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Clinical study on Moraxella-associated infectious keratoconjunctivitis (IKC) of small ruminants

Ahmed M. A. Zaitoun[1], Mohamed G.A. Elseleny[2], and Khaled A.S. El-Khabaz[1*]

[1]*Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Assiut university, Egypt.*

[2] *Assiut Directorate of Veterinary Medicine*

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ABSTRACT

Infectious keratoconjunctivitis (IKC) represents an important health problem in semi-desert and desert areas. The present study tries to investigate the possible role of *Moraxella* as a causative agent of IKC. A total number of 140 sterile conjunctival swabs from the diseased animals (93 sheep and 47 goats) of different ages showed signs of IKC were subsequently collected, bacteriologically examined to isolate *Moraxella spp,* which was successively isolated from 19 animals (11 sheep and 8 goats) with infection rate about 13.6%. In relation to age, a higher infection rate (19.2%) was recorded among sheep in the age group ≥ 12 to < 24 months and among goats in the age group ≥ 3 to < 6 months. Antimicrobial sensitivity test of obtained isolates revealed that *Moraxella* was 100% sensitive to Ciprofloxacin, Gentamicin, and Tylosin, and 79% to Oxytetracycline. All tested isolates were resistant to Vancomycin, Ampicillin plus Sulbactam, and Sulfamethoxazole plus Trimethoprim. Therapeutically, two therapeutic regimens were carried out to treat the diseased cases under field conditions and the results were illustrated in detail.

1. INTRODUCTION

Infectious keratoconjunctivitis (IKC) of small ruminants is an outstanding problem causing a considerable level of economic losses in sheep and goats flocks. Clinically the disease can be recognized by progressive cloudiness of eye, severe congestion of ocular mucous membrane with inflammation of cornea, ocular discharges vary from serous to purulent and temporary or permanent blindness (Baker et al., 2001 and Abdullah et al., 2014). There are several pathogens were incriminated as etiologic agent responsible for IKC. However, Moraxella with and/or without other pathogens was implicated as a major pathogen causes IKC in small and large ruminants (Abdullah et al., 2015; Gelormini, et al., 2017 and Zheng et al., 2019). Moreover, frequent isolation of different microorganisms from ruminants with signs of IKC may attributed to the synergistic action between environmental risk factors and opportunistic pathogens habitat eye (Sağlam etal., 2018). However, *Moraxella spp* was isolated as single pathogen from outbreaks of IKC in sheep and goats flocks (Karthik et al., 2017 and Athira et al., 2018).

Etiologically, *Moraxella* are Gram negative diplococci, non-motile and catalase and oxidase positive, easily transmitted by contact with nasal and ocular discharges and by flies as mechanical transmitter (Ojo et al., 2009 and Athira et al., 2018). *Moraxella ovis* was found to produce one or more heat-labile exotoxins causing both hemolysis for bovine RBCs and cytotoxic activities against corneal tissues (Cerny et al., 2006). *Moraxella spp* has the ability to form biofilm but in vivo, this character reduced by the action of lysozyme naturally present in tears (Ely et al., 2018). Different prevalence rates of *Moraxella* associated infectious keratoconjunctivitis have been observed by several authors (Van Halderen et al., 1994; Naglić et al., 2000 and Karthik et al., 2017). There are different outcomes of various therapeutic trials used for IKC. Variations in results of antimicrobials susceptibility were noticed between *Moraxella spp* isolates of different regions. this may refer to differences in genotyping characters of isolates (Loy and Brodersen, 2014). Cure rate of 100% can be achieved by using various types of antibiotics systemically by injection along with topical antibiotic eye preparations (Pandey, 2018 and Sağlam et al., 2018). Role of *Moraxella spp* in sheep and goats with signs of IKC is aimed. Epidemiological notes of IKC of both sheep and goats was monitored and antibiogram followed by field-therapeutic trials of the diseased cases were also carried-out.

2. MATERIAL AND METHODS

2.1. Study area:

The study was carried out on El-Ghaniem, Assiut governorate - Egypt. This city located next to Assiut's Western Mountain with hot weather and high humidity during summer months. In winter, there no rains and the climatic temperature is warm at day and cool at night. Topographically, El-Ghanien is semi-desert and desert land. Sheep and goats located in desert area of El-Ghaniem rather than the semi-desert areas.

* Corresponding author: Khaled A.S. El-Khabaz, Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Assiut university, Egypt.

2.2. Animals and sampling:

During the period of investigation (2019 – 2020), a total number of 93 sheep and 47 goats (140 animals) of different ages were clinically examined according to (Jackson and Cockcroft, 2002) for the presence of signs of IKC. Aseptically, conjunctival swab was taken from each diseased case. The swabs were immediately inoculated in sterile tubes containing Trypticase Soy broth (Titan media – India) and transported in ice tank to the bacteriological laboratory unit, Infectious Diseases, Faculty of Veterinary Medicine, Assiut University, with minimum of delays.

2.3 Bacteriological examination:

Tubes containing broth were incubated at 37 ◦C for 24 h. A bacteriological loopful from each tube was streaked onto 5% blood agar (Lab M limited-UK) plate and incubated at 37 ◦C for 24 - 48 h with daily examination for any colony growth. The suspected colonies were picked-up, recultured and thereafter subjected to biochemical identification based on criteria illustrated by Baron et al., (1994) and Quinn et al., (1994). These criteria include Gram staining followed by indole, oxidase, motility, sugar-fermentation, Simmon's citrate and Catalase tests. Morphologically, target colonies were being small (1mm), friable, grayish-white and surrounded with narrow zone of complete hemolysis. Biochemically, *Moraxella spp* were being positive for oxidase and catalase tests while being negative for Gram staining, indole, motility, Simmon's citrate and fermentation tests.

2.4. Antibiogram and therapeutic trails:

On Mueller-Hinton agar (Accumedia-India), all Moraxella isolates were tested for their antimicrobials sensitivity according to Bauer et al., (1966) using commercial antimicrobial discs (Mast Diagnostics - U.K.). Ampicillin plus Sulbactam 20 µg, Vancomycin 30 µg, Ciprofloxacin 5 µg, Gentamicin 10 µg, Oxytetracycline 30 µg, Sulfamethoxazole plus Trimethoprim 25 µg and Tylosin 30 µg were used.

Based on the results of sensitivity tests, two therapeutic regimens were used, the first regimen include: injection of long acting oxytetracycline I.M. (Alamycin L.A®) by 1 ml / 10 kg every 48 h, I.M injection of diclofenac sodium (Dicloprima®) 1 ml/25 kg (in feverish cases), I.M injection of AD3E (Devedry Med®) 1 ml/25 kg/12h for 3 successive days and after eye washing with Boric acid lotion 2%, topical eye ointment (Terramycin®) was applied 3 times daily for 3 successive days. The second therapeutic regimen include: I.M injection of Tylosin plus Gentamicin (Tylogent reforzado®) 1ml/ 25 kg /12 h, I.M injection of Flunixin meglumine (Finadyne®) 1ml/25 kg (for feverish cases), I.M injection of AD3E (Devedry Med®) 1 ml/25 kg/12h for 3 successive days and after eye washing with Boric acid lotion 2%, topical eye drop containing Tobramycin + Dexamethasone (Dexatobrin®) was applied 3 times daily for 3 successive days.

3. RESULTS

3.1. Clinical findings:

Of the examined animals, 19 (13.6 %) cases showed dramatic signs of conjunctivitis, keratitis with different degrees of corneal opacities either unilateral (6/19, 31.6 %) or bilateral (13/19, 68.4 %). The ocular discharge was mucopurulent and obviously blepharospasm (Fig 1). Feverish condition (10/19, 52.6 %), tachycardia and tachypnea, nasal discharge, corneal ulcers, restlessness, separation from the flock and reduction in feed intake were clinically monitored in some diseased animals.

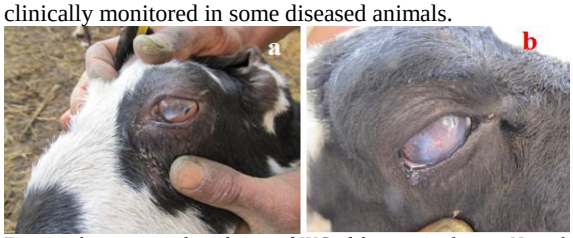

Figure 1 characteristic clinical signs of IKC of the examined cases. Note: the moderate degree of opacity in Left case (a) in contrast the right case (b) shows severe cloudiness corneal opacity.

3.2. Bacteriological and Antimicrobial sensitivity results:

The current bacteriological examinations and biochemical identification revealed that *Moraxella* species were successively isolated from 19 out of 140 clinically diseased cases with signs of IKC. All tested *Moraxella* strains were 100% sensitive to Ciprofloxacin, Gentamicin and Tylosin. the susceptibility to Oxytetracycline was 78.9% (15/19). The tested strains were resistant to Ampicillin plus Sulbactam, Vancomycin and Sulfamethoxazole plus Trimethoprim.

3.3. Therapeutic trail results:

The recovery rate for these *Moraxella spp* infected animals (n=19) with first therapeutic regimens was about 74% (14 out of 19), the 5 non-responsive cases when subjected to second therapeutic regimens, the cure recovery rate was 100%.

3.4. Epidemiological investigation:

Out of 140 tested animals with signs of IKC, *Moraxella spp* were isolated from 19 animals (11 sheep and 8 goats) with a prevalence rate about 13.6%. The prevalence rate of *Moraxella* infection among sheep was 11.8% (11 out of 93) while among goats 17% (8 out of 47). The relationship between the prevalence of *Moraxella* associated IKC and age was illustrated in table 1.

Table 1 Infection rate of Moraxella-positive cases of the examined sheep and goats in relation to age of examined animals.

Age groups per month	Sheep			Goats		
	No.	+ ve *Moraxella*		No.	+ve *Moraxella*	
		No.	%		No.	%
< 3	9	0	0.0	5	0	0.0
≥ 3 - < 6	23	1	4.4	7	2	28.6
≥ 6 - < 12	30	5	16.7	16	4	25
≥ 12 - < 24	26	5	19.2	16	2	12.5
≥ 24	5	0	0.0	3	0	0.0
Total	93	11	11.8	47	8	17

4. DISCUSSION

Sheep and goats occupied the second grade following buffaloes and cattle in Egypt as a source of Meat and economic incomes. However, in semi-desert and desert areas of Assiut Governorate like El-Ghaniem, the population density of sheep and goat are more than large ruminant. Currently, IKC representing a considerable health problem for sheep and goats flocks (Hidson and Winter, 2008). The observed clinical findings strongly refer to IKC. Similar clinical findings were reported previously (Ojo et al., 2009 and Karthik et al., 2017).

Although different microorganisms were encountered as causative agents of IKC in small ruminant, *Moraxella* and *Mycoplasma* appear to be the most important one (Åkerstedt

and Hofshagen, 2004). In the present study Moraxella was successively isolated from 19 cases of 140 examined animals (13.6 %). the low isolation rate of Moraxella may give an indication that IKC is a multi-complexes and Moraxella is not the only pathogen responsible for IKC in small ruminant. IKC caused by different microorganisms and the presence of risk factors is crucial (Van Halderen et al., 1994; Jansen et al., 2006; Åkerstedt and Hofshagen, 2004 and Pandey, 2018).

In the current study the prevalence of *Moraxella spp* among the diseased sheep and goats was 11.8% and 17% respectively which come in agree with Jansen et al., (2006) who reported prevalence of *Moraxella* among bighorn sheep and domesticated goats about 29%, and 18.8%, respectively. Also, Akerstedt and Hofshagen (2004) recorded a prevalence rate about 14% (6/43) among IKC diseased Norwegian sheep. *Moraxella ovis* was isolated from 85.7% of affected sheep with IKC (Sağlam, et al., 2018). Moraxella was isolated from 100% of diseased goats (n=8) (Ojo et al., 2009). Variation in prevalence rates may be attributed to differences in the number of examined animals and distinction in managemental and environmental conditions in different localities where the studies were conducted.

Epidemiologically, from geographical point of view, the study area located next to the western mountain increasing the chance for corneal dryness and corneal abrasion caused by aero-sandy particles carried by wind, as well as exposure to high percentage of ultraviolet rays which may predispose for *Moraxella* colonization. Different environmental predisposing factors are greatly needed for *Moraxella* to precipitate their characteristic clinical signs, furthermore, the presence of corneal hurt by mechanical factors or ultraviolet irradiation is important for occurrence of the disease (Dubay et al., 2000). However, *Moraxella* was isolated as a single pathogen from goats with characteristic signs of IKC (Athira et al., 2018).

In relation to age, the infection rate of Moraxella associated IKC was high in sheep in the age group ≥12 to <24 months while in goats in the age group ≥3 to <6 months. Zero prevalence was recorded in sheep and goats aged less than 3 months and more than 24 months. This may be due to the small number of studied animals in these age groups or due to a build-up immunological status as maternal immunity in young and developed acquired immunity in aged cases. Variation in the prevalence rate in relation to age was noticed by (Naglić et al., 2000), the authors could isolate Moraxella from 44% in the age group less than 1 year, 33% from sheep aged 1-3 years and 22% from those more than 3 years.

Therapeutically, the results of the first regimens of therapeutic trail that depends on usage of oxytetracycline systemically and topically come in accordance with the results of antimicrobial sensitivity test as the sensitivity of *Moraxella* to Oxytetracycline was 78.9%. The susceptibility rate of Moraxella of small and large ruminants to oxytetracycline ranged from 80% to 91% and the haphazard use of oxytetracycline in veterinary practice contributed to the emergence of resistant strains (Maboni et al., 2015). Using of oxytetracycline topically as eye ointment and systemically by injection gives better results with a rare relapse rate (Naglić et al., 2000 and Pandey, 2018). On the other side, the 5 non-responsive cases when shifting to the second therapeutic regimens, they were responded well with 100% cure recovery rate. this was harmonious with the results of antimicrobial sensitivity testing. Currently, *Moraxella* isolates were 100 % sensitive to Tylosin and Gentamicin. By disk diffusion technique, *Moraxella* were resistant to penicillin, ampicillin, cloxacillin and chloramphenicol while being 100% susceptible to gentamicin and ofloxacin (Ojo et al., 2009).

5. CONCLUSION

Infectious keratoconjunctivitis is serious problem of small ruminant located in semi-desert and desert areas. Moraxella infection was bacteriologically identified as an important pathogen causing IKC. However, further studies should be warranted to determine the other possible pathogens responsible for IKC in particular Mycoplasma infection and epidemiological determinants need further investigation. Systemic and topical antimicrobials increase the recovery rate. Tylosin, gentamicin or ciprofloxacin are drugs of choice.

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