

**CLINICAL; BACTERIOLOGICAL AND
THERAPEUTIC STUDIES ON MIXED INFECTION
WITH ACTINOMYCES BOVIS AND ACTINOMYCES
PYOGENSE IN BUFFALOES
IN ASSIUT- GOVERNORATE, EGYPT**
(With 2 Tables and 5 Figs.)

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دراسات إكلينيكية وبكتريولوجية وعلاجية عن العدوى المختلطة بميكروبي الفطر الشعاعي
والفطر الصددي في الجاموس في محافظة أسيوط / مصر

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في هذا البحث تم عمل الفحوصات الإكلينيكية على عدد (٩٠٠) من الجاموس وأظهرت النتائج بان عشرة حالات كانت مصابة بأورام كبيرة في الجزء الأسفل لعظمة الفك السفلي والمنطقة الوسطية بين الفك السفلي والغدد تحت الأذن (الغدة النكفية). صور الأشعة أظهرت خلل وأورام في الأنسجة العظمية للفك السفلي مع وجود فراغات بين الضروس الطاحنة للفك السفلي والعلوي. أفادت نتائج الفحوصات البكتريولوجية والبيوكيميائية والحقن في الأرناب بان الميكروبات المرضية المعزولة هما ميكروب الفطر الشعاعي والفطر الصددي. المشاهدات الإكلينيكية وصور الأشعة مدعمة بنتائج الفحوصات البكتريولوجية والبيوكيميائية والحقن في الأرناب وصلتنا إلى التشخيص الصحيح لهذه الحالات المصابة وأن المرض كان مرض الفطر الشعاعي وكانت المسببات المرضية الأساسية هما ميكروبي الفطر الشعاعي مرتبط مع ميكروب الفطر الصددي. أظهرت اختبارات الحساسية لاختبار أفضل المضادات الحيوية فاعلية على الميكروبات المغرولة بأن السيفالكسون والبنسلين هما أكثر المضادات الحيوية فاعلية على ميكروبي الفطر الشعاعي والفطر الصددي. علاج الحيوانات المصابة بالمضاد الحيوي سيفالكسين (١٠مجم/ لكل كجم من وزن الجسم) يوميا مع العلاج الجراحي ويوديد الصوديوم (١٠جم/ ١٠٠كجم) بتركيز ١٠% في المحاليل مع المحاليل المغوية أعطى نتائج جيدة في الإصابات الموجودة في الجزء الأسفل لعظمة الفك السفلي وعضلات الخد بينما كانت ضعيفة الاستجابة وتحتاج إلى وقت طويل في إصابات الأنسجة العظمية للفك السفلي. أفادت نتائج البحث بان نسبة الإصابة بهذا المرض كانت (١١,١%) وعلى هذا فان مرض الفطر الشعاعي في الجاموس يوجد بنسبة قليلة في محافظة أسيوط.

SUMMARY

Ten cases (1,11%) out of (900) clinically examined buffaloes showed swelling at the lower part of the mandible, intermandibular space and parotid lymphnodes. Radiographs of the affected lesions of a severe case revealed rarefaction, osteomyelitis, displacement of the molar teeth and enlargement at the lower part of the mandible of the infected animal. Bacteriological; Biochemical and pathogenicity tests in rabbits cleared that mixed infection of the collected samples and the purified isolated strains were *Actinomyces bovis* and *Actinomyces pyogenes*. Results obtained from clinical, radiographs, bacteriological examinations and pathogenicity tests conducted us to the correct diagnosis of infected cases and the disease was actinomycosis. Results of antibiotic sensitivity tests showed that cephalaxine "Velosef" and Penicillin were more effective on the purified isolated strains of *Actinomyces bovis* and *Actinomyces pyogenes*. Therapeutic trials of the infected cases by cephalaxine (10 mg/kg of BW) for 10 days with surgical treatment, sodium iodide (10 gm/100 kg) 10% solution and supportive therapy gave good results in the lesion of the lower part of the mandibles and lesions of cheek muscles, but the bony lesions in the mandibles were poor response and need long time. The obtained results from the present study pointed that the percentage of infection was (1,11%) and thus, the actinomycosis of buffaloes is present at low percentage in Assiut Governorate.

Key words: Actinomyces in Buffaloes

INTRODUCTION

Buffaloes has several advantages and it becomes the main dairy animals in Egypt. Bacterial infectious diseases of buffaloes were considered as one of the economic loss in the animal wealth in Egypt. *Actinomyces* is a genus whose normal habitat in the oral cavities of human and animals, (Slack and Gerencser, 1975). *Actinomyces bovis* infection in the mandible was recorded by (Patgiri *et al.*, 1985). *Actinomyces bovis* was isolated from granulomatous lesions in the tracheal lumen of a cow by Bertone and Rebhun, (1984) El-Sanousi *et al.* (1992) isolated *Actinomyces bovis* from abscesses in the lower jaw of cows. Ayyappan *et al.* (1993) reported that *Actinomyces bovis* was isolated from granulomatous nodules in the neck of buffaloes in India. On the other hand, Attia *et al.* (1958) in Egypt, mentioned that

Actinomyces pyogenes was isolated from a pus containing lymphnodes of Sudanese cattle that was suspected to be due to actinomycosis while, Khater *et al.* (1970) pointed that *Actinomyces pyogenes* and *Actinomyces lignieresii* were isolated from lesions of actinobacillosis of unusual case of cattle. The studies on actinomycosis of buffaloes are still brief in Assiut Governorate; hence it becomes necessary in the present work to study (1) the clinical picture of the disease in buffaloes. (2) Isolation and identification of the pathogenic aetiological agents responsible for swellings of the lower part of the mandible of infected buffaloes. (3) Therapeutic trials of infected cases with choice antibiotics according to the results of antibiotic sensitivity tests.

MATERIAL AND METHODS

1) Animals:

From July, (1999) to August, (2000) a total number of (900) buffaloes of different villages in Assiut-Governorate were clinically examined and the infected cases which were suffering from swellings in the lower part of the mandibles and parotid lymphnodes were subjected to detailed clinical examinations for describing the clinical findings. Owner's complaint and history of infected buffaloes including patient data also were taken. All examined buffaloes were at different age from (4-8) years and from both sexes.

2) Samples and Culturing:

Closed skin swelling in the lower part of the mandible of infected buffaloes were surgically incised and the material content were swabbed deeply and also tissue specimens were taken under complete aseptic conditions. The collected samples were directly inoculated into tubes containing brain heart infusion broth for 24-48 hours at 37 C⁰. The incubated tubes were plated on sheep blood agar media. One of each inoculated plates was put in anaerobic Jar (Macintosh) with 10% CO₂ and incubated at 37C⁰ for 2-5 days. The other inoculated plates were incubated aerobically at 37C⁰ for 2-5 days. Growth colonies were picked up and subjected to morphological and biochemical identification as described by Sneath *et al.* (1986).

3) X-Ray examinations:

Radiographs were carried out on affected regions of a severe case of infected buffaloes by exposure the animal to X-Ray in the student veterinary hospital Assiut University.

4) Pathogenicity tests:

Each purified isolated strains having morphological; cultural and biochemical characters was subjected to pathogenicity test. One ml of 24

hours broth culture was injected subcutaneously in rabbit and another rabbit was inoculated also subcutaneously by the same does of sterile broth and used as control. The two rabbits were put under observation. Broth culture was made from pus material of abscess of rabbit and plated on sheep blood agar media.

5) Antibiotic sensitivity test:

The purified isolated strains were tested for the presence of different members of Antibiotic discs and inhibition zone were measured and interpreted according to method of Baure *et al.* (1966). The choiced antibiotic was used for treatment of some infected buffaloes.

RESULTS

1) Clinical findings:

Ten (1,11 %) cases of the clinically examined buffaloes (900) showed swelling in the lower part of the mandible, intermandibular space and parotid lymphnode. Observed lesions were bony swellings particularly in the mandible. Lesions were very hard, immovable and painless. Swelling extended from the base of the ear along the base of the mandible till the lower lip. Swellings were elongated, elevated, firm, filling the intermandibular region of the head and pocket-like in shape in the cheek muscles, containing about one litter of yellowish fluids (Fig. 1) parotid lymphnodes were swelled, hard, immovable and painless. By incision, lesion were filled with odorless thick sticky yellowish exudate, oily in colour containing yellow-white granules (Sulfur granules) and hony shape tinged with blood. Infected buffaloes showed reduced of the appetite, difficulty in mastication, dropping of food from the mouth and loss of condition. Radiographs of the affected region in the head revealed swelling in the lower part of the mendibular space, rarefication, osteomyelitis of bone of mandible and displacement of the molar teeth, (Fig. 2).

2) Bacteriological examination:

Ten closed swellings at the lower part of the mandibles of the infected buffaloes were selected, sampled and bacteriologically cultured. Results of bacteriological samples yielded mixed infection with *Actinomyces bovis* and *Actinomyces pyogenes*. Colonies of *Actinomyces bovis* grow well anaerobically on sheep blood agar media at 37C⁰, white in colour and bread crumb in shape without any haemolysis on blood. The organisms were gram positive filamentous rods, non-motile, diphteroidal arrangements (Y, V and T) forms, present in palisades and the filamentous rods were found in wavy, straight short

chain and slender, and containing branching filaments (Fig. 3). Biochemical analysis showed that catalase, urease and nitrate reductions were negative. The carbohydrates were fermented with acids without gases production. *Actinomyces pyogenes* were grown well anaerobically within 2 days at 37°C. Colonies were grey in colour and produced complete-β-haemolysis on sheep blood agar media. The organisms were gram positive short bacilli, arranged in diphtheroid form, non-motile and were found in palisades, (Fig.4). The biochemical tests showed that catalase, indol, urease and nitrate reduction were negative reactions while the gelatin liquefaction and milk coagulation were positive.

Pathogenicity tests:

The inoculated rabbits showed subcutaneous abscess within 2-3 weeks, *Actinomyces bovis* and *Actinomyces pyogenes* were re-isolated from each abscess of each inoculated rabbit. (Fig.5)

Antibiotic sensitivity tests and therapeutic trials: -

Results of the antibiotic sensitivity test on the purified isolated strains of *Actinomyces bovis* and *Actinomyces pyogenes* were illustrated, in Tables (1 & 2). The therapeutic trials on infected buffaloes by using surgical incision, complete evacuation of the yellowish exudates, washing with potassium permanganate 5%, packing of cavities with a gauze soaked with tincture iodine 2%, intravenous injection of sodium iodide 10 gm/100 Kg, B.W. and intramuscular injection of velosef 10 mg/Kg, Bw, daily for 10 days with supportive therapy as dextrose-saline intravenous injection gave good results. Yellowish exudate inspissated, disappeared and the lesions were diminished in size but the bony swelling of the mandibles need long-time.

DISCUSSION

In the present study, swellings at the lower part of the mandibles, intermandibular space and parotid lymph nodes of the head, (Fig. 1) and rarefaction, osteomyelitis and displacement of the molar teeth (Fig.2) were the main clinical signs observed on the infected buffaloes. Similar clinical observations were recorded by Patgiri *et al.* (1985) in India and El-Sanousi *et al.* (1992) in Saudi Arabia. Results of morphological, biochemical and pathogenicity tests of the purified isolated strains (Fig.3 and 5) respectively were agreed with *Actinomyces bovis* as described by Slack and Gerencser (1975). Results of bacteriological and Pathogenicity tests of the other pathogenic purified isolated strains (Fig.4 and 5) were agreed typically with *Actinomyces pyogenes* as mentioned by Soliman *et al.* (1970) and Reddy *et al.* (1982). Results of

the clinical, X-Ray, bacteriological examination and pathogenicity tests in rabbits, conducted us to the correct diagnosis of the infected buffaloes and the disease was actinomycosis. The obtained results by bacteriological examination showed that *Actinomyces bovis* and *Actinomyces pyogenes* were considered the main aetiological pathogenic agents responsible for actinomycosis of buffaloes. Reddy *et al.* (1982) mentioned that the factors of pathogenicity and virulence which enable the actinomycetes, especially *Actinomyces Israeli* and *Actinomyces bovis* to invade the tissue and to cause necrosis and abscesses formation have not been elucidated. Only *Actinomyces pyogenes* has been shown to produce soluble toxic and hemolytic activities which are neutralized by antitoxin. Soliman *et al.* (1970) stated that corynebacterium pyogenes infection in farm animals is rather of an endogenous origin as a result of a stimulus provoking a latent infection. Results of antibiotic sensitivity test adverted that velosef had strong inhibitory effect on the most of the isolated strains of *Actinomyces bovis* and *Actinomyces pyogenes*. The therapeutic trials with antibiotic choic with surgical treatment, sodium iodide/ i/v, supportive therapy gave good results in the lesion in the cheek muscles while the bony lesions of mandible were poor response to treatment and need long-time. This opinion attributed to the myocelial masses of *Actinomyces bovis* reduce the rate of penetration of antibiotics and may physically protect the associated bacteria (Bowden and Goodfellow, 1990).

Table 1: Antibiotic sensitivity test on some isolated strains of *Actinomyces bovis*, (n = 10).

Antibiotic disc	Inhibition Zone									
	1	2	3	4	5	6	7	8	9	10
Streptomycin	2+	2+	1+	1+	2+	2+	1+	1+	2+	2+
Tetracycline	3+	2+	1+	2+	2+	1+	1+	2+	3+	3+
Penicillin	2+	3+	2+	2+	2+	3+	3+	3+	2+	3+
Cephalexine*	3+	2+	3+	3+	3+	2+	3+	3+	2+	3+
Gentamycine	2+	2+	3+	2+	3+	3+	3+	2+	2+	3+
Erythromycin	1+	1+	2+	2+	3+	2+	3+	3+	3+	2+
Neomycin	3+	3+	1+	2+	1+	2+	2+	3+	1+	2+
Ampicillin	1+	1+	2+	1+	1+	1+	2+	2+	1+	1+

* Cephalexine = Velosef (squibb-Egypt)

Table 2: Antibiotic sensitivity test on some isolated strains of Actinomyces pyogenes, (n = 10).

Antibiotic disc	Inhibition Zone									
	1	2	3	4	5	6	7	8	9	10
Streptomycin	1+	1+	2+	1+	1+	1+	1+	2+	1+	2+
Tetracycline	2+	3+	1+	2+	2+	1+	3+	1+	1+	3+
Penicillin	3+	2+	3+	3+	3+	2+	2+	2+	2+	3+
Cephalexine*	3+	3+	2+	2+	3+	3+	3+	3+	3+	3+
Gentamycin	3+	1+	2+	1+	2+	2+	3+	1+	2+	2+
Erythromycin	2+	3+	3+	2+	1+	1+	2+	2+	2+	3+
Neomycin	3+	3+	2+	2+	2+	1+	2+	2+	3+	1+
Ampicillin	1+	3+	1+	1+	2+	1+	1+	1+	1+	2+

* Cephalexine = Velosef (squibb-Egypt)

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Fig. (1): Clinical lesions of actinomycosis in buffaloes.

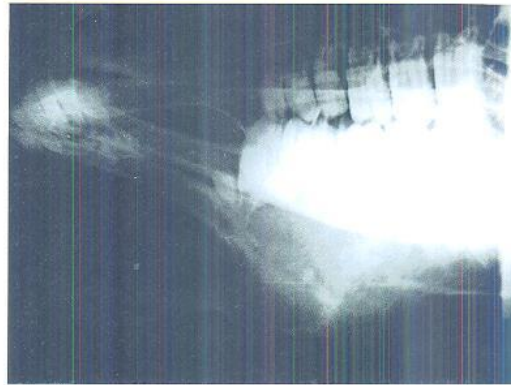


Fig. (2): Radiograph on right side of head of infected buffalo showing lesions of actinomycosis.

Fig. (3): isolated strains of actinomyces bovis stained with gram- stain

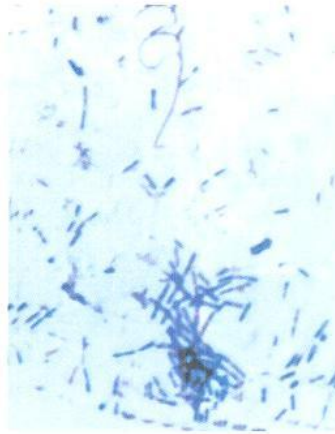


Fig. (4): isolated strains of actinomyces pyogenes stained with gram- stain.



Fig. (5): subcutaneous abscess formation due to injection of each isolated strains of actinomyces bovis and actinomyces pyogenes.