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## STUDY ON INFECTIOUS KERATOCONJUNCTIVITIS IN CATTLE AND BUFFALOES

(With 3 Fig. and 4 Tables)

By

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(Recieved at 31/7/1993)

### دراسة عن مرض التهاب القرنية والملتحمة المعدى فى الأبقار والجاموس

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أجريت هذه الدراسة على عدد ١٥ عجل بقرى تعاني من إصابات بالعين بمزرعة كلية الطب البيطرى - جامعة قناة السويس بالإضافة إلى ١٣ جاموسة واردة إلى مستشفى كلية الطب البيطرى - جامعة الزقازيق. أخذت مسحات من العيون المصابة وأسفرت الفحوصات البكتيرية والاختبارات البيوكيميائية أن الميكروب المسبب لهذا المرض هو موركسلا بوفس وبإجراء اختبار الحساسية على هذا الميكروب فى المعمل وجد أنه شديد الحساسية لكل من البنسلين والنايكسك أسيد والكلورمفينيكول والفيورازوليدون وأقل حساسية لكل من اريثروميسين والجنتاميسين والنيوميسين ويعلاج الحيوانات المصابة طبيعياً تبين أن المراهم المحتوية على المضاد الحيوى ومضاد الالتهابات أفضل من المراهم المحتوية على المضاد الحيوى منفرداً وكان لها تأثير كبير فى العلاج مع تحسن ملحوظ بعد ٥ - ١٠ أيام من العلاج ( امتدت إلى ٢١ يوم فى الحالات المتقدمة من المرض).

بالنسبة للحيوانات الخمس الضوابط حدث عى كامل مع انفجار للقرنية فى احدها وبالنسبة لباقي المجموعه فقد شفيت تدريجياً خلال ٣ أسابيع بدون علاج. فى محاولة لاجراء العدوى الصناعيه بتقطير ميكروب M.bovis المعزول من الجاموس فى العين اليمنى وترك العين اليسرى كضابط وجد تدميع مؤقت وتقلص للجفن فى العين المصابة صناعياً فى كل من الأرانب والفئران مع اعاده عزل الميكروب واختفت الأعراض تدريجياً خلال ٣ أيام ولم تظهر أى أعراض اكلينيكية واضحة على مجموعات الجرزان والخنزير الهنديه والدواجن التى تم عدواها تجريبياً.

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### SUMMARY

This study was carried out on naturally infected 15 out of 69 cattle calves at Faculty of Veterinary Med. Suez Canal Univ. and 13 buffalo cases that were admitted to the Vet. Clinic. Fac. of Vet. Med. Zagazig Univ. Cultural and biochemical examinations revealed that, the causative microorganism was *Moraxella bovis*. In vitro sensitivity test showed that the isolated *Moraxella bovis* was highly sensitive to penicillin, nalidixic acid, chloramphenicol, furazolidone, terramycin, gentamycin and neomycin. Field treatment of diseased animals revealed that Ophthalmic ointment containing either oxytetracycline, chloramphenicol, neomycin or gentamycin was highly effective for treatment with complete recovery within 5-10 days post treatment and up to 21 days in case of advanced stage of the disease. Out of 5 non-treated animals one became blind due to ulceration and pulging of the cornea, the other 4 were spontaneously recovered within 3 weeks. Experimental infection in rabbits, guinea-pigs, mice rats and chicken by instillation of *M. bovis* isolate in the conjunctival sac of eyes, resulted in transient lacrimation and blepharospasm in rabbits and mice, while rats, guinea pigs and chicken appeared quite normal without any clinical signs.

### INTRODUCTION

Infectious bovine keratoconjunctivitis (IBK) is commonly called "pink eye". Synonyms for IBK are infectious ophthalmia, infectious keratitis and keratitis solaris. The disease was described first in 1886 in the Netherlands and in 1899 in Nebraska. The history of the disease and its relationship to sunlight were reviewed by HUGHES et al. (1965). It is one of the most costly diseases affecting all ages of stocks particularly calves of up to one year old during their first summer grazing (BLOWEY, 1985).

*Moraxella bovis* has been incriminated as a main aetiological agent by several investigators (JACKSON, 1953, HUGHES et al., 1965; PUGH et al., 1970 and PUGH et al., 1977). Other agents had been associated with this condition as infectious bovine rhinotracheitis (PUGH and HUGHES, 1971) and *Mycoplasma* (GOURLAY et al., 1974). There might be dual



relationship between different organisms to produce the disease (HENSON and GRUMBLES, 1960).

The usual line of therapy for this disease includes antibiotics or chemotherapeutic in the form of powders, ointments or injection in conjunctival sac, suturing the eye closed and eye patches (STOREY et al., 1977).

Therefore the aim of this work was to study some diseased animals suffering from infectious keratoconjunctivitis in two different localities and species as regards secondly, isolation and identification of the causative agent and sensitivity of isolates to different antibacterials was also aimed. Field trails for treatment of infected animals were planned. In addition experimental infection trials of different groups of laboratory animals and chicken was carried out using *M. bovis* isolated from naturally infected buffaloes.

### MATERIAL and METHODS

#### Animals:

Fifteen out of 69 mixed breed cattle, calves with clinical signs of eye affection at Faculty of Vet. Med., Suez Canal University Farm at summer season and 13 individual buffaloes admitted to Clinic of Faculty of Vet. Medicine, Zagazig Univ. were also suffering from the same condition constituted the materials for the study. Case history and clinical examination revealed that the affection of 3 buffaloes was of traumatic nature and these were excluded from this study.

Clinical examination of all animals revealed conjunctivitis and lacrimation with watery or mucopurulent discharge. Two cow calves and five buffaloes showed bilateral infection, the remained animals were unilaterally affected.

#### Samples:

The samples were collected by sterile cotton swabs from the conjunctival sacs and over the cornea of each diseased animal. These swabs were cultured into nutrient broth and incubated at 37°C for 24 hours. Then subcultured on blood agar "5% sheep RBCs" and on MacConkey agar plates. The isolates were identified biochemically according to ELMER et al. (1988).

In vitro sensitivity test of isolates was done against different antibiotics which obtained from bio Merieux\* laboratories using the radial diffusion technique described by BAUER et al. (1966).

\*: bio Merieux: F6920 Charbnnieres Les Bains, France.



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The infected animals were subjected to different means of treatment and examined daily for 3 weeks.

### Experimental infection:

Five of each of rabbits, guinea-pigs, mice, rats and 3 weeks old chicken were used. The experimental infection was done by instillation of 0.1 ml of pure 24 hours old broth culture of *M. bovis* (buffaloe origin). The right eye was used for infection, while the left eye remained as a control. All animals and chicken were inspected daily for 21 days.

### RESULTS

Observed clinical abnormalities were congestion of the blood vessels of eye ball, Fig. (1), oedema of the conjunctiva, and watery or mucopurulent lacrimation. Different degrees of corneal opacity were also seen surrounded with bluish red zone of inflammation Fig. (2). The body temperature was normal in all animals except 3 cow calves showing a slight rise of temperature (39-39.5°C).

Cultural examination and biochemical identification revealed that *M. bovis* was isolated alone from 13 cow calves and 9 buffaloes while mixed infection of *M. bovis* with *Staph aureus* was recorded from 2 calves and one buffalo (Tables 2 and 3). The results of the antibiotic sensitivity test of the isolated strains are given in Table (4).

The effect of treatment measures of the affected animals showed clinical improvement and complete recovery at the 5<sup>th</sup> to the 10<sup>th</sup> days and up to 21 days in advanced cases as shown in Table (1). One calf out of non treated group became blind due to ulceration and pulging of the cornea, the remained 4 calves were spontaneously recovered within 3 weeks.

Experimental infection showed transient lacrimation and blepharospasm in rabbits and mice, while rats, guinea-pigs and chicken appeared quite normal without any clinical signs.

### DISCUSSION

Infectious kerato-conjunctivitis is an extremely painful condition infecting all ages of stock particularly calves of up to one year old during their first summer grazing.

The results of this study revealed that the principle isolated microorganism was *M. bovis* in almost pure culture (86.6% in calves and 90% in buffaloes). Similar results of isolation *M. bovis* from such cases were reported by several workers (BALDWIN, 1945; HUGHES *et al.*, 1965 and BUXTON & FREASER, 1977; AIKMAN *et al.*, 1985; MOAIED *et al.*, 1990;



MOUSTAFA, 1990 and MOUSTAFA et al., 1993). Also BLOOD et al. (1989) reported that *M. bovis* was not usually found in the conjunctival sacs of cattle with no history of pink eye and it had been incriminated as the aetiological agent of the disease and the introduction of pure cultures of *M. bovis* into the conjunctival sacs of cattle would cause mild disease even if the conjunctiva was not injured.

The sensitivity test trials in our work indicated that *M. bovis* was highly sensitive to penicillin, nalidixic acid, chloramphenicol, furazolidone, erythromycin, gentamycin and neomycin with varying degrees. Similar results were reported by BUCHANAN and GIBBONS (1974); WILSON and MILES (1975), BRAUDE et al. (1981), ELMER et al. (1988), BLOOD et al. (1989) and MOAID et al. (1990).

Trials for treatment of infected animals showed that clinical improvement and complete recovery were observed at 5<sup>th</sup> day post treatment in the first group which was treated with Terra-cortril eye ointment containing oxytetracycline Hcl with hydrocortisone acetate. While for the 2<sup>nd</sup> group treated with mephenicol eye ointment, complete recovery occurred 7<sup>th</sup> day post treatment. The 3<sup>rd</sup> and 4<sup>th</sup> groups which were treated with gentamycin or polyspectran ointment respectively were completely recovered 7-10 days post-treatment and up to 21 days in advanced cases (Table 1).

The complete recovery of members of the first group in a short period may be attributed to the anti-inflammatory action of hydrocortisone. Similar results were reported by SARMA et al. (1982) who noticed that a greater effectiveness was achieved when the antibiotic was given in combination with prednisolone. SLATTER et al. (1982) also observed that cortisone in combination with antibiotics would cause a marked improvement in ocular discomfort and more rapid resolution of the condition than when animals were treated with ointments antibiotic alone. Moreover MOUSTAFA et al. (1983) concluded that the use of cortisone with antibiotics would cause a marked decrease in severity of infection and rapid improvement of the condition. Complete recovery of animals in the 2<sup>nd</sup> group treated with mephenicol ointment was supported by SINHA et al. (1979) who concluded that chloramphenicol had been reported most frequently as the drug used for topical treatment of IBK and was regarded as the drug of choice.

It was observed in this study that one calf of non treated group became blind due to ulceration and pulping of the cornea whereas the remaining 4 calves were spontaneously recovered within 3 weeks. Such recovery may be attributed to the effect of tears in dilution and elimination of the bacteria and the



production of antibodies and antibacterial cells which would counteract the infection (BLOWEY, 1985).

In experimental infection, rabbits and mice showed transient lacrimation and blepharospasm. *M. bovis* was isolated from lacrimal secretion within 3-5 days post-infection. These animals were recovered spontaneously after 7 days without medication while guinea pigs, rats and chicken appeared normal without showing clinical signs for up to the 21 days of observation. Guinea-pigs could resist infection with *M. bovis* even after ultraviolet irradiation (CHANDLER *et al.*, 1982).

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Fig. 1: Congestion of blood vessels of eye ball and corneal opacity in buffalo.

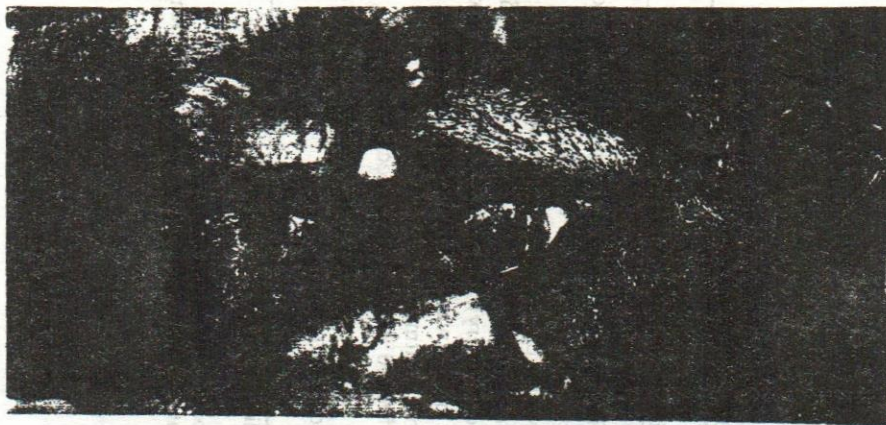


Fig. 2: Oedema of the conjunctiva, corneal opacity and watery lacrimation in buffalo.

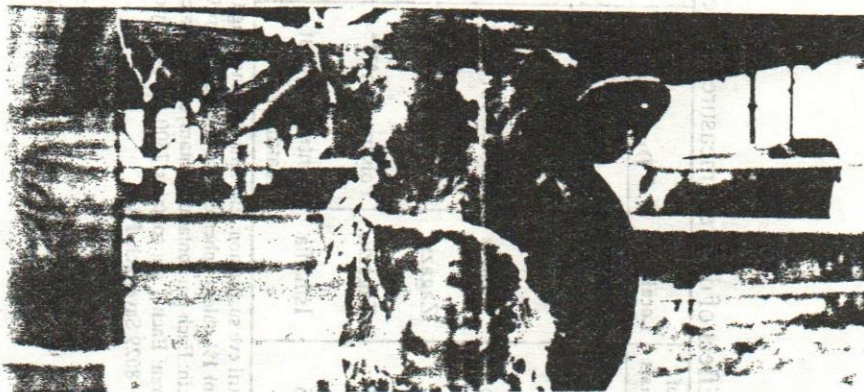


Fig. 3: Ulceration and pulping of the cornea of non treated calf.



Table (1): Effect of different measures of treatment on diseased animals.

Group No.	No. of animals	Locality	Drug used	Methods of application in all groups	Time of recovery
I	5	Ismailia	Terra-cortril suspension.*	The affected eyes were washed with 2% boric acid, then apply ribbon of ointment either to the margin of the eye lid or into the conjunctival sac three times daily for 5 to 7 successive days (complete recovery), the infected animals were kept in a dark place away from direct sunlight during the period of treatment, Each infected animal was injected with 150.000 i.u. Vit. A daily for 3 days. Untreated, "control"	5 days
II	5	Ismailia	Miphenicol oint.		7 days
III	5	Zagazig	Gentamycin oint.		7-10 days
IV	5	Zagazig	Polyspectran****		7-10 days } up to 21 days in advanced cases
V	5	Ismailia	Control without treatment		One calf became blind, the remaining 4 calves were spontaneously recovered within 3 weeks.

\* Terra-cortril eye suspension: Each ml contains 5 mg Oxytetracycline and 5 mg hydrocortisone acetate; (Pfizer).

\*\* Miphenicol 1% chloramphenicol eye ointment (Misr Co.).

\*\*\* Gentamycin: Each gm contains Gentamycin (sulphate) 3 mg (Laboratories Cust Spain).

\*\*\*\* Polyspectran: Each 1 gm contains 7500 i.u. polymyxin B sulphate; 5 mg neomycin sulphate and 0.02 mg gramicidin. (Thilo. Dr. Thi'o & Co. GmbH D. 8029 Saverlach, FRG).



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Table (2): The bacterial isolates from infected eyes of calves and buffaloes.

Species	M.bovis	M.bovis + Staph. aureus
Calves	13	2
Buffaloes	9	1

(With 3 Tables)

Table (3): Different features of the isolated *M. bovis* (Elmer et al., 1988).

Feature or test	Appearance or result
Shape	Coccobacilli
Gram stain	Gram Negative
Blood agar	B- haemolysis
Colony size	1-5 mm
Growth on McConkey agar	Poor growth or negative
Oxidase	Positive
Glucose oxidized	Negative
Urease	Negative
Indole	Negative
Nitrate reduction	Positive
Catalase	Positive
Litmus milk	Alkaline

Table (4): Sensitivity of *M. bovis* strains to different antibacterial agents.

Agent	Sensitivity
Nalidixic acid	+++
Chloramphenicol	+++
Penicillin	+++
Furazolidone	+++
Tetracycline	++
Gentamycin	++
Erythromycin	++
Neomycine	++
Trimethoprim	-
Colestin	