

## COREYNEBACTERIUM PSEUDOTUBERCULOSIS IN BUFFALOES AND SHEEP

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### SUMMARY

60 ( 9.74%) out of 616 examined buffaloes in Sharkia Governorate showed lameness in one limb or more, swellings in the shoulder and side of abdomen, the related lymph nodes were swollen and inflamed. Aspiration of closed lesions revealed bloody fluid and pus, while opened lesions yield necrotic ulcerated tissues with pus formation

Lymphadenitis was detected in 20 (13.16 %) out of 152 examined sheep and 1 (1.3%) out of 78 goats .The affections were characterized by severe emaciation of animals, loss of appetite and enlargement of the superficial lymph glands (retropharyngeal, parotid, prescapular and prefemoral lymph nodes. This affections were seen in adult sheep (over 2 years old). Those abscesses showed thick caseated creamy pus. Postmortem examination of 4 died ewes revealed visceral lesions involving the lung parenchyma, mediastinal and

mesentric lymph nodes and yielded thick creamy pus.

Nitrate reduction test was positive with *C. pseudotuberculosis* isolated from buffaloes and negative in *C. pseudotuberculosis* isolated from sheep and goat

Antibiotic sensitivity test revealed that most *C.pseudotuberculosis* isolates were highly sensitive to Gentamycin, Nitrofurantoin ,Doxycillin, Cephalocin and Erythromycin, and less sensitive to Terramycin and resistant to Trimethoprim sulphamid and Streptomycin

The therapeutic trials in buffaloes with drugs were successful particularly at early stage of the disease. Clinical improvement within 7-10 days in early stage of infection. Opened lesions healed within 3-4 weeks

The isolated bovine strains are similar to the ovine and caprine strains in its capacity to produce exotoxin with varying titer. Examination of *Corynebacterium pseudotuberculosis* isolated from bovine / ovine or caprine reacted against its homologous and heterologous antibodies on employing by the immunodiffusion technique .

Agar gel immunodiffusion test indicated positive results in 88.3 % of infected and 13.3% of contact buffaloes and 85.7% of infected and 20 % contact sheep, while the 84 contact buffaloes with infected sheep showed no clinical symptoms and Agar gel immunodiffusion test revealed negative results .

Parasitological examination of the blood films from buffaloes revealed negative results .

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## INTRODUCTION

Corynebacteria are pyogenic bacteria causing a variety of suppurative conditions. *Corynebacterium pseudotuberculosis* infections (Previous name *Corynebacterium ovis*; *C.ovis*) are global problems. Such agent infect several species including sheep, goats, deer , buffaloes , horses , camels and mules, ( Benhan et al., 1962 and Smith, 1966) . The chief among these are caseous lymphadenitis in sheep and ulcerative lymphangitis in horses, cattle and buffaloes.Two strains have been distinguished chemically and serologically a sheep / goat strain and equine / bovine strain (Bat-

ey,1986).Virulence of *C.pseudotuberculosis* is attributed to the haemolytic toxin which has phospholipase activity and to the cell wall lipids (Quinn et al.,1994)

The objectives of this investigation are: 1- Description of clinical picture of *Corynebacterium pseudotuberculosis* infection in buffaloes and sheep 2-Test of the isolated organisms against different antibiotics .3-Therapeutic trials in buffaloes according to the results of antimicrobial susceptibility test. 4-Detection of *Corynebacterium pseudotuberculosis* specific antibodies in the serum samples collected from both infected and contact animals by Agar Gel Precipitation Test (AGPT) .

## MATERIAL AND METHODS

**Animals:** 616 buffaloes and calves (aged from 6 month up to 7 years) at different villages in Sharkia Governorate were clinically examined; 60 cases showed abscessation or ulceration in skin and lymph nodes and lymphatic, in addition to 152 balady sheep and 78 goats live contact with 84 buffaloes belonged to Governmental farm in Wadi El- Mollak , Ismailia Governorat were clinically examined. 20 ewes and only one goat showed enlargement of the superficial lymph nodes. 4 out of the infected ewes died and was subjected to postmortem and bacteriological examinations.

**Samples:** Pus swabs: bacteriological swabs were collected aseptically by deep scrapings of the open lesions and a spirated fluid from closed lesions under aseptic condition. Post mortem and bacteriological examinations were carried out on the 4 died ewes.

**Bacteriological examinations:** direct smears were prepared and stained with Gram and Ziehl Neelsen stains. Each sample was subjected to bacteriological cultures and mycological examinations. All different colonies were selected and purified by the sub-culturing on the same type of media and each of them was considered as a separate isolate. Each isolate was tested morphologically by Gram stained film and then subjected for biochemical characterization according to Carter and Chengappa (1991).

**Blood samples:** peripheral blood smears from ear vein as well as citrated blood samples were collected from buffaloes for parasitological examinations for detection of filariasis (Soulsby , 1986) .

**Serum samples:** sera were collected from infected as well as 15 apparently normal contact animals in addition to 84 buffaloes contacted with infected sheep were subjected to agar gel immunodiffusion test according to Cameron and Smith (1970) against pooled ovine, caprine and bovine *C.pseudotuberculosis* exotoxin which pre-

pared as described by Arab et al. (1988). The obtained toxins were titrated by rabbit skin neutralization test of Doty et al. (1964).

**Antimicrobial susceptibility test:** In vitro sensitivity test of *C.ovis* isolates was done against different types of antibiotics obtained from bio Mérieux Laboratories (France) using radial diffusion technique according to Bauer et al. (1966). The zones of inhibition were measured. Interpretation of results was done according to the manufacture recommendation.

**Therapeutic trials in buffaloes:** infected buffaloes were classified into 2 groups and treated with different antibiotics according the results of sensitivity test and surgical interference in advanced and /or opened cases. Supportive treatment including vitaminsAD3E intramuscularly was also applied to all animals. All affected sheep were discarded from the flock.

**Statistical Analysis:** The obtained data were statistically analyzed according to Snedecor and Cochran (1967)

## RESULTS

60 (9.74%) out of 616 examined buffaloes showed lameness in one limb or more; swellings in the shoulder and side of abdomen. The swell-

ings varied in size (5 -30 cm diameter), related lymph nodes were swollen and inflamed (Table 1),(Fig.1,2, and 3). Aspiration of closed lesions revealed bloody fluid and pus, while opened lesions yield necrotic ulcerated tissues with pus formation

Lymphadenitis was detected in 20 (13.16 %) out of 152 sheep and in 1 (1.3% ) out of 78 goats. The affection was characterized by severe emaciation of animals, loss of appetite and enlargement of the superficial lymph glands (parapharyngeal, parotid and prescapular lymph nodes; Fig (3) and (4 ). This affection was seen in adult sheep (over 2 years old ) The size of abscess was as the size of chicken egg or more . Samples from those abscesses showed thick caseated creamy pus. Post-mortem examination of 4 died ewes revealed visceral lesions involving the lung parenchyma, mediastinal and mesentric lymph nodes which yielded thick creamy pus .

Bacteriological examination showed that cultural tubes of nutrient broth showed pelicle formation. Cultural plates revealed non-haemolytic pinpoint sized colonies that appeared on blood and nutrient agar. After further incubation for another 24 hours, these colonies were opalescent white and were encompassed by narrow zone of haemolysis.

No growth on MacKonky agar plate. The micro-organism which was Gram positive bacillus was identified as *C. pseudotuberculosis* on the basis of positive reactions for catalase, glucose, nitrate reduction and urea, and showed negative reactions with lactose and sucrose.

Results of bacteriological examinations are shown in Table (2).

The collected samples were negative for nocardia, mycotic infection and mycobacteria. Also parasitological examination revealed negative results

Results of, sensitivity test, therapeutic trials, titration of toxins and AGPT are shown in Tables 3,4,5 and 6.

The isolated bovine strains are similar to the ovine and caprine strains in its capacity to produce exotoxins with varying titer

Treatment trials in buffaloes with antibiotics showed clinical improvement in early stage of infection. Opened lesions which were treated with antibiotics and surgical interference healed within 3-4 weeks.

**Table (1):** incidence of the disease in relation to age of buffaloes and season.

Season	Total No.	Adult animals (over 2 years)		Young animals (under 2 years)		Diseases animals	
	No. of animals	No.	Diseases	No.	Diseases	No.	%
Summer	310	223	43	87	2	45	14.5
Autumn	105	84	4	21	0	4	3.8
Winter	80	43	3	37	0	3	3.8
Spring	121	79	7	42	1	8	6.6
Total	616	429	57	187	3	60	9.74

**Table (2) :** Results of bacteriological examinations :

Animals	Lesions	No.of cases	No.of C.ovis alone	C.ovis and Staph	C.ovis and Strept	C.ovis and E.coli	Staph and strept
Buffaloes	Closed	54	42	2	5	2	3
	Opened	6	0	1	3	1	1
Sheep	Closed	8	7	0	1	0	0
	Opened	12	0	8	0	2	2
Goat	Closed	1	1	0	0	0	0

56 isolates of *C. pseudotuberculosis* from buffaloes and 18 isolates from sheep and one isolate from goat.

**Table (3):** Results of sensitivity test *C.pseudotuberculosis* isolates from buffaloes and sheep

Drugs Used	56 <i>C.pseudotuberculosis</i> isolates from buffaloes		19 <i>C.pseudotuberculosis</i> isolates from sheep and goat	
	Sensitive isolates	%	Sensitive isolates	%
Doxycycline<30	54	96.4	17	89.5
Gentamycin <10	54	96.4	17	89.5
Cephalothin<30	53	94.6	16	84.2
Refampicin<30	52	92.9	16	84.2
Nitrofurans<300	50	89.3	15	78.9
Erythromycin<15	45	80.3	12	63.2
Ampicillin<10	30	53.6	10	52.6
Teracyclin<30	23	41.1	9	47.4
Penicillin<10	12	21.4	89	42.1
Streptomycin<10	0	0	0	0

**Table (4):** Therapeutic trials of diseased buffaloes.

Group No.	Type of lesions	No. of cases	Drug used
Group No.I	Closed	22	Gentamycin 10%*: 4ml/100kg.b.wt. im for 7 days. * produced by ADWIA, Egypt.
	Opened	8	
Group No.II	Closed	22	Velosef: Cephadrine semisynthetic Cephalosprin **: 5mg.Kg. B.wt i.m for 10 days.** produced by Bristol-Myers Squib Egypt.
	Opened	8	

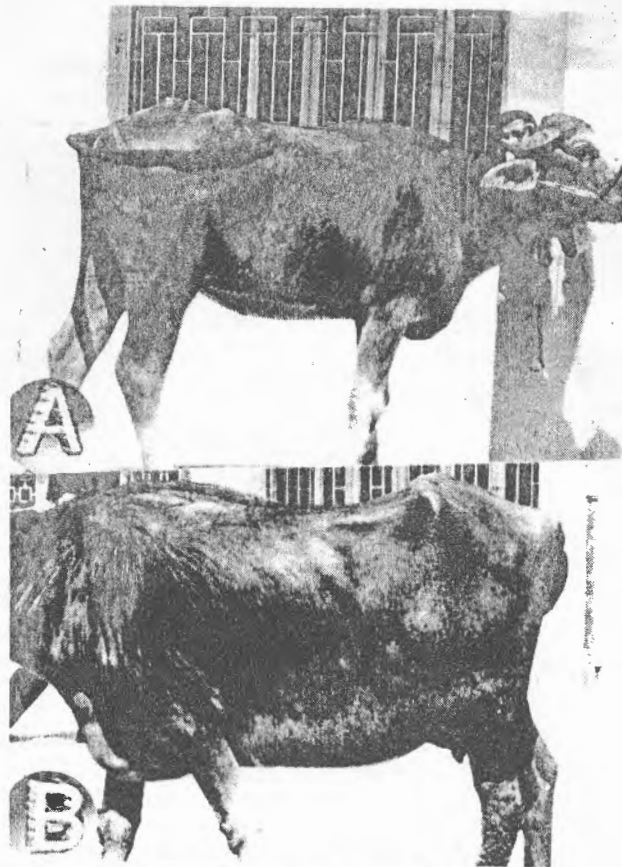
**Table (5):** Titration of the prepared *C.pseudotuberculosis* exotoxins by Rabbit skin neutralization test..

Strain No.	1 (Ovine)	2 (Ovine)	3 (Caprine)	4 (Bovine)
Titre	1:256	1:512	1:256	1:128

**Table (6) :**Results of AGPT .

Animals	Condition	No.	No. of Positive	%
Buffaloes	Infected	60	53	88.3
	Non infected	15	2	13.1
Buffaloes	No infected, contact with infected sheep	84	0	0
Sheep	Infected	20	17	85
	Contact sheep	15	3	20
Goats	Infected	1	1	100
	Non infected	15	1	6.7

**Fig. 1. A : Buffalo: enlarged pre-  
scapular lymph node.  
B: Buffalo: enlarged prefe-  
moral lymph node.**



**Fig. 2. A : Buffalo: enlarged prescapular lymph node.  
B: Buffalo: enlarged prefemoral lymph node.**



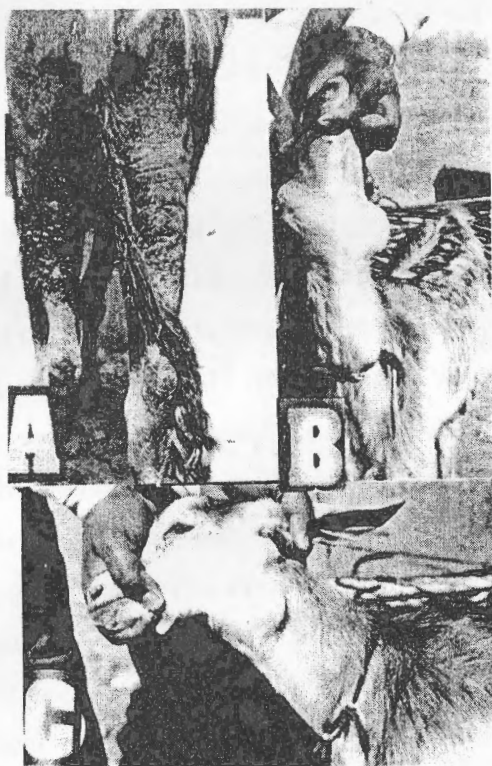


Fig. 3: A. Buffalo : ulceration in the hind limb.  
 B. Goat: bilateral enlargement in the parapharyngeal lymph node.  
 C. Goat: enlarged left parapharyngeal lymph node



Fig.4: A. Ewe: enlarged parotid, retropharyngeal and opened prescapular lymph nodes.  
 B: Ewe; enlarged right parapharyngeal lymph node.  
 C. Ewe : opened submandibular lymph node and yield caseated pus.

## DISCUSSION

In buffaloes, the observed clinical symptoms associated with isolation of 56 *C. ovis* isolates (42 in pure culture and 12 isolates associated with other bacteria). These findings were similar to the conditions called ulcerative lymphangitis in buffaloes and cows that described by Carne (1933) and Carpano (1934) and Soliman et al. (1963) who isolated 23 *C. ovis* strains in pure culture from 32 cases in primary isolation from buffaloes showed and same clinical symptoms They claimed that it

may be a type of ulcerative lymphangitis in cattle; however; they preferred the name Oedematous Skin Disease and., Barakat (1980); Ibrahim et al. (1983); Abd El Galil et al. (1986); Khalel et al. (1995); Zhagawa and ElGarib (1996) and Ali & Zaiton (1999). These findings were confirmed by the successful experimental reproduction of the disease in buffaloes calves when they were experimentally inoculated with pure culture of *C. ovis* (Hassan et al., 1983; Khater et al., 1983; Esmat, 1984 and Hassan (1988). *Corynebacterium ovis* produced a potent exotoxin which on in-

jection caused local area of suppurative inflammation and necrosis (Torky et al., 1982). The most important component are included in the pathogenesis of *Corynebacterium pseudotuberculosis*, the cell wall lipids which is the pathogenic factor and the exotoxin which probably serves to promote the local spread of the organism (Zaki, 1976).

It is cleared that the peak of infection occurred during the summer ( May, June, July and August) especially old age buffaloes. Similar results were reported by Esmat (1984); Al-Gaabary and Ammar (1999) and Ali & Zaitoun (1999). There is a relation between fly population during the hot months and the disease. This finding was supported with Braverman et al. (1999) who isolated *C. pseudotuberculosis* from 40 wild houseflies which had fed on a lesion on a cow and from 28 laboratory flies fed on contaminated milk from a cow infected with mastitis, they concluded that houseflies (*Musca domestica*) plays an important role in harboring and disseminating *C. pseudotuberculosis* in dairy herd. It is also probable that wallowing of buffaloes in the muddy channels as skin-wetting agent during the summer season should not be neglected as enhancing factor for growth of *C. pseudotuberculosis*. The relative degree of resistant of young animals may be due to persisting protective antibodies from an infection acquired when young (acquired herd immunity) while Zaghawa and El-Garib (1996) recorded

that the percentage of clinically diseased buffaloes under 2 years was 44 % while it was 9.8 % for those animals more than 2 years old.

Biochemical tests on the isolated strains of *C. pseudotuberculosis* revealed that most isolates were positive with citrate utilization, urea hydrolysis and negative with H<sub>2</sub>S production, gelatin liquification and Indol production and not ferment most sugars with production of acid only. Biotyping of the *C. pseudotuberculosis* isolates showed positive nitrate reduction test which came in accordance with Barakat (1984) and Stuman et al. (1999) who recorded that all the strains of *C. pseudotuberculosis* isolated from ulcerative lymphangitis in cattle were nitrate reductase positive.

Antibiotic sensitivity test revealed that most *C. pseudotuberculosis* isolates were highly sensitive to Gentamycin, Nitrofurantoin Doxycillin, Cephalocin and Erythromycin and less sensitive to Terramycin and Resistant to Trimethoprim sulphonamid and Streptomycin. These agreed with Abd El Galil et al. (1986); Khalel et al. (1995); Hamoda (1996); Ali and Zaitoun (1999) and Zaki (1999). On the other hand, Al-Gaabary and Ammar (1999) found that isolates of *C. pseudotuberculosis* were highly sensitive to Sulphonamide, Cephalothin, Oxytetracyclin, and Gentamycin and moderately sensitive to Penicil-

lin and Ampicillin and resistant to Neomycin .

Agar gel immunodiffusion test indicated positive results in 88.3 % of infected and 13.3% of contacted buffaloes. These results go hand to hand with that mentioned by Burell (1980), Robertson (1980), Arab et al. (1988). The presence of serological positive among normal contact buffaloes in the view of Anderson and Narin (1985) does not necessarily reflect active infection. Antibodies may be present in diseased animals, in animals exposed to infection but not diseased and in previously infected animals which have been recovered.

It is of interest to record that most infected buffaloes were previously injected with either penicillin or terramycin and were not respond to treatment. *Corynebacterium* are extremely sensitive to penicillin in vitro, however; the purulent exudate in the lesions of *C. pseudotuberculosis* prevent systemic penicillin from reaching to bacteria at a bacteriocidal concentration. (Brander and Pugh 1971)

The therapeutic trials in buffaloes with drugs were successful particularly at early stage of the disease. Therapeutic trials were not completely successful In buffaloes with marked suppurative lymphangitis, Similar results were recorded with Ali and Zaitoun (1999). The difficulty to treat-

ment may be due to the relative long course of the disease and the probability of recurrence in spite of early treatment may attributed to the specific pathogenesis of the disease which described by Carter and Chengappa (1991) assuming that macrophages migrate to the invasion site engulfing the organism in a phagosome fusion of phagosome and lysosome does not bring about the destruction of the organism owing to their ability to resist of lysosomal enzymes. This property is due to the high levels of specific cell wall- lipids This may be related to the high amount of pussy material which preventing the diffusion of the antibiotic (Quinn et al., 1994).

In sheep lymphadenitis was detected in 20 adult ewes (13.16 %) out of 152 sheep and 1 (1.3%) out of 78 goats Most infected sheep had history of sarcoptic mite infestation and /or shearing of wool which may cause skin abrasions as facilitating infection with *C. Pseudotuberculosis*. *C. Pseudotuberculosis* was isolated in pure culture from 7 samples and associated with other organism from 12 samples, similar observations were reported by Arab (1979); Ammar (1983); Seddik et al. (1983) Moustafa and Afifi (1996) and Sohair et al. (1999) in Egypt. Batey (1986) and Bergin (1987) in Australia, Abdel Hamid et al. (1992) in Saudia Arabia, Abo-El Hassan and Hagour (1995) in Lybia and Al-Rawashdeh and El-Qudah (2000) in Jordan who reported that the prevalence of

caseous lymphadenitis increase with age as well as young sheep after shearing and spreads among sheep more than goats.

Postmortem examination of 4 died ewes revealed visceral lesions which involving the lung parenchyma, mediastinal and mesentric lymph nodes which develop from haematogenous spread of bacteria from regional lesions as reported by Lloyd (1994) and Mubark et al. (1991).

Biochemical tests on the isolated strains of *C.pseudotuberculosis* revealed that most isolates were positive with citrate utilization, urea hydrolysis and negative with H<sub>2</sub>S production, gelatin liquefaction and Indol production and not ferment most sugars with production of acid only. Biotyping of the *C.pseudotuberculosis* isolates showed negative nitrate reduction test, a results which came in accordance with Biberstein et al. (1971) and Bergin (1987) in Australia and Conner et al. (2000) who found that all *C.pseudotuberculosis* isolates from caseous lymphadenitis were non reduce nitrate.

Antibiotic sensitivity test revealed that most *C.pseudotuberculosis* isolates were highly sensitive to Gentamycin, Nitrofurantoin Doxycillin, Cephalocin and Erythromycin and less sensitive to Terramycin and resistant to Trimethoprim sulphamid and Streptomycin. These findings agree to a certain extent with those reported by Lu et al.

(1987) and Abd El-Ghani et al. (1998). Moreover Zaitoun and Ali (1999) who indicated that all *C.pseudotuberculosis* isolates from caseous lymphadenitis were streptomycin resistant and highly sensitive to cephredine

Agar gel immunodiffusion test was positive in 85.% infected and 20 % contacted sheep. Similar findings were reported by Awad et al. (1977); Ammar et al. (1987); Arab et al. (1988) and Abo-El Hassan & Hagour (1995).

84 contact buffaloes with infected sheep showed no clinical symptoms and AGPT revealed negative results, this findings indicated absence of infection, the infection did not transmitted from sheep to buffaloes. Chikamatsu et al. (1989) in Japan concluded that immunodiffusion test was less sensitive than ELISA in detecting antibodies against *C. pseudotuberculosis*, it did not give any non-specific reactions, It may be of practical usefulness in detecting the disease in the field.

Both ovine and bovine strains of *Corynebacterium pseudotuberculosis* employing by the immunodiffusion technique revealed that each exotoxins not only reacted against its corresponding antigen but also against the other heterologous exotoxins, they are antigenically related as observed by Doty et al. (1964) and Lovell and Zaki (1966) who used rabbit skin neutralization test and mouse protec-

tion test respectively . They concluded that although the general characters of their bovine isolates were similar to those of other isolates from sheep, goats, buffaloes and equines, there were slight difference in the antigenic structure of their exotoxin and it is apparent that those exotoxins are similar, however, not identical.

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