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## Localization of *Lactobacillus plantarum* in bacterial profile of conjunctiva of clinically healthy cattle

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**Abstract:** The aim of this study was to determine the normal conjunctival bacterial flora and the effects of sex and age variations on them in healthy cattle in Urmia, Iran. The animals (n = 50) were selected from 2 sexes and divided into 2 age groups. Swabs were taken from the inferior conjunctival sac of both eyes (n = 100) and were cultured on blood and MacConkey agar. All (100%) of the specimens yielded positive bacterial isolations. The bacterial isolates (in order of decreasing frequency) were *Lactobacillus plantarum*, *Escherichia coli*, *Bacillus cereus*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Staphylococcus epidermidis*, and *S. aureus*. There was no significant difference in number of isolates between sexes and age groups ( $P > 0.05$ ). In the present study *L. plantarum*, as a normal flora organism, was reported from the conjunctival sac in cattle for the first time.

**Key words:** *Lactobacillus plantarum*, bacterial flora, conjunctiva, cattle

### 1. Introduction

Being rich in nutrients, several microorganisms are often found on the eye's surface, i.e. the ocular flora (1). The conjunctival sac's bacterial microbiota is involved in the maintenance of ocular health by preventing overgrowth of potentially pathogenic agents (2). The normal flora can become potential pathogens in the case of corneal damage or decreased resistance of the host to infection (3). The bacterial flora of the normal conjunctiva has been reported in various domestic and wild animals (4). Gram-positive bacteria were reported as the most common isolates. Isolation of a specific organism from an animal with an ocular surface disease does not solely imply a cause-and-effect association. Therefore, ocular flora identification is essential to interpret the results of microbial cultures and to understand surface ocular disease dynamics and microbial interactions (5). The ocular bacterial microorganisms in healthy cattle have been studied in various countries (6–10); however, detailed study in this field has not been carried out in Iran. The purpose of this investigation was to identify the bacterial flora in the conjunctival sac of clinically normal cattle, and to determine the effect of sex and age variations on them in the geographical region of Urmia, in northwestern Iran.

### 2. Materials and methods

Iranian Holstein crossbred cattle were examined from March to June 2011 in 4 dairy farms in 4 different points of an Urmia suburb. The population sizes of the farms were nearly 500 heads for each farm. A total of 100 specimens were collected from both eyes of 50 (11 males, 22%; 39 females, 78%) healthy cattle. Based on their estimated denture ages, the animals were categorized into 2 groups of below and above 2 years of age (11 cases, 22% and 39 cases, 78%, respectively) and were examined for signs of ophthalmic or general clinical disease. The healthy cattle did not have clinical evidence or recent history of external ocular inflammation or disease. Samples were obtained from the inferior conjunctival sac of each eye using separate premoistened sterile swabs per eye, avoiding swab contact with the eyelashes or skin of the eyelids. Swabs were placed in tubes containing a sterile transport medium (peptone broth; Merck, Germany; catalog number: 107228) and were transported immediately to the microbiology laboratory in a chilled thermal box. Immediately after arrival, samples were cultivated on 5% ovine blood agar (Merck; catalog number: 110886) and MacConkey agar (HiMedia, India; catalog number: M081), then incubated at 37 °C for 24–48 h in an atmosphere containing 5% CO<sub>2</sub>. Different colony types were further isolated on blood agar plates. All distinct isolated colonies were typed and identified using standard microbiological methods (11).

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Statistical analyses were performed with the use of SPSS 15.0 with the statistical significance set at  $P < 0.05$ . Student's t-test was used to compare the number of bacterial species between different sex and age groups.

### 3. Results

The isolated organisms and the frequencies of isolation based on sex and age groups are shown in the Table. Bacteria were recovered from all (100%) of the 100 specimens. Gram-positive bacteria (55.83% of isolates) predominated over those that were gram-negative (44.17% of isolates). *Lactobacillus plantarum* (33.33%) was the most frequent isolate, followed by *Escherichia coli* (20.83%).

There was no significant difference in the number of isolates between sexes and age groups ( $P > 0.05$ ).

### 4. Discussion

In the current study, bacteria were recovered from all (100%) of the 100 samples of healthy cattle. Positive bacterial growth has been reported in 88.8%, 74.6%, 67%, and 45% of samples from the normal conjunctival sac of Asian elephants (12), birds of prey (13), cats (14), and dogs (15), respectively. The difference between percentages of positive cultures in each species of animal has been ascribed to the sampling techniques (16), but the difference between various animal species may be due to variability in the different ocular surface niches and defense mechanisms (2).

Based on the results of other investigations of healthy eyes, gram-positive bacteria are the predominant flora (4). This is consistent with our data, where 55.83% of the isolates were gram-positive bacteria. *L. plantarum* was found to be the most frequent isolate of the ocular surface in our study and is reported here for the first time as normal flora in the eye of cattle. This organism had previously been reported with frequency of 4.76% from a study on only dogs (17). In studies conducted in cattle, *Bacillus cereus* (6), *Staphylococcus epidermidis* (10), and unidentified gram-positive cocci (9) were reported to be the most frequent isolates. These differences may be due to sampling techniques used in different studies, geographical location, climate, season, nutritional status, and breed of cattle (5).

In surveys comparing bacterial flora of healthy eyes and eyes with external eye infections, similar organisms were isolated (4). Therefore, members of the normal microbial flora could themselves become pathogens.

Reportedly potential pathogens that inhabit the ocular surfaces without complications include *S. aureus*, *Streptococcus* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Moraxella* spp., and the Enterobacter group. Additionally, coagulase-negative staphylococci, *Corynebacterium* spp., and *Bacillus* spp., which exhibit no pathogenicity, can, on occasion, cause ocular disease (4,18). Most of these bacteria have been isolated in our study.

**Table.** Bacteria recovered from conjunctival sacs of healthy Iranian Holstein crossbred cattle.

Microorganism	Total	Number of isolates (%)			
		Sex		Age group	
		M	F	1	2
Gram-positive bacteria					
<i>Lactobacillus plantarum</i>	40 (33.33)	11 (9.16)	29 (24.16)	12 (9.99)	28 (23.13)
<i>Bacillus cereus</i>	23 (19.66)	4 (3.33)	19 (15.83)	2 (1.66)	21 (17.49)
<i>Staphylococcus epidermidis</i>	3 (2.5)	2 (1.66)	1 (0.83)	2 (1.66)	1 (0.83)
<i>Staphylococcus aureus</i>	1 (0.83)	-	1 (0.83)	-	1 (0.83)
Gram-negative bacteria					
<i>Escherichia coli</i>	25 (20.83)	3 (9.09)	22 (18.33)	5 (4.16)	20 (16.66)
<i>Proteus mirabilis</i>	22 (18.33)	8 (6.66)	14 (11.66)	6 (4.99)	16 (13.33)
<i>Enterobacter aerogenes</i>	6 (5)	-	6 (5)	1 (0.83)	5 (4.17)

M: male, F: female, 1: below 2 years of age, 2: above 2 years of age.

*Moraxella bovis*, as a primary cause of infectious bovine keratoconjunctivitis (19), was not isolated in our study. This agent has been reported from healthy eyes of cattle (9,10). Powe et al. (20), Momeni (8), and Ghadrddan et al. (7) isolated *M. bovis* from 1.88% (3/160), 1.73% (4/231), and 4.5% (9/200), respectively, of the conjunctival sacs of healthy cattle. On the other hand, some workers have failed to report *M. bovis* from normal eyes of cattle (6). It seems that *M. bovis* is not a common isolate of the eye surface in unaffected cattle.

Culture results for microflora can potentially vary due to season and ambient temperature at collection time both in normal and diseased eyes (5). In a study done by Wilcox, *M. bovis* was isolated from healthy eyes of cattle in summer and autumn, but not from any during winter or spring (9). This may explain the absence of the species in our cultures, not to mention the small sample size in our study.

In addition, the presence of *L. plantarum*, the most frequent isolate and a friendly organism, in the ocular

surface of cattle may inhibit the growth of certain pathogens.

In humans, many strains of the genus *Lactobacillus* colonize specific parts of the body, e.g., the oral cavity, the gastrointestinal tract, and the urogenital tract, where they play an important role in the competitive exclusion of pathogens (21,22). Antimicrobial activity of *Lactobacillus* strains against bacterial pathogens emerges to be multifactorial and to include the production of hydrogen peroxide, lactic acid, bacteriocin-like molecules, and unknown heat-stable, nonlactic-acid molecules (23). Other mechanisms proposed for their activity are competition for nutrients (24), adhesion inhibition of pathogens to the surface, and simulation of the immune system (25).

In this study, *Lactobacillus plantarum* was reported for first time in the conjunctival sac of cattle. We suggest an increase of the test profiles and, more specifically, further investigation of the prevalence and potential significance of *L. plantarum* in the normal conjunctiva in cattle.

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