Global Veterinaria 10 (1): 09-12, 2013
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2013.10.1.71121

Bacterial Flora of Normal Conjunctiva of Native Goats in Northwestern Iran

[1]Abdullah Araghi-Sooreh, [2]Ali Hassanpour and [3]Sajad Fathollahi

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine,
Urmia branch, Islamic Azad University, Urmia, Iran
[2]Department of Clinical Sciences, Faculty of Veterinary Medicine,
Tabriz branch, Islamic Azad University, Tabriz, Iran
[3]Faculty of Veterinary Medicine, Urmia branch, Islamic Azad University, Urmia, Iran

Abstract: The purpose of this study was to identify the normal conjunctival bacterial flora and determine the effects of gender and age variations on them in healthy goats. In summer 2011, 100 swabs were taken from the inferior conjunctival sac of b oth eyes of 50 healthy goats. The animals were selected from two sexes (30 males, 20 females) and divided into two age groups, below 12 months of age (31 cases) and above 12 months of age (19 cases). The samples were inoculated on blood and EMB agar and examined for aerobic bacteria. A total of 132 bacterial isolates were identified in 99 out of 100 (99%) eyes. Single bacterial species were isolated in 66 eyes. Two bacterial species were isolated in 33 eyes. The isolates included 9 different species. Gram-positive bacteria (84.85 %) were isolated more frequently than Gram-negative bacteria. The most commonly isolated Gram-positive bacteria were Bacilli (45.54 %), followed by Staphylococci (19.69 %). *Escherichia coli* (12.12 %) was the most common isolated Gram-negative bacteria. According to the statistical analysis, isolation frequency of *E. coli* and *Bacillus brevis* was significantly ($P \leq 0.05$) higher in the males and in those below 12 months of age and isolation frequency of *B. cereus*, *Staph. aureus* and *Enterococcus* spp. was significantly ($P \leq 0.05$) higher in goats below 12 months of age.

Key words: Bacterial Flora · Conjunctiva · Goat · *Bacillus* sp · Iran

INTRODUCTION

The ocular surface is directly exposed to the external environment and is therefore endangered by a multitude of antigen and pathogenic microorganisms. Under normal circumstances, the conjunctiva supports a population of microorganisms that interact with each other and with the immune system of the host, regulating and preventing eye infection [1]. Isolation of a specific organism from the conjunctival sac of an animal with external ocular disease is not a definitive evidence of a cause and effect association. Therefore, knowledge of the normal surface ocular flora is pivotal to interpret the results of microbial cultures and understanding surface ocular disease dynamics. Bacterial flora of the normal conjunctiva has been reported in various domestic and wild animals [2-5]. Most reports of normal ocular flora in these animals showed a predominance of nonpathogenic, mainly Gram-positive organisms. The microbiota of ocular surface depend on seasonal variations, housing and geographical location [1,6]. Reports describing the surface ocular flora of domestic goat are sparse [7]. The population of goats in Iran is about 25 million heads (2.9% of world total) [8], of which 14% are genetically pure and 86% are categorized as either mixed or untitled. The purpose of this study was to document the normal aerobic bacterial conjunctival flora and to determine effects of gender and age variations on them in the healthy native goats in temperate geographical conditions of Urmia district, northwest of Iran.

MATERIALS AND METHODS

Fifty native goats (30 male, 60%; 20 female, 40%) that had been referred to the Urmia slaughterhouse in the summer of 2011 were included in this study. The

Corresponding Author: Abdullah Araghi-Sooreh, Islamic Azad University, Urmia branch,
Faculty of Veterinary Medicine, Department of Clinical Sciences, 57135-969 Urmia, Iran.

animals were divided into two age groups, below 12 months of age (31 cases; 62%) and above 12 months of age (19 cases; 38%). Apparently healthy eyes were examined by the direct ophthalmoscope to ensure that external ocular signs of disease did not exist, such as lesions or vessels in cornea and conjunctival injection. Samples were obtained from the inferior conjunctival sac of both eyes using dry sterile swabs. Special care was taken to ensure that the swabs did not come into contact with the eyelashes or skin of the eyelids. Swabs were placed in tubes containing sterile transport medium (peptone broth); and transported immediately in thermal box containing ice packs to the microbiology laboratory. Immediately after arrival, samples were cultivated on 5 % ovine blood agar (Merck, Germany) and eosin methylene blue agar (EMB) (HiMedia, India) and incubated aerobically at 37°C for 24-48 hours. Different colony types were further isolated on blood agar plates. Colonies isolated from all plates were identified by using standard microbiological and biochemical methods [9].

Statistical analysis was performed with the use of SAS ver. 9.1 with the statistical significance set at $P < 0.05$. Mann-Whitney test was used to compare the frequency of bacterial isolation between different gender and age groups.

RESULTS

The isolation and frequency of organisms according to sex and age groups was shown in Table 1. One hundred and thirty two bacterial isolates were identified in 99 (99%) out of the 100 specimens. Single bacterial species were isolated in 66 (66.7%) eyes. Two bacterial species were isolated in 33 (33.3%) eyes. Gram-positive organisms were the predominant bacteria, representing 84.85% of all 132 bacterial isolates. Bacilli (45.54%) were the most frequently isolated, followed by Staphylococci (19.69%). Gram-negative organisms comprised 15.15% of all 132 bacterial isolates, with *Escherichia coli* (12.12%) being the most prevalent. The other isolated species were *Enterococcus* spp. (15.15%), *Streptococcus agalactiae* (4.54%), *Klebsiella* (2.27%) and *Enterobacter aerogenes* (0.75%).

Statistical evaluation of sex and age impact on frequency of isolates showed that *E. coli* isolation was significantly higher in males and in those below 12 months of age ($P<0.01$), *Staphylococcus aureus* isolation was significantly higher in goats below 12 months of age ($P<0.05$), *B. brevis* isolation was significantly higher in males ($P<0.05$) and those below 12 months of age ($P<0.01$) and *B. cereus* and *Enterococcus* spp. isolation was significantly higher in goats below 12 months of age ($P<0.01$).

DISCUSSION

In our study nine different species of bacteria were cultured from the conjunctival sac of goats. Most species were Gram-positive, including species of *Bacillus* and *Staphylococcus*. These bacteria have been commonly isolated from the conjunctiva of other healthy domestic and wild mammals and birds [3, 10-12]. Similar to the findings in the present study, *Bacillus* sp. have been reported to be most common microbial isolates from clinically normal conjunctiva of cattle [10], sheep [13], bison [11] and camels [14]. *Staphylococcus* sp. were reported to be the predominant organisms in healthy eye of water buffalos [15], horses [16], dogs [17], cats [18], domestic rabbits [19], llama [20], Asian elephants [2], raccoons [3], opossums [4] and in the birds [12].

Table 1: Bacteria isolated from conjunctival sac of healthy native goats in Urmia, Iran and their frequency between sex and age groups

	Number of isolates (%)				
	Sex		Age		
Bacteria	Male	Female	1	2	Total
Bacillus cereus	17 (12.87)	13 (9.85)	22 (16.66)**	8 (6.06)**	30 (22.27)
Bacillus brevis	18 (13.63)*	12 (9.09)*	25 (18.94)**	5 (3.78)**	30 (22.27)
Enterococcus spp.	11 (8.33)	9 (6.81)	16 (12.12)**	4 (3.03)**	20 (15.15)
Staphylococcus epidermidis	10 (7.57)	7 (5.30)	11 (8.33)	6 (4.54)	17 (12.87)
Escherichia coli	12 (9.09)**	4 (3.03)**	12 (9.09)**	4 (3.03)**	16 (12.12)
Staphylococcus aureus	6 (4.54)	3 (2.72)	7 (5.30)*	2 (1.52)*	9 (6.81)
Streptococcus agalactiae	3 (2.27)	3 (2.27)	4 (3.03)	2 (1.52)	6 (4.54)
Klebsiella spp.	2 (1.52)	1 (0.75)	2 (1.52)	1 (0.75)	3 (2.27)
Enterobacter aerogenes	1 (0.75)	0 (0.0)	1 (0.75)	0 (0.75)	1 (0.75)

*:$p < 0.05$; **:$p < 0.01$; 1: below 12 months of age 2: above 12 months of age

Three of 9 bacterial species were Gram-negative; *E. coli* was most frequent. As in goats, *E.coli* was the organism most frequently found in sheep [13], water buffalos [15] and pigs [21]. It is worth noting that the composition of the ocular bacteria isolated from goats eye, especially the predominant Gram-positive, *Bacillus* sp. and the Gram-negative, *E. coli* are similar to that observed in domestic and wild ruminants [11,13,14].

In the present study, bacterial growth was found in 99% of 100 conjunctival samples. This rate of isolation is high compared to other species. In water buffalos [15], Asian elephants [2], horses [22] and birds of prey [12] this proportion was 91, 88.8, 79.7 and 74.6 %, respectively. The higher number of positive cultures in goats compared with other animals is probably due to variability in eye surface niches and defense mechanisms [1].

Out of 66.7% of eyes a single bacterial species and out of 33.3% of eyes two bacterial species were isolated. In birds of prey [12], dogs [17], horses [22], rabbits [19] and water buffalos [15] these values are, respectively, 87.6, 76.6, 45.5, 41 and 13.19% for one bacterial species and 12.4, 23.4, 34.5, 59 and 86.81% for two or more species.

In surveys comparing bacterial flora of healthy eyes and eyes with external disease, similar organisms were isolated [5, 23-26]. Therefore, members of the normal microbial flora can themselves become pathogens. Injury to the cornea or use of topical corticosteroids and antimicrobials may result in proliferation and potential pathogenicity of normal ocular flora [27].

Some of known bacterial pathogens as *Moraxella* ovis [26], *Moraxella bovis* [25], *Staph. aureus*, *Pseudomonas* spp. *Acinetobacter* spp. and Enterobacter group can inhabit the eye surface of healthy sheep and goats [7,25,28]. In our study from these, *E. coli* and *Staph. aureus* were isolated with frequency of 12.12 and 6.81 %, respectively. *Staph. aureus* usually as co-infection could be impressive in frequency and severity of infectious keratoconjunctivitis (IKC) in sheep and goats [24,25,29].

Several studies have showed effects of sex [20, 21] and age [19, 30] of host on frequency of conjunctival isolates. In our study, impact of goat gender and age on frequency of isolates was also proved. Additional studies are necessary to assess how the sex and age of the host affect the eye surface bacterial population.

In our study it was observed that amongst the conjunctival flora of healthy goats, *Bacillus* sp. usually non-pathogenic bacteria, were the most common organisms. Some cases showed presence of potentially bacterial pathogens with normal looking eyes. Frequency of conjunctival bacterial isolation can be significantly influenced by gender and age of host.

REFERENCES

1. McClellan, K.A., 1997. Mucosal defense of the outer eye. Survey of Ophthalmology, 42(3): 233-246.
2. Tantivanich, P., K. Soontornvipart, N. Tuntivanich, S. Wongaummuaykul and P. Brikawan, 2002. Conjunctival microflora in clinically normal Asian elephants in Thailand. Veterinary Research Communications, 26(4): 251-254.
3. Spinelli, T.P., E.F. Oliveira-Filho, D. Silva, R. Mota and F.B. Sa, 2010. Normal aerobic bacterial conjunctival flora in the Crab-eating raccoon (Procyon cancrivorus) and Coati (Nasua nasua) housed in captivity in pernambuco and paraiba (Northeast, Brazil). Veterinary Ophthalmolology, 13 Suppl: 134-136.
4. Pinard, C.L., A.H. Brightman, T.J. Yeary, T.D. Everson, L.K. Cox, M.M. Chengappa and H.J. Davidson, 2002. Normal Conjunctival Flora in the North American Opossum (*Didelphis virginiana*) and Raccoon (*Procyon lotor*). Journal of Wildlife Diseases, 38(4): 851-855.
5. Moore, C.P. and M.P. Nasisse, 1998. Clinical Microbiology. In Veterinary Ophthalmology, Eds. Gelatt, K.N. Lea & Febiger, pp: 259-289.
6. Eichenbaum, J.D., J.D.Lavach, G.A. Severin and M.E. Paulsen, 1987. Immunology of the ocular surface. Compendium on Continuing Education for the Practicing, 9(11): 1101-1107.
7. Mushi, E.Z., M.G.Binta, R.G. Chabo and K. Dintwe, 2007. Conjunctival flora of fifty healthy goats in Sebele farm,Gabarone, Botsowana. Journal of Animal and Veterinary Advances, 6(12): 1388-1389.
8. Abdel Aziz, M., 2010. Present status of the world goat populations and their productivity. Lohmann Information, 45(2): 42-52.
9. Quinn, P.J., W.J. Carter, M.E. Markey and G.R. Carter, 1994. Clinical Veterinary Microbiology. Wolfe, pp: 118-327.
10. Kojouri, G.A., A. Ebrahimi and F. Nikookhah, 2007. Systemic dexamethasone and its effects on normal aerobic bacterial flora of cow. Pakistan Journal of Biological Sciences, 10(12): 2095-2097.

11. Davidson, H.J. , J.G. Vestweber, A.H. Brightman, T.H. Van Slyke, L.K. Cox and M.M. Chengappa, 1999. Ophthalmic examination and conjunctival bacteriologic culture results from a herd of North America bison. Journal of American Veterinary Medicine Association, 215(8): 1142-1144.
12. Dupont, C., M. Carrier and R. Higgins, 1994. Bacterial and fungal flora in healthy eyes of birds of prey. Canadian Veterinary Journal, 35(11): 699-671.
13. Araghi-sooreh, A. and A. Mohammad-Aminzadeh, 2010. Bacterial flora of the conjunctival sac of healthy Makui and Ghezel sheep in Urmia. Veterinary Journal of Islamic Azad University-Tabriz Branch, 4(2):855-860.
14. Monteiro, A.J., M.F.S. Teixeira and J.J.C. Sidrim, 2005. Survey of bacterial microorganisms in the conjunctival sac of clinically normal dogs and dogs with ulcerative keratitis in Fortaleza, Ceara, Brazil. Veterinary Ophthalmology, 8(1): 33-37.
18. Espinola, M.B. and W. Lilenbaum, 1996. Prevalence of bacteria in the conjunctival sac and on the eyelid margin of clinically normal cats. Journal of Small Animal Practice, 37(8): 364-366.
19. Cooper, S.C., G.J. McLellan and A.N. Rycroft, 2001. Conjunctival flora observed in 70 healthy domestic rabbits (*Oryctolagus cuniculus*). Veterinary Record, 149(8): 232-235.
20. Gionfriddo, J.R., R. Rosenbusch, J.M. Kinyon, D.M. Betts and T.M. Smith, 1991. Bacterial and mycoplasmal flora of the healthy camelid conjunctival sac. American Journal of Veterinary Research, 52(7): 1061-1064.
21. Davidson, H.J., D.P.Rogers, T.J. Yeary, G.G. Stone, D.A. Schoneweis and M.M.Chengappa, 1994. Conjunctival microbial flora of clinically normal pigs. American Journal of Veterinary Research, 55(7): 949-951.
22. McLaughlin, S.A., A.H. Brightman, L.C. Helper, J.P. Manningand J.E. Tomes, 1983. Pathogenic bacteria and fungi associated with extraocular disease in the horse. Journal of American Veterinary Medicine Association, 182(3): 241-242.
23. Gerding, P.A., S.A. McLaughlin and M.W. Troop, 1988. Pathogenic bacteria and fungi associated with external ocular diseases in dogs; 131 cases (1981-1986). Journal of American Veterinary Medicine Association, 193(2): 242-244.
24. Egwu, G.O., W.B. Faull, J.M. Brandburry and M.J. Clarkson, 1989. Ovine infectious keratoconjunctivitis: A microbiological study of clinically unaffected and affected sheeps eye with special reference to Mycoplasma conjunctivae. Veterinary Record, 125(10): 253-256.
25. Ojo, O.E., O.A.Oluwole and A.J. Adetosoye, 2009. Isolation of Moraxella bovis from infectious keratoconjunctivitis in a flock of goats. Nigerian Veterinary Journal, 30(1): 56-59.
26. Dagnall, G.J.R., 1994. An investigation of colonization of the conjunctival sac of sheep by bacteria and mycoplasmas. Epidemiology Infection, 112(3): 561-567.
27. Moore, C.P., N. Heller, L.J. Majors, D. Whitley, E.C. Burgess and J. Weber, 1988. Prevalence of ocular microorganisms in hospitalized and stabled horses. American Journal of Veterinary Research, 49(6): 773-777.
28. Akerstedt, J. and M. Hofshagen, 2004. Bacteriological Investigation of Infectious Keratoconjunctivitis in Norwegian Sheep. Acta Veterinaria Scandinavica, 45(1-2): 19-26.
29 . Egwu, G.O. and W.B. Faull, 1993. Clinico-serological studies of ovine infectious keratoconjunctivitis in adult ewes experimentally infected with *Mycoplasma conjunctivae* and/or *Staphylococcus aureus*. Small Ruminants Research, 12(2): 171-183.
30. Andrew, S.E., A. Nguyen, G.L. Jones and D.E. Brooks, 2003. Seasonal effects on the aerobic bacterial and fungal conjunctival flora of normal thoroughbred brood mares in Florida. Veterinary Ophthalmology, 6(1): 45-50.