الجلد الأوديمى. بإجراء الفحص البكتريولوجي على جميع العينات – تم عزل ٣٧ عترة من ميكروب كورينى السل الكاذب. وبإجراء اختبار الحساسية كانت المعزولات حساسة لترای میثوبریم + سلفاميزوكزازول والأموكسيسلين والجنتاميسين والإنروفلوكساسين. ومن أجل التعرف على عترات كورينى السل الكاذب المفرزة لسم الدفتيريا تم إجراء اختبار الانتشار المناعى المزدوج على السموم الخارجية المركزة المحضرة من المعزولات ضد المصل المضاد لسم الدفتريا وكانت النتيجة كالتالي: ١٥(٤٠.٥٤%) إيجابي، ٢٢ (٥٩.٤٦%) سلبى لوجود السم الدفتيرى ، وعلى أساس الاختبار السابق تم قياس ضراوة المعزولات التى تحتوى على الفوسفوليباز (د) فقط والتى تحتوى على الفوسفوليباز (د) والسم الدفتيرى عن طريق حقن السموم الخارجية المحضرة من المعزولات تحت الجلد في أرانب غينيا وكانت النتيجة كالتالي: ٢٢(٥٩.٤٦%) ماتوا خلال ٤٨ ساعة، ١٥(٤٠.٥٤%) ماتوا خلال ١٨ ساعة ، و الجدير بالذكر قلة أو ندرة الأبحاث التى تعرفت على وجود السم الدفتيرى في عترات كورينى السل الكاذب المعزولة من الجاموس المصرى.