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Bacterial Conjunctivitis in Cattle and Antibiotic Sensitivity of the Isolates

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Abstract: A field study was carried out between June 2006 to July 2008, in order to determine the incidences of the pathogenic bacteria with its rule in conjunctivitis in cattle and the effective antibiotics for ocular administration. Three hundred- fifty cattle of different breeds, ages and sexes showing clinical signs of conjunctivitis were examined; 287 cattle were suffered from unilateral conjunctivitis (82%) and 63 cattle were suffered from bilateral conjunctivitis (18%). Conjunctival swabs from both eyes of 350 cattle were sampled; a total of 169 were bacteriologically positive (48%).Young cattle below 1 year old were significantly more susceptible to bacterial conjunctivitis than older (p value 0.002). Bacteria were isolated from 217 of total 700 conjunctival swabs (31%). *Streptococcus* sp. were the predominant bacteria isolated, 98 (31.3%); then *Staphylococcus* sp., 73 (23.3%), while *Moraxella* sp., *Escherichia coli* were the predominant gram negative bacteria isolated (12.1, 10% respectively). Isolated *Staphylococcus sp*. was highly resistant to antibiotics. Danofloxacin, lincomycin, enrofloxacin, gentamicin, erythromycin and kanamycin were effective whereas amoxicillin/clavulanic acid, ampicillin/sulbactam and oxytetracycline were moderately effective on most of the isolates. Most isolated bacteria were resistant to sulpha/Trimethoprim, chloramphenicol and Cefuroxime.

Key words: Conjunctiva, bovine, Bacteria, Antibiogram, screening .

INTRODUCTION

Conjunctivitis is the inflammation of bulber or palpebral conjunctiva[1]. It is characterized with erythema, hyperemia, chemozis, leukocyte infiltration, folliculitis, blepharospazm in eyelid, photophobia and tears in different composition[1,2]. Conjunctivitis can be broadly categorized according to duration of inflammation, composition of tear and predisposition and incriminated microorganism. Regardless of category of conjunctivitis, etiological diagnosis is the basic of the efficient treatment[3]. Conjunctival swab or scraping or biopsy techniques are used for diagnosis of microbial conjunctivitis[3]. Following isolation and identification of microorganism, antibiotic sensitivity results help us to choose the most effective antibiotics. As a replacement therapy, antiseptics, astringents and vasoactive agents can be administered as well[1,2]. This study was designed to screen the bacterial conjunctivitis and evaluate antibiotic sensitivity test.

MATERIALS AND METHODS

Animal and Sa mple Collection: Cattle of different age, breed and sex were examined during the period between June 2006 to July 2008 at El-Moonib and El-Bassatin abattoirs and from farms in Giza and El-Fayoum Governorates. Those suffered from different degrees of unilateral or bilateral conjunctivitis were 350 cattle (Table 1).

Conjunctival swabs were obtained from both eyes of 350 cattle using sterile cotton swabs. Each swab was placed into screw-capped tube containing Brain Heart Infusion broth then sent to laboratory in ice box in order to isolate and identify causing pathogens.

Isolation and Identif ication: Collected samples were cultivated on Blood agar, Chocolate Agar, EMB and MacConkey agar were used. All agar plates were incubated for 24-72 hours at 37°C, under aerobic and microaerophilic conditions. Colonies were identified using gram stainning, colony morphology and biochemical tests. Biochemical identification using oxidase, and catalase, urease, tube coagulase, nitrate reduction, indol and citrate tests[4].

For isolation of mycoplasmas; Brain Heart infusion (BHI) broth and agar media containing: Yeast extract 5%, Thallium acetate 2%, Penicillin G sodium 1000.0 i.u. / ml and Horse serum were used[5]. Swabs were placed in MacCartney bottles containing broth media. Six to 12 hours after incubation at 37°C a loopful was

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removed and inoculated on agar plates. The inocula which did not show growth on the agar were blindly passage twice. Digitonin test was used for differentiation between Mycoplasma and Acholeplasma Genera[6].

Antibiotic sensitivity discs provided by Oxoid were used in order to determine the sensitivity of the isolated and identified microorganisms to antibiotics. The used discs are enumarated in Table (2). Antibiotic resistance of the isolated and identified bacteria was assessed using the disc agar diffusion test[7]. To do so, colonies were placed into tubes containing Triptic Soy Broth. The tubes were incubated for 5-6 hours at 37°C. Following incubation, with standard 0.5 of Mc Farland, colony forming unit was determined if it was equal to 1x $(10)^8$per milliliter.Colonies were sprinkled to the surface area of Mueller Hinton Agar adjusted suspensions blurriness by sterile swabs. For accurate placement of antibiotic discs Oxoid disc dispenser were used then the plates incubated at 37°C for 18-24 h. After the incubation, the zone diameters surrounding the discs were measured. The zone diameters were evaluated by comparing the values (M2 A4)[7].

The antibiotic susceptibility of the mycoplasma isolates was done by the determination of the minimal inhibitory concentration (MIC) values instead the disc diffusion test[8,9].

Statistical Method: Chi square at confidence interval 95% was performed. (Statistix, version 8.0 copyright 1985-2003 analytical software).

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RESULTS AND DISCUSSION

Results: Out of 350 cattle suffered for conjunctivitis; 287 cattle showed unilateral conjunctivitis (82%) and 63 cattle showed bilateral conjunctivitis (18%). A total of 169 were bacteriologically positive (48%);Table 3. Young cattle ≤ 1 year old were significantly more susceptible to bacterial conjunctivitis than older (62% in young animals -105 out of 171- and 36% in older -64 out of 179-) p value 0.002.While both sex was similar in prevalence of bacterial conjunctivitis 50% in male (108 out of 216) and 46% in female (61 out of 134) p value 0.07.

Of 700 eye swabs, microorganisms were isolated from 217 (31%) as shown in Table 3. These positive eye swabs yield 313 isolates either as single or mixed infection with other bacteria.

Different isolated and identified microorganisms from both eyes of 350 cattle are listed in Table 4. *Streptococcus* sp. were the predominant bacteria isolated, 98 (31.3%); then *Staphylococcus* sp., 73 (23.3%). Also *Moraxella* sp., *Escherichia coli*, *Corynebacterium* sp., *Pseudomonas* sp., *Neisseria* sp., *Micrococcus* sp., *Klebsiella pneumoniae*, *Haemophilus* sp., *Enterobacter* sp., and *Mycoplasma* sp. were isolated and their percentage are shown in Table (4). It was also found that 18 samples of 73 infected by *Staphylococcus* sp. were *identified as S. aureus*. Among samples infected by *Streptococcus* sp. 98; 47 were ά-hemolytic streptococci, 20 were β-hemolytic streptococci and 31 were non-hemolytic streptococci. Table 5 summarizes the antibiotic sensitivity of isolated and identified gram-positive bacteria. There were differences in sensitivity and resistance to different antibiotics. Especially, *Staphylococcus* sp. was resistant to most of antibiotics.

Discussion: The limited number of studies is available for herd surveillance with respect to bacterial conjunctivitis and effective antibiotic to use against infectious bovine keratoconjunctivitis. Thus, recent surveys focus on screening and consequently herd-basis approach.

Because the isolation and identification of the causing agent may require a longer time and could be expensive, Broad spectrum antibiotics are commonly applied in ocular treatment. However, many studies concluded that use of effective antibiotics after antibiogram for isolation and identification of causing microorganisms increases likelihood of eradication of this problem. Thus, empirical treatment may not result in effective cure[3].

In present study 350 cattle suffered for different degrees of conjunctivitis were studied; of these 82% were suffered from unilateral conjunctivitis and 18% were suffered from bilateral conjunctivitis (Table 3). Age was significantly associated with the disease, young cattle ≤ one year old are more susceptible than older (*P* value 0.002). Nearly similar results were obtained where 110 cattle were studied with different degrees of infectious bovine keratoconjunctivitis. Of these, 94 (85.5 %) were unilateral and 16 (14.5 %) were bilateral. The prevalence of the disease was significantly ($P< 0.001$) influenced by age[10].

On the basis of isolation and identification, a high ratio of gram-positive bacteria from the conjunctival swabs was obtained. *Streptococci* (31.3%) and *Staphylococci* (23.3%) followed by and *Corynebacterium* species (5.1%).Ten isolates of *Mycoplasma* species were identified (3.2%). *Moraxella* species (12.1%) and *Escherichia coli* (10%) were the most predominant isolated gram negative bacteria (Table 4). Similar studies were conducted, Sarma *et al.*[11] isolated mostly *Streptococcus* sp. and to lesser extent, *Pasteurella haemolytica*, *Corynebacterium bovis*, *Micrococcus* sp. and *Candida* sp. from conjunctival flora of 27 cows in England. In a large-sample

epidemiological study carried out in Australia[3], it was shown that gram-positive bacteria predominated (54.4%). Other bacteria were *Corynebacterium* sp. (27.4%), *Moraxella nonliquefaciens* (26.9%), *Neisseria catarrhalis* (10.5%), *Acinetobacter sp*. (8.0), *Moraxella bovis* (6.5%), *Coliform sp.* (6.5%) and *Bacillus* sp. (1.3%). In France *Moraxella bovis* was isolated from conjunctival swabs of 16 cows with infectious bovine keratoconjunctivitis(82.3%)[12]. While in another research *Chalamydia* species was recovered from 103 (66%) among 156 cows' conjunctival samples and lesser extent, *Moraxella bovis*, *Neisseria catarrhalis*, and *Corynebacterium bovis*.[13]

Concerning the antibiotic sensitivity test, (Table 5) it was found that isolated *Staphylococcus* species were highly resistant. Danofloxacin, lincomycin, enrofloxacin, gentamicin, erythromycin and kanamycin were highly effective whereas amoxicillin/clavulanic acid, ampicillin/sulbactam and oxytetracycline were moderately effective. Most bacteria were resistant to sulpha/Trimethoprim, chloramphenicol and Cefuroxime. Nearly similar results were obtained where the sensitivity of bacteria to different antibiotics was different[14]. Isolated *Staphylococcus* species were highly resistant to antibiotics. Danofloxacin, gentamicin and kanamycin were sensitive whereas

Table 1: Total numbers of examined animals.

Animals	Total Numbers
Male calves ≤1 year old	101
Female calves ≤1 year old	70
Bull >1 year old	115
Heifers & cows > 1 year old	64
Total	350

Table 2: Antibiotic discs used for sensitivity test

Antibiotic	Symbol	concentration	Antibiotic	Symbol	concentration
Cefuroxime	CXM	30µg	Sulpha/Trimethoprim	SXT	23.75+1.25µg.
Danofloxacin	DAN	30µg	Enrofloxacin	ENR	10µg
Erythromycin	E	15µg	Kanamycin	K	30µg
Lincospectin	MY	10µg	Amoxicillin/Clavulanic acid	AMC	20/10 µg
Gentamicin	CM	10µg	Oxytetracyline	OT	30µg
Chloramphenicol	C	30µg	Ampicillin/Sulbactam	SAM	10/10 µg

Table 3: Proportion of positive animals to total number of examined animals with total number of positive samples.

Animals	Total numbers of positive animals		Total numbers of positive samples	
	----NO----------	----------%	----------NO----------	----------%
Male calves ≤1 year old	66	65%	96	48%
Female calves ≤1 year old	39	56%	41	29%
Bull >1 year old	42	37%	57	25%
Heifers & cows > 1 year old	22	19%	23	18%
Total	169	48%	217	31%

Table4: The Isolation rate of different microorganisms.

Microorganism	The number of microorganism	Isolation rate (%)
Staphylococcus sp	73	23.3
S. aureus	18	5.7
Streptococcus sp.	98	31.3
alpha hem strept	47	15
beta hem strept	20	6.4
non hem strept	31	9.9
Micrococcus sp	7	2.2
Corynebacterium sp	16	5.1

Table 4: Continue

Moraxella sp.	38	12.1
Escherichia coli	31	10
Pseudomonas sp.	15	4.8
Klebsiella pneumoniae	7	2.2
Neisseria sp.	10	3.2
Haemophilus sp	4	1.3
Enterobacter	4	1.3
Mycoplasma sp.	10	3.2
Total	313	100 %

Table 5: Antibiotic sensitivity test for the identified bacterial isolates.

Antibiotic Symbols Bacteria (no. isolates)	DAN (%)	CM (%)	K (%)	ENR (%)	E (%)	MY (%)	OT (%)	AMC (%)	SAM (%)	C (%)	SXT (%)	CXM (%)
Staphylococcus sp (55)	43 (78)	35 (63)	32 (58)	39 (70)	31 (56)	42 (76)	18 (33)	30 (54)	29 (53)	1 (2)	3 (5)	0 (0)
Staph. aureus (18)	10 (56)	6 (33)	5 (28)	7 (39)	5 (28)	8 (44)	2 (11)	4 (22)	3 (17)	0 (0)	0 (0)	0 (0)
α- hem strept sp (47)	40 (85)	38 (81)	37 (79)	39 (83)	36(77)	40 (85)	21 (45)	33 (70)	36 (77)	0 (0)	5 (11)	1 (2)
β- hem strept sp (20)	18 (90)	15 (75)	14 (70)	17 (85)	14 (70)	17 (85)	9 (45)	13 (65)	12 (60)	2 (10)	4 (20)	1 (5)
non hem strept sp.(31)	31 (100)	27 (87)	26 (83)	27 (87)	26 (83)	28 (90)	21 (67)	24 (77)	23 (74)	6 (19)	9 (29)	3 (10)
Micrococcus sp (7)	7 (100)	7 (100)	5 (71)	6 (86)	7 (100)	5 (71)	4 (57)	7 (100)	5 (71)	2 (29)	3 (43)	4 (57)
Corynebact. sp (16)	16 (100)	14 (87)	12 (75)	15 (93)	16 (100)	16 (100)	12 (75)	14 (87)	12 (75)	1 (6)	4 (25)	3 (19)
Moraxella sp. (38)	38 (100)	35 (92)	38 (100)	38 (100)	33 (87)	38 (100)	33 (87)	36 (94)	35 (92)	15 (39)	10 (26)	19 (50)
Escherichia coli (31)	26 (84)	29 (94)	27 (87)	26 (84)	26 (84)	26 (84)	28 (90)	27 (87)	23 (74)	12 (38)	10 (32)	16 (51)
Pseudomonas sp. (15)	14 (93)	14 (93)	13 (87)	13 (87)	14 (93)	14 (93)	4 (26)	10 (66)	5 (33)	1(7)	5 (33)	15 (100)
Kleb. Pneumoniae (7)	7 (100)	5 (71)	7 (100)	7 (100)	5 (71)	7 (100)	1 (14)	4 (57)	4 (57)	0 (0)	0 (0)	4 (57)
Mycoplasma sp. (10)	10 (100)	8 (80)	10 (100)	10 (100)	8 (80)	10 (100)	9 (90)	0 (0)	0 (0)	0 (0)	0 (0)	3 (30)
Neisseria sp. (10)	10 (100)	9 (90)	10 (100)	10 (100)	10 (100)	10 (100)	7 (70)	8 (80)	9 (90)	3 (30)	4 (40)	4 (40)
Haemophilus sp (4)	4 (100)	4 (100)	4 (100)	4 (100)	3 (75)	4 (100)	2 (50)	3 (75)	3 (75)	0 (0)	1 (25)	2 (50)
Enterobacter. sp (4)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	3 (75)	4 (100)	4 (100)	2 (50)	1 (25)	2 (50)

amoxicillin/clavulanic acid and ampicillin/sulbactam were moderately sensitive[14]. Most bacteria were resistant to penicillin G and cefaperazon. While it was found that chloramphenicol was the most effective antibiotic in other study[11]. Mycoplasma isolates were highly sensitive to Danofloxacin, kanamycin, lincomycin and enrofloxacin, moderately sensitive to oxytetracycline, gentamicin and erythromycin and highly resistant to amoxicillin/clavulanic acid, ampicillin/sulbactam and sulpha/Trimethoprim. All of the mycoplasmas are resistant to beta-lactames because of the lack of the cell wall. The same resistance can be observed to nalidixic acid, polymyxin, rifamycin, trimethoprim and to sulfonamides[15]. Mycoplasmas is sensitive to antibiotics, which inhibit the protein or nucleic acid synthesis. The most effective antibiotics are the pleuromutilins (tiamulin, valnemulin) and the fluoroquinolones[16]. The tetracyclines, the macrolids and the aminoglycosides used to be good against mycoplasma, but lately there are elevated numbers of resistant strains[17,18].

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