نتائج موجبة لأختبار الروزبنكال في الخيول

نبيه محمد عطا ، محمد جويد علوان ، سعدي أحمد غناوي السامرائي
شاكرمحمود مرهش ، حيدر بدري عبود ،أياد عبد الجبار
كلية الطب البيطري – جامعة بغداد

ملاحظات قصيرة

لوحظت حالات متفرقة في مجموعة من الخيول شملت ناسور الحارك، التهاب المفاصل وتورم كيس الصفن. سحبت عينات دم من بعض هذه الحالات ومن خيول تبدو سليمة لقياس خضاب الدم وأجراء اختبار الروزبنكال (Rose Bengal test) . كذلك تم سحب نموذج من السائل في كيس الصفن. أظهرت الفحوصات أن مستوى خضاب الدم كان ضمن الحدود الطبيعة (11.6- 16.2)غم/ 100سم^3 . وان خمسة خيول من عشرة (50%) أظهرت نتيجة موجبة لأختبار الروزبنكال وكانت من الجنسين وبأعمار مختلفة ، ولم تقتصر على الخيول التي أظهرت ناسور أو تورم . ولم تعزل جراثيم من السائل المصلي الرائق والحاوي على كريات دم حمراء وخلايا قيحية والمسحوب من كيس الصفن لأحد الخيول .

The Iraqi J. Vet. Med. Vol. 24, No. (2) 2000

NATURAL NOCARDIOSIS IN CATTLE : A SURVEY AND PATHOLOGIGIAL STUDY

M.J.ALWAN
Department of Vet. Pathology and poultry, College of Vet. Medicine, University of Baghdad, Iraq.

SUMMARY

This work was done to investigate Nocardiosis in cattle in order to evaluate the incidence of nocardiali mastitis in Al- dejiala station, bacteriological examination was conducted on 90 milk samples obtaited from mastitic cows Nocardia asteroides was found in 25 out of 90 (27. 77% mastitic cows. This M.O was recorered in pure culture in 5 cases and as mixed with other microorganisms in 20 cases .The gross examination of the affected udders showed multiple nodular lesions in firm fibrotic udders, some of them formed draining sinus which exuded pus to the exterior multifocal nodules of varying size were seen in the lungs Microscopic examination of the affected

udders revealed pyogranulomatous lesions in different areas of the glandular parenchyma multiple classical granulomatous lesions were also noticed in the lung tissues.

INTRODUCTION

Since the early isolate of Nocardia asteroides in 1888 by Nocard, many naturally occurring condition have been recorded in different animal species, such as dog, sheep, horse and wild animals (1,2,3)

in cattle, the disease was shown in three main form, skin farcy, mastitis and pulmonary form Bovine skin farcy was frequently observed in Africa. Awad9 (4) described the disease an externl form of farcy in sudoness cattle characterized by chronic subcutaneous abscesses. The internal form of skin farcy may or may not be accompanied by the external form and is characterized by a Localized or diffuse pyogranul omatous lesion of the internalorgana and tissues (5) .

In the polmonary form, the lungs are involved primary or secondary to skin farcy or mastitis (6,7) . Dissemination of the lesions were recegnized internal organs (8) Nocardial mastitis have been reprorted as sporadic cases or in outboreaks over the world (9)%. Hibbs et al . , (10)

reported outbeaks of nocardial mastitis in 3300 dairy cows ehere 450 cows died and further 500 were culled .
In this work , the author aimed to evaluate the occurrence of nocardial mastitis in dairy cattle and to describe the pathological changes accompanied .

MATERTALS AN D METHODS

This stydy was carried out on cattle at the AL-Dejiala station Bacteriological examination was conducted on 90 milk samples obtained from mastitic cows . Five mastitic cows which positive for nocardial examination were slaughtered . Post- mortem examination were carried out specimens from different organs were fixed in 10% normal buffer formalin for histopathological examination . Milk , pus and tissue samples were collected aseptically for bacteriolegical examination .

Bacterial examination

Soon after collection , samples were cultured into brain heart infusion agar , containing 7% sheep RBC and blood agar .
They were incubated at 37c˚ for three days . Suspected colonies were subjected to gram and acid fast stains

followed by necessary confirmating biochemical and physiological examination chosen according to Alwan (4) and Collins and Anne (II)

Animals inoculation

In order to determine the pathogenicity of the isolates, five guinea pigs were used . They were inoculated intrapulmonary with IXI0^9 CFU of N.asteroides , taken from a suspension of 3 days old culture in sterile saline . thermodurability of the isolated M.O was checked by incubating at 50c ˙ f0r shrs .

RESULTS

N. asteroides was found in 25 out of 90 (27.77%) mastitic cows as wellas milk tank . Isolation of this M.O. was found in pure culture in 5 cows . In other remaining samples M.O including candida spp , streptococeeus spp, staphylococcus spp , klebseila and Bacillus spp were also isolated . N. asteroides were also isolated from the lung and supramammery lymph nodes of the necropsied cows .

Colonies of N. asteroides wre seen on blood agar and brain heart infusion agar after 72 hrs of aerobic inoeubation colonies of N. asteroides were seen on blood agar and brain heart infusion agar after 72 hrs of aerobic inocubation at 37C,They were opaque, chalky

white firmly adherent to the medium, and not haemolytic (Fig:I) . in 72 hrs of incubation, the colour of colony was turned in to yellowish- orgage. In broth, a pellicle of waxy growth was noticed afters 48 hrs of incubation. The M.O. were partially or complelly acid fast Some beaded appearance due to irregular staining. These M.O. remained alive more then 8hrs at 50c.The isolates were fermented glucose, producing acid and gass, mannitole, sucrose and fractose were not fermented . They reduced nitrate to nitrite, produced urease and hydrolysed aseculine but not casein (Table:1)The inoculated guinea pigs died within 5-6 days post infect infection . N.asteroides was isolated from all internal organs. All infected animals showed massive pmns and mnsinfiltration in the examined organs .

Pathological changes

At early stage, the infected udders were swollen and painful but later on , they become firm and fibritic with palpable nodular lesions . Some of then ruptered or formed draining sinus which exuded whitish pus to the exterior (Fig:2) in addition marked thick walled abscesses were observed One 0r more quarter of the udder were involved In the lung , necropsy findings revealed firm yellowish- white multifocal nodules of

varying size ranging from 1-1. 5cm in diameter. Tthey are uniformly scattered throughout the parenchyma with their cut section revealed dry purulent center. The supramammary lymph nodes were enlarged and edematous mediasinal and bronchial L.N. were also showed the same lesions Histopathological examination of mammary glands showed massive inflammatory cells infiltrated the interacinar tissuc which lead to induce pressure atrophy the adjacent acini and lactiferous duct which showed in addition of epithelial sloughing. Multiple classical granulomatous lesions were also noticed in different areas of the udder. They were characterized by purulent necrotic center surrounded by a zone of epithelioid cells, mononuclear and langhans, giant cells and outer zone of fibrous connective tissuc (Fig:3) .The skin at the sinus tract area showed cellulitis. The adjacent udder skin was acanthotic and hyperkeratotic (Fig:4). Micreoscopic examination of lung revealed pyogranulomatousmatous lesions identical to those described in the mammary glands.The interalveolar and interlobular septa were thickened due to mononuclcar cells and neutrophils infiltration as well as fibroblast proliferation (Fig:5) .

DISCUSSION

The results showed that among 90 mastitic cows in the AL- dejiala station , 25 (27.77%) were shedding N. asteroides in their milk . such high incidence was also reported by Argente et. al. (12) who found a total number of 227 cows in Briton , 41 casrs of nocardial mastitis . Tarable et. al. , (13) have also reported the existence of N. asteroides in the milk of 22 out of 25 (84%) mastitic cows in Argentina .

The significantly high percentage of nocardial isolates observed in this study indication that nocardiosis is not rare in dairy herds in Iraq . The affect cows showed drop in milk yeild with loss condition . This exentually should lead to culling of affected . On the other hand , the mangment of such cows was costy of continuous treatment with no promising responses . N.asteroides has been identified as a problem of economic significace in certain dairy herds (Blood et. al. , (14) .

The isolation of N.asteroides in pure culture from 5 cases is a strong evidence to be an important independent cause of bovine mastitis . previous reports stated that the isolation of this M.O in pure and mixed from mastitic cows (15) . The existence of mastitis in

outbreak and the occurance of the disease lactating heifers might be attributed to the improper hygienic measures employed in AL- dejiala station . The existence of the M.O . in soil and possible in tick facilitates its transmission and infection (16) . Although the initial infection might occur during the dry peroid where a focus of infection remain clinically unrecognized till parturation as the cow an start to lactate and udder is full with milk , the Nocardial foci probably become disrupted and the M.O. spreads through the lacteferous tree resulting in massive infection . The existence of this organism in milk tank may indicate improper , non hygienic handling of the milk or that might be due to bacteremia . pulmonary lesions might be due to heamatogenous metastasis (17) .

Table . I : Some biochemical and physiological characteristic of N. asteroides isolated

From mastitic cows

1-	Gram stain Reaction	±	6-	Aesculine Hydrolysis	+
2-	Acid fast staining	Partial	7-	Nitrate reduction	+
3-	Growth at 50	±	8-	Urease activity	+
4-	Guinea pig inoculation	Died after 5-6 days	9-	REC hemolysis	–
5-	Fermentation of Mannitole	–	10-	Gelation liquefaction	–
	Sucrose	–	11-	Casein hydrolysis	–
	Fructose	–			
	Glucose	+			

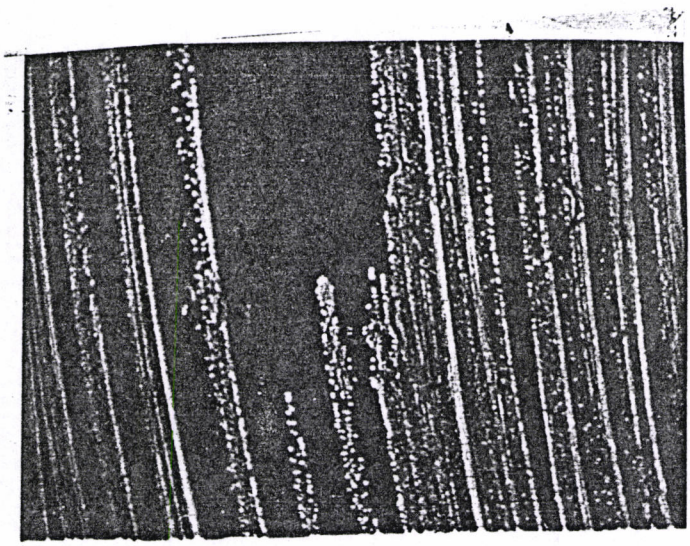

Fig.i : colonies of N. asteroides after 72 hr of incubation on blood agar . Notice opaqu , chalky white , non hemolytic firmly adherent colonies .

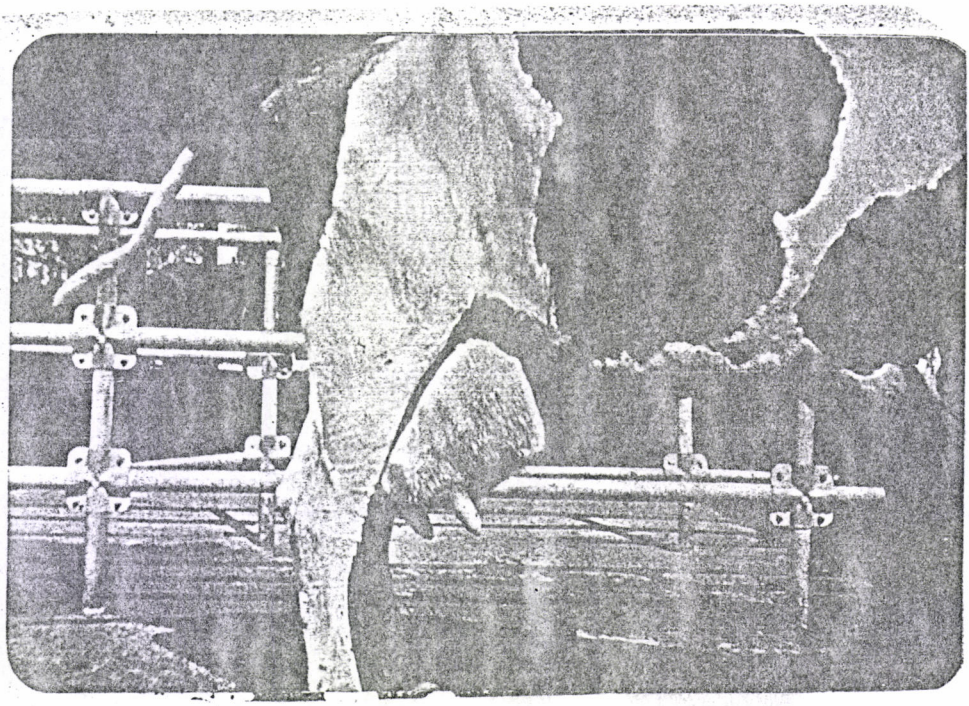

Fig . 2 : udder of mastitic cow , swollen with sinus tract .

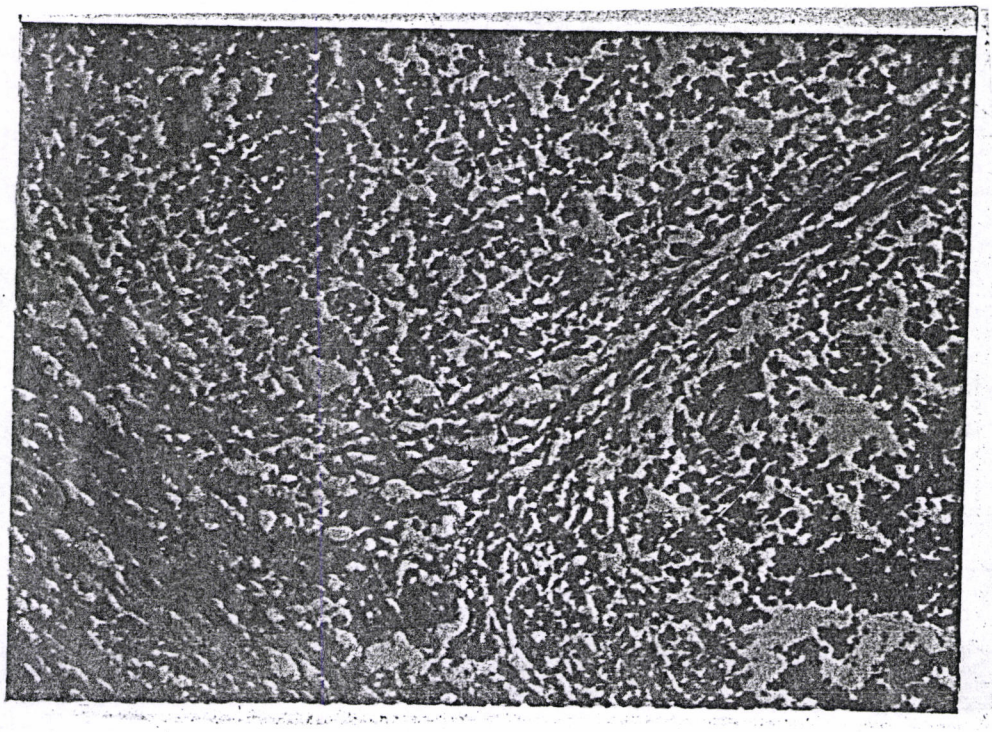

Fig . 3 : Udder of mastitic cow . pyogranulomatous lesion with purulent necrotic surrounded by epitheloid cells , mononuclear cells , giant cells and enveloped by fibtous connective tissues (H.E. IOOX)

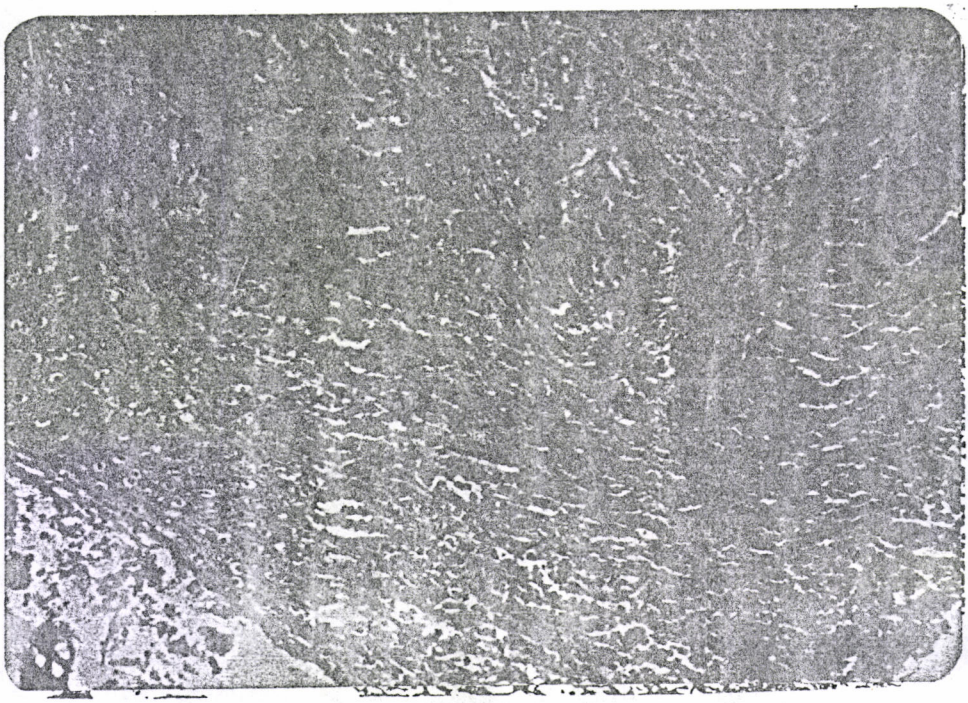

Fig. 4: Skin of mastitic udder. Notice cellulitis adjacent to the sinus tract. (H.E.IOOX) .

Fig . 5: Bovine nocardiosis . Lung showing fibrotic thiokening of the interalveolar septa due to fibrous connective tissue proliferation and mononuclear cells infiltration
(H. & E. IOOX) .

References

1-Maccallum , M.G.(1902) On the life history of actinomyces asteroides zenlralblatt fur bakteriologic and parasiterk unde . 3L: 528-547.

2-Sharma , D.N. and Dwivadi , J.N. (1977) Pulmonary mycesis of sheep and goat in india .Lnd. J.Anim. Sc. 47: 808-815 .

3-Confer , A.W,; Coxttu, K. and ecter, C.C.(1981) Disseminated Nocardiosis in an arabian foal . J.Am. Vet. Med. Ass. 179:40-48 .

4-Awad , F.I. (1960) Some observation on bovine farcy . Vet. Rec. 73:515-516 .

5-Mostafa , I.E. (1967 a) Studies of bovine farcy in the Sudan . Pathology of the disease . J.Com. Path. 77: 223-229.

6-Norton , J.M. (1976) Tuberculosis like granulomas in cattle caused by actinomycetes . Aust. Vet. J, 52: 455-457.

7-Osiyemi , J.J.O.; Okewole, P.M. and Lukden , W.D.(1985) Nocardiosis in a friesian cow . hull. Anim. Hlth. Prod. Afr. 33: 23-24.

8-Argente, G.; Jullo, A.Lemoi, E.H. and Lorant , J.M. (1983) Mammite a nocardia chez la voche enquete et description de la maladie a propose de II troupeaux infectes Le point Veterinarre . 14: 7-12 .

9-Collins, G.H. and Anne, H.C. (1988) Presumptive identification of Nocardia in a clinical laboratory . J.Appl. Baet. 65: 55-59 .

10-Tarable , H.D.; Zurbrigger, M.R.; Canavesio , V.R. and calvunli,L.F. (1993) ocardial mastitis in a small argentinian herd . Vet. Rec. 20: 303-303 .

11-Biood , D.C.; Henderson , J,A, and Radostits , O.M. (1989) Veterinary medicine 15th ed. Bailliere , Tindall and casseli .

12-Shigidi , M.T.A. and Momoun , I.E. (1981) Isolation of N.asteroides from cattle with mastitis in the Sudan . Bull . Hith. Prod. (Africa) . 29: 275-278 .

13-AL- Janabi , B.M.; Brananagar , R. and Panskin , D.(1975) he trans – stadial transmission of the Amblyomnavariegatum Trop . Ainm. H ith. Prod. & : 205-209.

14-Pier, A.C.; Willers , F.H. and Mejia, M.J.(1961) N. 6 asteroides as mammary pathogen of cattle II. The source of N. asteroides in cow experimental reproduction of disease . Am .J. Vet. Rec. 22: 698-703 .

الخلاصة

مرض داء النوكارديا في الأبقار
مسح ميداني ودراسة التغيرات المرضية

محمد جويد علوان
فرع الأمراض والداجن / كلية الطب البيطري
جامعة بغداد – العراق

استهدفت هذه الدراسة بحث إصابة الأبقـــــار بجراثيم Nocardia asteroides ولمعرفة مدى انتشار ووبائية المـرض فـي الأبقـار الحليـب مـن محطة الدجيل من خلال فحص (90) نموذج حليب جمعت من حيوانات مصابـة بالتهاب الضرع ،أوضحت الدراسة وجود داء النوكارديا بشكل وبائي في محطـة أبقار الدجيل حيث عزلت جرثومة N. asteroides من (20) حالـة وشـكلت نسبة الإصابة 77و27 % . لقد تعززت هذه النتائج مـن خـلال ظـهور عـزلات نقية للجرثومة من (5) حيوانـات إضافـة إلـى وجودهـا مـع عـزولات جرثوميـة أخرى من (20) حالة .أظهر الفحص المجهري وجود الورم الحبيبي القيحي في ضرع الحيوانات المصابة بالتهاب الضرع إضافة إلى وجودهـا فـي رئـات نفـس الحيوانات .