2026, Scienceline Publication
World's Veterinary Journal
World Vet J, 16(2): 468-475.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2026.wvj44
PII: S232245682600044-16

Cytological Analysis of Tracheal Wash and Bronchoalveolar Lavage Fluid in Healthy and Asthmatic Horses

Zakaria Abdullah[1], Saleh Alsayed[1], Abdulaziz Almuhanna[1], Mohammad Alsalman[1], Basel Hamoud[2], and Turke Shawaf[1]*

[1]*Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia*
[2]*Department of Surgery and Gynecology, College of Veterinary Medicine, Hama University, Syria*

*Corresponding author's Email: tshawaf@kfu.edu.sa

ORIGINAL ARTICLE
Received: March 05, 2026
Revised: April 06, 2026
Accepted: April 19, 2026
Published: June 30, 2026

ABSTRACT

Although numerous recent studies have explored the cytology of equine asthma, its cytological profile in Arabian horses is still insufficiently described. This study compared the differential cell counts in tracheal lavage (TW) and bronchial lavage fluid (BALF) samples between healthy and asthmatic Arabian horses in Saudi Arabia. Seventeen horses (5 healthy, 12 asthmatic), with an average age of 7 ± 5.5 years, and an average weight of 325 ± 120 kg, enrolled in this study to collect and analyze tracheal wash (TW) or bronchoalveolar lavage fluid (BALF) samples. The affected horses were taken from the respiratory cases admitted to the veterinary teaching hospital of Faisal University, while the healthy horses were taken from the horse research center in Faisal University (Saudi Arabia). Differential counts were performed on TW and BALF samples using microscopic examination after staining the samples with Diff Quick stain for evaluation of macrophages, lymphocytes, neutrophils, mast cells, eosinophils, and epithelial cells. A significant increase in neutrophils was observed in the affected group in both TW and BALF, compared to the control group. In contrast, a marked decrease in the percentage of lymphocytes was observed in asthmatic BALF, while macrophage proportions decreased in affected and control groups. TW eosinophils were elevated in asthmatic horses compared to healthy horses, though BALF eosinophil counts did not differ between healthy and asthmatic horses. The percentage of epithelial cells was decreased in TW but modestly increased in the BALF of asthmatic horses. No change in the number of mast cells was observed in any of the groups, as it was low. These findings confirmed that equine asthma in Arabian horses enrolled in this study is characterized by pronounced neutrophilic inflammation with concomitant lymphocytic and macrophagic depletion, particularly in the BALF samples.

Keywords: Bronchoalveolar, Cytology, Horse, Respiratory, Tracheal, Wash

INTRODUCTION

Equine asthma is a common disorder in horses, particularly in dusty and allergen-rich environments (Couetil et al., 2020; Diez de Castro and Fernandez-Molina, 2024). Equine asthma is a chronic, non-contagious inflammatory condition affecting the lower respiratory tract, impacting horses globally (Hermange et al., 2019; Lendl and Barton, 2024). Equine asthma causes a decline in respiratory function, leading to decreased athletic performance, high treatment costs, and the progression of many cases to a chronic form requiring ongoing veterinary care (Rossi et al., 2018; Sundman et al., 2020; Lendl et al., 2025).

Since its initial description as equine asthma (Lowell, 1964), it was later referred to as inflammatory airway disease, chronic obstructive pulmonary disease, and recurrent obstructive airway disease (Ainsworth et al., 2003; Gehlen et al., 2008; Couetil et al., 2020). The clinical manifestations of Equine asthma vary, ranging from mild respiratory allergies and inflammation to moderate inflammation and typical respiratory symptoms, culminating in severe symptoms accompanied by significant respiratory distress and hypoxia, as well as increased inflammation due to infiltration of inflammatory cells into the airways, mucus accumulation, and acute airway constriction resulting from contraction of the surrounding smooth muscles (Bosshard and Gerber, 2014; Lendl and Barton, 2024; Drespling et al., 2025).

Despite the clinical significance of this disease and its impact on performance, data detailing the cellular characteristics of equine asthma in Arabian horse breeds in Saudi Arabia, including those exposed to various respiratory allergens such as sand and date palm pollen, remain relatively scarce (Shawaf, 2019). Early diagnosis of respiratory infections is crucial for determining appropriate treatment to prevent disease progression and reduce the chances of recovery (Kozłowska et al., 2022). Accurate diagnosis of equine asthma relies on several methods, including clinical assessment, endoscopic examination of airway secretions, and cytological analysis of airway samples obtained via tracheal wash (TW) or bronchoalveolar lavage fluid (BALF, Wysocka and Kluciński, 2015; Morini et al., 2023;

To cite this paper: Abdullah Z, Alsayed S, Almuhanna A, Alsalman M, Hamoud B, and Shawaf T (2026). Cytological Analysis of Tracheal Wash and Bronchoalveolar Lavage Fluid in Healthy and Asthmatic Horses. *World Vet. J.*, 16(2): 468-475. DOI: https://dx.doi.org/10.54203/scil.2026.wvj44

Drespling et al., 2025). Tracheal wash and BALF samples are valuable mirrors of different areas of the airway and can be used in combination with other methods, such as radiography or ultrasound, for the diagnosis of lower respiratory diseases (Wysocka and Kluciński, 2015; Rossi et al., 2018). Cytological examination of the lower airways is considered the gold standard for differentiating inflammatory subtypes and counting cells such as neutrophils, eosinophils, and mast cells, which reflect the severity of inflammation (Pickles et al., 2002a; Rossi et al., 2018). Tracheal wash sampling and analysis, as a less invasive and cost-effective procedure, complement this important approach to understanding the nature and extent of inflammatory changes in the airways of affected horses (Pickles et al., 2002b; Cullimore et al., 2018; Jocelyn et al., 2018). Dély et al. (2024) found that cytological examination of TW and BALF samples helps differentiate between healthy and asthmatic horses and the severity of the condition based on characteristic changes in the number and type of inflammatory cells. Horses with mild to moderate asthma showed an increase in neutrophils and mast cells in their BALF compared to healthy horses (Sage et al., 2024; Meiseberg et al., 2025), while in horses with severe asthma, a marked increase in neutrophils, often exceeding the established threshold values (≥ 20–25% neutrophils, characteristics of BALF cytological analysis), and exceeding 55% in BALF samples from affected horses was observed (Janssen et al., 2022; Lee et al., 2022).

Given the varying climatic conditions and diverse respiratory allergens in animals in Saudi Arabia (Shawaf, 2019; Shawaf et al., 2021), it is crucial to identify and compare the basic cytological characteristics of healthy and asthmatic horses. Therefore, the present study aimed to compare the analysis of TW and BALF cytology in healthy and asthmatic horses.

MATERIALS AND METHODS

Ethical approval

All experimental procedures used in this study, including TW and BALF sample collection, were approved by the Ethics Committee at King Faisal University, Saudi Arabia (Permission number KFU-REC / 2025-121).

Animals

A total of 17 Arabian horses (5 healthy controls and 12 affected with asthma) were included in the cytological analysis of TW and bronchoalveolar lavage fluid (BALF) between September 2025 and January 2026. Horses involved in this study were (7 stallions and 10 mares) of average aging (7 ± 5.5) and average weight (Kg, 325±120). Horses were fed a diet of hay and barley, while their training consisted of daily walking and cantering. The control group was selected randomly from animals maintained at the Horse Research Center, King Faisal University, Eastern Province, Saudi Arabia. Healthy horses selected based on physical examination (no fever or signs of abnormal respiratory signs like cough, nasal discharge, dyspnea, or abnormal lung sounds as well as normal white blood cell counts and biochemistry panel; Couetil et al., 2020). Horses with chronic respiratory symptoms were purposively selected from those brought to the Veterinary Teaching Hospital, College of Veterinary Medicine, King Faisal University. The horses were selected based on case history, clinical respiratory symptoms characteristic of the chronic form of the disease, such as coughing for more than three weeks, nasal discharges, abnormal breathing sounds, and the appearance of a heavy line on the abdomen (Gehlen et al., 2008). Horses with other diseases were excluded, and only those with chronic respiratory signs were included.

Sample collection

Samples were collected from horses without prior exercise in the standing position following mild sedation with Xylazine 2% (Rompun; Bayer Health Care, Germany) intravenously at a dose of 1 mg/kg body weight (Wiederkehr et al., 2021). The TW was taken during endoscopy, while BALF was collected immediately after endoscopy (Rossi et al., 2018). A twitch was used to restrain the horses when needed. A flexible endoscope (EVIS Olympus, OLYMPUS AUSTRIA GmbH., Vienna) with a 10 mm diameter and 140 cm long was used in endoscopy and for TW collection. The endoscope was passed via the nostril and advanced through to the mid-cervical trachea. 15 mL of sterile saline at room temperature was instilled through a plastic catheter (EQUIVET; 2.3 mm × 350 cm, Italy) to dilute mucous fluids and make them easier to withdraw. About 10 ml of tracheal fluid was immediately suckled back from the curvature of the distal trachea into a syringe. Samples were submitted for laboratory analysis (Veterinary teaching hospital lab, King Faisal University, Saudi Arabia) and were processed within 30 min of collection. Bronchoalveolar lavage fluid (BALF) collection was performed immediately following TW sampling. To prevent coughing while collecting BALF, 40 mL of 1% lidocaine (Lidocaine hydrochloride; Hikma, Canada) was infused as local anesthesia into the lower airway walls using a BALF catheter (EQUIVET B.A.L. catheter 240 cm, KRUUSE, Denmark, Rossi et al., 2018). The bronchoalveolar lavage fluid (BALF) tube was gently but securely wedged, as detected by resistance to further

advancement. The cuff was then gently inflated using a 10 ml syringe with 5-10 ml of air. This cuff assures a complete wedge to prevent the backflow of infused fluid. Prewarmed (37 ^{o}C) sterile physiologic saline solution, 0.9% NaCl (300-350 ml) was administered in one single volume in the distal airways and alveoli, followed by immediate aspiration. Syringes with the retrieved about 60% of infused fluid, which were immediately placed on ice and submitted to laboratory analysis within 30 min after collection (Shawaf, 2019).

Cytological analysis

To prepare the samples for differential cell counting, the TW and BALF samples were centrifuged at 1500 rpm for 15 minutes, and the supernatant was discarded. Smears of the cell precipitate were prepared on glass slides, air-dried, and stained with Diff Quick (Hemal Stain Co. Inc., Danbury, USA). Using an oil immersion lens at 1000x magnification and a standardized counting protocol (De Brauwer et al., 2002), the percentages of phagocytes, lymphocytes, neutrophils, mast cells, eosinophils, and epithelial cells were counted after 400 cells were counted using a microscope (Olympus BH2; Olympus Optical, Tokyo, Japan).

Statistical analysis

Differences between means were analyzed using one-way analysis of variance (ANOVA) with Bonferroni's post-hoc test for multiple comparisons using GraphPad Prism 7 statistical software. The D'Agostino-Pearson cross-test for normality was also used to assess normality. Differential cell counts were expressed as mean percentages ± standard deviation (SD) alongside the observed range. Differences between groups were considered statistically significant if the P-value was less than 0.05.

RESULTS

Marked differences in cellular profiles were observed both between healthy and asthmatic horses and between sampling techniques (TW versus BALF), concerning neutrophil, epithelial cell, macrophage, and lymphocyte distributions. The high percentage of neutrophils in horses with asthma compared to healthy horses in TW and BALF samples is considered one of the most important findings of this study. In TW samples, neutrophils constituted (57.29 ± 13.93%) of the total cell count in asthmatic horses, whereas healthy horses exhibited considerably lower values (22.11 ± 6.88%) with a significant difference ($p < 0.05$, Table 1 and Figure 3).

Similarly, in BALF, neutrophil percentages were increased in asthmatic horses (37.29 ± 13.32%) compared to their healthy horses (4.55 ± 2.06%) with a significant difference ($p < 0.05$, Table 1and Figure 4). In contrast, a decrease in macrophage percentages was observed in asthmatic horses in both TW and BALF samples compared to healthy horses. In TW, mean macrophage values decreased from 45.44 ± 13.24% in healthy horses to 15.29±7.44% in asthmatic horses ($p < 0.05$; Table 1 and Figure 3). This decline was consistent with BALF samples, where macrophage percentages decreased from 40.67 ± 6.01% in healthy animals to 29.57 ± 14.97% in affected horses, where the P value was recorded as $p < 0.05$ (Table 1 and Figure 4). A significant decrease in the percentage of lymphocytes was also observed in affected horses, particularly in BALF samples, compared to healthy horses. Healthy horses showed a lymphocyte percentage in BALF of 41.33 ± 6.42%, while the asthmatic horses exhibited BALF lymphocyte percentages of only 14.86 ± 6.09% ($p < 0.05$). In TW samples, lymphocyte percentages were also decreased in affected horses (6 ± 3.59%) compared to controls (9.44 ± 3.78%; $p < 0.05$), although this difference was less obvious than in BALF. In TW samples, epithelial cells were reduced in affected horses (10.93 ± 6.79%) compared to controls (18.44 ± 7.45%; $p < 0.05$).

Conversely, in BALF, epithelial cell percentages were increased in affected horses (10 ± 5.23%) compared to healthy horses (6.88 ± 3.22%). Eosinophil percentages were increased in TW samples from affected horses (10.21 ± 5.24%) compared to healthy controls (3.89 ± 2.42%, $p < 0.05$). However, BALF eosinophil percentage was nearly identical between healthy and affected groups (healthy horses: 6 ± 3.09%; asthmatic horses: 6.5 ± 3.57%, 0-12%) with no significant difference. Mast cell percentages recorded low values across both groups in TW and BALF samples (< 1.1% mean), with no significant differences.

Comparing the cell composition of TW and BALF in healthy horses revealed high proportions of macrophages, neutrophils, and epithelial cells in the tracheal lavage samples, while high proportions of lymphocytes and eosinophils were recorded in the alveolar lavage samples (Table 1; Figures 1 and 2). Similarly, affected horses showed higher values for neutrophils, eosinophils, and epithelial cells in TW, while elevated levels of phagocytes and lymphocytes were observed in BALF samples.

A notable finding was the absence of a difference in the percentage of epithelial cells among TW and BALF samples in affected horses, whereas a significant increase in epithelial cells was observed in TW samples compared to BALF samples in healthy horses (Figures 1 and 2).

Table 1. The differential cell counts (Mean ± SD) of tracheal wash and bronchoalveolar lavage fluid in healthy and asthmatic horses in Saudi Arabia

Cells	Tracheal wash (TW)			Bronchoalveolar Lavage Fluid (BALF)		
	Healthy Mean ± SD	Affected Mean ± SD	P value	Healthy Mean ± SD	Affected Mean ± SD	P value
Macrophages (%)	45.44 ± 13.24	15.29 ± 7.44**	0.006	40.67 ± 6.01	29.57 ± 14.97*	0.04
Lymphocytes (%)	9.44 ±3.78	6 ±3.59*	0.04	41.33 ± 6.42	14.86 ±6.09**	0.009
Neutrophils (%)	22.11± 6.88	57.29 ± 13.93**	0.008	4.55 ± 2.06	37.29 ± 13.32***	0.0009
Mast cells (%)	0.66 ± 1	0.42 ± 0.85	0.071	066 ± 0.71	1.07 ± 1.14	0.065
Eosinophils (%)	3.89 ± 2.42	10.21 ± 5.24*	0.045	6 ± 3.09	6.5 ± 3.57	0.076
Epithelial cells (%)	18.44 ± 7.45	10.93 ± 6.79*	0.03	6.88 ± 3.22	10 ± 5.23*	0.45

SD: Standard deviation; NS, Not significant. *p < .05; **p < .01; ***p < .001.

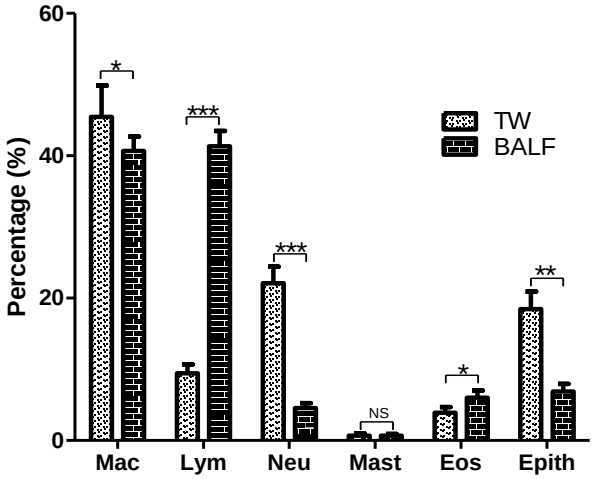

Figure 1. The differential cell counts (%) of tracheal wash (TW) and bronchoalveolar lavage fluid (BALF) from healthy horses. Mac: Macrophages, Lym: Lymphocytes, Neu: Neutrophils, Mast: Mast cells, Eos: Eosinophils, Epith: Epithelial cells, NS: not significant. *p < .05; **p < .01; ***p < .001.

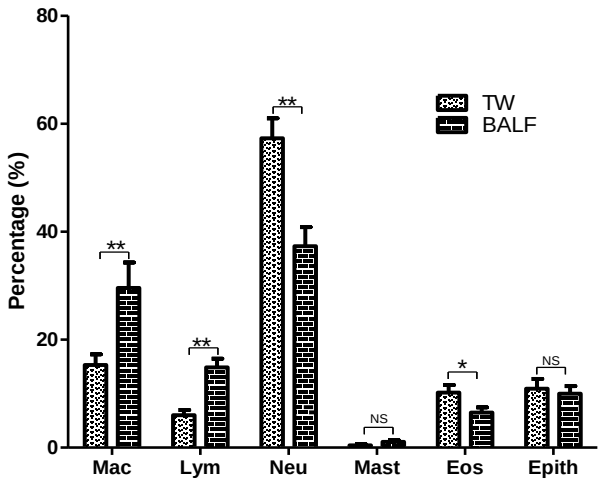

Figure 2. The differential cell counts (%) of tracheal wash (TW) and bronchoalveolar lavage fluid (BALF) from asthmatic horses. Mac: Macrophages, Lym: Lymphocytes, Neu: Neutrophils, Mast: Mast cells, Eos: Eosinophils, Epith: Epithelial cells, NS: not significant. *p < .05; **p < .01.

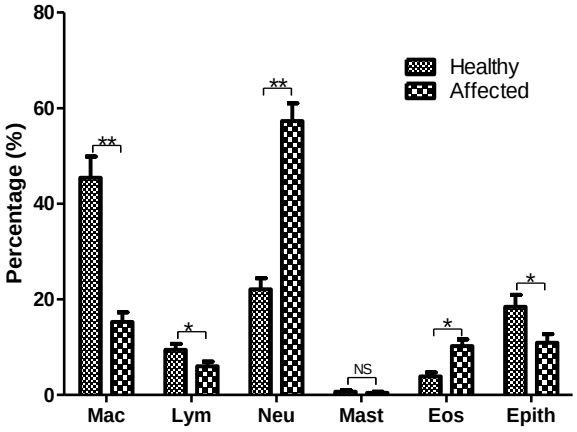

Figure 3. The differential cell counts (%) of tracheal wash (TW) from healthy and asthmatic horses. Mac: Macrophages, Lym: Lymphocytes, Neu: Neutrophils, Mast: Mast cells, Eos: Eosinophils, Epith: Epithelial cells, NS: not significant. *p < .05; **p < .01.

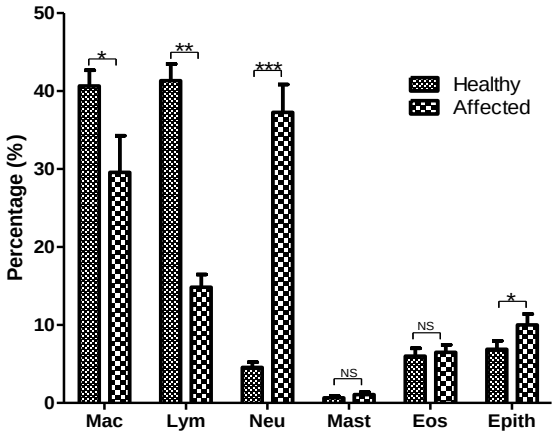

Figure 4. The differential cell counts (%) of bronchoalveolar lavage fluid (BALF) from healthy and asthmatic horses. Mac: Macrophages, Lym: Lymphocytes, Neu: Neutrophils, Mast: Mast cells, Eos: Eosinophils, Epith: Epithelial cells, NS: not significant. *p < .05; **p < .01; ***p < .001.

DISCUSSION

The results in this study showed similar values for differential cell counts in tracheal wash (TW) and bronchoalveolar lavage (BALF) of Arabian horses, with differences in some cells, which may be due to different allergens and diseases in dry and desert environments (Shawaf et al., 2021). Similar values for neutrophils were shown, which are very important in differentiating between healthy and affected horses that need treatment (Sage et al., 2020). However, the most significant finding in the present study was a marked increase in the number of neutrophils in both TW and BALF in asthmatic horses, exceeding the percentages established for healthy horses and equine asthma cases in previous studies (Rossi et al., 2018; Couetil et al., 2020).

A significant increase in neutrophil infiltration was observed in the tracheal space (57.29%) compared to the alveolar space (37.29%). These findings were previously documented by Rossi et al. (2018), who noted that the neutrophil count in tracheal fluid often exceeds that in TW from both healthy and asthmatic horses. A higher percentage of neutrophils could be attributed to the extrusive migration of neutrophils from distant sites of inflammation towards the trachea (Lendl et al., 2025) or to longer duration of neutrophil placement in the tracheal lumen (Uberti and Morán, 2018). The increased number of neutrophils in TW compared to BALF could also be explained by the persistent contamination resulting from the trachea's proximity to the pharynx, which serves as a food passageway (Shawaf et al., 2021).

It is important to note that findings in the current study that the mean neutrophil percentage in the BALF of asthmatic cohort (37.29%) significantly exceeded the diagnostic threshold of 10% for mild to moderate asthma and approached the 25% threshold typically cited for severe asthma (Janssen et al., 2022; Lee et al., 2022), suggesting that the affected horses included in this study predominantly represented moderate to severe cases. The concurrent decrease in the lymphocyte percentage in the BALF from 41.33% in healthy horses to 14.86% asthmatic horses indicates a marked increase in neutrophils, which affected the percentage of lymphocytes (Davis et al., 2019). This finding is consistent with that of Sage et al. (2024), who recently demonstrated, using single-cell transcription analysis, that severe equine asthma is characterized not only by a marked increase in neutrophils but also by a T-cell immune response that subsequently leads to a quantitative decrease in total lymphocyte recovery (Kang et al., 2022; Morini et al., 2023). Meiseberg et al. (2025) reported previously that the reason for the sharp decline in lymphocyte count in severe asthma may be due to lymphocyte death within the inflammatory microenvironment. In the present study, the percentage of macrophages decreased significantly in both TW and BALF of asthmatic horses, with the greatest decrease occurring in the TW. This decrease is likely attributable to the relative dilution effect of neutrophil infiltration, which was markedly elevated, consistent with the documented impaired phagocytic function of airway phagocytic cells in asthmatic horses (Hansen et al., 2020b; Wilson et al., 2020). The wide variation in macrophage percentages observed in the BALF of asthmatic horses may be attributed to differences in the type and stage of airway inflammation, leading to heterogeneity in the inflammatory phenotype (Lendl and Barton, 2024). The pattern of epithelial cell percentage changes in both TW and BALF in this study warrants particular attention. The reduced number of epithelial cells in TW of asthmatic horses may reflect ongoing debridement and regeneration of the epithelium due to chronic inflammation, a process previously described in acute equine asthma (Bosshard and Gerber, 2014). Alternatively, the reduction of epithelial cells in TW in affected horses may represent a relative attenuation of the high proportion of inflammatory cells, as noted in previous studies (Kang et al., 2022). Conversely, the slightly increased number of epithelial cells in BALF in asthmatic horses (from 6.88% to 10.00%) is surprising, but consistent with the findings reported previously (Drespling et al. 2025).

They hypothesized that the use of a plastic catheter for sampling, together with its potentially traumatic effect on the airway mucosa, could contribute to epithelial damage. This effect may be exacerbated by bronchial hyperresponsiveness and airway constriction during bronchoalveolar lavage catheter insertion in inflamed lungs. Consequently, increased epithelial disruption may occur, leading to the shedding and aspiration of epithelial cells into the cytological sample (Pickles et al., 2002a; Wysocka and Kluciński, 2015).

A marked increase in eosinophils was observed in the TW of asthmatic horses, while eosinophil counts in BALF remained similar between the two groups. This difference between TW and BALF samples from affected horses suggested that eosinophilic inflammation may be primarily concentrated in the large conducting airways rather than in the alveolar interstitial tissue (Couetil et al., 2020). The result of eosinophil counts in the current study supports the concept of individual phenotypic variability in equine asthma and the multi-factorial nature of equine asthma, as a subset of affected horses exhibit mixed granulomatous inflammation (Wysocka and Klucinski, 2015; Couetil et al., 2020). The lower values of eosinophils in BALF in affected horses in the present study differ from some European studies that have indicated eosinophil-predominant patterns of equine asthma (Hermange et al., 2019; Rasmussen et al., 2024), which may reflect differences related to geographical location, weather conditions, and asthma-associated allergens (Couetil et al., 2020).

The arid, sandy desert environment of Saudi Arabia, with its prevalence of date palm pollen and desert dust rather than mold spores and other pollen types common in temperate climates, may stimulate neutrophilic rather than eosinophilic airway responses (Shawaf, 2019). Mast cell proportions in the present study remained uniformly low in both TW and BALF from healthy and diseased animals. The absence of significant differences in mast cell counts between healthy and asthmatic horses may be attributed to the predominantly neutrophilic nature of inflammation in the affected animals. This type of inflammatory response is not typically associated with marked mast cell proliferation on the airway surfaces, as previously reported (Hansen et al., 2020a). Furthermore, the Diff-Quik staining method used may not be sufficient for staining mast cells (Pickles et al., 2002b).

CONCLUSION

The proportion of cellular changes in TW and BALF samples in asthmatic Arabian horses is similar to those studied in other breeds. TW and BALF analysis in this study also indicate some cytological differences from previous studies, which may be attributed to allergens and diseases in arid and desert environments. Further research is needed to study a larger sample size than this study and to investigate the impact of weather and seasons on the cellular composition of the airways in Arabian horses.

DECLARATIONS

Acknowledgements

The researchers thank the Deanship of Scientific Research at King Faisal University for funding this research (Grant No. KFU 261665). The authors are also thankful to the veterinary teaching hospital at King Faisal University, Saudi Arabia, for providing the horses.

Authors' contributions

Zakaria Abdullah, Abdulaziz Almuhanna, and Turke Shawaf did the examination; Saleh Alsayed, Mohammad Alsalman, Basel Hamoud, and Turke Shawaf did laboratory work. Zakaria Abdullah, Abdulaziz Almuhanna, and Turke Shawaf drafted the manuscript. The final manuscript was approved by all of the authors.

Availability of data and materials

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

Conflict of interests

The authors have not declared any conflict 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 confirm that no AI tools were used in conducting and preparing this study.

Funding

This study was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Grant No. KFU 261665).

REFERENCES

Ainsworth DM, Grunig G, Matychak MB, Young J, Wagner B, ErbHN, and Antczak DF (2003). Recurrent airway obstruction (RAO) in horses is characterized by IFN-gamma and IL-8 production in bronchoalveolar lavage cells. Veterinary Immunology and Immunopathology, 96(1-2): 83-91. DOI: https://www.doi.org/10.1016/S0165-2427(03)00142-9

Bosshard S and Gerber V (2014). Evaluation of coughing and nasal discharge as early indicators for an increased risk to develop equine recurrent airway obstruction (RAO). Journal of Veterinary Internal Medicine, 28(2): 618-623. DOI: https://www.doi.org/10.1111/jvim.12279

Couetil L, Cardwell JM, Leguillette R, Mazan M, Richard E, Bienzle D, Bullone M, Gerber V, Ivester K, Lavoie JP et al. (2020). Equine asthma: Current understanding and future directions. Frontiers in Veterinary Science, 7: 450. DOI: https://www.doi.org/10.3389/fvets.2020.00450

Cullimore AM, Secombe CJ, Lester GD, and Robertson ID (2018). Bronchoalveolar lavage fluid cytology and airway hyper-reactivity in clinically normal horses. Australian Veterinary Journal, 96(8): 291-296. DOI: https://www.doi.org/10.1111/avj.12721

Davis KU and Sheats MK (2019). Bronchoalveolar lavage cytology characteristics and seasonal changes in a herd of pastured teaching horses. Frontiers in Veterinary Science, 6: 74. DOI: https://www.doi.org/10.3389/fvets.2019.00074

De Brauwer EI, Jacobs JA, Nieman F, Bruggeman CA, and Drent M (2002). Bronchoalveolar lavage fluid differential cell count. How many cells should be counted?. Analytical and Quantitative Cytology and Histology, 24(6): 337-341. Available at: https://pubmed.ncbi.nlm.nih.gov/12508692

Dély S, Gerber V, Peters LM, and Sage SE (2024). Association between equine asthma and fungal elements in the tracheal wash: An environment-matched case-control study. PlOS one, 19(9): e0309835. DOI: https://www.doi.org/10.1371/journal.pone.0309835

Diez de Castro E and Fernandez-Molina JM (2024). Environmental management of equine asthma. Animals, 14(3): 446. DOI: https://www.doi.org/10.3390/ani14030446

Drespling J, Berwanger L, Kühn H, Schwarz B, Doherr M, and Mundhenk L (2025). Endoscopically assessed mucus parameters in equine asthma: Relationship to clinical history and cytological findings data. Equine Veterinary Journal, 58(30): 767-778. DOI: https://www.doi.org/10.1111/evj.70002

Gehlen H, Oey L, Rohn K, Bilzer T, and Stadler P (2008). Pulmonary dysfunction and skeletal muscle changes in horses with RAO. Journal of Veterinary Internal Medicine, 22(4): 1014-1021. DOI: https://www.doi.org/10.1111/j.1939-1676.2008.0111.x

Hansen S, Fjeldborg J, Hansen J, and Baptiste K (2020). Reliability of cytological evaluation of mast cells from bronchoalveolar lavage fluid in horses: Intraobserver agreement and mast cell identification. Equine Veterinary Education 32: 47-52. DOI: https://www.doi.org/10.1111/eve.13112

Hansen S, Otten ND, Birch K, Skovgaard K, Hopster-Iversen C, and Fjeldborg J (2020). Bronchoalveolar lavage fluid cytokine, cytology and IgE allergen in horses with equine asthma. Veterinary Immunology and Immunopathology, 220: 109976. DOI: https://www.doi.org/10.1016/j.vetimm.2019.109976

Hermange T, Le Corre S, Bizon C, Richard EA, and Couroucé A (2019). Bronchoalveolar lavage fluid from both lungs in horses: Diagnostic reliability of cytology from pooled samples. Veterinary Journal, 244: 28-33. DOI: https://www.doi.org/10.1016/j.tvjl.2018.12.002

Janssen P, Tosi I, Hego A, Maréchal P, Marichal T, and Radermecker C (2022). Neutrophil extracellular traps are found in bronchoalveolar lavage fluids of horses with severe asthma and correlate with asthma severity. Frontiers in Immunology, 13(1): 921077. DOI: https://www.doi.org/10.3389/fimmu.2022.921077

Jocelyn NA, Wylie CE, Lean M, Barrelet A, and Foote AK (2018). Association of neutrophil morphology with bacterial isolates in equine tracheal wash samples. Equine Veterinary Journal, 50(6): 752-758. DOI: https://www.doi.org/10.1111/evj.12837

Kang H, Bienzle D, Piché É, Viel L, Odemuyiwa, SO, and Beeler-Marfisi J (2022). Flow cytometric analysis of equine bronchoalveolar lavage fluid cells in horses with and without severe equine asthma. Veterinary Pathology, 59(1): 91-99. DOI: https://www.doi.org/10.1177/03009858211042588

Kozłowska N, Wierzbicka M, Jasiński T, and Domino M (2022). Advances in the diagnosis of equine respiratory diseases: A review of novel imaging and functional techniques. Animals, 12(3): 381. DOI: https://www.doi.org/10.3390/ani12030381

Lee GC, Beeler-Marfisi J, Viel L, Piché É, Kang H, Sears W, and Bienzle D (2022). Bronchial brush cytology, endobronchial biopsy, and SALSA immunohistochemistry in severe equine asthma. Veterinary Pathology, 59(1): 100-111. DOI: https://www.doi.org/10.1177/03009858211048635

Lendl and Barton AK (2024). Equine asthma diagnostics: Review of influencing factors and difficulties in diagnosing subclinical disease. Animals, 14(23): 3504. DOI: https://www.doi.org/10.3390/ani14233504

Lendl L, Wirth C, Merle R, and Barton AK (2025). Influence of a standardized lunging exercise test on balf cytology in horses suffering from mild-moderate equine asthma. Animals, 15(16): 2428. DOI: https://www.doi.org/10.3390/ani15162428

Lowell FC (1964). Observations on heaves. An asthma-like syndrome in the horse. The Journal of Allergy, 1(35): 322-330. DOI: https://www.doi.org/10.1016/0021-8707(64)90095-4

Meiseberg LK, Mergani A, Delarocque J, Imker R, Köhn D, Wanes D, Bonilla MC, Veldhuizen EA, Von Köckritz-Blickwede M, Ohnesorge B et al. (2025). Equine asthma is characterized by severity-dependent correlations between blood neutrophil cholesterol content and net formation. European Journal of Immunology, 55(10): e70072. DOI: https://www.doi.org/10.1002/eji.70072

Morini M, Gobbo F, Rinnovati R, Romagnoli N, Peli A, Massarenti C, Spadari A, and Pietra M (2023). Bronchoalveolar Lavage cytology in severe equine asthma: Cytocentrifugated versus sediment smear preparations. Veterinary Sciences, 10(8): 1-13. DOI: https://www.doi.org/10.3390/vetsci10080527

Pickles K, Pirie RS, Rhind S, Dixon PM, and McGorum BC (2002a). Cytological analysis of equine bronchoalveolar lavage fluid. Part 1: Comparison of sequential and pooled aliquots. Equine Veterinary Journal, 34(3): 288-291. DOI: https://www.doi.org/10.2746/042516402776186137

Pickles K, Pirie RS, Rhind S, Dixon PM, and McGorum BC (2002b). Cytological analysis of equine bronchoalveolar lavage fluid. Part 2: Comparison of smear and cytocentrifuged preparations. Equine Veterinary Journal, 34(3): 292-296. DOI: https://www.doi.org/10.2746/042516402776186155

Rasmussen N, Karlsen P, Otten ND, Fjeldborg J, and Hansen S (2024). Bilateral bronchoalveolar lavage cytology profiles in a warmblood horse population during a 1-year period. Journal of Veterinary Internal Medicine, 38(4): 2391-2398. DOI: https://www.doi.org/10.1111/jvim.17118

Rossi H, Virtala AM, Raekallio M, Rahkonen E, Rajamäki MM, and Mykkänen A (2018). Comparison of tracheal wash and bronchoalveolar lavage cytology in 154 horses with and without respiratory signs in a referral hospital over 2009-2015. Frontiers in Veterinary Science, 5(61): 1-9. DOI: https://www.doi.org/10.3389/fvets.2018.00061

Sage SE, Leeb T, Jagannathan V, and Gerber V (2024). Single-cell profiling of bronchoalveolar cells reveals a Th17 signature in neutrophilic severe equine asthma. Immunology, 171(4): 549-565. DOI: https://www.doi.org/10.1111/imm.13745

Shawaf T (2019). Cytological analysis of tracheal washing and bronchoalveolar lavage fluid obtained from donkeys with chronic lung disease. Alexandria Journal of Veterinary Sciences, 60(1): 15-21. DOI: http://www.doi.org/10.5455/ajvs.20051

Shawaf T, Almathen F, Meligy A, El-Deeb W, and Al-Bulushi S (2017). Biochemical analysis of some serum trace elements in donkeys and horses in Eastern region of Kingdom of Saudi Arabia. Veterinary World, 10(10): 1269-1274. DOI: https://www.doi.org/10.14202/vetworld.2017.1269-1274

Shawaf T, Almubarak A, Alhumam N, Almathen F, and Hussen J (2021). Cytological analysis of tracheal wash and bronchoalveolar lavage fluid in health and respiratory disease in dromedary camels. PeerJ, 9(1): e11723. DOI: https://www.doi.org/10.7717/peerj.11723

Sundman EA, Chigerwe M, and Hunyadi LM (2020). A retrospective study of bronchoalveolar lavage fluid analysis in barrel racing horses with exercise-induced pulmonary hemorrhage and asthma in Texas from 2016 to 2018. Research in Veterinary Science, 132(1): 338-341. DOI: https://www.doi.org/10.1016/j.rvsc.2020.07.001

Uberti B and Morán G (2018). Role of neutrophils in equine asthma. Animal Health Research Reviews, 19(1): 65-73. DOI: https://www.doi.org/10.1017/S146625231800004X

Wiederkehr A, Barbarossa A, Ringer SK, Jörger FB, Bryner M, and Bettschart-Wolfensberger R (2021). Clinical Randomized comparison of medetomidine and xylazine for isoflurane balanced anesthesia in horses. Frontiers in Veterinary Science. 8(1): 603695. DOI: https://www.doi.org/10.3389/fvets.2021.603695

Wilson ME, McCandless EE, Olszewski MA, and Robinson NE (2020). Alveolar macrophage phenotypes in severe equine asthma. Veterinary Journal, 256: 105436. DOI: https://www.doi.org/10.1016/j.tvjl.2020.105436

Wysocka B and Kluciński W (2015). Cytological evaluation of tracheal aspirate and broncho-alveolar lavage fluid in comparison to endoscopic assessment of lower airways in horses with recurrent airways obstruction or inflammatory airway disease. Polish Journal of Veterinary Sciences, 18(3): 587-597. DOI: https://www.doi.org/10.1515/pjvs-2015-0076