

Aerobic Bacterial Flora of Conjunctival Sac in the Healthy Iranian Water Buffalo (*Bubalus bubalis*)

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Abstract: This study was aimed to identify the normal aerobic bacterial flora of the conjunctiva in the Iranian water buffalo and to determine the effect of gender, age and ecotype variations on the flora. Fifty healthy Iranian water buffalos (33 females, 17 males), aged 1-15 years, of four different ecotypes-Azeri, Mazandarani, Khuzestani and Guilani were selected and divided into 4 age groups. Swabs were taken from the inferior conjunctival sac of both eyes (n = 100) and cultured on blood and MacConkey agar in aerobic condition. Forty seven buffalos out of 50 (94%) were positive for bacteria; 44/50 (88%) had positive culture from both eyes. The most frequent isolates were *Staphylococcus* sp., *Escherichia coli* and *Bacillus cereus*. Statistical analysis did not show significant difference in frequency of isolates between sexes, ecotypes and age groups (p>0.05). A correlation was found between the buffalo age and number of species isolated per eye (p<0.01). In this study *Dermatophilus congolensis* and *Edwardsiella tarda* were reported for the first time in the conjunctiva of animals. Gram-positive aerobes were most commonly cultured from the conjunctival sac of healthy water buffalos.

Keywords: Bacterial flora, conjunctiva, *Dermatophilus congolensis*, *Edwardsiella tarda*, water buffalo

INTRODUCTION

The microbial flora of the ocular surface consists of resident and transient bacterial, fungal (Andrew *et al.*, 2003; Dupont *et al.*, 1994), mycoplasmal (Dagnall, 1994; Barber *et al.*, 1986; Rosendal, 1973; Campbell *et al.*, 1973) and chlamydial (Pinard *et al.*, 2002; Davidson *et al.*, 1994) organisms that exist in a balance between themselves and immune system of the host. The resident microbial flora aids in maintaining ocular surface health by preventing overgrowth of potentially pathogenic agents. However in some circumstances (e.g., when surface defense mechanisms are weakened) this normal flora may become opportunists and cause infection (McClellan, 1997; Gilger, 2008). Therefore, characterization of normal flora of conjunctiva may be useful in treating eye infections. Bacterial flora of the normal conjunctiva has been reported in various animals (Moore and Nasisse, 1998). Gram-positive bacteria were reported as the most common isolates. However microbiota of the ocular surface depends on season of sampling, geographic location, housing conditions and the age of the animal (Ramsey, 1998).

Normal conjunctival bacterial flora has been reported in swamp buffalo (Tantivanich *et al.*, 1988) and bison (Davidson *et al.*, 1999), but to the authors' knowledge, conjunctival flora of healthy riverine buffalos has not been reported.

In Iran, almost half a million water buffaloes (river-type) are farmed, with 75% of its population concentrated in the northwest (Azeri ecotype), 18% in the southwest (Khuzestani ecotype) and 7% in the north of the country (Guilani and Mazandarani ecotypes) (Naserian and Saremi, 2007).

The purpose of this study was to identify the conjunctival aerobic bacterial flora and to determine the effect of gender, age and ecotype variations on this flora in the healthy Iranian water buffalo.

MATERIALS AND METHODS

This study was carried out in the summer of 2009 at the buffalo breeding and extension training center of Urmia, northwest of Iran. Fifty clinically normal Iranian water buffaloes (33 females, 66%; 17 males, 34%) with an age range of 1 to 15 years old in 4 age groups-yearlings 19 cases (3%), 2-5 years 8 cases (16%), 6-10 years 2 cases (4%) and 11-15 years 21 cases (42%) were used. The animals were of 4 Azeri (25 cases; 50%), Mazandarani (13 cases; 26%), Khuzestani (8 cases; 16%), and Guilani (4 cases; 8%) ecotypes. The buffalos were run through a specially designed chute. The eyes were examined by direct ophthalmoscopy to rule out the presence of any signs of ocular disease. Samples were taken from the inferior

conjunctival sac of both eyes using a sterile swab for each eye, avoiding swab contact with the eyelashes or skin of the eyelids. Swabs were placed in individual sterile test tubes and submitted immediately to the microbiology laboratory of center. In the laboratory swabs were cultivated on 5% ovine blood agar (Merck, Germany) and MacConkey agar (Himedia, India) and incubated at 37°C for 24-48 h, in aerobic condition. Different colony types were further isolated on blood agar plates. Colonies isolated from all plates were identified by using standard microbiological and biochemical methods (Quinn *et al.*, 1994).

Spearman's correlation test was used to measure of relationship the age of buffalo with number of bacterial species isolated per eye. ANOVA test was used to compare the number of isolates between ecotypes and age groups of buffalos and Student's t-test to compare the number of isolates between sexes using a software package (SPSS 16.0) with the statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSION

A total of 91 (91%) eyes were positive for bacteria. No bacteria were isolated in 3 cases (6 eyes; i.e., 6%). Single bacterial species were isolated in 12 (13.19%) eyes. Two different bacterial species were isolated in 54 (59.34%) eyes; 3 different bacterial species were isolated in 25 (27.47%) eyes. Based on Spearman's correlation test, there was a positive correlation between age of buffalo and number of the bacterial species isolated per eye ($R = 0.449$; $p = 0.001$).

The bacterial isolates consisted of 10 species belonging to 8 genera (Table 1). Gram-positive organisms were the predominant bacteria, representing 59% of isolates. Members of the genus *Staphylococcus* (29.20%) were the most prevalent. Genera of *Dermatophilus* and *Streptococcus* were less commonly isolated gram-positive organisms. Gram-negative organisms comprised 41% of isolates, with *Escherichia coli* being the most prevalent. *Edwardsiella*, *Klebsiella* and *Proteus* were infrequently isolated gram-negative genera. *E. coli* was the most frequent isolate in the 2-5 years group and Mazandarani ecotype, but in both sexes, other age groups and ecotypes, *Staphylococcus* sp., were the predominant isolates. Difference in quantity of isolates between sexes, ecotypes and age groups was not significant ($p > 0.05$).

Many studies have shown that gram-positive bacteria are usually predominant over gram-negative microorganisms in healthy eyes. This is consistent with our data where 59% of the isolates were gram-positive bacteria and *Staphylococcus* sp., predominated. Similar results have been found in healthy eye of horses (Vidal *et al.*, 2010), sheep (Dagnall, 1994), dogs (Prado *et al.*, 2005), cats (Espinola and Linelbaum, 1996), domestic rabbits (Cooper *et al.*, 2001), llamas (Gionfriddo *et al.*, 1991), Asian elephants (Tantivanich *et al.*, 2002), mule deers (Dubay *et al.*, 2000), raccoons (Spinelli *et al.*, 2010), opossums (Pinard *et al.*, 2002), iguanas (Taddei *et al.*, 2010), birds (Dupont *et al.*, 1994; Zenoble *et al.*, 1983; Wolf *et al.*, 1983) and in the swamp buffalos (Tantivanich *et al.*, 1988).

Table 1: Aerobic bacteria recovered from conjunctival sac of clinically normal Iranian water buffalo (*Bubalus bubalis*)

Bacteria	Number of Isolates (%)										
	Total	Sex		Ecotype				Age group			
		1	2	3	4	5	6	7	8	9	10
Gram-positive											
<i>Bacillus cereus</i>	43 (22.05)	14 (7.18)	29 (14.87)	17 (8.72)	3 (1.54)	8 (4.10)	5 (2.56)	15 (7.69)	5 (2.56)	1 (0.51)	22 (11.28)
<i>Staphylococcus epidermidis</i>	36 (18.46)	11 (5.64)	25 (12.82)	15 (7.69)	4 (2.05)	11 (5.64)	6 (3.08)	12 (6.15)	7 (3.59)	2 (1.02)	15 (7.70)
<i>Staphylococcus intermedius</i>	17 (8.72)	6 (3.08)	11 (5.64)	7 (3.59)	1 (0.51)	3 (1.51)	6 (3.08)	4 (2.05)	2 (1.05)	1 (0.51)	10 (5.13)
<i>Dermatophilus congolensis</i>	14 (7.18)	7 (3.59)	7 (3.59)	11 (5.64)	1 (0.51)	1 (0.51)	1 (0.51)	8 (4.10)	3 (1.53)	1 (0.51)	2 (1.05)
<i>Staphylococcus aureus</i>	4 (2.05)	1 (0.51)	3 (1.53)	-	2 (1.05)	-	2 (1.05)	-	-	-	4 (2.05)
<i>Streptococcus zooepidemicus</i>	1 (0.51)	-	1 (0.51)	-	-	-	1 (0.51)	-	-	-	1 (0.51)
Gram-negative											
<i>Escherichia coli</i>	52 (26.67)	16 (8.2)	36 (18.47)	20 (10.25)	6 (3.10)	9 (4.61)	17 (8.71)	11 (5.64)	10 (5.12)	3 (1.54)	28 (14.37)
<i>Edwardsiella tarda</i>	14 (7.18)	5 (2.56)	9 (4.61)	5 (2.56)	2 (1.02)	4 (2.05)	3 (1.53)	3 (1.53)	2 (1.02)	1 (0.51)	8 (4.10)
<i>Klebsiella</i> spp	9 (4.61)	4 (2.05)	5 (2.56)	5 (2.56)	-	1 (0.51)	3 (1.53)	4 (2.05)	1 (0.51)	-	4 (2.05)
<i>Proteus</i> spp	5 (2.55)	2 (1.05)	3 (1.53)	3 (1.53)	-	-	2 (1.05)	3 (1.53)	-	-	2 (1.05)

1: Male; 2: Female; 3: Azeri; 4: Guilani; 5: Khozestani; 6: Mazandarani; 7: yearlings; 8: 2-5 years of age; 9: 6-10 years of age; 10: 11-15 years of age

The most common gram-negative organisms present in the eyes vary among the species. *E.coli* was reported to be the most common gram-negative organism in pigs (Davidson *et al.*, 1994) and camels (Fahmi *et al.*, 2003), as well as in the present study in water buffalos.

D. congolensis with frequency of 7.18% is one of isolates that is reported for the first time from conjunctiva of animals in the present study. This organism is primarily known as a cutaneous pathogen in buffalos (Pal, 1995; Sharma *et al.*, 1992) and other animals (Pal, 1995), but it is able to cause disease in other tissues of various animals, such as oral cavity in buffalos (Kharole *et al.*, 1976) and cattle (Ibu *et al.*, 1987), lymph node in goats (Singh and Murty, 1978) and subcutaneous tissue in cats (Cararostas *et al.*, 1984). In Iran, there are reports of cutaneous form of disease in cattle (Jafari Shoorijeh *et al.*, 2008) and sheep (Hashemi *et al.*, 2004).

E. tarda with frequency of 7.18% was isolated from conjunctiva of animals for the first time in this study. This organism is principally associated with freshwater sources and animals that inhabit these ecosystems, such as amphibia, reptile and fish (Janda *et al.*, 1991). The buffalo is also one of those animals that like aquatic environments. The infections associated with this organism include gastroenteritis, septicemia, meningitis and wound infections such as cellulitis or gas gangrene associated with trauma to mucosal surfaces in humans (Nelson *et al.*, 2009; Janda *et al.*, 1991), septicemia, chronic enteritis and emphysematous putrefactive disease in animals (Galal *et al.*, 2005; Meyer *et al.*, 1973).

Considering the presence *D. congolensis* and *E. tarda* in ocular surface of buffalos, external eye infections with these opportunistic pathogens can be expected in situations where the normal defense mechanism of the ocular surface is damaged.

In our study diversity of nonpathogenic and potentially pathogenic bacteria was isolated, but, *Moraxella bovis* as a primary cause of infectious bovine keratoconjunctivitis (Brown *et al.*, 1998) was not isolated. This agent has been reported from healthy eyes of cattle (Powe *et al.*, 1992; Barber *et al.*, 1986; Wilcox, 1970) and bison (Davidson *et al.*, 1999), but not in swamp buffalo (Tantivanich *et al.*, 1988). In addition, this potentially pathogen has not been isolated from diseased eye of riverine buffalos (Rajesh *et al.*, 2009). It seems that *M. bovis* is not a common isolate of ocular surface in both affected and unaffected water buffalos.

It had previously been confirmed that age has various effects on the conjunctival normal flora in humans (Liu *et al.*, 2011; Rubio, 2006) and animals

(Andrew *et al.*, 2003; Cooper *et al.*, 2001; Whitley, 2000). Liu *et al.* (2011) gave evidence that age has a significant correlation with number of species per eye in humans (Liu *et al.*, 2011). Our study produced similar results to Liu *et al.* (2011), as the number of species of conjunctival bacterial flora from the older buffalos was greater than that of younger. This may be due to aged buffalos having a lower resistance from depressed ocular defense mechanisms (McClellan, 1997).

CONCLUSION

The results of this study were in agreement with previous studies indicating *Staphylococcus* spp as the most common inhabitant of the eyes of most animal species. This is first report of presence of *D. congolensis* and *E. tarda*, pathogenic and opportunist organisms, in the normal conjunctiva of animals. Incidence of bacterial species of conjunctival flora can be influenced by age of buffalo.

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