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Conjunctival Cytology and Bacterial Isolation from Arabian Horses with Conjunctivitis in Saudi Arabia

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ABSTRACT

Cytological and bacterial analyses are vital for diagnosing equine conjunctival diseases. These analyses facilitate accurate pathogen detection and enable targeted treatments. The present study aimed to describe and compare the conjunctival cytology and bacterial flora of clinically healthy Arabian horses and those with conjunctivitis in the Eastern Province of Saudi Arabia. Eighteen Arabian horses, including 5 healthy and 13 with conjunctivitis, were referred to King Faisal Hospital and examined. Healthy horses were distinguished from those with conjunctivitis through case history and an ocular examination. Conjunctival swabs were collected for cytological analysis and for aerobic bacterial culture and identification, with Diff-Quik staining. Differential cell counts were compared across the groups, and the findings were correlated with bacterial isolates. Biochemical identification of bacterial isolates was performed using conventional methods, including Gram staining and catalase and oxidase tests. Healthy horses exhibited a cytological profile primarily composed of superficial epithelial cells (59 ± 12.0%), with only a small number of inflammatory cells, including neutrophils and eosinophils. In contrast, horses with conjunctivitis exhibited a significant decrease in superficial epithelial cells (28 ± 17%) and a significant increase in basal cells (5 ± 3.5%). Conjunctival samples exhibited marked neutrophilia (32 ± 12.5%) and a significant increase in eosinophils (12 ± 9.5%) compared to healthy controls, indicating an inflammatory condition in the conjunctiva. Bacterial growth was found in 40% of healthy horse samples and in 92% of infected horse samples. *Staphylococcus aureus* was the most common isolate in infected horse samples. Gram-negative bacteria, including *Moraxella* sp., *Escherichia coli*, and *Klebsiella* sp., were isolated from 7 of 13 affected horses and were associated with a severe neutrophilic response (39 ± 6.5%) and significant epithelial disruption. Conjunctivitis in Arabian horses in the Eastern Province of Saudi Arabia was characterized by neutrophilic and eosinophilic inflammation with concurrent epithelial disruption.

Keywords: Bacteria, Conjunctivitis, Cytology, Equine, Ocular, Swab

INTRODUCTION

Equine populations, especially those residing in arid regions of the Middle East, such as Saudi Arabia, are persistently exposed to environmental stressors, including hot, dry, and dusty conditions (Beech et al., 2003). Although horses lack the same specialized ocular adaptations for harsh and dusty environments, their ocular adnexa, including the eyelids and the third eyelid (nictitating membrane), are essential for protecting the eyeball from environmental particulate matter and desiccation (Williams and Miller, 2006). The third eyelid of horses, composed of T-shaped cartilage and associated lymphoid tissue, serves to distribute the tear film and provide a mechanical barrier against foreign bodies (Brooks, 2010). Ocular disorders are a significant and common problem in horses, frequently resulting in different degrees of discomfort, pain, and potential vision impairment (Stoppini et al., 2005).

In intensive veterinary management, the prevalence of ocular infections and inflammation, particularly conjunctivitis, is significantly high (Ferreira et al., 2017). This increased rate of conjunctivitis is frequently associated with several factors, including the widespread presence of dust and sand, large populations of flies serving as mechanical carriers of pathogens, and, importantly, poor management and hygiene practices in some stables that weaken the eye's natural defenses (Shawaf and Hussen, 2023). The conjunctiva is a mucous membrane that lines the inner eyelids and covers the anterior surface of the eye. Conjunctiva comprises non-keratinized stratified squamous epithelium containing goblet cells, as well as stratified columnar and stratified cuboidal epithelium (Henriksen et al., 2020; Kovalcuka et al.,

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2023). Conjunctival cytology offers a valuable, non-invasive method for rapid assessment of cellular responses within the epithelium, as well as for detecting foreign bodies, infectious agents, and inflammatory processes. This technique facilitates a greater understanding of the pathogenesis and prognosis of numerous ocular diseases (Henriksen et al., 2020; Moshtaghion et al., 2021; Kovalcuka et al., 2023).

Cytologic features of the eye have been widely documented in veterinary literature, especially in equine, bovine, canine, and feline species (Henriksen et al., 2020; Sebbag et al., 2020; Lucyshyn et al., 2021). Cytologic samples are typically studied for the presence of inflammatory cells, infectious agents, intracellular inclusion bodies, and neoplastic cells (Brooks, 2010; Kayat et al., 2017). A typical cytologic sample consists mainly of conjunctival epithelial cells with no cytoplasmic or nuclear abnormalities. In contrast, inflammatory ocular cytology demonstrates an elevated number of inflammatory cells, including neutrophils, lymphocytes, eosinophils, and mast cells (Calonge et al., 2004; Donaldson and Matas, 2011; Henriksen et al., 2020). The ocular surface naturally contains a community of microorganisms that constitute its normal microflora (Ferreira et al., 2017; Hampson et al., 2019). However, resident microorganisms, such as *Staphylococcus* spp., *Streptococcus* spp., and *Bacillus* spp., are often involved in opportunistic ocular infections, particularly when external factors such as trauma, stress, or environmental disturbances interfere with the normal commensal microbiota (Ferreira et al., 2017; Zak et al., 2018). Conjunctivitis, commonly known as pink eye, may result from different conditions affecting the eye (Stoppini et al., 2005; Saberinia et al., 2025).

While general equine ocular pathology is well documented, limited information is available on the specific cytological assessment of the equine conjunctiva under the distinct environmental and management conditions in Saudi Arabia. The present study aimed to characterize and compare the cytological analysis of conjunctival swabs from healthy horses and those with conjunctivitis in Saudi Arabia, focusing on how these findings relate to risk factors such as dust, flies, and management practices.

MATERIALS AND METHODS

Ethical approval

All experimental procedures carried out in the present study were approved by the ethics committee at King Faisal University, Saudi Arabia (Permission number KFU-REC-256002).

Animals

The present study examined 18 Arabian horses. Five horses were apparently healthy based on a general clinical ocular examination, while thirteen horses were affected by conjunctivitis. The healthy horses, comprising two males and three females, had a median age of 7 ± 5.2 years and a median weight of 320 ± 105 kg and were selected from a stable located at the Equine Research Center, King Faisal University, Al-Ahsa, Saudi Arabia. The study was conducted from October 2025 to February 2026, during which ambient temperatures fluctuated between 18°C and 38°C. To ensure the health status of the control group, a systematic and comprehensive clinical and ophthalmic examination was conducted on each horse. The assessment was performed under both ambient and darkened lighting to facilitate a comprehensive evaluation of all ocular structures and to ensure horses were free of general or ocular disorders, including signs of discharge, swelling, discomfort, or abnormalities (Brooks, 2010). The affected horses, including five males and eight females, had a median age of 5 ± 3.5 years and a median weight of 330 ± 145 kg. The affected horses, exhibiting signs such as chemosis, squinting, blinking, and ocular discharge, were brought to the Veterinary Teaching Hospital and the Equine Research Center at King Faisal University, Saudi Arabia.

Sampling

Samples were collected for bacterial analysis by swabbing the lower conjunctival sac of the affected eye without using a local anesthetic. The process employed swabs specifically designed for bacterial collection and was conducted under strict sterile conditions (Donaldson and Matas, 2011). Precautions were implemented to ensure that the swab did not contact the upper eyelid margin or adjacent facial skin, thereby reducing the risk of contamination. Subsequently, the swab was placed within the protective tube of the double-capped swab. The samples were transported to the bacterial diagnostic laboratory at the College of Veterinary Medicine, King Faisal University, Saudi Arabia, at room temperature in a modified medium and cultured within 24 hours. Conjunctival samples for cytological evaluation were obtained by carefully removing mucus and debris with sterile saline-moistened gauze. The samples were subsequently evenly and delicately smeared onto clean microscope slides, allowed to air-dry at ambient temperature, and stained using the Diff-Quik method (Hemal Stain Co., Inc., Danbury, USA).

Cytological examination

The slides were prepared for microscopic examination after staining with the Diff-Quik method and examined at different magnifications under a light microscope (Nikon Eclipse, Tokyo, Japan) to identify and perform a differential cell count. Differential cell counts of the target cells were performed using an oil-immersion lens (1000x) to highlight each cell's morphological features. Differential counts were expressed as percentages and mean values (with ranges) for neutrophils, eosinophils, lymphocytes, macrophages, and epithelial cells (including surface, mesoderm, and basophils; Henriksen et al. 2020).

Bacterial isolation and identification

To identify the bacterial species in the samples, individual smears were cultured on Columbia agar (CM331; Oxweed, Basingstoke, UK) with 5% citrate-treated sheep blood and on MacConkey agar (Oxweed, Basingstoke, UK). After inoculation, plates were incubated aerobically and anaerobically at 37°C for 18-24 hours, with an additional 24 hours when no growth was observed. Cultures with dissimilar colonies and no growth were excluded. Bacterial isolates grown on nutrient, blood, and MacConkey agar after 24-48 hours at 37°C were identified following Quinn et al. (2002). Identification of Gram-negative and Gram-positive bacteria involved biochemical tests such as catalase, coagulase, oxidase, citrate utilization, triple sugar iron, indole, methyl red, urease, H_2S production, and motility analysis.

Statistical analysis

The differences between means were analyzed employing Student's t-test with GraphPad Prism 7 statistical software to compare the healthy and affected groups. Normality was assessed using the D'Agostino and Pearson omnibus normality test. Differences with a probability level (p) of 0.05 or less were considered statistically significant, utilizing Duncan's multiple range test for mean separation.

RESULTS

The cytological evaluation of conjunctival smears revealed distinct differences between the healthy and affected groups, and microbiological culture identified a diverse range of aerobic bacteria from the conjunctival sacs. The differential cell counts derived from the conjunctival cytology of both healthy and affected horses are presented in Table 1. In healthy horses, the conjunctival epithelium mainly consisted of superficial epithelial cells, accounting for 59 ± 12.0%, while intermediate and basal cells were less common. The healthy group demonstrated a minimal inflammatory cell presence, with low mean percentages of neutrophils (8 ± 5.5%) and lymphocytes (11 ± 4.5%). Eosinophils and macrophages were rarely seen, with mean values of 1 ± 1.5% and 1 ± 0.5%, respectively. In contrast, horses with clinical signs of conjunctivitis demonstrated a significantly different cytological profile. There was a statistically significant decrease in the percentage of superficial epithelial cells (28 ± 17%) compared to the healthy group ($p < 0.05$). This was accompanied by a significant increase in basal epithelial cells (5 ± 3.5%; $p < 0.05$), indicating epithelial hyperplasia or increased cellular turnover in response to inflammation. The percentage of intermediate epithelial cells, while slightly lower in the affected group (14 ± 7.8%), did not differ significantly from the healthy group ($p > 0.05$). The most pronounced differences were observed in the inflammatory cell infiltrate. Horses with conjunctivitis exhibited a significant increase in the percentage of neutrophils (32 ± 12.5%) compared to healthy horses ($p < 0.01$). Eosinophils were significantly elevated in the affected group (12 ± 9.5%; $p < 0.001$). Conversely, the percentage of lymphoid cells was significantly lower in the conjunctivitis group (4 ± 3.1%) than in the healthy group ($p < 0.05$). The percentage of macrophages was minimal in both groups and did not exhibit a significant difference ($p > 0.05$).

A total of 18 conjunctival swabs were subjected to aerobic bacterial culture. Bacteria were isolated from 2 out of 5 (40%) healthy horses and from 12 out of 13 (92%) horses diagnosed with conjunctivitis. The isolated microorganisms are detailed in Table 2. In the healthy group, *Staphylococcus aureus* (*S. aureus*) and *Moraxella* sp. were detected. No bacterial growth was observed in three healthy horses. Among horses with conjunctivitis, a wider variety of bacterial species was isolated. Gram-positive bacteria were the most frequently identified, with *S. aureus* being the most common, detected in five horses.

Other Gram-positive isolates included *Corynebacterium* sp., *Streptococcus* sp., and *Bacillus* sp. Gram-negative bacteria were isolated from 7 out of 13 affected horses, either alongside Gram-positive bacteria in mixed cultures or as single isolates. These bacteria included *Moraxella* sp., *Escherichia coli*, and *Klebsiella* sp. The relationship between conjunctival cytology and the type of bacteria isolated is presented in Table 3. In healthy horses, ocular samples that yielded Gram-positive bacteria exhibited a cellular profile, superficial cells at 62 ± 5.5% and neutrophils at 9 ± 4.5%, similar to the average for the healthy group. Ocular samples from Gram-negative isolates demonstrated a slightly lower mean percentage of superficial cells (49 ± 8.0%) and a lower neutrophil count (5 ± 3.5%), although the limited sample

size precluded statistical analysis. In horses with conjunctivitis, ocular samples with Gram-positive bacterial isolates demonstrated marked neutrophilia (28 ± 11.5%) and eosinophilia (11 ± 7.5%), along with a reduced superficial epithelial cell count (30 ± 14%). Ocular samples from which Gram-negative bacteria were isolated exhibited an even stronger inflammatory response, characterized by the highest mean percentage of neutrophils (39 ± 6.5%) and a similarly high eosinophil count (12 ± 7.5%). These samples had the lowest mean percentage of superficial epithelial cells (25 ± 13%) and the highest percentage of basal cells (7 ± 2.5%), indicating a more severe epithelial injury associated with Gram-negative infections.

Table 1. Ocular samples from healthy horses and those affected with conjunctivitis from October 2025 to February 2026 in the Eastern province of Saudi Arabia

Cells		Healthy horses (%)	Affected horses (%)	
		Mean ± SD	Mean ± SD	P-value
Epithelial	Superficial	59 ± 12.0	28 ± 17	0.01
	Intermediate	17 ± 8.2	14 ± 7.8	0.12
	Basal cells	2 ± 0.5	5 ± 3.5	0.03
Leukocytes	Neutrophils	8 ± 5.5	32 ± 12.5	0.001
	Lymphoid cells	11 ± 4.5	4 ± 3.1	0.01
	Eosinophils cells	1 ± 1.5	12 ± 9.5	0.001
Macrophages		1 ± 0.5	2 ± 0.5	0.2

SD: Standard deviation. Data are expressed as Mean ± SD

Table 2. Microorganisms isolated from ocular horse samples from October 2025 to February 2026 in the Eastern province of Saudi Arabia

Animal numbers		Gram-positive bacteria	Gram-negative bacteria
Healthy horses	1	*Staphylococcus aureus*	-
	2	-	-
	3	-	-
	4	-	*Moraxella* sp.
	5	-	-
Horses with conjunctivitis	6	*Streptococcus sp.*	-
	7	*Staphylococcus aureus*	*Klebsiella* sp.
	8	-	*Moraxella* sp.
	9	-	-
	10	*Staphylococcus aureus*	*Moraxella* spp.
	11	*Corynebacterium sp.*	*Escherichia coli*
	12	*Staphylococcus aureus*	-
	13	*Bacillus sp.*	*Klebsiella* sp.
	14	-	*Escherichia coli*
	15	*Staphylococcus aureus*	
	16	*Corynebacterium sp.*	-
	17	*Staphylococcus aureus*	-
	18	-	*Escherichia coli*

Table 3. Relationship between conjunctival cytology and cell types and isolated bacteria from healthy horses and horses affected with conjunctivitis from October 2025 to February 2026 in the Eastern Province of Saudi Arabia

Cells		Healthy horses (Mean ± SD [%])		Affected horses (Mean ± SD [%])	
		Gram-positive bacteria	Gram-negative bacteria	Gram-positive bacteria	Gram-negative bacteria
Epithelial	Superficial	62 ± 5.5	49 ± 8.0	30 ± 14	25 ± 13
	Intermediate	14 ± 5.1	18 ± 7.5	12 ± 5.5	16 ± 6.6
	Basal cells	1 ± 0.8	2.2 ± 0.3	4 ± 3.4	7 ± 2.5
Leukocytes	Neutrophils	9 ± 4.5	5 ± 3.5	28 ± 11.5	39 ± 6.5
	Lymphoid cells	12 ± 2.5	8 ± 5.5	5 ± 2.5	4 ± 2.8
	Eosinophils cells	1.5 ± 1	0.5 ± 0.5	11 ± 7.5	12 ± 7.5
Macrophages		1.5 ± 0.5	0.5 ± 0.5	1.5 ± 0.5	2.5 ± 0.8

SD: Standard deviation. Data are expressed as Mean ± SD

DISCUSSION

The present study provided a detailed comparative analysis of conjunctival cells and aerobic bacteria in Arabian horses in the Eastern Province of Saudi Arabia, a region characterized by a dry climate that poses distinct challenges to ocular health and exposes individuals to inflammatory and allergenic agents. The present results revealed clear differences in the cellular composition of the conjunctiva between healthy and infected horses and identified several potential pathogenic bacteria associated with conjunctivitis. The cellular composition of the healthy horses in the present study was consistent with that of normal equine conjunctiva, with surface squamous epithelial cells being the predominant cell type and inflammatory cells being rare (Bonsembiante et al., 2019; Berzina et al., 2022). The low number of neutrophils and lymphocytes observed in healthy horses in the present study was consistent with previous findings on the healthy ocular surface (Braus et al., 2017; Henriksen et al., 2020). Conversely, horses with conjunctivitis exhibited a notable change in their cellular profile (Brooks, 2010; Kari and Saari, 2014). The marked decrease in surface epithelial cells, coupled with the marked increase in basal epithelial cells in the present study, indicated epithelial damage followed by regeneration. This change might be due to inflammation in the surface layers, which then triggers a proliferative response in the basal layers to rebuild the epithelial structure, as previously reported by Calonge et al. (2004) and Stoppini et al. (2005).

The principal characteristic of the inflammatory response observed in the conjunctiva of the affected horses in the present study was a marked increase in neutrophil count (Maggs, 2008; Bruschi et al., 2020). This elevation of neutrophils is a cytological indicator consistent with bacterial conjunctivitis, as these cells are the first line of defense against bacterial activity (Donaldson and Matas, 2011; Johns et al., 2011). A particular finding in the present study was the markedly elevated eosinophil count in the affected horses. While eosinophils are often associated with parasitic infections or hypersensitivity reactions, they were observed in equine conjunctival cells (Henriksen et al., 2020; Lucyshyn et al., 2021). This eosinophilic infiltration could be associated with the environmental and unsanitary conditions in which the horses resided, as well as with contact allergens such as insects, dust, and pollen (Shawaf and Hussein, 2023). The cytological findings in the present study indicated that the conjunctivitis observed in the horses might have a mixed etiology, involving infectious and hypersensitivity factors. Persistent irritation from airborne particles could be an exacerbating factor, aligning with previous findings (Williams and Miller, 2006; Zak et al., 2018). The marked decrease in lymphocytes in the affected horses compared to healthy horses in the present study was unexpected, as lymphocytes are key mediators of the adaptive immune response (Henriksen et al., 2020). This decrease could be due to the acute nature of the inflammation, in which the immediate neutrophil response surpasses the lymphocyte response, or it might result from the specific sampling method and the characteristics of the surface infiltrate (Stoppini et al., 2005). Low macrophage counts in affected and healthy horses suggested that the conjunctivitis was primarily acute and did not progress to chronic granulomatous inflammation (Lamagna et al., 2015; Moshtaghion et al., 2021). Bacterial isolation from 60% of healthy horses confirmed the presence of normal ocular microbiota, consistent with the findings of Hampson et al. (2019).

The detection of opportunistic pathogens such as *S. aureus* and *Moraxella* spp. from healthy ocular samples suggested that these commensal organisms can cause disease when ocular surface defenses are compromised (Zak et al., 2018). In horses with conjunctivitis, the bacterial isolates were more diverse. The dominance of Gram-positive bacteria, particularly *S. aureus*, was consistent with the findings of Ferreira et al. (2017) and emphasized their role as the main opportunistic pathogens. The isolation of *Escherichia coli* and *Klebsiella* sp. from affected horses was clinically

substantial. These Gram-negative bacteria are frequently associated with more severe ocular disease due to their virulence factors and capacity to cause rapid tissue damage (Brooks, 2010). The presence of these pathogens, along with *Corynebacterium* sp. and *Moraxella* sp., indicated that conjunctivitis in the affected population can be caused by a broad range of bacteria. This finding highlighted the importance of performing culture and sensitivity analyses to determine the most effective antimicrobial treatment (LaFrentz et al., 2020; Seeger et al., 2021). The correlation between cytology and bacteriology offered valuable insights into the host response to different types of infection (Shawaf and Hussen, 2023). Although the sample size in the healthy group was too small for statistical comparison, the trend in horses with conjunctivitis helped clarify the relationship between cytological changes and bacterial pathogens. Infections involving Gram-negative bacteria were associated with a stronger neutrophilic response and greater epithelial disruption (fewer superficial cells, more basal cells) than infections involving only Gram-positive isolates.

These findings supported the clinical understanding that Gram-negative bacteria frequently trigger a more severe inflammatory response, likely due to endotoxins in their cell walls (Lamagna et al., 2015), and cause more extensive tissue damage (Bourges-Abella et al., 2007). The elevated eosinophil counts observed in both bacterial types in the present study suggested that environmental allergens, such as insects and dust, might be a common underlying factor in Eastern Saudi Arabia. This could increase horses' susceptibility to developing more severe clinical signs (Shawaf and Hussen, 2023).

CONCLUSION

The current study demonstrated that conjunctivitis in Arabian horses in Eastern Saudi Arabia had a distinctive cytological pattern of neutrophilic and eosinophilic inflammation, accompanied by epithelial cell regeneration. The present findings highlighted the importance of conjunctival cytology as a rapid, readily accessible tool for characterizing the inflammatory response, particularly when combined with bacterial culture. Nevertheless, the current study encountered several limitations, notably the small sample size, particularly in the healthy control group, which constrained the statistical power and the broader applicability of the current results. The investigation was confined to a specific period and did not account for potential seasonal variations. Additionally, the study focused solely on aerobic bacteria, overlooking anaerobic bacteria, fungi, and viruses, which may contribute to conjunctivitis. Further studies are needed with larger equine samples and across different time periods, including the investigation of fungal and viral agents, to fully elucidate the multiple causes of equine conjunctivitis in arid regions.

DECLARATIONS

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Authors' contributions

Zainab Al-Mashabirah, Maryam Al-Shehab, and Turke Shawaf did the examination. Maryam Al-Shehab, Khalid Elbager, Mohammad Asvad, and Mohammad Alsalman contributed to laboratory investigations. Zainab Al-Mashabirah, Moustafa Salouci, and Turke Shawaf drafted the manuscript. The final edition of the manuscript was read and approved by all authors.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declared no conflicts of interest.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. The authors confirmed that no AI tools were used in conducting and preparing the present study.

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