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Research Article

Isolation of *Moraxella bovis* in Cattle and Detection of Antibiotic Susceptibilities

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ABSTRACT

The scope of this study was determination of isolation rates of *Moraxella bovis* from infectious bovine keratoconjunctivitis cases in cattle and antibiotic susceptibility of strains. The animal material of research, consisted of 100 units of existing animals and different age groups with infectious bovine keratoconjunctivitis clinical findings found in the cattle business in Aydin Province. Isolation of the agent was done by taking sterile swab samples from the conjunctival sacs of cattle. Ten (5.12%) *M. bovis* were identified by phenotypic identification in 80 ocular swabs samples. Also 44 *Proteus* sp., 25 *Streptococcus* sp., 16 *Staphylococcus* sp., 8 *Bacillus* sp., 2 *Corynebacterium* sp., 1 *E. coli* and 1 *Candida* sp. have been identified. Antibiogram test results revealed that *M. bovis* isolates were sensitive to amoxicillin to clavulanic acid in the ratio of 70%, florfenicol in the ratio of 80%, cefoperazone in the ratio of 60% oxytetracycline and enrofloxacin in the ratio of 50%, gentamicin 40%, resistant to clarithromycin in the ratio of 80%, erythromycin in the ratio of 100%.

Key words: *Moraxella bovis*, Identification, Antibiotic susceptibility

INTRODUCTION

Infectious bovine keratoconjunctivitis (IBC) is a contagious disease characterized by blepharospasm, conjunctivitis, excess lacrimation, varying degrees of corneal opacity and ulceration in cattle. The disease is intensely seen in temperate climate countries and causes economic loss. The disease was defined as pinkeye, new forest, infectious ophthalmia, infectious keratitis and finally IBC. Although many microorganisms are implicated in the aetiology of IBC, *Moraxella bovis* is regarded as the primary cause of the disease (Erdeger and Aydin, 1991). *M. bovis* is an aerobic bacterium with 0.5-1 µm width, 1-3 µm length, Gram (-), non-motile, non-spore, and seen in the bacillus or diplobacilli morphology in microscopy. Haemolytic and non-haemolytic strains were identified previously (Jacinta *et al*., 2001). It has been reported that the virulence of *M. bovis* is related to haemolytic, leukocytic and pilus strains. It has been reported that these agents are present only in the eyes of sick cattle however; non-haemolytic strains without pilus antigens are found in healthy cattle and likely to be present in the normal conjunctival flora (Luke *et al*., 2004). The relationship between autoagglutination, hemagglutination activity and pathogenicity of *M. bovis* has been investigated and it has been stated that strains positive for autoagglutination and hemagglutination are pathogenic (Samsar *et al*., 1993). Many researchers report that there are different serotypes of *M. bovis* in the field (Ruehl *et al*., 1993; Conceição *et al*., 2004; Isik, 2008; Angelos *et al*., 2014). *M. bovis* was detected susceptible to ampicillin, bacitracin, chloramphenicol, gentamicin, kanamicin, nitrifuroson, oxytetracycline, penicillin G and polymixin B, cephalosporin and resistant to streptomycin, lincomycin, erythromycin, cloxacillin and tylosin in previous research (Gokce *et al*., 2002; Isik 2008). Although infectious bovine keratoconjunctivitis is widely known around the world poses a risk for its infectious nature, which is seen in cattle, studies in the veterinary field in our country are not at a satisfactory level. The scope of this study is to investigate the presence of the disease in the cattle establishments in Aydin province and its surrounding areas and present a regional data for future studies to use appropriate antibiotics for veterinarians to take necessary precautions in the farms where the disease is identified.

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MATERIALS AND METHODS

This research was carried out between March and September 2017 by visiting 21 cattle breeding enterprises in 16 different localities in and around Aydin province. The animal material of research consisted of 80 cattle (including 51 animals unilaterally, 29 animals with bilateral symptoms), which were found in such enterprises and had conjunctivitis, excessive lacrimation, corneal opacity clinical symptoms. In total, 109 swab samples were taken from 80 animals. The information on the animals being sampled is given in Table 1. The age scale of the animals used in this study was presented in Table 2.

Table 1: The information on the animals being sampled

The districts	Number of animals	Number of specimen	Number of positive sample
Baltakoy	6	8	-
Pinardere	5	9	
Karahayit	7	8	1
Dalaman	3	3	1
Armutlu	6	9	
Kocagur	14	19	3
Mesutlu	5	7	
Atca	1	2	
Kardeşkoy	1	1	
Efeler	1	1	
Golhisar	6	7	
Musluca	2	2	
Savrandere	2	4	
Umurlu	6	9	
Kozalakli	9	12	5
Sahnali	6	8	
Total	80	109	10

Table 2: Age scale of the animals and isolation rate

Age	Number of animals	Number of positive animals	Isolation rate (%)
0-12 months	51	1	1,25
12-48 months	27	8	10
48 months older	2	1	1,25
Total	80	10	12.5

Samples were taken from the conjunctival sacs of the animals to swabs and sent to the routine diagnostic laboratory of the Department of Microbiology, Department of Veterinary Medicine, Adnan Menderes University under cold chain. These specimens were investigated for the presence of *M. bovis* and antibiotic resistance. Adnan Menderes University Animal Experiments Local Ethics Committee (ADU-HADYEK) stated that this research did not show any penalty about 25.08.2016 dated and 64583101/2016/139 numbered document.

The swab samples were brought to the laboratory and were streaked onto blood agar. Blood agar plates were left in aerobic incubation for 48 hours at 37°C. Reproduction on Oxidation/Fermentation medium, MacConkey agar growth, carbohydrate fermentation, gelatine hydrolysis, colony morphology, haemolysis test, catalase, oxidase, motility test, nitrate reduction test, MR-VP test, autoagglutination, haemagglutination, autoagglutination inhibition (Samsar *et al*., 1993; Arda, 2000; Bilgehan, 2009) and antibiotic susceptibility tests were used for identification of *M. bovis* strains. *Moraxella bovis* ATCC® (10900), obtained from the manufacturer, was used as a positive control in this study.

RESULTS AND DISCUSSION

As a result of the phenotypic identification, *M. bovis* was identified from 10 (12.5%) of 80 ocular swab samples examined. 8 (80%) of the *M. bovis* isolates were identified as haemolytic and 2 (20%) were detected as nonhaemolytic. In addition, 44 *Proteus* sp., 25 *Streptococcus* sp., 16 *Staphylococcus* sp., 8 *Bacillus* sp., 2 *Corynebacterium* sp., 1 *E. coli* and 1 *Candida* sp. have been identified as a result of biochemical tests of other isolates except *M. bovis.*

Antibiotic susceptibilities of 10 *M. bovis* strains isolated in this study were examined. Antibiogram tests showed that *M. bovis* strains were susceptible to amoxicillin-clavulanic acid in the ratio of 70%, susceptible to florfenicol in the ratio of 80%, cefoperazone in the ratio of 60%, oxytetracycline and enrofloxacin in the ratio of 50%, gentamicin in the ratio of 40%, resistant to clarithromycin in the ratio of 80% and erythromycin in the ratio of 100%. The antibiotic susceptibility results were given in Table 3.

Table 3: The antimicrobial susceptibility results

Antimicrobial agent	S	I	R
Amoxycilli-clavulanic acid	7	-	3
Cefoperazon	6	-	4
Gentamycin	4	3	3
Oxytetracyclin	5	2	3
Clarithromycin	2	-	8
Enrofloxacin	5	-	5
Erythromycin	-	-	10
Florfenicol	8	-	2

The scope of this study was to investigate the presence of *M. bovis* in the animals in the region by identification of the agent in cattle enterprises around Aydin province. The results of the study showed that 10 *M. bovis* strains were identified by morphologic, gram staining and biochemical tests. Antibiotic susceptibilities of all identified strains were examined by disk diffusion method.

There are conclusions regarding the isolation of *M. bovis* from IBK symptomatic animals in the world (Angelos *et al*., 2014).

The virulence factors of *M. bovis* were reported as haemolysin and pili in a previous study (Ruehl *et al*., 1993; Angelos *et al*., 2014). In Brazil, Argentina and Uruguay, 30 strains of ocular lesions were reported to be isolated from cattle between 1974 and 2001 (Conceição *et al*., 2004).

In Turkey, *M. bovis* was isolated from 41 of the 208 materials obtained from 168 IBK (Infectious Bovine Keratoconjunctivitis) suspected cattle (Erdeger and Aydin, 1991). *M. bovis* was isolated from the whole of 51 IBK symptomatic cattle (Samsar *et al*., 1993). Thirty *M. bovis* isolates were obtained from IBK outbreaks occurring in Swiss brown cattle in the study conducted in and around Kars province (Gokce *et al*., 2002). It has been reported that *M. bovis* was isolated from 26 (17.9%) of 145 cattle with IBK symptoms in Erzurum and its districts

(Isik, 2008). The 12.5% isolation rate obtained in our study was consistent with previous findings.

In another study, 22 haemolytic (75.8%) and 7 (24.2%) nonhemolytic *M. bovis* strains were identified from 7% sheep blood agar. It is seen that the colony formations of isolation obtained from this study are in accordance with literature data (Isik, 2008; Angelos *et al*., 2014).

All isolated strains in this study showed positive oxidase and catalase reactions. Researchers report that the positive reaction of *M. bovis* to the oxidase reaction is a constant feature. The catalase test was negative for some investigators, positively for some investigators, and found variable strains in some investigators. It is suggested that this is probably due to geographical strain differences and the findings obtained in the research overlap with the findings of the researchers. None of the isolates were grown on MacConkey agar. MR-VP test, glucose, xylose, arabinose, fructose, galactose, sucrose, lactose, mannitol, dulcitol, inositol salisin and indole fermentation, urease activation, hydrogen sulphide and gas formation were found negative. The results obtained are found to be appropriate for the previous studies (Erdeğer *et al*., 1991; Isik *et al*., 2008; Isler *et al*., 2008).

In this study, the nitrate reduction test was found to be negative for all isolates, it is predicted that the method or strain variation used in the literature plays a role for expressing variable nitrate reduction feature of *M. bovis* (Whittier, 2007).

M. bovis were incubated for 1 day at 37°C after inoculation instead of nutrient gelatine agar, and all strains isolated after incubation had hydrolysed nutrient gelatine. These findings are consistent with the literature data (Whittier, 2007; Isik, 2008).

One of the most important identification criteria of pathogen *M. bovis* is the absence of autoagglutination and hemagglutination by the effect of haemagglutination, autoagglutination and $MgCl_2$ of *M. bovis*. Strains giving haemagglutination and autoagglutination are considered pathogenic. In this study, 8 isolated haemolytic strains were found positive for haemagglutination and autoagglutination test. Haemolytic *M. bovis* strains all underwent autoagglutination in a 0.85% salt suspension, and all of the autoagglutination positive strains were also found to be hemagglutination-positives and autoagglutination-positive strains lost this feature by addition of 10% $MgCl_2$. Two nonhaemolytic strains were negative for haemagglutination and autoagglutination test. These findings support the findings of researchers (Erdeger and Aydin, 1991; Isik, 2008).

Findings related to breed and age sensitivity of cattle against IBK were found to be low in 0-12 month old animals in this study, while showing compliance with the studies conducted (Erdeger, 1991; Isik, 2008). It is predicted that the reason for the low isolation rate in 0-12 month old animals used as material is that they are kept in closed stables, which are usually up to 6 months in Aydin province, and that they are not exposed to some predisposing factors such as UV rays.

It has been reported that 12% of eye problems detected in a study investigating the incidence of eye diseases seen in cattle in Hatay province are infectious bovine keratoconjunctivitis (Isler *et al*., 2008). When eye diseases were compared in terms of seasonal factors, the disease rate was highest in spring (39.33%) and followed by summer (26.8%), autumn (21.84%) and winter (11.95%). In our study, *M. bovis* isolates were identified from the samples collected in spring and summer, similar to these findings.

The susceptibility of *M. bovis* to antibiotics has been studied by many investigators. It is reported that *M. bovis* is susceptible to ampicillin, bacitracin, gentamycin, kanamycin, nitrification, oxytetracycline, penicillin G and polymyxin B, cephalosporin, trimetoprim-sulfonamide and streptomycin, erythromycin and tylosin (Whittier, 2007; Angelos *et al*., 2014). The antimicrobial susceptibility of 10 *M. bovis* strains isolated in the study was investigated with disc diffusion method. It was determined that isolated strains were not effective in cefoperazone, amoxicillin clavulanic acid, erythromycin, on the contrary sensitive to florfenicol, and clarithromycin.

Conclusion

When treatment is delayed in infectious bovine keratoconjunctivitis disease, corneal scarring can result in decreased vision, rupture of bulbus oculi and permanent blindness. When an increase of disease in herd-base level is observed about clinical findings, the disease treatment and control program needs to be revised. Conjunctival samples should be collected for bacterial culture and antibiogram testing if a previously administered antibiotic treatment does not appear to be effective. Samples should be collected from untreated early cases as well as from other healthy animals. It is recommended to take as many specimens as possible from the majority of affected animals, and 20% from animals that appear healthy as an ideal ratio. For the bactericidal identification of the agent from the collected samples, the microbiology laboratories may save time in terms of cost.

With a successful treatment, animals affected by IBD disease are generally more likely to have the ability to keep their eyelids open, resulting in improvements in welfare level as well as in efficiency. In cases of possible outbreaks, it is important to take early action for treatment failures. Herd-based disinfection procedures should be followed immediately, and suspected animals with subclinical disease should be immediately isolated from healthy animals. It is necessary to emphasize the importance of timely vaccination during periods when the IBK is typically seen in many cases. In any herd environment, IBC should not be estimated as an individual animal problem.

Although the isolation rate of *M. bovis* is 12.5% in this study, this ratio should be considered. In particular, the economic loss that the IBK has caused to cattle breeding needs to be determined. To protect the animals from disease, the necessary precautions must be taken in order to protect the animals from the disease and the possible risk factors for diseases (flies, fomites, plant grasses, dust, and other bacterial, viral or mycotic infections) should be transferred to the producers in all aspects, including the reduction and control of the IBK.

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