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1. ABSTRACT

Infectious Bovine Keratoconjunctivitis in Al-Silate Area

Abstract

Infectious bovine keratoconjunctivitis was prevalent in Al-Silate area, Khartoum State. The disease was encountered in farms kept under poor hygienic and management conditions. The disease was encountered in 30 animals of different age groups but was most prevalent among calves. Infectious bovine keratoconjunctivitis was characterized by copious watery lacrimation, closure of the eyelids, photophobia and blepharospasm. Some animals showed hyperemia and edema of the conjunctiva. Others showed mucopurulent ocular discharge, in addition to white or yellow opacity of the cornea, scleritis, keratitis and corneal ulceration.

Thirty isolates of virulent hemolytic and fimbriated *Moraxella bovis* were obtained in pure culture on bovine blood enriched agar. The isolates were non-motile, catalase and oxidase positive and hydrolysed Tween agar.

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 (19) were weakly sensitive to Co-Trimoxazole.

Five calves were successfully treated and recovered after five-day treatment with Ciprofloxacin and topical application of chloramphenicol eye drops.

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.

Key words : *Moraxella bovis* , Isolation and identification, Drug sensitivity, controlling the disease.

ملخص البحث

التهاب القرنية والملتحمة المُعدي في الأبقار

التهاب القرنية والملتحمة المُعدي في الأبقار منتشر في منطقة السلييت بولاية الخرطوم في المزارع التي تفتقر لكافة المعايير الصحية و الإدارية. تم الحصول على 30 حالة مرضية في مختلف الأعمار ولكن كانت نسبة الإصابة عالية بين العجول. تميز التهاب القرنية والملتحمة المعدي بادماع مائي غزير، وإغلاق جفون العين، و رُهابُ الضوء، وتشنج الجفون. بعض الحيوانات ظهر عليها إحمرار ووذم في الملتحمة. كما ظهر على حيوانات اخرى انسياب افراز مخاطي قيحي من العيون. وظهر على العديد من الحيوانات عتامة بيضاء أو صفراء في قرنية العين، والتهاب صلبة العين والقرنية وتقرحات في قرنية العين.

تم عزل بكتيريا الموركسيلة البقرية وكانت من النوع الضار المُحمل من جميع الحالات المصابة في المستنبتات التي تحتوي على دم الأبقار. تميزت الموركسيلة المعزولة بانها ساكنة وغير متحركة، وكانت موجبة الكاتالاز والاكسيداز والتحليل المائي لمستنبت اجار التوين.

تم إجراء اختبار حساسية بكتيريا الموركسيلة البقرية للمضادات الحيوية المختلفة حيث تبين ان جميعها شديد الحساسية لمضاد السبروفلوكسسين، ومتوسط الحساسية لمضاد الكلورامفينيكول، وضعيف الحساسية لمضاد التتراسايكلين. كما تبين أن جميع بكتيريا الموركسيلة البقرية قد أظهرت حساسيات متفاوتة في بقية المضادات تراوحت بين الشديدة والمتوسطة. وكانت أغلبها (19) ضعيفة الحساسية لمضاد الكوترايمكسزول.

تم علاج خمسة عجول وشفيت تماماً بعد مضي خمسة أيام من العلاج بمضاد السبروفلوكسسين عن طريق -----و موضعياً باستعمال قطرة مضاد الكلورامفينيكول.

تم تقديم النصح والإرشاد لاصحاب الماشية لاتباع الطرق المثلى للسيطرة على أمراض العيون وغيرها من الأمراض أو تقليل نسبة إنتشارها وذلك بتفادي عوامل الخطر التي

تزيد من حدة المرض أو تهيئ لانتشار الأمراض وعلى وجه الخصوص عوامل الخطر التي تهيئ لانتشار مرض التهاب القرنية والملتحمة المعدية في الأبقار.

2. INTRODUCTION AND LITREATURE REVIEW

Definition:

Infectious keratoconjunctivitis is a highly contagious disease of cattle. It is characterized by blepharospasm, conjunctivitis, lacrimation, and varying degrees of corneal opacity and ulceration.

Etiology:

The gram-negative rod *Moraxella bovis* is the only organism reported to cause IBK in cattle. Seven different serogroups of *M. bovis* are currently recognized.

Epidemiology:

Occurrence:

Infectious bovine keratoconjunctivitis (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 calves being most susceptible, but in a susceptible population, cattle of all ages are likely to be affected (Radostits, Gay, Hinchcliff and Constable, 2007; Kahn, Line, Aiello, Allen, Anderson, Jeffcott, Queensberry, Radostits, Reeves and Wolf, 2011). 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.

Source of infection:

Cattle are the reservoir of infection, and the organism is carried on the conjunctiva, nares and vagina. Persistence of the disease from year to year is by means of infected animals, which can act as carriers for periods exceeding 1 year.

Transmission:

The disease reaches epizootic proportions (summer and autumn) when flies and dust are abundant and grass is long. The disease is thought to be transmitted by these agents contaminated by the ocular and nasal discharges of infected cattle. Under experimental conditions, transmission is unusual in the absence of the face fly (*Musca autumnalis*) and the Asian fly (*Musca bezzii*) because of the feeding preference of these flies for the area around the eyes being important vectors of *Moraxella bovis*. It is worth of mention that *Musca autumnalis* remains infected for periods of up to 3 days, and *M. bovis* can be isolated from the crops of this fly after feeding on the eyes and lacrimal secretions of infected cattle.

Other ocular infections of cattle:

Most other ocular infections of cattle are characterized by conjunctivitis with minimal or no keratitis. The primary differential diagnosis of infectious bovine rhinotracheitis (IBR) is the severe conjunctivitis and corneal edema originating near the limbus; but corneal ulceration is uncommon. *Mycoplasma* spp. may cause conjunctivitis of cattle, either alone or in conjunction with *M. bovis*.

Infection with IBR virus or other microbes may increase the severity of infection with *M. bovis*. Another *Moraxella* species that has been frequently isolated from eyes of cattle with IBK is *Moraxella bovoculi*. However, recent research does not support the role for *Moraxella bovoculi* as a primary pathogen in causing the corneal ulceration associated with IBK (Radostits *et al.*; 2007; Kahn *et al.*, 2011).

Moraxella bovoculi has also been reported in cases of infectious keratoconjunctivitis in reindeer.

Risk factors:

Plant awns, face flies, ultraviolet radiation from bright sunlight, dry and dusty environmental conditions, and shipping stress are all risk factors associated with IBK in cattle. It is likely that *Moraxella bovoculi* may act as a risk factor for IBK by causing conjunctivitis similar to other risk factors for infectious agents such as *Mycoplasma* spp. and IBR virus. Additional risk factors that should be considered when making herd management decisions include trace mineral deficiencies such as selenium and copper (Webber and Selby, 1981; Radostits *et al.*, 2007). *Moraxella bovis* was found in association of *Demodex ghanensis* infecting the meibomian glands of 8012 cattle Abu-Samra and Shuaib, 2014).

Moraxella bovis:

Moraxella bovis is a Gram-negative rod that causes infectious bovine keratoconjunctivitis, a devastating ocular disease of cattle which occurs

worldwide (Brown, Brightman, Fenwick, and Rider, 1998). The organism is an opportunistic pathogen whose virulence is influenced by both host and environmental factors. The virulence of *M. bovis* is attributed to fimbriae, which allow adherence of the organisms to the cornea, and during replication, haemolysin and other lytic enzymes were produced playing a significant role in virulence (Webber and Selby, 1981; Brown *et al*, 1998; Quinn, Markey, Carter, Donnelly, and Leonard, 2002; Davidson and Stokka, 2003; Hess and Angelos, 2006; Radostits *et al*, 2007).

Pathogenesis:

The pathogenesis of *Moraxella bovis* was described by many workers (Webber and Selby, 1981; Brown *et al*, 1998; Davidson and Stokka, 2003, Hess and Angelos, 2006) who reported that it 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 aminopeptidases. Receptors for I-pili may be found on tissues other than the cornea and facilitate colonization of non corneal tissue and in apparent infections. The initial production of the corneal ulceration is due to the direct cytotoxic activity of the organism. This is followed by focal loss of corneal epithelium, degeneration of keratocytes, and invasion of the corneal stroma with fibrillar destruction. An inflammatory reaction occurs several days post infection and results in enlargement of the corneal ulcers with deeper stromal involvement, corneal edema, and corneal vascularization.

The lesions are localized in the eye and there is no systemic infection. Abu-Samra and Shuaib (2014 b) concluded that *Moraxella bovis* possessed an array of virulence factors (toxins and enzymes) causing severe meibomian gland deterioration and damage facilitating the establishment of *Demodex ghanensis* mites in the lesions produced and provided an excellent microclimate for the mites to propagate and reproduce, resulting in a severe and progressive disease as observed in natural field cases. Furthermore, the “highturnover” granulomatous reaction which characterized the histopathological changes proved that *Demodex* mites and associated *Moraxella bovis* are both persistent and immunogenic.

Lesions:

Infectious kertoconjunctivitis lesions vary in severity. In cattle, one or more small ulcers typically develop near the center of the cornea. Initially, the cornea around the ulcer is clear, but within a few hours a faint haze appears that subsequently increases in opacity. Lesions may regress in the early stages or may continue to progress. After 48–72 hr in severe cases, the entire cornea may become opaque, blinding the animal in that eye. Blood vessels may invade the cornea from the limbus and move toward the ulcer at ~1 mm/day. Corneal opacity may result from edema (hazy white to blue corneas), which is a part of the inflammatory process, or leukocyte infiltration (milky white to yellow corneas), which indicates severe infection. Continued

active ulceration may cause corneal rupture. Relapse may occur at any stage of recovery.

Clinical Findings:

The disease usually is acute and tends to spread rapidly. In all species early signs are accompanied by a copious watery lacrimation. One or both eyes may be affected. The earliest clinical signs are photophobia, blepharospasm, and epiphora; later, the ocular discharge may become mucopurulent. Conjunctivitis, with or without varying degrees of keratitis, is usually present. The color of the opacity varies from white to deep yellow. As the acute inflammation subsides, the ocular discharge becomes purulent and the opacity begins to shrink, complete recovery occurring after a total course of 3-5 weeks. The appetite may be depressed because of ocular discomfort or visual disturbance that results in inability to locate food. The usual clinical course varies from a few days to several weeks. Most corneal ulcers in cattle with IBK heal without loss of vision. However, in severe cases the cornea becomes conical in shape, may rupture and permanent blindness can occur in the most severe cases.

Diagnosis:

In all species, presumptive diagnosis is based on ocular signs and concurrent systemic disease. It is important to distinguish that the lesions are not due to foreign bodies or parasites. In IBR, upper respiratory signs and conjunctivitis predominate, while keratitis accompanied by ulceration is rare. In bovine

malignant catarrhal fever, respiratory signs are prominent with primary uveitis and associated keratitis (Radostits *et al*, 2007; Kahn *et al*, 2011). Microbial culture may be beneficial in confirming the causative organisms. *Chlamydia* and *Mycoplasma* spp. require special media; the diagnostic laboratory should be consulted before sample collection. Cytological evaluation of stained slides prepared from conjunctiva scrapings of cattle may reveal *Chlamydia* organisms; however, intracytoplasmic inclusion bodies can be difficult to recognize. PCR analysis can be used to detect *Chlamydia* and *Mycoplasma* spp. (Radostits *et al*, 2007; Kahn *et al*, 2011).

Differential diagnosis:

Traumatic conjunctivitis is usually easily differentiated because of the presence of foreign matter in the eye or evidence of a physical injury. *Pasteurella multocida* (capsular type A) has been isolated from the eyes of housed heifers that experienced outbreaks of severe keratitis with severe loss of corneal stroma within 72 hours. *Mycoplasma bovis* has been isolated from the eyes of steers with an outbreak of severe conjunctivitis with corneal opacity and ulceration. Conjunctivitis is prominent in other mycoplasmal infections that produce keratoconjunctivitis. *Listeria monocytogenes* iritis. Infectious bovine rhinotracheitis. Bovine malignant catarrh. Thelaziasis Chlamydial keratoconjunctivitis presents with identical clinical findings but has a protracted course despite treatment and a higher morbidity (Radostits *et al*,

2007; Kahn *et al*, 2011). Chlamydiophila DNA can be detected by PCR in conjunctiva swabs.

Clinical pathology:

The organism can be identified by culture or fluorescent antibody. The hemolytic form of the bacterium is noticeably more pathogenic than the non-hemolytic form. Serum agglutinins (1:80 to 1:640) are present 2-3 weeks after clinical signs commence, and modified gel diffusion precipitin test is capable of detecting *M. bovis* antibodies (Radostits *et al*, 2007; Kahn *et al*, 2011). An ELISA test is also used for antibody detection in experimental studies; however, neither agglutinating antibody nor antibody detected by ELISA correlates well with individual animal resistance to infection. There is little indication for serological examinations in clinical practice. Necropsy examinations are not usually necessary.

Treatment:

Topical therapy:

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 field conditions. The organism is sensitive to most antibiotics and sulfonamides but is resistant to erythromycin, lincomycin, and tylosin (Quinn *et al*, 2002; Radostits *et al*, 2007; Kahn *et al*, 2011).

Parenteral therapy:

Biberstein and Hirsh (1999) reported that sulphonamides (sulfamethazine, sulfadimethoxine, sulfaquinoxaline) may be given prophylactically in feed or water. Penicillin G, tetracycline, erythromycin and novobiocin are used therapeutically. Corticosteroids administered along with antibiotics (penicillin G) 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 (Radostits *et al*, 2007; Kahn *et al*, 2011).

Prevention:

Infectious bovine kertoconjunctivitis (IBK) is frequently a self-limiting disease although early treatment will reduce the incidence of scarring of the eyes. Good management practices are of paramount importance to reduce or prevent spread of infection in cattle. Separation of infected animals is beneficial when possible (Radostits *et al*, 2007; Kahn *et al*, 2011). 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 (Biberstein and Hirsh, 1999; Quinn *et al*, 2002). Dust bags or insecticide impregnated ear tags can be used to reduce

the number of face flies (*Musca autumnalis*), an important vector for *M. bovis*. *Moraxella bovis* bacterins are available and can be administered before the beginning of the fly season. Cattle should be started on *M. bovis* vaccine series 6–8 weeks before the anticipated first cases of IBK to allow time for adequate immune responses to develop. The efficacy of current commercially available *M. bovis* bacterins is controversial and likely varies because of vaccinal versus outbreak strains of *M. bovis* and varying degrees of cross-protection afforded by vaccination. Biberstein and Hirsh (1999) reported that vaccination with killed or live avirulent bacteria of appropriate serotypes is beneficial. Vaccination may reduce the severity and duration of infection in affected animals (Davidson and Stokka, 2003). IBR may predispose cattle to infection with *M. bovis*; thus, vaccination of herds against IBR may reduce outbreaks of *M. bovis*. The use of modified live IBR vaccines has been associated with outbreaks of IBK in cattle; IBR vaccination must be appropriately timed with cattle shipments so that these events do not coincide. Vaccination of cattle with a modified live IBR vaccine could likely exacerbate an outbreak of IBK associated with *M. bovis* and/or *Moraxella bovoculi* because of increased ocular and nasal secretions spreading bacteria between herd mates as well as corneal epithelial damage.

3. OBJECTIVES OF THE STUDY

The objectives of this study were as follows:

- a) Conduct a survey to investigate the occurrence and prevalence of infectious bovine keratoconjunctivitis in Al-Silate area.**
- b) Study the clinical picture and epidemiology of the disease.**
- c) Isolation and identification of the causative gent (*Moraxella bovis*).**
- d) Study *in vitro* drug sensitivity of the organism.**
- e) Conduct pilot attempts to treatment some of the infected animals.**
- f) Put forward suggestions and recommendations for future research for treatment, prevention and control of this highly contagious and devastating disease of cattle at the National level.**

4. MATERIALS AND METHODS

4.1. Area of the Study:

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

4.2. Survey:

Eight herds of dairy cattle belonging to different owners were surveyed. A total of 30 cattle composed of 13 lactating cows and 17 calves of both sexes. The animals were kept under poor hygienic conditions in an open area exposed to wind and dust. They are housed in enclosures made of metal rails and wood. The calves are 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 domestica* and *Stomoxys calcitrans* flies were seen swarming in the enclosures and around the face of the animals.

Thirty animals especially calves showed eye affection of one or both eyes. The affected eye/eyes 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. 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 (Fig. 3). In many calves the cornea became conical in shape surrounded by a hyperemic zone, and showed ulceration involving the upper part of the cornea (Fig. 3). *Musca domestica* and *Stomoxys calcitrans* were seen feeding on eye secretions of all animals. Most of the animals resented examination of the eyes. They had depressed appetite because of ocular discomfort or visual disturbance that results in inability to locate food.

The disease was tentatively diagnosed as infectious keratoconjunctivitis subject to laboratory confirmation by isolation of the causative agent (*Moraxella bovis*).



Figure 1: A calf with severe infectious keratoconjunctivitis in Al-Silate area. Note: Severe conjunctivitis and edema resulting in lateral deviation of the eyeball, corneal opacity, copious lacrimation matting the hair on the lateral aspect of the face, and *Musca domestica* and *Stomoxys calcitrans* flies feeding on eye secretions.



Figure 2: A calf with severe infectious keratoconjunctivitis in Al-Silate area. Note: Scleritis, keratitis, and white opacity of the cornea and matting of the eye lashes with copious lacrimation.



Figure 3: A calf with severe infectious keratoconjunctivitis in Al-Silate area. Note: Mucopurulent ocular discharge, edema of the medial canthus, yellow opacity of the cornea, ulceration involving the upper part of the cornea, and *Musca domestica* and *Stomoxys calcitrans* flies feeding on eye excretions.

4.3. Collection of Samples:

Thirty swab samples were collected from the infected eyes by two methods (Fig. 4):

- 1) Gentle streaking of the swab in a horizontal manner through the eye ball.**
- 2) Gentle dipping of the swab in the medial canthus of the eye.**

4.4. 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 (1993).



Figure 4: Collection of samples from the infected eyes of calves with eye infection in Al-Silate area.

4.4.1. Preparation and Inoculation of Nutrient Broth (Transport Medium):

Sterile nutrient broth transport medium was prepared by dissolving 13 grams of nutrient broth powder (Oxoid, CM 67) in 1 Liter of distilled water in a sterile flask and autoclaved at 121°C for 15 minute. After cooling 5 ml of the nutrient broth was transferred aseptically in sterile tubes. Each of the collected swabs was aseptically dipped in the nutrient broth, and the tubes were labeled. The tubes were put in racks, kept cool in an ice box and transported to the Bacteriology Laboratory, Faculty of Veterinary Medicine, the Sudan University of Science and Technology. In the laboratory the inoculated tubes were incubated at 37 °C for 24 hr.

4.4.2. Preparation of Smears from the Nutrient Broth Cultures:

- 1) A drop of each of the 24 hr cultures of the organism in nutrient broth was aseptically transferred onto the middle of clean, defatted and sterile microscope slides using sterile Pasteur pipettes and smears were made.**
- 2) The slides were labeled and left to dry at room temperature, Gram stained and examined under the microscope using oil immersion lens.**

4.4.3. Preparation and Culture of the Organism on Blood Agar:

Blood agar was prepared by dissolving 37 grams of blood agar base (Oxoid, CM 55) in 1 Liter of distilled water in sterile media bottles and autoclaved at 121 °C for 15 minute. The media were cooled to 50 °C in a water bath and 100 ml of sterile bovine blood was added. The blood agar so prepared was then dispensed aseptically (15 ml) in each of sterile disposable plates using a Bunsen burner flame to ensure sterility of the media surface. The media in the plates was left to set at laboratory temperature and stored in the refrigerator at 4-8 °C for 24 hr.

The nutrient broth cultures of the 30 samples were each subcultured aseptically on the 10 % bovine blood enriched agar. 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.

4.4.4. Preparation of Smears from the Blood Agar Cultures:

Smears were made from 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. 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.

4. 4. 5. Preparation of Tween 80 Medium:

Hydrolysis of Tween media is a special identification medium used for the identification of *Moraxella bovis*. The medium is prepared as follows: 10% Tween 80 was made by adding 10ml Tween to 90 ml distilled water, and sterilized at 121 °C for 10min. If the Tween separates the flask is rotated to ensure mixing. Then 10 % of Tween 80 was mixed with an equal volume of 30% H₂O₂. 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.

4.4.6. Motility Test:

A drop of sterile normal saline is placed in the middle of a microscope slide coverslip using a sterile Pasteur pipette. A small piece of a pure colony from each isolate was aseptically mixed in the drop of saline. A thick film of Vaseline was smeared all around the borders of the coverslip; a clean microscope slide was put carefully on the cover slip (Fig. 5), and the organisms were examined for motility under X 100 objective lens.



Figure 5: Motility testing of the bacterial growth.

4.4.7. Biochemical Tests:

4.4.7.1. Slide Catalase Test:

The test is performed by placing a small amount of the bacterial growth of each isolate in the middle of a clean defatted microscope slide. One drop of 3 % hydrogen peroxide (H_2O_2) was added using a Pasteur pipette to the culture under test. Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. The release of a bubble of oxygen within a few seconds is indicative that the tested organism is catalase positive (Catalase +ve bacteria) because it produces the enzyme catalase.

4.4.7.2. Oxidase Test:

Oxidase is a class of enzymes that catalyze the reduction of molecular oxygen independently of hydrogen peroxide. Oxidase test is used to identify bacteria that contain cytochrome oxidase (*Moraxella*). The test was performed by soaking a piece of filter paper with a few drops of oxidase reagent (10g/L

solution of tetra methyl-p phenylenediaminedihydrochloride). A colony of the bacteria to be tested was put on the soaked filter paper. A positive reaction will result in oxidizing the phenylenediaimine in the reagent changing its color to a deep purple (blue purple) within few seconds. If the test was negative there will be no reaction.

4.4.8. *In Vitro* Drug Sensitivity:

A pilot study was conducted on *in vitro* drug sensitivity. A small amount of bacteria from pure cultures of each isolate was taken using a sterile swab and spreaded all over the culture plates, and the plates were labeled. Drug sensitivity discs were then placed on the inoculated media (Fig. 6) and incubated at 37 °C for 24 hr, after which period the plates were examined for inhibition zones around the drugs included in the sensitivity discs (Table 1). The following drugs were used:

- 1) BA: Co-Trimoxazole 25mcg, antibiotic preparation made of a mixture of trimethoprium and sulphamethoxazole.
- 2) CH: Chloramphenicol 30 mcg, a broad spectrum antibiotic with specific therapeutic activity against gram-positive and gram-negative bacteria, rickettsiae, chlamydiae 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.

- 3) CP: Ciprofloxacin 5mcg, a fluoroquinolone antibiotic with particularly good activity against gram-negative bacteria including enterobacteriaceae and *Pseudomonas aeruginosa*. It is used mainly in urinary tract infection.
- 4) CR: Ceftriaxone 30 mcg, a third generation cephalosporin antibiotic.
- 5) TZP:Tazobactam/piperacillin 100/10 mcg, penicillanic acid sulfone derivative and an extended-spectrum semisynthetic penicillin active against a wide range of gram-negative, gram-positive and anaerobic bacteria.
- 6) CF: Cefotaxime 30mcg, a second generation cephalosporin antibiotic.
- 7) TE: Tetracycline 30 mcg, an antibiotic effective against many different microorganisms, including rickettsiae, viruses and both gram-negative and gram-positive organisms.

The inhibition zones were rated as follows:

- +++: Highly sensitive.
- ++: Moderately sensitive.
- +: Weakly sensitive.



Figure 6: Drug sensitivity disc on culture plates inoculated with *Moraxella bovis*.

4.5. Trials on Treatment:

Five infected animals were treated with Ciprofloxacin and topical application of 0.5 % chloramphenicol eye drops to be applied three times daily.

4.6. Prevention and Control:

The owners of cattle were advised on the best measures that should be implemented in controlling the disease or reducing its occurrence in their herds.

4.7. Statistical Analysis:

The data were analyzed using SPSS software (Statistical Package for the Social Sciences, version 16.0, SSPS Inc., Chicago, IL, USA). All bacterial counts were analyzed by ANOVA. Statistical significance was set at a *P-value* of ≥ 0.05 .

5. RESULTS

5.1. Nutrient Broth Culture:

Culture of the organism in nutrient broth revealed uniform turbidity in all 30 swab samples.

5.2. Smears from the Nutrient Broth Cultures:

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.

5.3. Subculture of the Organism on Blood Agar:

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. 7). The remaining six samples produced in addition, 2-4 mm in diameter, β -hemolytic, circular, yellow and convex colonies.



Figure 7: Bovine blood enriched agar showing 3-4 mm in diameter, grey, smooth, circular and translucent colonies surrounded by a clear zone of hemolysis.

5.4. Smears from the Blood Agar Subcultures:

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 (1993) 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. 8). Pure cultures of this organism were obtained from the 30 samples through subculture and purification of the six samples that showed mixed organisms.

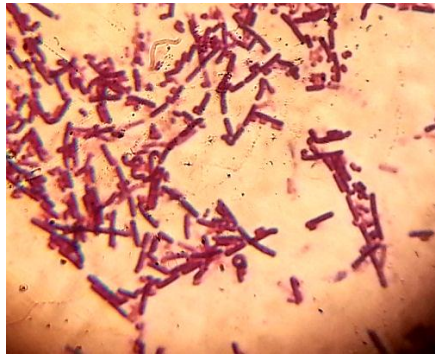


Figure 8: Gram-negative diplobacilli occurring in pairs or end to end.

5.5. Identification of *Moraxella bovis*.

The organism was non-motile, catalase and oxidase positive, and hydrolyzed Tween (Fig. 9). According to its growth characteristics, morphology, staining and biochemical tests it was identified as *Moraxella bovis* – the etiological agent of infectious bovine keratoconjunctivitis in accordance to Barrow and Feltham (1993); Biberstein and Hirsh (1999); Quinn, Markey, Carter, Donnelly and Leonard (2002).



Figure 9: Culture of *Moraxella bovis* on Tween medium, showing positive hydrolysis of Tween.

5.6. *In Vitro* Drug Sensitivity:

As shown in table 1 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 (19) were weakly sensitive to Co-Trimoxazole.

Table 1: Drug sensitivity testing of 30 isolates of *Moraxella bovis* isolated from cases of infectious bovine keratoconjunctivitis in Al-Silate area

Drug Symbol	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)

5.7. Prevention and Control:

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:

- 1) Improving the enclosures of their animals and provide the animals with good water and feed troughs.**
- 2) Provide their herds with salt licks, green roughage and concentrates.**
- 3) Removal of 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.**
- 4) Provision of enough shade for adult cattle and calves.**
- 5) 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.**
- 6) Establish wind and sand breaks all around the animals' enclosures; preferably by planting trees.**
- 7) Spray the enclosures with non-toxic fly repellents at regular intervals.**
- 8) Inspection of the animals in the morning, during feeding and in the evening.**
- 9) 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.**

6. DISCUSSION

The current study is probably first reports 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 agree with the findings of many workers (Biberstein and Hirsh, 1999; Quinn, Markey, Carter, Donnelly, and Leonard, 2002; Radostits *et al*, 2007; Kahn *et al*, 2011). 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 *Moraxella bovis* – the etiological agent of infectious bovine keratoconjunctivitis. The criteria on the basis of which the bacterium isolated in the current study was identified as *Moraxella bovis* were exactly similar to the criteria on the basis of which this organism was identified by previous workers (Barrow and Feltham, 1993; Biberstein and Hirsh, 1999; Quinn *et al.*, 2002). The organism is an opportunistic pathogen whose virulence is influenced by both host and environmental factors (Webber and Selby, 1981; Brown *et al.*, 1998; Radostits *et al*, 2007; Abu-Samra and Shuaib, 2014 a). 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 fimbriated. These findings authenticated the findings of many workers (Webber and Selby, 1981; Brown *et al.*, 1998; Biberstein and Hirsh, 1999; Quinn *et al.* 2002; Davidson and Stokka, 2003, Hess and Angelos, 2006; Abu-Samra and Shuaib, 2014 a) who reported that virulent strains of *Moraxella bovis* are fimbriated and hemolytic and possessed an array of toxins and enzymes. Webber and Selby (1981) Brown *et al.* (1998) Davidson and Stokka (2003) Hess and Angelos (2006), added that *Moraxella 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 aminopeptidases.

In the current research, the severe clinical picture observed in all affected animals caused by a virulent strain of *Moraxella 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 domestica* and *Stomoxys calcitrans* 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 (Webber and Selby, 1981; Brown *et al*, 1998; Biberstein and Hirsh, 1999; Quinn, *et al.*, 2002; Davidson and Stokka, 2003; Hess and Angelos, 2006; Radostits *et al*, 2007; Kahn *et al*. 2011; Abu-Samra and Shuaib, 2014 a, b) who enumerated many factors which exacerbate or predispose to outbreaks of infectious bovine keratoconjunctivitis such as:

- 1) Age: Young cattle less than 2 years of age are particularly susceptible to infection.
- 2) Breed: *Bos Taurus* breeds appear to be more susceptible than *Bos indicus* breeds.
- 3) Fly activity: Flies can act as vectors of *Moraxella bovis*.
- 4) Ocular irritants: Dust, tall grasses, grass seeds, wind, ultraviolet light.
- 5) Concurrent infections: Infection with bovine herpesvirus 1 or thelazia species.
- 6) Vitamin deficiency: Vitamin A.
- 7) 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 *Moraxella bovis* to antibiotics and sulfonamides (Biberstein and Hirsh,1999; Quinn *et al.*,

2002; Radostits *et al*, 2007; Kahn *et al*, 2011). 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 (19) 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 five 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 this antibiotic. Chloramphenicol is a well known broad spectrum antibiotic with specific therapeutic activity against gram-positive and gram-negative bacteria, rickettsiae, chlamydiae 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.

7. CONCLUSIONS

It is concluded from this research work that infectious bovine keratoconjunctivitis 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 *Moraxella 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.

8. 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 that 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 - *Moraxella 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 the enlightenment of cattle owners about 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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