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Oedematousskin Disease in Buffaloes and Cows

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BUFFALOES examined during summer 2006, out of 176 buffaloes out of 176 buffaloes examined 46 animals proved to be contract proved to be contract the disease representing a morbidity rate of 26.1%. On the other hand, out of 120 examined cows, 20 proved to be infected with the disease representing a morbidity rate of 16.6%. Concerning the clinical signs that observed in this epidemic, the disease occurred in 4 forms. The first is the edematous form which appeared in buffaloes more than in cattle. The second form was the ulcerative form, and it was more common in cattle than buffaloes. The 3^{td} was the nodular form and the 4^{ln} form was the mixed, the bacteriological examination, showed symptoms of edematous skin disease *Corynebacterium pseudotuberculosis* was isolated as a single infection. The phospholipase D toxin bands of the isolated pathogens were in a molecular size of 37 and 39 KDa. Serological investigation by using agar gel immunodiffusion test was strongly positive in serum samples. On the other hand, the OD reading of ELISA above the cut off point (0.28) was recorded in serum samples.

Keywords: *Corynebacterium pseudotuberculosis*, Cows, Buffaloes, Phospholipase D toxin.

Buffaloes may consider the principle sector of livestock in Egypt. It represents the main source for meat and milk production (Radostits *et al.,* 2007). 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 edematous skin disease(Sood *et al.,* 2012) . It was reproduced experimentally in buffalo calves by pure colonies of *C. pseudotuberculosis* (Khater *et al.,* 1984). Therefore, the disease was reported in a cyclic nature at different governorates of Egypt, Sharkia (Mostafa, 1984, Abdel Galil *et al.,* 1986 and Abu Zaid, 2001), Assiut (Khalil *et al.,* 1995) and Gharbia (Al-Gaabary & Ammar, 1999 and Mahmoud, 2009).

Edematous skin disease is also named ulcerative lymphangitis which often appeared as seasonal outbreaks during late spring .and early summer months where insects may play a role in the dissemination of the disease (Fouad *et al.,* 1974, Barakat, 1980, Addo, 1983 and Braverman *et al.,*1999). In between the cycles of epidemic it may appeared sporadically (Mostafa, 1984 and Al-Gaabary & Ammar, 1999).

The disease was characterized by low mortality and high morbidity (Khalil *et al.,* 1995) and clinically by hot painful inflammatory swelling appeared at different areas of the skin and the lymph vessels draining the inflamed area appeared as cord. The local lymph node was swollen reaching the size of watermelon. Necrosis and ulceration of the skin were also seen (Soliman *et al.,* 1970 and Hamoda, 1996). The condition of diseased animals is fair with little change in appetite, decreased milk yields and slight rise in body temperature. In addition, lameness may be noticed (Al-Gaabary & Ammar, 1999 and Abu Zaid, 2001).

Material and Methods

Animals

Field animals

A total of 176 buffaloes and 120 cattle aged from 6 months up to 7 years at El-Fauom Governorate was investigated in this study. Some of them were suffering from skin lesions in the form of diffuse edematous swelling in different parts of skin, ulceration and/or nodular lesions.

Experimental animals

Eight male guinea pigs of about 250-350 g body weight were obtained from the serum and Vaccine Research Institute, Abbasia, Cairo. Egyptwere used in isolation f the *Coryne bacterium pseudoiitberculosis*as well as determination of its pathogenicity.

Sensitivity discs

Bacteriological examination

Double samples were collected from each lesion either by aspiration from closed lesions or via cotton swabs from open lesions. All samples were taken under complete aseptic conditions and used for both direct smear and isolation of the causative agent by culturing onto 10% sheep blood agar, nutrient agar and MacConkey's agar plates then incubated at 37°C for 48 hours aerobically as well as in CO_2 incubator for the first isolation according to the method described by Bailey and Scott (1990).

Pathogenicity test in guinea pigs

Six guinea pigs were inoculated subcutaneously with 0.25 ml of 24 hrs broth culture of the isolated microorganisms. At the same time 2 animals (as controls) were inoculated with sterile broth by using the same dose and route of inoculation according to El-Far (1976).

- Detection of phospholipase D-antigen, which is produced by *C. pseudotuberculosis* cells. The isolates were cultivated in brain heart infusion broth, incubated and agitated at 37^{o}C for 36 hrs. the bacteria were removed by centrifugation at 5000 rpm for 10 minutes at 4C in cooled centrifuge and then filtered. Culture supernatant fluids were dialyzed overnight.

- Detection of *C. pseudotuberculosis* antibodies by:
  a. Agar gel immunodiffusion test according to Muckle and Menzies (1992). Nobel agar with sodium azide were prepared. The central well of each hexagonal pattern was filled with prepared (PLD) toxins and the peripheral wells were filled with tested serum. After incubation at 37^{o}C for 24 hrs, the plates were examined for the presence of sharp white precipitation lines.
  b. Sodium dodocylesuphate (SDS) – polyacrylamide gel electrophoresis (SDS-PAGE)**.**
  c. ELISA according to Menzies *et al.* (1994). Indirect ELISA performed by coating microtiter plates with phosphorlipase D (PLD).

Results and Discussion

Edematous skin disease is an endemic infectious disease that appears mainly among buffaloes and occasionally cattle in Egypt. The disease is usually confined to Lower Egypt as a result of the suitable climatic conditions (low temperature and high relative humidity) especially during late spring and early summer months. On the contrary the disease is less frequent in Upper Egypt due to the considerably high temperature and low relative humidity (Selim, 2001).

Out of the examined 176 buffaloes, 46 showed clinical signs of edematous skin disease and 2 of these infected animals were died representing a morbidity rate 26.1%, Similar results were reported by Barakat (1980), Al-Gaabary & Ammar (1999) and Abu-Zaid (2001). On the other hand out of the. examined 120 cattle, 20 showed clinical signs of the disease representing a morbidity rate 16.6% whereas no mortalities were recorded (Table 1). Similar results were recorded by Shpigel *et al.* (1993) and Abu-Zaid & Hammam (1996). So, it was obvious that the disease was more prevalent in buffaloes than in cattle. These results were in agreement with the results of Soliman *et al.* (1970), Barakat (1980), Khalil *et al.* (1995) and Abu Zaid (2001). The variations in the disease frequency between the different studies may be attributed to the endemic nature of the population variable degrees of immunity.

Concerning the age susceptibility, animals from 1-4 years in both buffaloes and cattle reported by Barakat *et al.* (1984), Al-Gaabary & Arnrnar (1999) and Shpigel *et al.* (1999) who described the epidemiological feature of the edematous : skin disease in Egypt. On the other hand, Zaghawa and El-Gharib (1996) found that edematous skin disease was more prevalent in animals lower than 2 years in comparison to those more than 2 years.

The clinical picture associated with the disease appeared in 4 forms (edematous, ulcerative, nodular and mixed forms (Table 2). The described clinical signs and forms of the disease had been previously reported by Al-Gaabary and Ammar (1999).

The edematous form was more obvious in buffaloes than in cattle, where it represented 35% of the cases in buffaloes and 5% in cattle. On the other hand, the ulcerative form was more prevalent in cattle than that in buffalo where it represents 85% of the cases in cattle and 6.5% of the cases in buffaloes (Table 2).

TABLE 1. Morbidity rates in examined animals.

Animal species	Number of examined animals	No. of diseased animals	No. of dead animals	Morbidity rate
Buffaloes	176	46	2	26.1%
Cattle	120	20	-	16.6%
Total	296	66	2	22.4%

TABLE 2. Percentage of clinical forms of edematous skin disease.

Age	Buffaloes			Cattle			Total		
	No. of examined animals	No. of diseased animals	%	No. of examined animals	No. of diseased animals	%	No. of examined animals	No. of diseased animals	%
Edematous form	46	35	76	20	1	5	66	36	54.5
Ulcerative form	46	3	6	20	17	85	66	20	30.3
Nodular form	46	3	6	20	2	10	66	5	7.6

Khater *et al.* (1983) reported that the skin of cattle especially the epidermis and dermal layers were relatively thinner than that in buffaloes. So, the great thickness of the dermis in buffaloes especially at shoulder region and posterior aspect of thigh with the presence of high vascularity and plenty of macrophage might create a favorable media for *Corynebacterium pseudotuberculosis* infection in severe form (edematous form).

The edematous swelling observed in I the edematous form might be occurred as a result of inflammatory activity of exotoxin mainly phospholipase D (PLD) produced by the causative organism. This effect is not, only locally but also systemically through the circulation to cause toxemia (Selim, 2001).

The phospholipase D toxin of the examined isolated strains of *C. pseudotuberculosis* was resolved by SDS-PAGE. The toxin bands were in a molecular size of 37 and 39 KDa as shown in (Fig. 1).

Locally the activity of PLD as its affect on its biological and enzymatic characters. The biological activity is expressed by development of severe inflammation in the surrounding blood vessels and lymphatics. The local edema is increased by the direct enzymatic effect of PLD on the phospholipids in the

mast cells resulting in the formation of arachidonic acid which is acted upon by certain enzymes to produce histamine like substances such as leukotrins and prostaglandins (Crinio *et al.,* 1998).

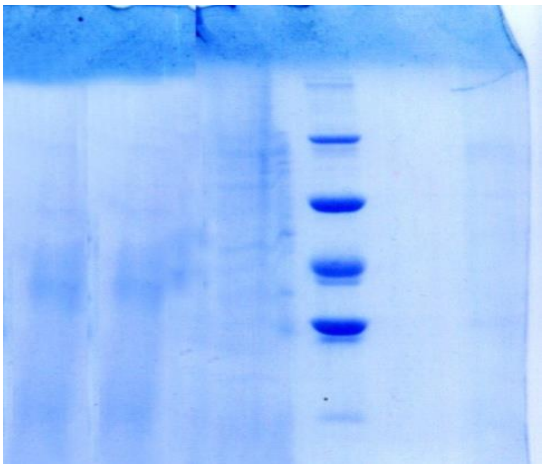

Fig.1. Characterization of phospholipase-D (of *C. pseudotuberculosis*) of buffalo and cow by SDS (immun blot).

Selim (2001), Aleman & Spire (2002) and Yeruham *et al.* (2003) referred the inflammatory process and local edematous swelling around lesions which occurred usually during edematous skin disease to the organism's putative virulencefactors mainly to the direct effect of phospholipase D I exotoxin in the surrounding blood vessels and lymphatics. Also, they reported that the phospholipase Dexotoxin has a general toxemic influence on internal organs in addition the cell wall lipid of *Corynebactermm pseiidotuberculosis* have a potent necrotic effect on the surrounding tissues (Amir and Mahmoud, 2012).

Brown & Olander (1987) and Lioyd (1994) showed that the protective nature of the cell capsule enables the bacterium to persist in the environment for extended periods under favorable conditions: damp, shady areas and low temperatures (Amagai *et al.,*2006).

The microbiological examination, *Corynebacterium pseudotuberculosis* was isolated as a single infection from 53 cases in both buffaloes and cattle samples (80.3%), These results were coincided with the results of Soliman *et al.* (1970), Abu-Zaid (2001) and El-Sawah (2002).

Concerning the biochemical characters of the isolated *Corynebacterium pseudoluberculosis,* all the isolated strains from buffaloes and cattle were nitrate positive. This comes in agreement with Biberstein *et al.* (1971), Hassan (1988),

Galila (1998), Zaki (1999) and Selim (2001). While, other authors have reported that both types (nitrate positive and nitrate negative) might be isolated from cattle (Yeruham *et al.*, 1997 and Steinman *et al.*, 1999).

Concerning guinea pigs inoculation for studying both pathogenesis and re-isolation, all the inoculated isolates of *Corynebacterium pseudotuberculosis* killed guinea pigs within 2-4 days post injection where the dead guinea pigs showed congestion and maceration of muscles at site of injection in addition to congestion of the internal organs. This comes in agreement with the findings of Barakat (1984), Khater *et al.* (1984), Tawfik (1990), Galila (1998) and El-Sawah (2002).

Serological examination, using agar gel immune diffusion test (Table 3), gave strong positive results. ELISA of examined serum samples (Table 4) revealed (OD) reading above the cut off point (0.28). These results suggest that the ELISA is more sensitive for detecting antibodies against *C. pseudotuberculosis* (Abramovits, 2005).

Soliman *et al.* (1970), Mostafa (1984) and Spiegel (1993) found that the isolated strains of *Corynebacterium pseudotuberculosis* were highly sensitive to penicillin *in vitro.* While Abd El-Galil *et al.* (1986), Khalil *et al.* (1995) and Al-Gaabary & Ammar (1999) found the isolated strains was highly sensitive *in vivo* and *in vitro* to gentamycin. Whereas Zaki (1999) found that the isolated strains was highly sensitive to enrofloxacin. Sehra *et al.* (2008).

These variations in the results of sensitivity tests and also in .recovery rates might be due to the development of some resistance against certain antimicrobial drugs which used in previous outbreaks.

TABLE 3. Agar gel immunodiffusion of buffalo and cow sera using (PLD).

Serum samples examined	Positive		Negative	
	No.	%	No.	%
Buffalo (46)	31	67.4	15	32.6
Caws (20)	11	55	9	45

TABLE 4. Optical density (OD) values of buffalo and cow sera using (PLD) as coating antigen.

Antigen PLD	Max.	Min.	Mean	Cut point
Buffalo	0.843	0.345	0.751663	0.28
Cow	0.653	0.382	0.643551	0.28

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مرض الجلدى الاوديمى فى الجاموس و الابقار

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ان فى عام ٢٠١٢ فى فصل الصيف قد تم فحص الجاموس و كان عددها ١٧٦ وكان من بينهم ٤٦ حيوان مصاب بالمرض وكان معدل حدوث الاصابة ٢٦,١٪، بينما تم فحص عدد ١٢٠ من الابقار و كان من بينهم ٢٠ حيوان مصاب بالمرض وكان معدل حدوث الاصابة ١٦,٦٪. وتظهر الاعراض الوبائية المرضية فى اربعة اشكال ، النوع الاول: عبارة عن اديما تظهر غالبا على الجاموس اكثر من الابقار، النوع الثانى عبارة عن تقرحات وتظهر فى الابقار اكثر من الجاموس ، النوع الثالث عبارة عن درنات (اورام صغيرة على الحيوان) والنوع الرابع خليط من الانواع السابقة. تم عزل الميكروب (الكورينى) المسبب للسل الكاذب بالفحص البكتيرى وكان الحجم الجزيئى (phospholipidD toxin) ما بين ٣٧-٣٩ kda. تم الفحص السيرولوجى لعينات السيرم باستخدام اختبار (agar gel immunodiffusion test) وكانت النتائج ايجابية للفحص وتم استخدام اختبار الاليزا وكان تسجيل العينات للكثافة الضوئية عند نقطة (٠,٢٨).