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Conjunctival cytological examination, bacteriological culture, and antimicrobial resistance profiles of healthy Mediterranean buffaloes (*Bubalus bubalis*) from Southern Italy

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ABSTRACT

Objective: To assess normal conjunctival cytological and bacteriological/fungal flora features in the Mediterranean buffalo (*Bubalus bubalis*).
Methods: Swabs were taken from the inferior conjunctival sac of both eyes of 57 healthy female buffaloes aged 24–36 months, with no evidence of ocular disease, farmed in Campania region (Southern Italy), for microbiological analysis. Conjunctival eye specimens of both eyes were subsequently obtained by a cyto-brush, for cytological analysis. The antimicrobial susceptibility of bacterial isolates was also determined using the disk-diffusion method on Mueller Hinton agar plates.
Results: Cytological examination of conjunctival swab specimens (114 eyes) revealed epithelial cells (basal, intermediate, columnar and superficial) in all samples, whereas neutrophils, lymphocytes and plasma cells were present in 70%, 10% and 2% of samples, respectively. Microorganisms, for a total of 261 aerobic bacteria and 6 fungi, were isolated from 112/114 conjunctival samples [98.25%; 95% confidence interval (*CI*): 93.18–99.70]. Only two conjunctival swabs did not yield bacteria and/or fungi (2/114, 1.75%; 95% *CI*: 0.30–6.82). Gram-positive aerobes were most commonly cultured (181/261, 69.35%; 95% *CI*: 63.31–74.81), with *Enterococcus faecium* and *Staphylococcus lentus* predominating. *Escherichia coli* was the most frequently isolated as Gram-negative bacteria (80/261, 30.65%; 95% *CI*: 25.19–36.69). The antimicrobial resistance patterns of the isolated bacteria showed amoxycillin/clavulanic acid and cephalothin as the least sensitive antibiotics for both Gram-positive and Gram-negative bacteria.
Conclusions: These results provided first information on normal conjunctival ocular microflora and cytological features in Mediterranean buffalo.

1. Introduction

Buffalo-breeding in Italy is an important zootechnical and economical reality, especially in Campania Region where buffalo herds represent 80% of the national buffalo assets.

Characterization of bacterial ocular flora of Mediterranean buffaloes is of special interest for animal and human health, since microorganisms can be transmitted to human populations that are in direct contact with these animals and can also be responsible for meat and milk deterioration. In addition, these animals have a potential role as vectors of antimicrobial resistant bacteria [1].

To our knowledge, there are very few reports describing morphological and functional features of buffalo eye [2], rare reports on congenital ocular abnormalities [3], and no reports on conjunctival microflora cytological features and/or incidence of ocular diseases in this species. Knowledge of the conjunctival flora of healthy buffaloes may be useful in the treatment of external ocular diseases. The aim of this study

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was to evaluate cytologically and microbiologically the ocular conjunctiva of healthy Mediterranean buffaloes to determine the aerobic bacterial and fungal flora population and cytologic features of normal eyes. The antimicrobial susceptibility of bacterial isolates was also determined.

2. Materials and methods

2.1. Sample collection

Fifty-seven (114 eyes) clinically healthy female Mediterranean buffaloes, 24–36 months old, were sampled in November 2014, in a farm in the province of Salerno (Campania Region, Italy). Temperature was about 15 °C and all Mediterranean buffaloes were housed outdoors in paddocks.

Each Mediterranean buffaloes underwent a thorough physical and ophthalmic examination including slit-lamp biomicroscopy and indirect ophthalmoscopy, to exclude animals with clinical signs of systemic and/or ocular diseases. The buffaloes were physically restrained by a skilled operator in a stock. The palpebral rim and the conjunctival sac of the lower lid were cleaned of mucus, debris and fluid. Pharmacologic induction of mydriasis was not necessary because of incomplete pupillary constriction.

For microbiology purpose, swabs were taken from the inferior conjunctival sac of both eyes of each buffalo. Topical anesthetics were not used. The microbiological sterile swab was removed aseptically from protective cover and was used dry until processing. The inferior eyelid was everted and specimens were collected by rotating the sterile swab.

Conjunctival eye specimens of both eyes were subsequently obtained by a cyto-brush, that was rotated in the inferior conjunctival fornix in a single direction five times and then lightly rolled onto a clean glass slide, as already described [4]. Slides were air-dried.

Swabs, in Amies transport medium (Oxoid Ltd, UK), and slides were sent to the laboratory on ice packs (4 °C) within 4 h from collection.

2.2. Cytological examination

Slides were stained with May-Grünwald-GiemsaQuik stain (Bio-Optica, Milan, Italy), observed with light microscope (E–600; Nikon Eclipse, Tokyo, Japan) at different magnifications and then examined at 40× magnification to differentiate and count the epithelial and inflammatory cells. The images were captured at the microscope coupled to a videocamera (DXM 1200F; Nikon Digital Camera, Tokyo, Japan), stored in the digital memory, and shown on the monitor.

Epithelial cells were classified as already described and quantified by counting 600 cells for each eye [5]. Inflammatory cells were counted for 20 high power field. The results were expressed as percentage of each epithelial cell and as median (range) of each inflammatory cell type.

2.3. Microbiological analysis

For conventional bacteriological detection, samples were cultured on blood agar base supplemented with 5% sheep blood, selective medium used for the isolation of Gram-positive microorganisms, on mannitol-salt agar, selective medium to identify staphylococci, and on MacConkey agar, selective and differential medium to grow Gram-negative bacteria. All agars were incubated for 24–48 h at 37 °C under aerobic conditions. Colonies were then subjected to classic methods including Gram staining, colony morphology and biochemical tests for identification (Api System bioMèrieux), according to manufacturer's instructions.

The samples were also cultured for fungi on Sabouraud dextrose agar with chloramphenicol and incubated under normal atmospheric conditions at 30 °C up to 7 days. The plates were all microbiological media from Oxoid Ltd, UK.

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the disk-diffusion method on Mueller Hinton agar plates as recommended by the Clinical and Laboratory Standards Institute guidelines for veterinary isolates (CLSI 2008).

Antimicrobial agents were: amoxycillin/clavulanic acid (30 μg), amikacin (30 μg), bacitracin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), ceftazidime (30 μg), clindamycin (2 μg), ceftriaxone (30 μg), cefuroxime (30 μg), cefoperazone (30 μg), doxycycline (30 μg), ceftiofur (30 μg), cephalothin (30 μg), enrofloxacin (5 μg), gentamicin (10 μg), neomycin (30 μg), sulphamethoxazole/trimethoprim (25 μg), tetracycline (30 μg), and tobramycin (30 μg). Antimicrobial disks were purchased from Oxoid Ltd, UK. For analysis purposes, isolates with intermediate susceptibility were recorded as resistant.

3. Results

3.1. Cytological findings

In all slides (100%) conjunctival cells identified as epithelial cells of various types (basal, intermediate, columnar and superficial) were observed (Figure 1). Inclusion bodies were not noticed in any specimen. Basal (42%) and intermediate (40%) cells were the most represented, while columnar (11%) and superficial (7%) cells were rarely seen.

Neutrophils were observed in 80 eyes (70%) (median 3; range 0–30 cells): in 34 (30%) eyes they had a low incidence (1–

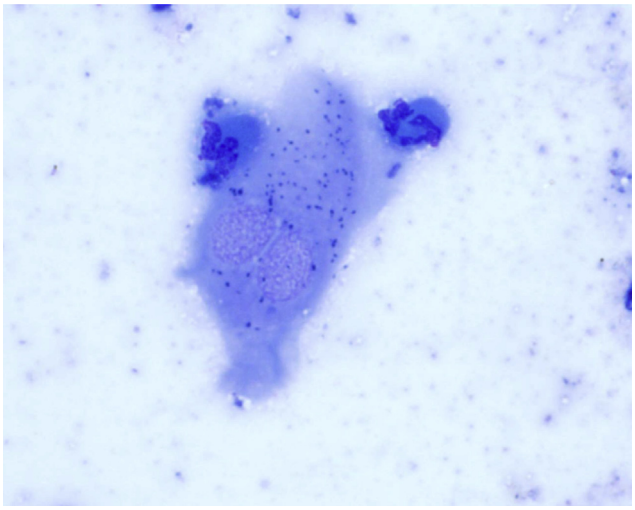

Figure 1. Photomicrograph of a conjunctival swab specimen from a healthy buffalo illustrating a superficial epithelial cell with two neutrophils. (May-Grünwald-GiemsaQuik stain, 40×).

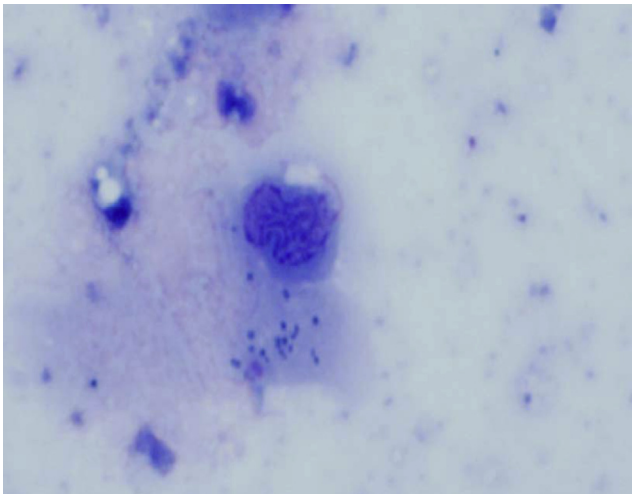

Figure 2. Photomicrograph of a conjunctival swab specimen from a healthy buffalo illustrating a degenerated neutrophil associated to bacteria. (May-Grünwald-GiemsaQuik stain, original magnification 40×).

5 cells), in 20 eyes (17%) they had a moderate incidence (5–10 cells) and in 26 eyes (23%) they had a high incidence (> 10). In 25 eyes (22%) neutrophils were degenerate, with swollen pale nuclei (Figure 2).

The lymphocytes were present in eleven eyes (10%) (0; 0–18), always in association with neutrophils. Plasma cells were observed only in two eyes (2%) (0; 0–3). In 22 slides (19%) extracellular bacteria were found. In one slide a *Malassezia* spp. was identified.

3.2. Microbiological findings

From 114 conjunctival swabs, 181 Gram-positive aerobic bacteria (181/261, 69.35%; 95% *CI*: 63.31–74.81), and 80 Gram-negative aerobic bacteria (80/261, 30.65%; 95% *CI*: 25.19–36.69) were cultured (Table 1). Only two conjunctival swabs did not yield bacteria (2/114, 1.75%; 95% *CI*: 0.30–6.82). All animals had a positive eye culture for one or both eyes. Moreover, we revealed 3/114 (2.63%; 95% *CI*: 0.68–8.07) specimens as negative for Gram-positive bacteria culture, whereas 32/114 (28.07%; 95% *CI*: 20.25–37.39) specimens as negative for Gram-negative bacteria culture.

Gram-positive were most commonly isolated with *E. faecium* and *S. lentus* predominating and showing the same percentage value (63/181, 34.81%; 95% *CI*: 27.99–42.28). The other isolates as Gram-positive bacteria were as follow: *S. capitis* (14), *S. uberis* (13), *S. xylosus* (12), *G. sanguinis* (9), *A. viridans* (1), *S. hyicus* (1), *E. durans* (1) and *Micrococcus* spp. (1). *E. coli* was the most frequently isolated as Gram-negative bacteria (55/80, 68.75%; 95%

Table 1

Microorganisms isolated from 114 conjunctival swabs of Mediterranean buffaloes.

Animals	Ocular swabs	Gram-positive bacteria	Gram-negative bacteria	Mycetes
1	Right	*S. lentus/E. faecium*	*E. coli*	Negative
	Left	*S. lentus/G. sanguinis*	*E. coli*	Negative
2	Right	*S. lentus/G. sanguinis*	*E. coli*	Negative
	Left	*S. lentus/E. faecium*	*E. coli*	Negative
3	Right	*S. lentus*	*E. coli*	Negative
	Left	*S. lentus/E. faecium*	*E. cloacae*	Negative
4	Right	*A. viridans*	*E. cloacae*	Negative
	Left	*S. lentus/G. sanguinis*	Negative	Negative
5	Right	*S. lentus/E. faecium*	Negative	Negative
	Left	*S. lentus/E. faecium*	*E. cloacae*	Negative
6	Right	*S. xylosus/E. faecium*	Negative	Negative
	Left	*S. hyicus/Enterococcus durans*	Negative	Negative
7	Right	*S. lentus/E. faecium*	*E. sakazakii*	Negative
	Left	*S. lentus/G. sanguinis*	*E. coli*	*Malassezia* spp.
8	Right	*S. xylosus*	*E. coli*	Negative
	Left	*S. xylosus/E. faecium*	*E. sakazakii*	Negative
9	Right	*S. lentus/G. sanguinis*	*C. koseri*	Negative
	Left	*S. lentus/G. sanguinis*	*C. koseri*	Negative
10	Right	*S. lentus/E. faecium*	*E. coli*	Negative
	Left	*S. lentus*	*Pantoea* spp.*3*	Negative
11	Right	*E. faecium*	*E. coli*	Negative
	Left	*E. faecium*	*E. coli*	Negative
12	Right	*S. lentus/E. faecium*	*E. coli*	Negative
	Left	*S. capitis*	*E. coli*	Negative
13	Right	*S. xylosus*	*E. cloacae*	Negative
	Left	*S. xylosus/Micrococcus* spp.	*E. cloacae*	Negative
14	Right	*S. lentus*	*E. cloacae*	Negative
	Left	*S. xylosus/E. faecium*	*E. cloacae*	Negative
15	Right	*S. lentus/E. faecium*	*E. coli*	Negative
	Left	*S. lentus/E. faecium*	*E. coli*	Negative
16	Right	*S. lentus/S. uberis*	*E. cloacae*	Negative
	Left	*S. xylosus/G. sanguinis*	*E. cloacae*	Negative
17	Right	*S. capitis/E. faecium*	*E. coli*	Negative
	Left	*S. lentus/E. faecium*	*E. coli*	*Alternaria* spp.
18	Right	Negative	*E. coli*	Negative
	Left	*S. capitis*	*C. baumanii*	Negative
19	Right	*S. capitis*	Negative	Negative
	Left	*E. faecium*	*Pantoea* spp.*4*	Negative

(*continued on next page*)

Table 1 (*continued*)

Animals	Ocular swabs	Gram-positive bacteria	Gram-negative bacteria	Mycetes
20	Right	*S. capitis*	Negative	Negative
	Left	*S. capitis*	*E. sakazakii*	Negative
21	Right	*E. faecium*	Negative	Negative
	Left	*S. capitis/E. faecium*	*E. coli*	Negative
22	Right	*S. lentus*	Negative	Negative
	Left	*S. lentus*	*E. coli*	Negative
23	Right	*S. capitis/E. faecium*	*E. coli*	Negative
	Left	*S. capitis/E. faecium*	*E. coli*	*Alternaria* spp.
24	Right	*S. lentus*	*E. coli*	Negative
	Left	*S. lentus/A. viridans*	*E. coli*	*Alternaria* spp.
25	Right	Negative	Negative	Negative
	Left	*S. lentus*	*E. coli*	Negative
26	Right	*S. lentus/E. faecium*	Negative	Negative
	Left	*S. lentus/E. faecium*	*E. cloacae*	Negative
27	Right	*G. sanguinis*	*E. coli*	Negative
	Left	*G. sanguinis*	*E. coli*	Negative
28	Right	*E. faecium*	*E. coli*	Negative
	Left	*E. faecium*	*E. coli*	Negative
29	Right	*S. xylosus*	Negative	Negative
	Left	*S. xylosus*	Negative	Negative
30	Right	*S. lentus*	Negative	Negative
	Left	*S. lentus*	Negative	Negative
31	Right	*S. lentus/E. faecium*	*E. coli*	Negative
	Left	*S. lentus/E. faecium*	*E. coli*	Negative
32	Right	*S. lentus/S. uberis*	Negative	Negative
	Left	*S. lentus/S. uberis*	Negative	Negative
33	Right	*S. lentus/S. uberis*	*E. coli*	Negative
	Left	*S. lentus/S. uberis*	*E. coli*	Negative
34	Right	*S. capitis/E. faecium*	*E. coli*	Negative
	Left	*S. capitis/E. faecium*	*E. coli*	Negative
35	Right	*S. capitis/E. faecium*	*E. coli*	Negative
	Left	*S. capitis/E. faecium*	*E. coli*	Negative
36	Right	*S. lentus/E. faecium*	*E. coli*	Negative
	Left	*S. lentus*	*E. coli*	Negative
37	Right	*S. lentus/E. faecium*	Negative	Negative
	Left	*S. lentus/E. faecium*	*E. coli*	Negative
38	Right	*S. lentus/E. faecium*	*E. coli*	Negative
	Left	*S. lentus/E. faecium*	*E. coli*	Negative
39	Right	*S. lentus/E. faecium*	Negative	Negative
	Left	*S. lentus/E. faecium*	*E. coli*	Negative
40	Right	*S. lentus/E. faecium*	*E. coli*	Negative
	Left	*S. lentus/E. faecium*	Negative	Negative
41	Right	*S. uberis*	Negative	Negative
	Left	*S. uberis*	Negative	Negative
42	Right	*E. faecium*	*E. coli*	Negative
	Left	*E. faecium*	*E. coli*	Negative
43	Right	*S. xylosus/E. faecium*	*E. cloacae*	Negative
	Left	*S. xylosus/E. faecium*	*E. cloacae*	*Candida* spp.
44	Right	*S. lentus/E. faecium*	*E. coli*	Negative
	Left	*S. capitis/E. faecium*	*E. coli*	*Candida* spp.
45	Right	*S. lentus/E. faecium*	*E. coli*	Negative
	Left	*S. lentus/E. faecium*	*E. coli*	Negative
46	Right	*S. lentus/S. uberis*	Negative	Negative
	Left	*S. lentus/S. uberis*	Negative	Negative
47	Right	*S. lentus/E. faecium*	*E. cloacae*	Negative
	Left	*S. lentus/E. faecium*	*E. cloacae*	Negative
48	Right	*S. lentus/S. uberis*	*E. sakazakii*	Negative
	Left	*S. lentus*	Negative	Negative
49	Right	*S. lentus/S. uberis*	Negative	Negative
	Left	*S. uberis*	Negative	Negative
50	Right	*E. faecium*	*E. coli*	Negative
	Left	*E. faecium*	*E. coli*	Negative
51	Right	*E. faecium*	*E. coli*	Negative
	Left	*E. faecium*	*E. coli*	Negative
52	Right	*S. xylosus/S. uberis*	Negative	Negative
	Left	*S. xylosus/S. uberis*	Negative	Negative
53	Right	*S. lentus*	Negative	Negative
	Left	Negative	Negative	Negative
54	Right	*E. faecium*	*Serratia odorigera*	Negative
	Left	*E. faecium*	Negative	Negative
55	Right	*S. lentus/E. faecium*	Negative	Negative
	Left	*S. lentus/E. faecium*	*Raoutella ornithinolytica*	Negative

(*continued on next page*)

Table 1 (*continued*)

Animals	Ocular swabs	Gram-positive bacteria	Gram-negative bacteria	Mycetes
56	Right	*S. lentus/E. faecium*	*E. coli*	Negative
	Left	*S. lentus/E. faecium*	*E. coli*	Negative
57	Right	*S. lentus/E. faecium*	Negative	Negative
	Left	*S. lentus/E. faecium*	Negative	Negative

S. lentus: *Staphylococcus lentus*; *E. faecium*: *Enterococcus faecium*; *G. sanguinis*: *Globicatella sanguinis*; *A. viridans*: *Aerococcus viridans*; *S. xylosus*: *Staphylococcus xylosus*; *S. hyicus*: *Staphylococcus hyicus*; *S. capitis*: *Staphylococcus capitis*; *S. uberis*: *Streptococcus uberis*; *E. coli*: *Escherichia coli*; *E. cloacae*: *Enterobacter cloacae*; *E. sakazakii*: *Enterobacter sakazakii*; *C. koseri*: *Citrobacter koseri*; *C. baumanii*: *Citrobacter baumanii*.

Table 2

Resistance to nineteen antimicrobial agents among 181 Gram-positive isolates.

Antibiotics	No. of resistant isolates	%	95% *CI*
Amoxycillin/clavulanic acid	30	16.57	11.63–22.98
Ceftazidime	28	15.47	10.69–21.76
Cephalothin	27	14.92	10.23–21.14
Cefuroxime	21	11.60	7.49–17.40
Ceftriaxone	17	9.39	5.73–14.85
Gentamicin	16	8.84	5.30–14.20
Clindamycin	15	8.29	4.87–13.55
Cefoperazone	15	8.29	4.87–13.55
Amikacin	14	7.73	4.45–12.89
Doxycycline	14	7.73	4.45–12.89
Tobramycin	14	7.73	4.45–12.89
Ceftiofur	13	7.18	4.04–12.23
Tetracycline	12	6.63	3.63–11.57
Bacitracin	11	6.08	3.23–10.89
Enrofloxacin	10	5.52	2.83–10.21
Sulphamethoxazole/ trimethoprim	9	4.97	2.45–9.53
Ciprofloxacin	8	4.42	2.07–8.83
Chloramphenicol	7	3.87	1.71–8.12
Neomycin	7	3.87	1.71–8.12

Table 3

Resistance to nineteen antimicrobial agents among 80 Gram-negative isolates.

Antibiotics	No. of resistant isolates	%	95% *CI*
Amoxycillin/clavulanic acid	12	15.00	8.32–25.13
Cephalothin	12	15.00	8.32–25.13
Cefuroxime	11	13.75	7.39–23.69
Cefoperazone	10	12.50	6.48–22.24
Ceftriaxone	9	11.25	5.59–20.76
Bacitracin	7	8.75	3.89–17.75
Tobramycin	7	8.75	3.89–17.75
Ceftiofur	7	8.75	3.89–17.75
Neomycin	7	8.75	3.89–17.75
Ceftazidime	6	7.50	3.09–16.20
Clindamycin	6	7.50	3.09–16.20
Doxycycline	6	7.50	3.09–16.20
Gentamicin	5	6.25	2.32–14.62
Chloramphenicol	4	5.00	1.61–12.99
Amikacin	4	5.00	1.61–12.99
Tetracycline	3	3.75	0.97–11.32
Sulphamethoxazole/ trimethoprim	1	1.25	0.07–7.73
Ciprofloxacin	1	1.25	0.07–7.73
Enrofloxacin	0	–	–

CI: 57.29–78.39). Furthermore were identified *E. cloacae* (14 strains), *E. sakazakii* (4), *C. koseri* (2), *Pantoea* spp.4 (1), *Pantoea* spp.3 (1), *Serratia odorigera* (1), *C. baumanii* (1) and *Raoutella ornithinolytica* (1).

Most conjunctival specimens were negative for growth of fungi. Fungal genera were recovered only in six specimens (6/114, 5.26%; 95% *CI*: 2.16–11.57), and we identified *Candida* spp. (2), *Alternaria* spp. (3), and *Malassezia* spp. (1).

Moreover, we observed 23 animals (23/57, 40.35%; 95% *CI*: 27.84–54.16) with bilateral positive eye culture presenting the same conjunctival bacteriological/fungal flora feature in both eyes.

3.3. Antimicrobial susceptibility testing

The antibiotic sensitivity of isolated and identified Gram-positive and Gram-negative are summarized in Tables 2 and 3, respectively. Amoxycillin/clavulanic acid, ceftazidime and cephalothin were the least sensitive antibiotics for Gram-positive bacteria, whereas amoxycillin/clavulanic acid and cephalothin were the least sensitive antibiotics for Gram-negative bacteria. No Gram-negative bacteria showed enrofloxacin-resistance.

4. Discussion

Normal cytology findings of the eyes are rarely reported in the veterinary literature except for the canine, feline and equine conjunctival cells that are well described [4–6].

The differential cell count of conjunctival epithelial cells, herein described, showed the highest mean densities in cylindrical and basal cells than intermediate and superficial cells. In contrast with our results in Mediterranean buffaloes, the average differential cell count in horses showed a majority of basal cells [6], whereas a predominance of superficial cells was recorded in dogs [5].

Furthermore, the presence of neutrophils and lymphocytes in 70% and 10% of samples, respectively in Mediterranean buffaloes differs from the data from normal eyes of cats and dogs, where the presence of lymphocytes and neutrophils resulted to be in low numbers [5].

In animals that lack any clinical evidence of conjunctivitis, lymphocytes and plasma cells have been considered normal cytologic findings, while other leukocytes were supposed to be consistent with blood contamination, due to sampling [7]. However, in this study the high number of neutrophils and the high percentage of degenerate neutrophils without erythrocytes, observed in some slides, were consistent with a typical pattern of ocular surface inflammation, in absence of clinical signs.

In the present study only two conjunctival swabs did not yield bacteria. In cattle, a higher incidence (55%) of negative samples was reported in Turkey [8], while bacteria were cultured from all tested eyes in North American bison [9]. Probably the recovery of bacteria in all conjunctival swabs from bison and Mediterranean buffaloes compared with cattle may be due, in part, to the different environmental conditions. These latter could play a critical role in conjunctival contamination. On the other hand, several parameters may influence the prevalence of certain microorganisms, such as geographical area, climate, season, species, hygienic conditions [10].

Aerobic bacterial culture of conjunctival swab specimens in the present study yielded predominantly Gram-positive bacteria (69.35%) with *Staphylococcus* spp. and *Enterococcus* spp. most frequently isolated. Gram-negative bacteria were less commonly isolated (30.65%). A significant presence of Gram-positive bacteria was recently reported in neotropical primates from Salvador, Brazil [11].

In Turkey the most common bacteria isolated from the eyes of healthy cattle were *Streptococcus* and *Staphylococcus* spp. [8]. In USA, Hare *et al.* used molecular biology-based methods to determine the composition of bacterial flora in the conjunctivae of normal dairy and beef cattle from Maryland, and *Staphylococcus crocelyticus*, *Staphylococcus sciuri* and Gram-negative bacteria from the family Enterobacteriaceae were most commonly isolated [12]. In an Iranian study *Bacillus cereus* and *Corynebacterium pseudotubercolosis* predominated in conjunctival sac of normal cows [13]. Finally, conjunctival bacteriologic culture results from a herd of North American bison identified *Bacillus* spp. and *Micrococcus* spp. as the prevalent organisms in bison [9].

However, *Staphylococcus* spp. show up as the most common Gram-positive bacteria isolated in our study (90/181, 49.72%; 95% *CI*: 42.25–57.21%), as already reported in many mammalian species [10]. *Staphylococcus* are ubiquitous microflora of the skin and mucous membranes and they are common etiologic agent of suppurative ocular and periocular infections [10]. We identified as Gram-positive bacteria a single strain of *S. hyicus*, which role as commensal on the skin and mucosal surfaces is well known. In any case, also *S. hyicus* is a potential pathogen, characterized by positive reactions to the coagulase test, and may cause local lesions and serious opportunistic infections in animals and humans [14]. Of course, commensals can become pathogens in the right setting, such as debilitated or immunocompromised patients, or when the resident microbiota is altered by physical or chemical injuries of the eye.

The environmental conditions and behaviours of the buffaloes may explain the recovery of *Enterococcus* spp. and many bacteria of Enterobacteriaceae family (in particular genera: *Escherichia* and *Enterobacter*) from the conjunctivae of these animals. In many farms in Campania, buffaloes are kept in paddocks, in humid environments, all year long, and they have straight contact with their feces. *E. faecium* can be a commensal in the human intestine, but it may be also a pathogen, causing diseases like neonatal meningitis and sepsis [15,16].

Furthermore, we found a relatively high presence of Gram-negative bacteria (80/261, 30.65%; 95% *CI*: 25.19–36.69) with high prevalence of *E. coli* (55/80, 68.75%; 95% *CI*: 57.29–78.39) that in the Mediterranean buffaloes can be attributed to their habitats.

Numerous fungi infect the eye either by direct introduction through trauma, by extension from infected adjacent tissues, or by hematogenous dissemination to the eye, but the percentage of these cases is usually low. Fungal infections of the eye are important amongst the clinical conditions responsible for ocular morbidity and blindness. Our specimens revealed the presence of fungal strains in the lowest percentage (6/114, 5.26%; 95% *CI*: 2.16–11.57). In Florida fungi were isolated from 100% of the healthy cows tested [17]. In Italy the total number of eyes of healthy cows positive for fungi ranged from 85% to 100%, from 65% to 95%, and from 55% to 95% on the basis of different management of the farms in which cows were housed [18].

On the basis of the antimicrobial test, amoxycillin/clavulanic acid and cephalothin were the least sensitive antibiotics for both Gram-positive and Gram-negative bacteria. No Gram-negative bacteria showed enrofloxacin-resistance which has been already reported for *E. coli* isolated from dogs and cats [19].

Monitoring antibiotic resistance patterns may serve as an early indicator of changes in antibiotic susceptibility of clinical isolates. The growing problem of antimicrobial resistance has become a significant public health concern worldwide, and it involves practically all types of bacteria both pathogenic and non pathogenic bacteria.

In conclusion, a different conjunctival flora in buffaloes in Campania compared to previous reports regarding cattle in various geographic area and North American bison was demonstrated; these differences may be correlated with environmental conditions and behaviours of the buffaloes; knowledge of normal ocular flora in buffaloes is very important in order to improve understanding about the comparative ocular flora, to help an immediate therapy of external ocular disease and to discern pathogens from contaminant.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Oliveira AAF, Pineiro JW Jr, Mota RA, Cunha MLRS, Lopes CAM, Rocha NS. Phenotype characterization of *Staphylococcus* species strains isolated from buffalo (*Bubalus bubalis*) milk. *J Vet Diagn Invest* 2011; **23**: 1208-11.

[2] Di Pietro S, Pugliese M, Pugliese A. Clinical features of normal buffalo eye (*Bubalus bubalis*): reference ranges. *Large Anim Rev* 2012; **18**: 299-304.

[3] Sarrafzadeh-Rezaei F, Farshid AA, Saifzadeh S. Congenital ocular dermoid cyst in a river buffalo (*Bubalus bubalis*) calf. *J Vet Med A* 2007; **54**: 51-4.

[4] Featherstone HS, Heinrich CL. Ophthalmic examination and diagnostics. In: Gelatt KN, Gilger BC, Kern TJ, editors. *Veterinary ophthalmology*. 5th ed. Hoboken, NJ: Wiley-Blackwell Publishing; 2013, p. 576-7.

[5] Prasse KW, Winston SM. The eyes and associated structures. In: Valenciano AC, Cowell RL, editors. *Cowell and Tyler's diagnostic cytology and hematology of the dog and cat*. 4th ed. Chicago: Mosby; 2013.

[6] Bourges-Abella N, Raymond-Letron I, Diquelou A, Guillot E, Regnier A, Trumel C. Comparison of cytologic and histologic

evaluations of the conjunctiva in the normal equine eye. *Vet Ophthalmol* 2007; **10**: 12-8.

[7] Coster ME, Stiles J, Krohne SG, Raskin RE. Results of diagnostic ophthalmic testing in healthy guinea pigs. *J Am Vet Med Assoc* 2008; **232**(12): 1825-33.

[8] Okumus Z, Kirecci E, Kisa F, Kaya M. Screening conjunctival bacterial flora and antibiogram tests in cattle. *J Anim Vet Adv* 2005; **4**: 845-7.

[9] Davidson HJ, Vestweber JG, Brightman AH, Van Slyke TH, Cox LK, Chengappa MM. Ophthalmic examination and conjunctival bacteriologic culture results from a herd of North American bison. *J Am Vet Med Assoc* 1999; **215**(8): 1142-4.

[10] Gould D, Papasouliotis K. Clinical microbiology and parasitology. In: Gelatt KN, Gilger BC, Kern TJ, editors. *Veterinary ophthalmology*. 5th ed. Hoboken, NJ: Wiley-Blackwell Publishing; 2013, p. 300-50.

[11] Oriá AP, Pinna MH, Almeida DS, da Silva RM, Pinheiro AC, Santana FO, et al. Conjunctival flora, Schirmer's tear test, intraocular pressure, and conjunctival cytology in neotropical primates from Salvador, Brazil. *J Med Primatol* 2013; **42**: 287-92.

[12] Hare WR, Hoyt PG, Hohn C, Higgins JA. Ribosomal RNA-based analysis of the bacterial flora from the conjunctivae of cattle with bovine keratoconjunctivitis (BKC). *Vet Microbiol* 2008; **131**(3–4): 358-68.

[13] Kojouri GA, Ebrahimi A, Nikookhan F. Systemic dexamethasone and its effect on normal aerobic bacterial flora of cows. *Pak J Biol Sci* 2007; **10**(12): 2095-7.

[14] Casanova C, Iselin L, von Steiger N, Droz S, Sendi P. *Staphylococcus hyicus* bacteremia in a farmer. *J Clin Microbiol* 2011; **49**(12): 4377-8.

[15] Willems RJL, van Schaik W. Transition of *Enterococcus faecium* from comensal organism to nosocomial pathogen. *Future Microbiol* 2009; **4**: 1125-35.

[16] Knoll BM, Hellmann M, Kotton CN. Vancomycin-resistant *Enterococcus faecium* meningitis in adults: case series and review of the literature. *Scand J Infect Dis* 2013; **45**(2): 131-9.

[17] Samuelson DA, Andresen TL, Gwin RM. Conjunctival fungal flora in horses, cattle, dogs, and cats. *J Am Vet Med Assoc* 1984; **184**: 1240-2.

[18] Sgorbini M, Barsotti G, Nardoni S, Brombin M, Sbrana A, Mancianti F, et al. Seasonal prevalence of fungi in the conjunctival fornix of healthy cows during a 2-year study. *Vet Ophthalmol* 2010; **13**: 227-34.

[19] Shaheen BW, Nayak R, Foley SL, Boothe DM. Chromosomal and plasmid-mediated fluoroquinolone resistance mechanisms among broad-spectrum-cephalosporin-resistant *Escherichia coli* isolates recovered from companion animals in the USA. *J Antimicrob Chemother* 2013; **68**(5): 1019-24.