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Original Research

Diagnosis of Lower Respiratory Tract Diseases with the Help of Bronchoalveolar Lavage Fluid in the Equines

G. S. Ubhare[1]*, R. V. Suryawanshi[1], A. H. Ulemale[1], A. B. Mali[2], C. S. Mote[3], U. M. Tumlam[4], P. V. Mehere[5], S. B. Swami[6], Y. B. Jadhav[6] and P. B. Chougale[1]

[1]Department of Veterinary Surgery and Radiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, INDIA
[2]Department of Veterinary Gynecology and Obstetrics, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, INDIA
[3]Department of Pathology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, INDIA
[4]Department of Veterinary Microbiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, INDIA
[5]Department of Veterinary Physiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, INDIA
[6]Department of Veterinary Clinical Medicine, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, INDIA

*Corresponding Author: gauriubhare0504@gmail.com

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Abstract

The clinical study was carried out in the ten horses age between 4-8 years having history of chronic respiratory symptoms during the winter season presented for the second opinion. The aim of this study was to diagnose lower respiratory tract diseases with the help of bronchoalveolar lavage and to study the pathological alteration present in the mentioned study. The bronchoalveolar lavage fluid was collected using BALF catheter and it was processed for cytological and bacteriological alterations. The results included total nucleated cell count (0.67±0.07%), alveolar macrophages (31.3±3.84%), lymphocytes (47.6±5.89%), neutrophils (8.1±1.66%), mast cell (1.6±0.52%) and eosinophils (1.6±0.22%). The bacteria like E. coli were also isolated. Thus, bronchoalveolar lavage cytology technique serves as important tool for diagnosing lower airway diseases.

Keywords: Bronchoalveolar, Cytology, Microbiology

Introduction

Young athletic horses are most commonly affected with Inflammatory Airway Disease (IAD) due to pressure exerted on lungs during moderate to heavy exercise and presented with history of cough, increased respiratory secretions and exercise intolerance. Recurrent Airway Obstruction (RAO) is an allergic disorder in adult or mature horses characterized by cough, mucopurulent secretion, abnormal breath sound, increased respiratory efforts and exercise intolerance according to Couetil (2014). Adult horses are susceptible for development of pneumonia when a bacterium is aspirated from environment, via nose and oropharynx then reach the lower airway and impairs the pulmonary defense mechanism (Carvallo *et al.*, 2017). Coughing was prominent signs of lower respiratory tract. However, horses that coughed were very likely to have lower airway disease for more than one month and housed on straw bedding (Burrell *et al.*, 1996).

The most common diagnostic modalities used to evaluate equine respiratory tract diseases includes endoscopy (Jean *et al.*, 2011), radiography (Couetil *et al.*, 2016 and Estell *et al.*, 2016), ultrasonography (Hussein *et al.*, 2018 and Siwinska *et al.*, 2019), bronchoalveolar lavage for cytological evaluation (Hermange *et al*, 2019 and Siwinska *et al.*, 2019), bacterial culture (Buckley *et al.*, 2007 and Estell *et al.*, 2016) and histopathological evaluation of respiratory mucosa and pulmonary parenchyma.

Bronchoalveolar Lavage (BAL) is a technique for collection of respiratory secretions that lines the peripheral airways or alveoli and it is considered to be very safe and sufficiently sensitive tool to detect chronic obstructive pulmonary diseases. (McGorum *et al.*, 1993); exercise induced pulmonary hemorrhages (Roy and Lavoie, 2003); inflammatory airway disease (Couetil *et al.*, 2007; Mazan, 2018 and Hansen *et al.*, 2019); inflammation at the cytologic level (Hoffman, 2008); recurrent airway obstruction (Kutasi *et al.*, 2011) and equine asthma (Hermange *et al.*, 2019).

Materials and Methods

Ten horses, suspected with lower respiratory diseases which were not diagnosed via thoracic radiography or ultrasonography were subjected for routine cytological examination of lower respiratory secretions via bronchoalveolar lavage fluid cytology technique with the help of Bronchoalveolar Lavage (BAL) catheter having diameter 10 mm x length 240 cm (Figure 1).Each horse was restrained and sedated with Inj. Xylazine @ 0.5 mg/kg and Inj. Butorphanol @ 0.02 mg/kg intravenously (Hare and Viel, 1989 and Davis and Sheats, 2019) for easy passage of catheter.

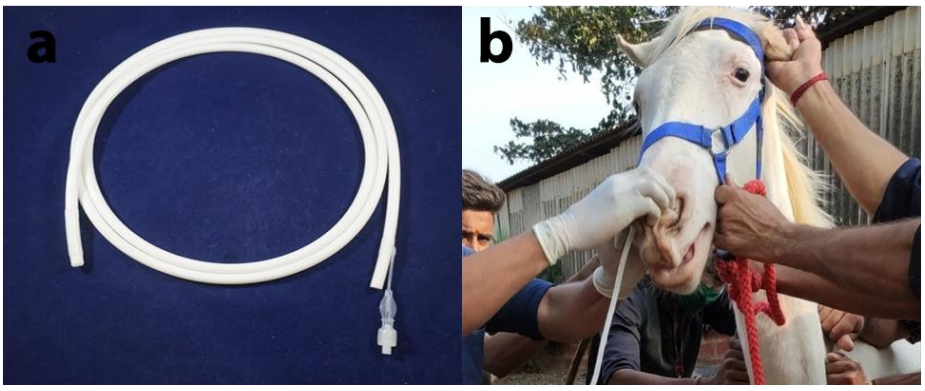

Figure 1: Broncho-alveolar lavage catheter of horses (a) and placement of BAL catheter into carina during collection of BALF.

The outer surface of catheter was smeared with 2% lignocaine jelly and 0.5% lignocaine solution was sprayed on the carina to prevent bronchoalveolar irritation. The catheter was passed smoothly through nasal meatus into lower airways and instillation of about 250-300 ml of lukewarm normal saline was performed. With the help of outlet cock, about 50-100 ml fluid was aspirated in sterile syringe to evaluate its transparency, color and presence of foamy layer and recorded.

The BAL catheter was sterilized with 2% glutardehyde solution and flushed with distilled water between two examinations to avoid cross contamination. Total nucleated cell count was performed to estimate the number of

cells with the help of Neubauer's hemocytometer by using standard cytological criteria as described by Davis and Sheats, (2019) which was used to evaluate the chronicity of various lung diseases in horses (Table No.1). BAL sample was processed for cytological examination within 24 hours (Hermange *et al.*, 2019) and cytocentrifugation was performed in centrifuge machine for 10 minutes at 75-RPM. The pellet was smeared on glass slide and was stained with Wright & Giemsa stain for 20 minutes and differential cell count (epithelial cell, lymphocytes, macrophages, polymorphonuclear cells (PMN), eosinophils, neutrophils and mast cell) under the 100 X microscope was carried out to assess the inflammatory status of lungs.

Table 1: Cytological criteria (differential cell count) used for grading of various lung diseases (RAO/IAD) in horses (Davis and Sheats, 2019)

Normal Count	Mild/ Moderate Neutrophilic	Mild/Moderate Mastocytic	Mild/Moderate Mixed	Severe
≤6% Neutrophils	7-19% Neutrophils	≥3% Mast Cells	7-19% Neutrophils	≥20% Neutrophils
≤2% Mast Cells	≤2% Mast Cells	≤6% Neutrophils	≥3% Mast Cells	Or
≤1% Eosinophils				↑Respiratory Rate/ Effort at Rest

In addition, bronchoalveolar lavage samples collected from horses for cytological evaluation were subjected for microbiological investigation.

Result and Discussion

Ten horses aged between 4-8 years underwent for bronchoalveolar lavage cytological (BALF) sampling during the winter season to evaluate cytologic lesions associated with chronic lung diseases (RAO or IAD). In present study, BAL catheter was found to be easier tool for collection of BAL sample in horses (Sweeney and Beech, 1991 and Couroucé-Malblanc *et al.,* 2010) for diagnosis of chronic lung disease.

In present study, about 50-100 ml volume of BAL samples were collected blindly from level of carina with BAL catheter. The recovery of BAL samples may vary i.e., 30 ml (Koterba, 1991); 100 ml (Sweeney and Beech, 1991) and 44.0±10.6 ml (Hansen *et al.,* 2019) according to technique used for BAL sampling. Grossly, all BALF samples were appeared to be colorless, clear transparent with white foamy layer on the surface i.e., surfactant and drawn (Figure 2). Similar observation made by Couetil (2014) and Mair and Rush (2013) and stated that, presence of surfactant in BAL sample indicates good sample retrieval. In present study, the mean average recorded values from the BALF samples included total nucleated cell count (0.67±0.07 x10^6/L) (Figure 3), alveolar macrophages (31.3±3.84%) (Figure 4), lymphocytes (47.6±5.89%) (Figure 5), neutrophils (8.1±1.66%) (Figure 6), mast cell (1.6±0.52%) (Figure 7) and eosinophils (1.6±0.22%) showed reduced cell count might be due decrease in cell count in BALF sample after 24 hrs at 40°C. Pickles *et al.* (2002) reported that, significant decrease in cell viability occurs by 24 hrs at 40°C in BALF sample.

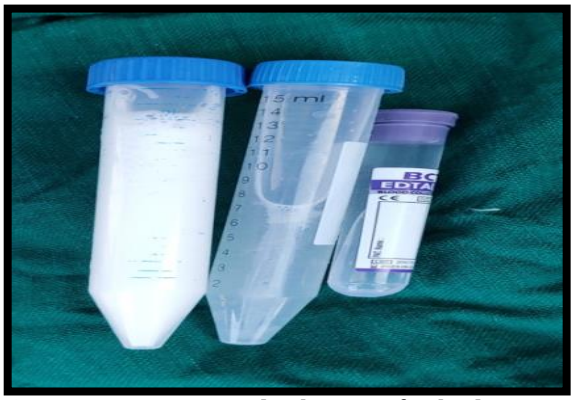

Figure 2: BALF sample showing frothy layer on top with few specks. Aliquots collected in EDTA vials for cytological examination and plain vials for microbiological examination.

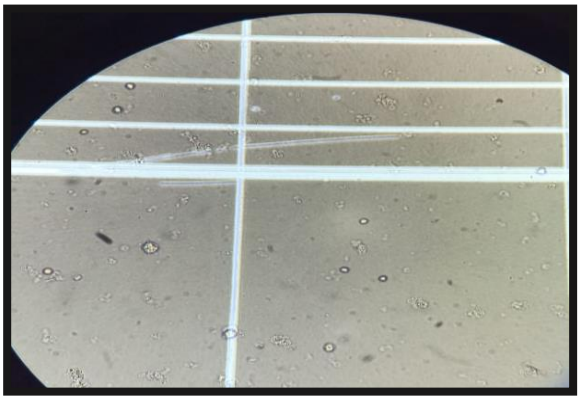

Figure 3: Total Nucleated Cell Count of BALF obtained from horse

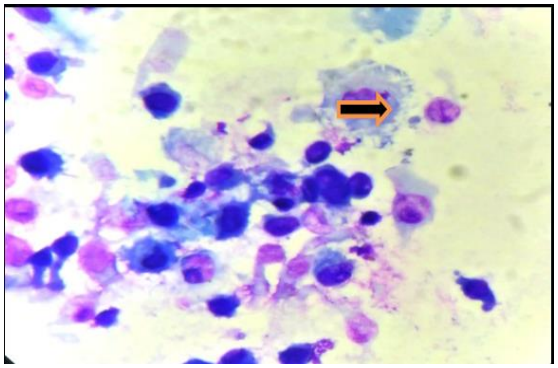

Figure 4: Cytology of BAL from horse showing alveolar macrophages(arrow)(Giemsa stain, X100)

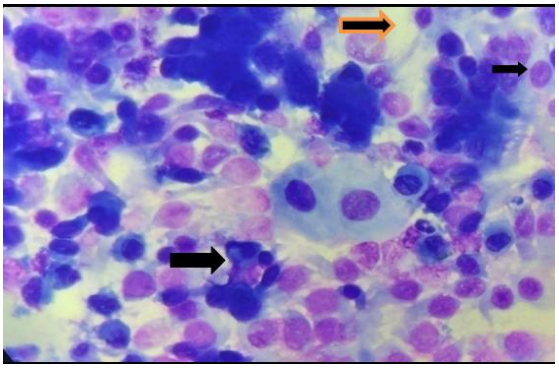

Figure 5: Cytology of BAL from horse showing lymphocytes(arrow). (Giemsa stain, X100)

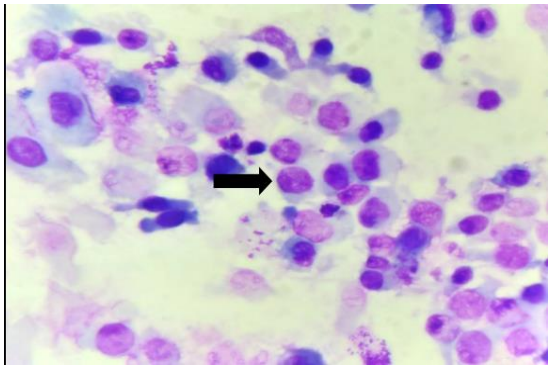

Figure 6: Cytology of BAL from horse showing neutrophils(arrow)(Giemsa stain, X100).

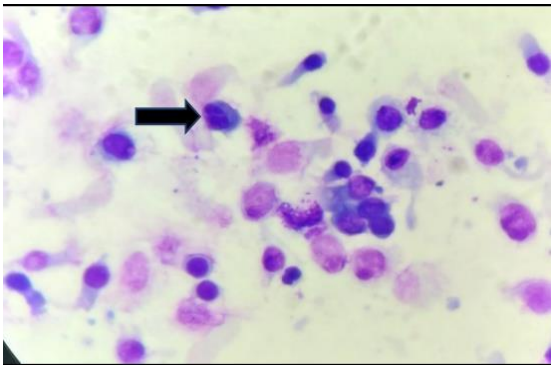

Figure 7: Cytology of BAL from horse showing mast cell (arrow) (Giemsastain, X100)

There are many researchers concluded that, >5% eosinophilia was considered for allergic lung disease (Hare and Viel, 1998) and > 10% neutrophils, >2% mast cells, >1% eosinophils indicate inflammatory airway disease (Richard, 2010). In another study, increase in amount of non-degenerative neutrophils which comprises of 20% and 40% of all cells indicates inflammatory airway disease and chronic obstructive pulmonary disease, respectively (Siwinska *et al.*, 2019).

In one horse, BALF sample showed very dense fibrillar mucus indicative of chronic neutrophilic inflammatory airway disease (Figure 8) whereas in another sample of BALF showed colonization of bacteria indicative of mixed bacterial infection associated with chronic lung disease (Figure 9). The present findings were corroborated with Hoffman, (2008) who stated that, presence of dense fibrillar mucus indicates chronic inflammatory airway disease.

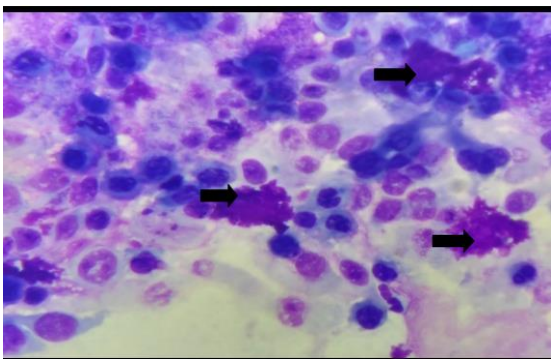

Figure 8: Cytology of BAL from horse showing very densefibrillar mucus indicative of chronic neutrophillic inflammatory airway disease.(arrows). (Giemsa stain, X100)

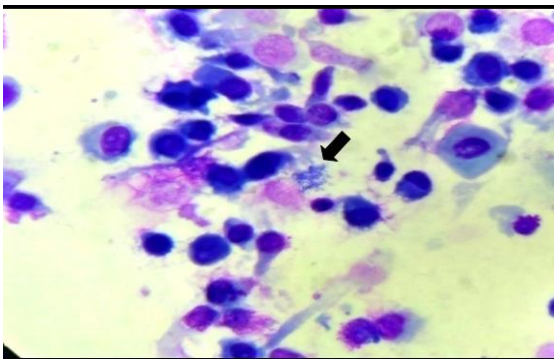

Figure 9: Cytology of BAL from horse showing presence of bacterial colony arrow)(Giemsa stain, X100)

In another study, counting minimum 200 cells which further were expressed as percentage of total leukocyte count (Fraipont *et al,* 2011) helped for pinpointing airway disease. Out of 10 BALF samples, 4 horses showed *E.coli*

(66.66%) (Figure 10) and each one horse showed *Enterobacter spp.* (16.67%) and gram negative bacilli organisms (16.67%), respectively. In present study, regardless exact mechanism predisposing to bacterial colonization of lower respiratory tract, large influx of inflammatory cells causes chronic inflammation of lung and parenchymal consolidation indicative of inflammatory airway disease. The 6 BALF antibiotic sensitivity results (Figure 11) showed highest sensitivity for Enrofloxacin (100%) followed by ciprofloxacin (75%), Amikacin (75%) and Gentamicin (50%) while resistance was reported to amoxicillin clavulanic acid (100%), Cefotaxime (100%), Amoxicillin-Sulbactam (100%) and Ceftriaxone tazobactam (100%) followed by Azithromycin (75%), Penicillin (75%) and Doxycycline (75%). The present findings of antibiotic sensitivity of nasal and BAL collected from horses with lower respiratory lung diseases was in accordance with Muktha *et al.* (2015) who reported, 66.66% *E. coli* isolates in horses and found that, ciprofloxacin and gentamicin has a highest sensitivity.

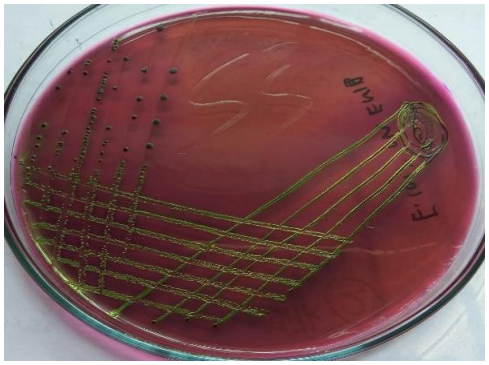

Figure 10: *E.coli* pigmented growth on EMB agar

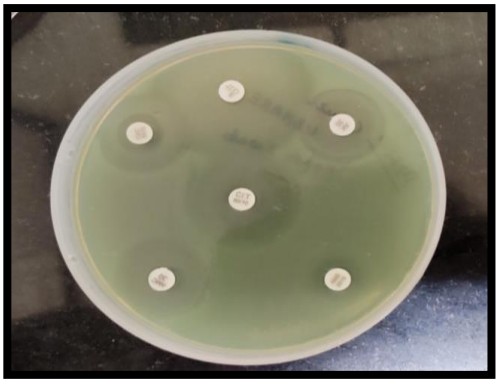

Figure 11: Antibiotic sensitivity test by agar disc diffusion method showing resistant and sensitive zones.

Table 2: Total nucleated cell count and differential cell count of BALF collected from horses (n=10) suffering with chronic lung diseases (IAD /&RAO)

S. No.	Brand No./Name of Horse	Total Nucleated Cell Count (X106/L)	Differential Cell Count							
			Alveolar macrophages (%)	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)	Mast cells (%)	Epithelial cells	Bacteria	Other incidental findings
1	Tag No.12	Very few cells	-	-	-	2	0	Present	Present	Very Dense Fibrillar Mucus
2	New Punjab	0.7	34	61	2	1	2	Present	-	Very Dense Fibrillar Mucus
3	Tag No.107	0.45	36	54	4	2	4	Present	Present	Cellulose Fibers
4	Tag No.110	62	32	60	6	1	1	Present	Present	Cellulose Fibers
5	Tag No.24	0.95	30	61	7	2	0	Present	Present	Very Dense Fibrillar Mucus
6	Kesar	0.82	24	61	14	1	0	Present	Present	Cellulose Fibers
7	Tag No.1	0.55	41	37	16	2	4	Present	Present	Ciliocytophthoria
8	Tag No.2	0.96	38	46	11	3	2	Present	Present	Cellulose Fibers
9	Tag No.3	0.54	37	50	12	1	0	Present	-	Cellulose Fibers
10	Tag No.4	0.42	41	46	9	1	3			
Mean average± SE		0.67±0.07	31.3±3.84	47.6±5.89	8.1±1.66	1.6±0.22	1.6±0.52	Present	-	Cellulose Fibers

Conclusion

Bronchoalveolar samples were collected in 10 horses with history of chronic respiratory signs to evaluate cytologic lesions in lung diseases. Grossly, about 50-100 ml volume of sample were retrieved and appeared to be colorless, transparent with foamy surfactant indicating good sample retrieval. Cytological findings include total nucleated cell count (0.67±0.07%), alveolar macrophages (31.3±3.84%), lymphocytes (47.6±5.89%), neutrophils (8.1±1.66%), mast cell (1.6±0.52%) and eosinophils (1.6±0.22%). One horse showed very dense fibrillar mucus and bacterial colonization suggestive of chronic neutrophilic inflammatory airway disease.

Conflict of Interests

There is no conflict of interest.

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References

1. Buckley T., E. McManamon and S. Stanbridge (2007). Resistance studies of erythromycin and rifampin for Rhodococcus equi over a 10 years period. *Irish Veterinary Journal*.60 (12), 728-731.
2. Burrell M. H., J. L. N. Wood, K. E. Whitwell, N. Chanter, M. E. Mackintosh and J. A. Mumford (1996). Respiratory disease in thoroughbred horses in training: The relationships between disease and viruses, bacteria and environment, *Veterinary Record*, 139(13), 308–313.
3. Carvallo F.R, F.A. Uzal, S.S. Diab, A.E. Hill and R.M. Arthur (2017). Retrospective study of fatal pneumonia in racehorses. *Journal of Veterinary Diagnostic Investigation*. 29(4), 450-456.
4. Couetil L. (2014). Inflammatory diseases of the lower airway of athletic horses. Equine sports medicine and surgery. Second edition, chapter 28, Saunders Elsevier publications, 605-625.
5. Couëtil L. L., J.M. Cardwell, V. Gerber, J.-P. Lavoie, R. L Eguillette and E.A. Richard (2016). Inflammatory Airway Disease of Horses-Revised Consensus Statement, *Journal of Veterinary Internal Medicine*, 30(2), 503–515.
6. Couetil L., A. Hoffman, J. Hodgson, V. Buechner-Maxwell, L. Viel, J. Wood and Lavoie (2007). Inflammatory Airway Disease of Horses. *Journal of Veterinary Internal Medicine*.21, 356-361.
7. Couroucé-Malblanc A. S. Pronost, G. Fortier, R. Corde, F. Rossignol (2002). Physiological measurements and upper and lower respiratory tract evaluation in French Standard bred Trotters during a standardised exercise test on the treadmill. *Equine veterinary journal*. Supplement (34), 402–407.
8. Davis K. U. and M. K. Sheats (2019). Bronchoalveolar lavage cytology characteristics and seasonal changes in a herd of pastured teaching horses, *Frontiers in Veterinary Science*. 6,1–7.
9. Estell K. E., A. Young, T. Kozikowski, E.A. Swain, B.A. Byrne, C.M. Reilly, P.H. Kass and M. Aleman (2016). Pneumonia Caused by Klebsiella spp. in 46 Horses, *Journal of Veterinary Internal Medicine*, 30(1), 314–321.
10. Fraipont A., E. Van Erck, E. Ramery, E. Richard, J-M. Denoix, P. Lekeux and T. Art (2011). Subclinical diseases underlying poor performance in endurance horses: Diagnostic methods and predictive tests, *Veterinary Record*, 169(6), 154.
11. Hansen S., N. D. Ottenb, K. Bircha, K. Skovgaardc, C. Hopster-Iversena and J. Fjeldborga (2019). Bronchoalveolar lavage fluid cytokine, cytology and IgE allergen in horses with equine asthma. *Veterinary Immunology and Immunopathology*, 220,109976.
12. Hare J. and L. Viel (1989). Pulmonary Eosinophilia Associated with increased responsiveness in young Racing horses. *Journal of Veterinary Internal Medicine* 12,163-170.
13. Hermange T., S.L. Corre, C. Bizon, E. A. Richard and A. Courouce (2019). Bronchoalveolar lavage fluid from both lungs in horses: Diagnostic reliability of cytology from pooled samples, *Veterinary Journal*. Elsevier, 244, 28-33.

14. Hoffman A. (2008). Bronchoalveolar lavage: Sampling Techniques and Guidelines for cytologic preparation and interpretation. *The Veterinary Clinics of North America. Equine Practice*.24 (2), 423-435.
15. Hussein H., C. Binici and R. Staufenbiel (2018). Comparative evaluation of ultrasonography with clinical respiratory score in diagnosis and prognosis of respiratory diseases in weaned dairy buffalo and cattle calves. *Journal of Animal Science and Technology*. 60:29.
16. Jean D., G. Beauchamp and J. Lavoie (2011). Lavage Fluid in Horses with Recurrent Airway Obstruction, *Analysis*, 72(6).
17. Koterba A. (1991). Disorders of the neonatal foal, Equine Respiratory Disorders, chapter 22, Lea & Febiger publication, 403-429.
18. Kutasi O., N. Balogh, Z. Lajos, K. Nagy and O. Szenci (2011). Diagnostic approaches for the assessment of equine chronic pulmonary disorders. *Journal of Equine Veterinary Science*.31, 400-410.
19. Mair T. and B. Rush (2013). Lower respiratory tract, Equine medicine, surgery and reproduction, second edition, chapter 6. Saunders Elsevier publications, 111-132.
20. Mazan M. R. (2018). Lower Airway Disease in the Athletic Horse, *Veterinary Clinics of North America - Equine Practice*. Elsevier Inc, 34(2), 443–460.
21. McGorum B., Dixon P. and R. Halliwell (1993). Evaluation of intradermal mould antigen testing in the diagnosis of equine chronic obstructive disease. *Equine Veterinary Journal*. 25(4), 273-275.
22. Muktha Z., Kabir S. and Rahman M. (2015). Isolation and identification of bacterial flora from respiratory tract of healthy horses. *Journal of Bangladesh Agricultural University*, 13(2), 239-246.
23. Pickles K., Pirie R., Rhind S., Dixon P. and B. McGorum (2002). Cytological Analysis of equine bronchoalveolar lavage fluid. Part 3: the effect of time, temperature and fixatives. *Equine Veterinary Journal*, 34(3), 297-301.
24. Richard E. A (2010). Sub-clinical diseases affecting performance in Standard bred trotters: Diagnostic methods and predictive parameters. *Veterinary Journal*.184, 282-289.
25. Roy M.F. and J.P. Lavoie (2003). Tools for Diagnosis of Equine Respiratory Disorder. *Veterinary Clin Equine*. 19,1-17.
26. Siwinska N., A. Zak, M. Slowikowska, P. Krupinska and A. Niedzwiedz (2019). Prevalence and Severity of Ultrasonographic Pulmonary Findings in Horses with Asthma- A Preliminary Study. *Polish Journal of Veterinary Sciences*. 22(4) ,653-659.
27. Sweeney C. and J. Beech (1991). Bronchoalveolar Lavage. Equine respiratory disorders. Chapter 4, 55-61.

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