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Infectious bovine keratoconjunctivitis in Al-silaite area, Khartoum state

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Abstract: Infectious bovine keratoconjunctivitis (IBK) was prevalent in Al-Silate area, Khartoum State. The disease was encountered in 30 animals kept under poor hygienic and management conditions. All infected animals showed copious watery lacrimation, closure of the eyelids, photophobia and blepharospasm and some animals showed hyperemia and edema of the conjunctiva. Thirty isolates of virulent hemolytic and fimbiriated 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 30 isolates of M. bovis were highly sensitive to Ciprofloxacin, moderately sensitive to Chloramphenicol and weakly sensitive to Tetracycline. For the rest antibiotics, varied susceptibilities ranging from high, moderate to weak. 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: kerato conjunctivitis, cattle, Moraxella bovis, clinical signs, Sudan

INTRODUCTION

Infectious keratoconjunctivitis (IBK) is a highly contagious disease of cattle. It is characterized by blepharospasm, conjunctivitis, lacrimation, and varying degrees of corneal opacity and ulceration cattle[1]. The gram-negative rod Moraxella bovis is the only organism reported to cause IBK in cattle. Seven different sero groups of M. bovis are currently recognized[1].

IBK is the most common ocular disease of cattle and is cosmopolitan in distribution. The disease occurs in most countries of the world and, although it can occur in all seasons, it is more prevalent in summer and autumn. Young claves being most susceptible, but in a susceptible population, cattle of all ages are likely to be affected[2]. The disease is not fatal, and cases in which there is permanent blindness or loss of an eye are rare. However, the morbidity rate can be as high as 80%, with the peak infection rate at weeks 3-4 of the outbreak. Severe outbreaks can be experienced in winter, especially if the cattle are confined in close quarters such as barns or intensive feedlots [1, 2]. Early, acute cases respond to treatment with ophthalmic ointments and solutions containing antibiotics but they need to be instilled in the conjunctival sacs at frequent intervals, which may be impractical under bad field conditions. The organism is sensitive to most antibiotics and sulfonamides but is resistant to erythromycin, lincomycin, and tylosin[3]. Radostits *et al.* [1], Kahn *et al.* [2] and Biberstein and Hirsh[4] reported that sulphonamides might be given prophilactically in feed or water. Penicillin G, tetracycline, erythromycin and novobiocincan also are used. Corticosteroids administered along with antibiotics do not influence resolution of lesions. Parenteral therapy with sulfadimidine at the normal dose rate of 100 mg/kg BW is an effective parenteral treatment[1, 2].

Good management practices are of paramount importance to reduce or prevent spread of infection in cattle. Separation of infected animals is beneficial when possible [1, 2]. Gloves and protective clothing should be worn and then disinfected between animals when affected individuals are being handled. Temporary isolation and preventive treatment of animals newly introduced to the herd may be helpful, because some of these animals may be asymptomatic carriers. Ultraviolet radiation from sunlight may enhance disease (particularly in cattle) therefore, affected animals should be provided with shade[3, 4]. This study is reporting the clinical picture of some cases of IBK and treatment outcome in Al-Silaite area.

MATERIALS AND METHODS

Study area

The study was conducted in Al-Silate area, East of Khartoum State; about 20 kilometers from Khartoum town. Al-Silate is densely populated with animals of different species especially cattle.

Clinical investigations

Eight herds of dairy cattle belonging to different owners were surveyed including a total of 30 cattle composed of 11 lactating cows and 19 calves of both sexes.

The main complaint according to the owner was eye problems in adult and young animals observed some weeks ago. After carrying out a thorough clinical examination according to Jackson and Cockcroft (2002), swab samples were collected the infected eyes.

Collection of samples

Thirty swab samples were collected from the infected eyes by gentle streaking of the swab in a horizontal manner through the eye ball and gentle dipping of the swab in the medial can thus of the eye.

Bacteriological Examination

The procedures adopted for the preparation of culture media and media for biochemical tests were according to the standard methods and techniques of Barrow and Feltham [5]. All of the collected swab samples were immediately and aseptically dipped in a 5 ml sterile nutrient broth transport medium(Oxoid, CM 67), then 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).

In the laboratory, the inoculated tubes were incubated at 37 ˚C for 24 hr. After that, the nutrient broth cultures of the 30 samples were each subcultered aseptically on the 10 % bovine blood enriched agar (Oxoid, CM 55). The culture plates were labeled and incubated at 37 ˚C for 24 hr; after which period the plates were examined for growth, colony morphology and hemolysis. Smears were made from the transport media and colonies on blood agar on clean, defatted and sterile microscope slides. The slides were labeled and left to dry at room temperature, Gram stained and examined under the microscope using oil immersion lens. The cultures were purified by subculture of the colonies on a new set of bovine blood enriched agar. The plates were labeled and again incubated at 37 ˚C for 24 hr.

The 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 labeled and incubated at 37 ˚C. The culture plates were daily examined. An opaque halo of precipitation around the growth was evident after three days indicating positive hydrolysis of Tween. Motility and biochemical tests including slide catalase and oxidase were conducted.

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, and the plates were labeled. Drug susceptibility discs were then placed on the inoculated media and incubated at 37 ˚C 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 used: Co-Trimoxazole (BA) 25mcg, Chloramphenicol (CH) 30 mcg, Ciprofloxacin (CP) 5mcg, Ceftriaxone (CR) 30 mcg, a third generation cephalosporin antibiotic, Tazobactam/piperacillin (TZP) 100/10 mcg, Cefotaxime (CF) 30mcg, 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.

RESULTS

Survey

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 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 domestic and Stomoxy scalcitrans flies were seen swarming in the enclosures and around the face of the animals.

The surveyed animals especially 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 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.

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

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

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

Other calves had mucopurulent ocular discharge, edema of the medial can thus and yellow opacity of the cornea. In many calves the cornea became conical in shape surrounded by a hyperemic zone, and showed ulceration involving the upper part of the cornea. Musca domestica and Stomoxy scalcitrans 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 results in inability to locate food.

Fig. 3: A calf with edematous medial can thus and yellowish opacity of the cornea. Musca domestica and Stomoxy scalcitrans flies feeding on eye excretions

Tentative diagnosis

Based on the history and the observed clinical symptoms, the disease was tentatively diagnosed as infectious keratoconjunctivitis.

Laboratory investigations

Culture of the organism in nutrient broth revealed uniform turbidity in all 30 swab samples. Subculture of the organism on bovine enriched agar revealed 3-4 mm in diameter colonies 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 2-4 mm in diameter, β-hemolytic, circular, yellow and convex colonies.

Fig.4: Bovine blood enriched agar showing the translucent colonies and hemolysis zone.

Gram stained smears from the nutrient broth culture showed Gram-negative diplobacilli occurring in pairs or end to end in 24 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 [5] 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.

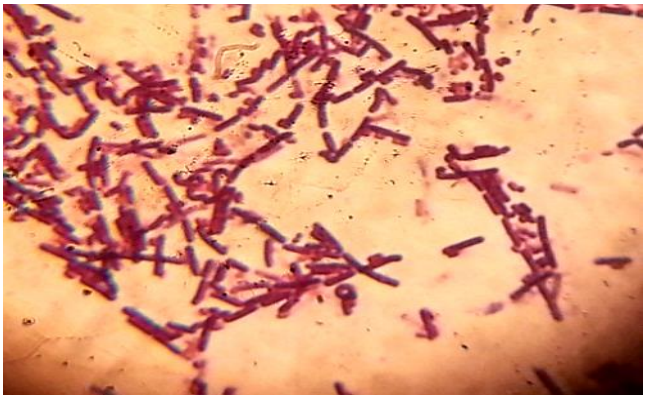

Fig.-5: Gram-negative diplobacilli occurring in pairs or end to end.

The organism was non-motile, catalase and oxidase positive, and hydrolyzed Tween (Fig. 6).

Fig.6: Culture of Moraxella bovis on Tween medium, showing positive hydrolysis of Tween.

As shown in table 1, all of the isolates were highly sensitive to Ciprofloxacin, moderately sensitive to Chloramphenicol and weakly sensitive to Tetracycline. For the rest antibiotics, varied susceptibilities ranging from high, moderate to weak.

Table 1: Drug susceptibility testing of the isolates recovered from cases of infectious bovine keratoconjunctivitis in Al-Silate area

Antibiotics	Number of Isolates ( ) and Inhibition Zone		
	+++	++	+
BA	(--)	(11)	(19)
CH	(--)	(30)	(--)
CP	(30)	(--)	(--)
CR	(11)	(19)	(--)
TZP	(20)	(10)	(--)
CF	(13)	(9)	(8)
TE	(--)	(--)	(30)

+++ = Highly sensitive, ++ =moderately sensitive, and + =weakly sensitive

Final diagnosis

According to the noted growth characteristics, morphology, staining and biochemical tests the isolated bacteria were identified as Moraxella bovis.

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.

Advises for control and prevention

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. The given advises were improving the enclosures of their animals and provide the animals with good water and feed troughs, providing 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 enclosure floor 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 preferably at a reasonable distance from the rest of the herd and immediately call the veterinarian.

DISCUSSION

The current study is probably the first report of infectious bovine keratoconjunctivitis in the Sudan. In Al-Salite area the disease 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/eyes showed scleritis, keratitis, corneal ulceration and yellow opacity of the cornea. These clinical findings agreed with the findings of many workers[2-4]. 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 infectious bovine kerato conjunctivitis. 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[5, 4,3]. The organism is an opportunistic pathogen whose virulence is influenced by both host and environmental factors[6- 9]. All 30 isolates were highly virulent as recognized by the clear zone of hemolysis produced on the blood agar and capability of corroding the agar on being fabricated. These findings authenticated the findings of many workers who reported that virulent strains of Moraxella bovis are fimbriated and hemolytic and possessed an array of toxins and enzymes[5-9]. Some worker added that M. bovis adhered to the cells via its fimbriae and pili proteins, produced β-haemolysin toxins which lysed the corneal epithelial cells, and secreted cytotoxic toxin and pathogenic fibrinolysin, phosphatase, hyaluronidase, and amino peptidases[10-11].

In the current research, 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 infectious bovine keratoconjunctivitis were encountered in all farms in Al-Silate area in which: 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 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 domestic and Stomoxy scalcitrans flies were seen swarming in the enclosures and around the face of the animals feeding on ocular secretions of infected animals. These findings agree with the reports of previous workers [1-8] who enumerated many factors which exacerbate or predispose to outbreaks of infectious bovine keratoconjunctivitis such as: age; young cattle less than 2 years of age are particularly susceptible to infection, breed; Bos Taurus breeds appear to be more susceptible than Bosindicus breeds, fly activity; Flies can act as vectors of M. bovis, ocular irritants; dust, tall grasses, grass seeds, wind, ultraviolet light, concurrent infections; Infection with bovine herpes virus 1 or thelazia species, vitamin deficiency; Vitamin A, and trace mineral deficiencies; 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 [1,2,6]. In the current research the pilot study conducted on drug sensitivity showed that all 30 isolates of Moraxella 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 Moraxella bovis isolates [12] 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 agree and disagree with their reports. For these reasons fifteen infected calves were successfully treated and recovered after five-day treatment with Ciprofloxacin and topical application of chloramphenicol eye drops. This was because in the current investigation, all isolates were found highly sensitive to Ciprofloxacin, and chloramphenicol was used because all 30 isolates were moderately sensitive to this antibiotic. Chloramphenicol is a well-known broad spectrum antibiotic with specific therapeutic activity against gram-positive and gram-negative bacteria, rickettsiae, chlamydia and anaplasmae. 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 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. Needless mention that all the objectives proposed for conducting this research work were satisfactorily and successfully achieved.

CONCLUSIONS

It is concluded from this research work that infectious bovine kertoconjunctivitis is quite prevalent in Al-Silate area and probably in other areas in Khartoum State, and the remaining States of the Sudan. Variable degrees of severity of the disease were recorded. The disease was encountered among different age groups of cattle but was most prevalent among calves. It is caused by a very virulent strain of M. bovis. Poor hygienic and management conditions generate many risk factors which exacerbate or predispose to outbreaks of infectious bovine keratoconjunctivitis. Successful treatment and recovery from the disease was obtained after five-day treatment with parenteral Ciprofloxacin and topical application of chloramphenicol eye drops.

Recommendations

Infectious keratoconjunctivitis is a highly contagious disease of cattle. It is characterized by blepharospasm, conjunctivitis, lacrimation, and varying degrees of corneal opacity and ulceration. The disease causes serious economic losses because of depressed appetite resulting from ocular discomfort or visual disturbance those results in inability to locate food, and in severe cases the cornea becomes conical in shape, may rupture and permanent blindness can occur. For all these reasons an extensive future research is warranted

on infectious bovine keratoconjunctivitis targeting at the following goals:

1. Conduct extensive surveys at the National level, in the different States of the Sudan to determine the magnitude and prevalence of this devastating disease.
2. The Central Veterinary Laboratories in the different States should collaborate in this program for the isolation of the causative agent –M. bovis. They should coordinate with each other for the media, biochemical tests and drug sensitivity discs that are to be used for the purpose of obtaining uniform data.
3. Research workers should conduct extensive treatment trials (parenteral and topical with and without steroid therapy) based on the uniform data compiled from the central laboratories in the different States. The uniform results obtained for the successful treatment of the disease should be widely circulated to all Veterinary Units. The Ministry of Animal Resources and Fisheries should contribute effectively by making available the specific drugs for a reasonable price.
4. At this point extensive extension programs should be conducted for cattle owners about the danger and losses resulting from the disease and the necessity for improving food, housing and management of their cattle. Moreover, cattle owners should receive practical demonstration on the ideal methods for keeping their herds under excellent hygienic and management conditions.
5. Through extension programs cattle owners are made aware that raising their animals under excellent hygienic and management conditions will drastically reduce or prevent risk factors that may exacerbate or predispose to outbreaks of infectious bovine keratoconjunctivitis and other disease.

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