Infectious bovine keratoconjunctivitis in Al-silaite area, Khartoum state


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Abstract: This study was conducted to investigate infectious bovine keratoconjunctivitis (IBK) in cattle in Al-Silat area, Khartoum State. Eight herds of dairy cattle belonging to different owners were surveyed for clinical IBK. The disease was seen in animals which were kept under poor hygienic and management conditions. All infected animals showed copious watery lacrimation, closure of the eyelids, photophobia and blepharospasm, whereas some of the infected animals showed hyperemia and edema of the conjunctiva. Thirty isolates of virulent hemolytic and fimbriated Moraxella bovis were obtained in pure culture on blood agar. The isolates were non-motile, catalase and oxidase positive and hydrolyzed Tween agar. Drug susceptibility testing showed that all of the isolates of M. bovis were highly sensitive to ciprofloxacin, moderately sensitive to chloramphenicol and weakly sensitive to tetracycline. Likewise to the other tested antibiotics. Five calves were successfully treated and recovered after a five-day treatment regimen with ciprofloxacin and topical application of chloramphenicol eye drops. The owners of cattle were advised on the best measures that should be implemented to control IBK and others ocular diseases or at least reduce their prevalence by avoiding predisposing factors.

Keywords: Keratoconjunctivitis, cattle, Moraxella bovis, clinical signs, Sudan

INTRODUCTION

Infectious keratoconjunctivitis (IBK) is a highly contagious disease in cattle [1]. It is characterized by blepharospasm, conjunctivitis, lacrimation, and varying degrees of corneal opacity and ulceration [2]. The gram-negative rod Moraxella bovis is the most not uncommon organism incriminated to cause IBK in cattle and the most frequently isolated. Seven different serogroups of M. bovis are currently recognized [1, 2]. Other causes like M. bovoculi, M. ovis, M. catarrhalis, Neisseria ovis, and Aspergillus flavus were also isolated from IBK in cattle and other ruminants [3, 4, 5, 6, 7].

IBK is the most seen ocular disease of cattle and is cosmopolitan in distribution [1]. It can be seen any time of the year but mostly in summer and autumn. Furthermore, calves are more susceptible to IBK when compared to adult cattle but a considerable number of adults is infected during outbreaks [1, 8]. The disease is not fatal and cases of permanent blindness or loss of an eye are rare. However, in naïve populations, the morbidity rate can be as high as 80% in 3-4 weeks from the beginning of the outbreak. Severe outbreaks can be experienced if the cattle are confined in close quarters such as barns or intensive feedlots [1, 8]. During the early acute phase of IBK, cases usually respond to treatment with ophthalmic ointments and solutions containing antibiotics [1]. These drugs should be installed in the conjunctival sacs at frequent intervals, which may be impractical under bad field conditions. The organism is sensitive to most antibiotics but resistance to erythromycin, lincomycin, and tylosin has been observed [2, 8, 9]. Sulphonamides might be given prophylactically in feed or water [10]. Penicillin G, tetracycline, and novobiocin can also be used. Corticosteroids administered along with antibiotics do not influence resolution of lesions at most. Parenteral therapy with sulfadimidine at the normal dose rate of 100 mg/kg is an effective parenteral treatment [2, 8].

Good management practices are of paramount importance to reduce or prevent spread of infection in cattle. Whenever applied, separation of infected animals is beneficial [2, 8]. Personal hygiene by wearing gloves and protective clothing helps to reduce the disease when affected individuals are being handled. Temporary isolation and preventive treatment of newly introduced animals to the herd may be useful, since some of these animals might be asymptomatic carriers. Ultraviolet radiation from sunlight could probably enhance disease, therefore, affected animals should be provided with shade [9, 10].

IBK has been reported in the Sudan previously by Iman [3], Mossaad et al. [4], Maria et al. [5], El Sanousi et al. [11], El Sanousi et al. [12], and El Sanousi et al. [13]. This study is reporting the clinical picture of some cases of IBK and treatment outcome in Al-Silat area.
MATERIALS AND METHODS

Study area

This study was carried out in Al-Silate area, East Nile (Sharg-Alneel) locality in the north eastern area of Khartoum state; about 20 kilometer from Khartoum town. This area is densely populated with animals of different species especially cattle and it is an important area in the locality. Worth mentioning, the locality covers a total area of about 8,000 square km, equivalent to more than one-third of the size of the state, and consisting of eight local administrative units, namely: East Nile, Hajj Yousef, Wadi Soba, Alaelfoon, Um-Dawan-Ban, Al-Seilat, Wadi Abu Saleh and Abu Dileiq. The area of land utilized for agriculture is about 400 thousand acres. The population inhabiting the locality is about 868,147 people. Most of these people practice farming, small business and government employees.

Study population

Eight herds of dairy cattle belonging to different owners were surveyed for clinical IBK. Animals of these herds were from both sexes (male and female), different breeds (local and cross), and different age groups (young and adults).

Clinical diagnosis

Clinical examination including history and visual and physical examinations were done as described by Kelly [14] and Jackson and Cockcroft [15]. The main complaint according to the owner was eye problems in adult and young animals observed some weeks ago.

Samples collection

Swab samples were collected from the infected eye(s) by gentle streaking of the swab in a horizontal manner through the eye ball and gentle dipping of the swab in the medial canthus of the eye [14].

All of the collected swab samples were immediately and aseptically dipped in a 5 ml sterile nutrient broth transport medium (Oxoid, CM 67), labeled, put in racks, kept cool in an ice box and transported to the Bacteriology Laboratory, College of Veterinary Medicine (CVM), Sudan University of Science and Technology (SUST).

Laboratory diagnosis

Media preparation

The procedures adopted for the preparation of the culture media and media for biochemical tests were according to the standard methods and techniques of Barrow and Feltham [16].

Culturing and sub-culturing

In the laboratory, the inoculated nutrient broth transport media were incubated at 37ºC for 24 hrs. After that, they were each sub-cultured aseptically onto 10% bovine blood enriched agar (Oxoid, CM 55) [17]. The culture plates were labeled and were examined for growth, colony morphology and hemolysis after a 24 hrs incubation at 37ºC. The cultures were purified by sub-culturing of the colonies on a new set of bovine blood enriched agar.

Gram stained smears were prepared from the transport media and colonies on blood agar and examined under the microscope using oil immersion lens.

Biochemical tests

Motility and other biochemical tests including slide catalase and oxidase were conducted according to Barrow and Feltham [16].

The Tween 80 test was conducted by streak inoculation of the test culture on the surface of 10% Tween 80 in sterile nutrient agar. The inoculated plates were incubated at 37ºC and were daily examined.

Drug susceptibility testing

For drug susceptibility testing, a small amount of the identified bacteria from pure cultures of each isolate was taken using a sterile swab and spread all over the culture plates. Drug susceptibility discs were then placed on the inoculated media and incubated at 37ºC for 24 hrs, after which period the plates were examined for inhibition zones around the drugs included in the sensitivity discs. The following drugs were tested: co-trimoxazole (BA) 25 mcg, chloramphenicol (CH) 30 mcg, ciprofloxacin (CP) 5mcg, ceftriaxone (CR) 30 mcg, cephalosporin, tazobactam/piperacillin (TZP) 100/10 mcg, cefotaxime (CF) 30 mcg, and tetracycline (TE) 30 mcg.

Treatment of diseased animals

Fifteen infected animals were treated with ciprofloxacin and topical application of 0.5% chloramphenicol eye drops to be applied three times daily for 5 days.

Results

General observations

The animals were kept under poor hygienic conditions in an open area exposed to wind and dust. They were housed in enclosures made of metal rails and wood. The calves were separated from adults in enclosures made of bushes and dry branches of trees. The animals were provided with minimum shade being exposed to the heat of the sun and direct sunlight (UV light) during most of the day. The floor of the enclosures was not clean with plenty of dung and mud from urine and water flooding from the drinking water troughs. Plenty of Musca domestica and Stomoxys calcitrans flies were seen swarming in the enclosures and around the face of the animals.

Visual and physical examination

Thirty of the surveyed animals, including 19 calves, were suffering from eye affections in one or
both eyes. The affected eye(s) showed copious lacrimation, closure of the eyelids, photophobia and blepharospasm. Some calves showed copious watery discharge from the affected eye and matting the hair on the lateral aspect of the face (Fig. 1). There was severe conjunctivitis and edema resulting in lateral deviation of the eyeball, and white opacity of the cornea.

Many calves showed scleritis, keratitis, and white opacity of the cornea and matting of the eye lashes with copious lacrimation (Fig. 2).

Other calves had mucopurulent ocular discharge, edema of the medial canthus and yellow opacity of the cornea. In many, the cornea became conical in shape surrounded by a hyperemic zone, and showed ulceration involving the upper part of the cornea. *Musca domestica* and *Stomoxys calcitrans* were seen feeding on eye secretions of all animals (Fig. 3). Most of the animals resented examination of the eyes and had depressed appetite because of ocular discomfort or visual disturbance that resulted in inability to locate food.

### Tentative diagnosis

Based on the history and the observed clinical symptoms, the problem was first (tentatively) diagnosed as infectious keratoconjunctivitis.

### Laboratory investigations

Culture of the organism in nutrient broth revealed uniform turbidity in all swab samples. Subculture of the organism onto bovine enriched agar revealed colonies that were surrounded by a clear zone of hemolysis. The colonies were grayish in color, smooth, circular and translucent and corroded the agar (Fig. 4). In addition, some samples produced circular, yellow and convex colonies with β-hemolysis.

Gram stained smears from nutrient broth cultures showed Gram-negative diplobacilli occurring in pairs or end to end in 80% (24/30) of the samples. The remaining six cultures revealed mixed Gram-positive cocci dispersed or in irregular clusters and Gram-negative diplobacilli occurring in pairs or end to end. While smears from the circular, yellow and convex colonies showing β-hemolysis revealed Gram-positive cocci dispersed or in irregular clusters or in characteristic bunches of grapes. The organism was identified in accordance to Barrow and Feltham [16] as *Staphylococcus aureus*, and was completely excluded from this study. Smears from the gray, smooth, circular and translucent colonies showing a clear zone of hemolysis revealed Gram-negative diplobacilli occurring in pairs or end to end (Fig. 5). Pure cultures of this organism were obtained from the 30 samples through subculture and purification of the six samples that showed mixed organisms.

The organism was non-motile, catalase and oxidase positive. Moreover, an opaque halo of precipitation around the growth was evident after three days indicating positive hydrolysis of Tween (Fig. 6).

### Final diagnosis

According to the noted growth characteristics, morphology, staining and biochemical tests the isolated bacteria was identified as *Moraxella bovis* confirming our tentative diagnosis; infectious keratoconjunctivitis.

### Drug susceptibility testing

As shown in Table 1, all of the isolates were highly sensitive to ciprofloxacin, moderately sensitive to chloramphenicol and weakly sensitive to tetracycline. Likewise to the other tested antibiotics.

### Treatment of diseased animals

The 15 infected animals that were treated with ciprofloxacin and chloramphenicol eye drops, perfectly recovered and IBK disappeared.

### Extension messages and advises

The owners of the affected animals were advised on the best measures that should be implemented to control IBK and others ocular diseases or at least reduce their prevalence. Avoiding predisposing factors, improving animals’ enclosures, providing animals with good water and enough feed, salt licks, green roughage and concentrates, removing of the dung and mud resulting from urine and over flooding of water from drinking troughs at regular intervals and replacement of the floor of the enclosure with clean dry sand, providing of enough shade for adult cattle and calves, separation of the animals in groups according to age and stage of production (i.e. calves, heifers, pregnant, lactating and dry) in improved well ventilated enclosures and housing, establishing wind and sand breaks all around the animals’ enclosures; preferably by planting trees, spraying the enclosures with non-toxic fly repellents at regular intervals, inspecting of the animals in the morning, during feeding and in the evening, and separation of sick animals from healthy ones in a separate enclosure, if possible, at a reasonable distance from the rest of the herd and immediately call the veterinarian were highlighted and stressed as important good farming practices.

### Discussion

In Al-Silate area, IBK was encountered among different age groups of cattle but was most prevalent among calves. The disease was characterized by copious lacrimation, closure of the eyelids, photophobia and blepharospasm. The conjunctivae were hyperemic and edematous and the cornea showed white opacity. The ocular discharge was purulent in some animals and the affected eye(s) showed scleritis, keratitis, corneal ulceration and yellow opacity of the cornea. These clinical findings agreed with the findings of many workers [2, 8, 9, 18].

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Fig. 1: A calf with severe conjunctivitis and edema resulting in lateral deviation of the eyeball, and corneal opacity

Fig. 2: A calf with scleritis, keratitis, white opacity of the cornea and matting of the eye lashes with copious lacrimation

Fig. 3: A calf with edematous medial canthus and yellowish opacity of the cornea. *Musca domestica* and *Stomoxys calcitrans* flies feeding on eye excretions
Fig. 4: Bovine blood enriched agar showing the translucent colonies and hemolysis zone.

Fig. 5: Gram-negative diplobacilli occurring in pairs or end to end.

Fig. 6: Culture of *Moraxella bovis* on Tween medium, showing positive hydrolysis of Tween.
The bacterium isolated from the infected eyes of 30 animals was non-motile, catalase and oxidase positive, and hydrolyzed Tween. According to its growth characteristics, morphology, staining and biochemical tests it was identified as *M. bovis* – the etiological agent of IBK. In the current investigation; the criteria on the basis of which the bacterium was isolated and identified as *M. bovis* were exactly similar to the criteria on the basis of which this organism was identified by previous workers [9, 16, 18]. The organism is an opportunistic pathogen and its virulence is influenced by both host and environmental factors [2, 19, 20, 21, 22]. All 30 isolates were highly virulent as recognized by the clear zone of hemolysis produced on the blood agar and the capability of corroding the agar on being fimbriated. These findings authenticated the findings of many workers [18, 19, 20, 23, 24] who reported that virulent strains of *M. bovis* are fimbriated, hemolytic and possessed an array of toxins and enzymes. Webber and Selby [19], Brown et al. [20], Davidson and Stokka [23], and Hess and Angelos [24], added that *M. bovis* adhered to the cells via its fimbriae and pili proteins, produced β-hemolysin toxins which lysed the corneal epithelial cells, and secreted cytotoxic toxin and pathogenic fibrinolysin, phosphatase, hyaluronidase, and aminopeptidases. In the Sudan, *M. bovis* has previously been isolated from infected eyes of humans and cattle [3, 5].

In the current investigation, the severe clinical picture observed in all affected animals caused by a virulent strain of *M. bovis* was not surprising because all factors which exacerbate or predispose to outbreaks of IBK were encountered in all farms in Al-Silate area. The animals were kept under poor hygienic conditions in an open area exposed to wind and dust. The enclosures were made of metal rails and wood, while calves enclosures were made of bushes and dry branches of trees. However, these enclosures provided minimum shade to the animals and were exposed to the heat of the sun and direct sunlight (UV light) during most of the day time. The floor of the enclosures was not clean with plenty of dung and mud from urine and water flooding from the drinking water troughs. Plenty of *Musca domestica* and *Stomoxys calcitrans* flies were seen swarming in the enclosures and around the faces of the animals feeding on ocular secretions of infected animals. These findings agreed with the reports of previous workers [2, 8, 19, 20, 21, 22, 23, 24] who enumerated many of the above mentioned factors which exacerbate or predispose to outbreaks of IBK and further listed the following: age where young cattle less than 2 years of age are particularly more susceptible to the infection, and breed with *Bos Taurus* breeds appear to be more susceptible than *Bos indicus* breeds. Moreover, fly activity; flies can act as vectors of *M. bovis*, ocular irritants like dust, tall grasses, grass seeds, wind, ultraviolet light, concurrent infections such as infection with bovine herpesvirus 1 or thelazia species, and nutritional deficiencies including vitamin A, and trace minerals (selenium and copper).

Contradicting reports were encountered in the available literature with regards to topical and parenteral therapy, and the sensitivity of *M. bovis* to antibiotics and sulfonamides [2, 8, 19, 20]. In the current pilot study conducted on drug sensitivity, all isolates of *M. bovis* were highly sensitive to ciprofloxacin, moderately sensitive to chloramphenicol and weakly sensitive to tetracycline. However, all isolates showed variation in sensitivity from high to moderate for the remaining drugs, but the majority of the isolates were weakly sensitive to co-trimoxazole. In spite of the fact that many of the drugs advocated by previous investigators were not tested in the current research, yet the results obtained, coincided with some and differed with some of their reports. For these reasons five infected calves were successfully treated and recovered after five-day treatment with ciprofloxacin and topical application of chloramphenicol eye drops. All isolates were found highly and moderately sensitive to ciprofloxacin and chloramphenicol, this was why they were used in the treatment trials. Chloramphenicol is a well-known broad spectrum antibiotic with specific therapeutic activity against gram-positive and gram-negative bacteria, rickettsiae, chlamydiae and anaplasmiae. It is used for the treatment of a wide variety of eye infections such as bacterial conjunctivitis, mucopurulent conjunctivitis, keratitis, trachoma, and as a prophylactic use after eye operations or eye trauma.

The owners of cattle were advised on the best measures that should be implemented for controlling the

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**Table 1: Drug susceptibility status of the isolates recovered from cases of IBK in Al-Silate area**

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<th>Antibiotics</th>
<th>Inhibition Zone</th>
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<td>13</td>
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<td>TE</td>
<td>0</td>
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i = no. of highly sensitive (+++), ii = no. of moderately sensitive (++), iii = no. of weakly sensitive (+) and iv = no. of resistant

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disease (ocular and others) or reducing the occurrence of these diseases in their herds by avoiding the risk factors which exacerbate or predispose to outbreaks of many diseases especially those involved in outbreaks of infectious bovine keratoconjunctivitis.

Conclusions and recommendations

It was concluded from this study that IBK is prevalent in Al-Silate area, Khartoum state. Variable degrees of severity of the disease were recorded and it was encountered among different age groups of cattle but was mostly in calves. *M. bovis* was isolated from all infected animals. Poor hygienic and management conditions generated many risk factors which exacerbated or predisposed the animals to IBK. Successful treatment and recovery from the disease was obtained after five-day treatment with parenteral ciprofloxacin and topical application of chloramphenicol eye drops. An in-depth and area-wide investigation of this disease is warranted alongside extension programs on good farming practices.

References

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