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Exercise-induced pulmonary hemorrhage in barrel racing horses in the Pacific Northwest region of the United States

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Background: Exercise-induced pulmonary hemorrhage (EIPH) refers to bleeding from the lungs in association with strenuous exercise. It has been documented in race horses but little information exists on EIPH in barrel racing horses.

Hypothesis/Objectives: Our goals were to evaluate the presence of EIPH in barrel racing horses and estimate its prevalence in the Pacific Northwest.

Animals: 149 barrel racing horses enrolled at events in WA (11), ID (3), and MT (33).

Methods: Observational cross-sectional study. Data collected included signalment, history of illness, respiratory disease, race division, and pre-race medications. Endoscopy was performed and tracheobronchoscopic (TBE) EIPH score was assigned based on quantity of blood in the trachea (0 = no blood to 4 = abundance of blood within the trachea). After TBE, bronchoalveolar lavage (BAL) was performed. Erythrocyte (red blood cell, RBC) counts were obtained from bronchoalveolar lavage fluid (BALF). Statistical analysis included linear and logistic regression, Fisher's exact *t* test, and calculation of correlation coefficient. Significance was set at $P < .05$.

Results: The prevalence of EIPH based on TBE EIPH score was 54%. When based on BALF RBC count >1,000 cells, EIPH prevalence was 66%. Race time did not significantly affect the presence of EIPH. A significant ($P < .0001$) positive linear relationship between the TBE and BAL erythrocyte count was identified, but its strength was poor ($r^2 = .15$).

Conclusions and Clinical Importance: EIPH occurs in over 50% of barrel racing horses in the Pacific Northwest. Precise determination of the impact of EIPH on health of barrel racers requires further study.

KEYWORDS
bronchoalveolar lavage, endoscopy, equine, respiratory

1 | INTRODUCTION

Exercise-induced pulmonary hemorrhage (EIPH) is bleeding that occurs in the lungs during exercise, after exercise or both and can have a negative impact on performance.[1,2] It is diagnosed by detecting the presence of blood in the airways during the post-exercise period by either tracheobronchoscopy (TBE), bronchoalveolar lavage (BAL) or both.[2]

Abbreviations: BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; EIPH, exercise-induced pulmonary hemorrhage; IAD, inflammatory airway disease; ; PBS, phosphate buffered 0.9% sodium chloride; RBC, red blood cell; TBE, tracheobronchoscopic EIPH exam; WBC, white blood cell.

The work was performed at barrel racing events in Idaho, Montana and Washington State. A synopsis of the work described in this manuscript was presented at the ACVIM Forum 2017.

Despite the fact that this condition occurs in a large number of performance horses[1] and is associated with substantial economic losses, its pathogenesis and impact on athletic performance and the long-term health of the horse still needs clarification.[2]

Barrel racing horses (predominantly American Quarter Horses or related breeds) are required to run for short periods of time (usually <20 seconds/run) at maximal intensity. This exercise is similar to that of sprinting Thoroughbreds or Quarter Horses, which are breeds known to experience performance-limiting EIPH.[2] Surprisingly, only 1 peer-reviewed report describes the incidence and severity of EIPH in barrel racing horses.[3] In the population studied, prevalence of EIPH was high (77/170 horses [45.3%]) but the study design did not allow thorough evaluation of effect on performance. Poor performance is a primary presenting complaint to veterinarians in 39%-41% of clinical cases involving barrel racing horses.[4,5] The purpose of our study was to assess the presence of EIPH in barrel racing horses, estimate its prevalence in the Pacific Northwest, and determine if relationships exist between EIPH and barrel racing performance.

2 | MATERIALS AND METHODS

In this observational study, veterinary researchers travelled to different barrel races in Washington, Idaho, and Montana in 2016. The events typically were 2–4 days long with a variety of levels of competition. The races were run in designated divisions, 1D through 6D, which reflected the competitive abilities of the horses. The 1D classes were for the fastest horses. The National Barrel Racing Association uses increments of 0.5 seconds to determine individual divisions or classes. The fastest time is a 1D. The 2D cut-off time is 0.5 seconds slower than 1D time, and so on until all 6 divisions are established for an event. The 6D class typically is for horse and rider that run the course more slowly, or for schooling young horses. A booth was set up at the races, and announcements at the event and via social media encouraged participation in the study.

Interested horse owners filled out a questionnaire requesting information regarding age, breed, barrel racing division, history of any illness, respiratory disease, previous diagnosis with EIPH, cough at rest or with exercise, and medications administered before racing. To be included in the study, horses must have run at least once on the day of evaluation. Some of the horses had a reported history of EIPH, whereas others had no history of previous bleeding. Horses were not excluded from the study if they had a history of EIPH nor were they excluded for use of race day medications or nasal flare strips. The owners had the risks and benefits of the evaluative procedures explained before participation in the study and consent forms were signed before performing TBE and BAL. The study was approved by Washington State University Animal Care and Use Committee, ASAF # 04778-002, 01/06/2016.

All horses received a complete physical examination after they ran followed immediately by a TBE and BAL. The physical examination was performed at least 45 minutes post-race with some being performed > 90 minutes post-race. Heart rate, respiratory rate, temperature, and elapsed time since the race were recorded. The horses were sedated with xylazine (0.5-1 mg/kg IV) and acepromazine (0.02-0.6 mg/kg IV) after the physical examination and just before TBE and BAL. Both TBE and BAL were sequentially performed immediately after sedation.

2.1 | Endoscopic procedure and scoring

Tracheobronchoscopy, including recording of videos was performed using a 12.9 mm diameter 3-meter video-endoscope (Vetel Diagnostics, San Luis Obispo, California) as previously described.[3,6,7] Horses were considered positive for EIPH if the TBE EIPH score was ≥ 1. The TBE EIPH score was based on the quantity of blood in the trachea: Grade 0 = no blood; Grade 1 = 1 or more flecks of blood or 2 or fewer short narrow streams of blood in the trachea or mainstem bronchi; Grade 2 = 1 long stream of blood or more than 2 short streams of blood occupying less than a third of the tracheal circumference; Grade 3 = multiple distinct streams of blood covering more than a third of the tracheal circumference, with no blood pooling at the thoracic inlet; and Grade 4 = multiple, coalescing streams of blood covering more than 90% of the trachea with blood pooling at the thoracic inlet.[3,6,7] Tracheobronchoscopy and BAL were performed as close to 60 minutes-post racing as possible. Scores were assigned for the presence of blood in the trachea after review of the videos (Figure 1).[6]

2.2 | Bronchoalveolar lavage

Bronchoalveolar lavage was performed through the endoscope immediately after TBE. The purpose of using the endoscope was to select the bronchi in which blood was visible. The endoscope was passed into the right or left main stem bronchus depending upon whether blood was noted in the right, left, both, or neither bronchus. When blood was noted in each mainstem bronchus, the bronchus exhibiting the greatest amount of blood was entered. If blood was not observed, typically the right mainstem bronchus was selected. The endoscope was advanced until it was wedged in a smaller bronchus. Three hundred milliliters of phosphate buffered 0.9% sodium chloride (PBS) was instilled through a 2.3 mm diameter 320 cm catheter (Endoscopy Support Services, Brewster, New York) which had been passed down the biopsy channel of the endoscope. The initial 60 cc syringe of PBS also contained 15 mL of lidocaine to anesthetize the airways. Once all of the fluid was infused, fluid was aspirated through the catheter. The initial 10 mL of retrieved bronchoalveolar lavage fluid (BALF) was discarded and the rest of the fluid was aspirated until negative pressure was obtained. A portion of the retrieved PBS was saved in a 5 mL EDTA tube for red blood cell (RBC) count; the remainder of the fluid was stored in sterile 50 mL containers on ice until processing.

2.3 | Bronchoalveolar lavage red cell count

The number of erythrocytes (RBC) in the BALF was determined using a hemocytometer and a microscope under ×40 power. If there were initially too many RBCs to count on the hemocytometer, the BALF was diluted and the dilution factor incorporated into calculation of the total number of RBCs. The exact number RBCs considered positive for EIPH

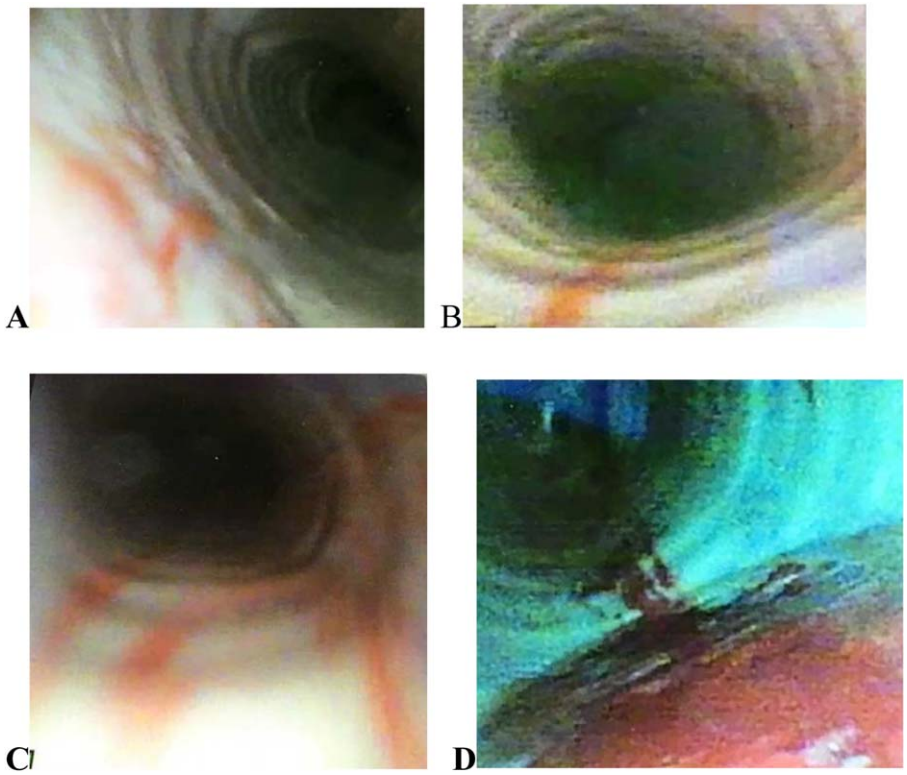

FIGURE 1 Images of tracheobronchoscopic scores grading exercise-induced pulmonary hemorrhage: A: Grade 1, B: Grade 2, C Grade 3, D Grade 4. Grade 1 = 1 or more flecks of blood or 2 or fewer short narrow streams of blood in the trachea or mainstem bronchi; Grade 2 = 1 long stream of blood or more than 2 short streams of blood occupying less than a third of the tracheal circumference; Grade 3 = multiple distinct streams of blood covering more than a third of the tracheal circumference, with no blood pooling at the thoracic inlet. Grade 4 = multiple, coalescing streams of blood covering more than 90% of the trachea with blood pooling at the thoracic inlet. Grading system used according to Hinchcliff[8]

has not been definitively established, and RBC >1,000/μL was utilized to classify horses as positive for EIPH in accordance with a previous recommendation.[8]

2.4 | Bronchoalveolar lavage cytology

The BALF was centrifuged for 10 minutes at 300*g* for cytologic evaluation and standard cytologic analysis of a stained slide was performed by a board-certified veterinary clinical pathologist. Cell type percentages were calculated and presence of hemosiderin or hemosiderophages was noted. Prussian blue stain was used to quantify presence of iron in hemosiderin (classified as percentage of iron-containing cells/sample).

2.5 | Statistical analysis

The statistical analysis was based on the 158 barrel races performed by 149 horses enrolled in the study. Where appropriate, data were expressed as mean ± standard deviation. The association between TBE EIPH score, BALF RBC count, and potential predictor variables was assessed using linear (BALF RBC count versus numeric variables) or logistic (TBE EIPH score ≥ 1 versus numeric or categorical variables) regression analysis of variance (ANOVA; BALF RBC versus categorical variables), or Fisher's exact test (TBE EIPH score ≥ 1 versus categorical variables).

A logarithmic transformation was applied to the BALF RBC count to satisfy normality and homogeneity of variance assumptions. All statistical analyses and descriptive statistics such as mean, standard deviation, and frequency were performed using SAS v9.4 (Washington State University, Pullman Washington). The significance level was $P < .05$.

3 | RESULTS

A total of 8 barrel racing events was attended during the 2016 summer and fall barrel racing season. The lengths of the barrel races were: 1 event utilized a short pattern (230 feet), 3 events utilized medium patterns (260 and 287 feet), and 4 events utilized long patterns (330 feet and 335 feet). Two of the events were outdoors, and 6 events were indoors. There were 128 TBE EIPH scores and BALs performed after indoor races and 30 evaluations after outdoors races (Table 1).

One-hundred forty-nine horses (mean age, 8.0 ± 11.5 years; range, 3–23 years) were enrolled in the study and they collectively completed 158 runs. Eight horses had TBEs and BALs performed at 2 different events. One horse had TBE and BAL performed at 3 different events. There were 2 stallions, 92 geldings, and 55 mares. There were 133 Quarter Horses, 7 Appendix Quarter horses, 7 Paint horses, and 2 Appaloosas.

TABLE 1 Number of horses with TBE and BAL performed at barrel racing events.

Event # and Place	Number of horses enrolled
Event 1 Pasco, WA	20
Event 2 Walla Walla, WA	32
Event 3 Deer Park, WA	3
Event 4 Pasco, WA	37
Event 5 Lewiston, ID	3
Event 6 Kennewick, WA	11
Event 7 Kalispell, MT	33
Event 8 Kennewick, WA	19

With 2 exceptions, horses were assessed on the same day after they performed at least 1 barrel race. Two horses had TBE and BAL performed 12 hours after they ran, because of a late-night performance and evidence of epistaxis the night they ran. Three horses ran twice on the same day before TBE and BAL were performed. The horses that ran twice had similar TBE EIPH scores and similar RBC/μL as horses that ran once, but because only 3 horses ran twice, no comparison could be made between horses that ran twice in 1 day and horses that ran once.

The prevalence of EIPH using TBE EIPH score ≥1 was 54% (85/158) of runs and when using a BALF RBC count of > 1,000 RBCs/μL was 66% (104/158) of runs. Some horses that were EIPH positive on TBE EIPH score (n = 17 horses) were not positive on BALF RBC count. Conversely, some horses that were BALF RBC positive were not EIPH positive with TBE EIPH score (n = 37 horses). The relationship between the 2 scoring systems was linear and significant ($P < .0001$) but the correlation was weak ($r^2 = .15$, Figure 2). The distribution of EIPH positive horses (n = 84) by TBE EIPH score was: grade 1 = 37%, grade 2 = 39%, grade 3 = 18%, and grade 4 = 6% (Table 2). For the 104 horses with BALF RBC counts > 1,000 cells/μL, 83 horses had between 1,000 and 25,000 RBC/μL; 6 horses had from 25,000 to 50,000 RBC/μL; 3 horses had between 50,000 and 100,000 RBC/μL; and 12 horses had >100,000 RBC/μL (Table 3).

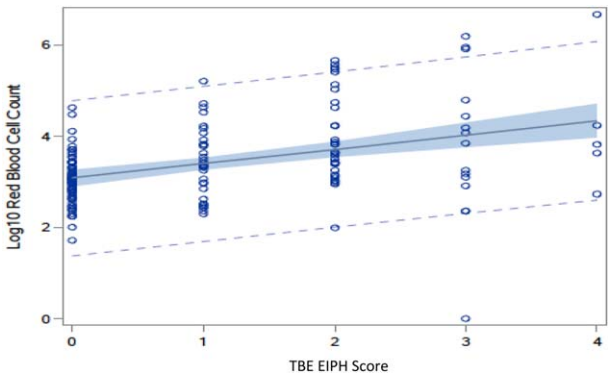

FIGURE 2 Red blood cell count correlation with TBE EIPH Score. The linear relationship was significant $P < .001$, but the correlation was poor $r^2 = .155$

TABLE 2 Tracheobronchoscopic positive EIPH scores in 85 barrel racing horses compared to those reported in thoroughbred racehorses.[2,8]

Tracheal score	TBE EIPH score for racehorses (%)	TBE EIPH score for barrel racing horses (%)
1	37	37
2	38	39
3	19	18
4	6	6

Horses had the TBE and BAL performed as close to 60 minutes post-race as possible, but variation still occurred in the exact time post-exercise when they were performed mainly because of the time when owners presented their horses for physical examination and EIPH evaluation. Twenty-seven runs performed by horses had TBE and BALs performed 45–60 minutes post-race. One-hundred and twenty runs performed by horses had TBE and BALs performed between 60–90 minutes post-race. Eleven runs performed by horses had TBE and BALs performed > 90 minutes post-race. A significant relationship was found between the actual time post-exercise when BAL was performed and the BALF RBC numbers ($P = .0004$), but the correlation ($r^2 = .10$) was poor. No relationship was found between the elapsed post-exercise time of the TBE and TBE EIPH score ($P = .07$; $r^2 = .03$; Figure 3).

No significant associations were found between the race time or distance of races with respect to TBE EIPH score ($P = .15$) or BALF RBC >1,000/μL ($P = .94$), or if the race was inside versus outside (TBE EIPH score, $P = .11$; BALF RBCs/μL, $P = .67$).

Thirty-seven horses competed on various IV dosages of furosemide (150–500 mg) administered 1–4 hours before racing. Of these, 25/37 (67%) had a TBE EIPH score ≥ 1, BALF RBC count > 1,000 cells/μL, or both. Sixteen horses received clenbuterol PO, with a range of doses from 362.5 μg to 725 μg before racing. Thirty-two horses had other drugs administered which included firocoxib, phenylbutazone, flunixin meglumine, chlorpromazine, albuterol, dexamethasone, acepromazine, ketoprofen, glycopyrrolate, aspirin, zylekene, omeprazole, and interferon before racing. Seventy-five horses received no pre-race medications. Medications before competition had no significant effect on BALF RBC count > 1,000 cells/μL ($P = .31$), but a significant relationship was found in the TBE EIPH score of horses that received clenbuterol. These horses had higher TBE EIPH scores ≥ 1 ($P = .002$).

TABLE 3 BALF RBC count for the 104 horses/149 horses with RBCs > 1,000 cells/μL

# of Horses-104	Red blood cells/μL	Mean + Standard Deviation
83	1,000–25,000	4,336 ± 4,177
6	25,000–50,000	35,817 ± 6,928
3	50,000–100,000	54,953 ± 4,524
12	>100,000	823,655 ± 1,259,274

Horses with RBCs > 1,000 cells/μL were considered positive for EIPH/

TABLE 4 Number of horses that coughed at rest or with exercise and pre-race medication.

Cough/Pre-race treatment	# of Horses
Coughed at rest	48
Coughed during exercise	74
Coughed during rest and exercise	40
Pre-race furosemide	37
Pre-race clenbuterol	16
Pre-race other	32
No pre-race medication	75

The horses with other included firocoxib, phenylbutazone, flunxin meglumine, chlorpromazine, albuterol, dexamethasone, acepromazine, ketoprofen, glycopyrrolate, aspirin, zylekene, omeprazole, and interferon.

Thirty-two percent (49/149) of the horses reportedly coughed at rest and 51% (77/149) of the horses coughed with work. Twenty-seven percent (40/149) of horses coughed both at work and during rest. Coughing, whether during exercise or while at rest, had no correlation with either TBE EIPH score ≥ 1 ($r^2 = .00$, for exercise; $r^2 = .00$, at rest) or BALF RBC count $>$ 1,000 cells/μL, ($r^2 = .02$ during exercise; $r^2 = .001$, at rest).

A board-certified veterinary clinical pathologist evaluated BALF slides from 83 of the 158 BALF samples. Evaluation of results of the BALF cytologic examination of WBC on 83 horses found no correlation between EIPH (using both TBE EIPH score and BALF RBC count $>$ 1,000 cells/μL) and the percentages of eosinophils, neutrophils, macrophages, lymphocytes, and hemosiderophages or the presence of inflammatory airway disease (IAD) but, 39/83 (47%) of these BALF had signs of chronic bleeding as indicated by the presence of hemosiderophages on Prussian blue-stained slides. Inflammatory airway disease was classified according to the ACVIM consensus statement of 2016 wherein $>$ 10% neutrophils, $>$ 5% mast cells and $>$ 5% eosinophils were considered consistent with IAD.[8]

4 | DISCUSSION

Our study describes the prevalence of EIPH in a selected population of actively competing barrel racing horses in the Pacific Northwest using 2 different methods of detecting EIPH. Interestingly, 37 (17%) of the runs performed by horses would not have been considered positive for EIPH if determined by TBE EIPH score alone. On the other hand, 17 (11%) runs made by horses would not have been regarded as positive for EIPH if using BALF RBC count $>$ 1,000 cells/μL. Classically, TBE EIPH score has been the preferred method for detecting EIPH because of ease of performance and its less invasive nature compared to BAL. Further studies are needed to determine the best method of diagnosing EIPH as well as the best post-exercise time period in which to perform TBE, BAL or both for diagnosing EIPH.

The prevalence rate in our study was higher (54% by TBE EIPH score and 66% by BALF RBC count $>$ 1,000 cells/μL) than that in another study that found a prevalence of 45.3% in barrel racers in Alberta Canada.[3] The prevalence rates obtained in our study[24–26] and the previous study[3] were similar to those of large studies of Thoroughbred[24–26] racehorses in which prevalence ranged from 43 to 75%.[6,7] Results as high as 85% have been reported with repeated TBE EIPH scores.[9,10] Thus, the results for the barrel racers are similar to those of other strenuously exercising horses despite the very short duration of exercise associated with barrel racing.

The pathophysiologic cause of EIPH currently is thought to be stress failure of the pulmonary capillaries caused by excessive transmural pressure resulting from a combination of high pulmonary intracapillary blood pressure and high negative peak inspiratory pressure during exercise.[11] During maximal exercise on a treadmill at speeds $\geq$ 14 m/s on a 5% incline, mean pulmonary arterial pressures in horses increased from 25 mmHg to $>$ 95 mmHg.[11–13] Peak inspiratory pressures can approach −60 cm H_2O (approximately 45 mmHg).[11–17] We could find no reports regarding heart rate during barrel racing. However, given the speeds at which these horses compete in 1D and 2D races (mean speeds in 10 horses in another study ranged from

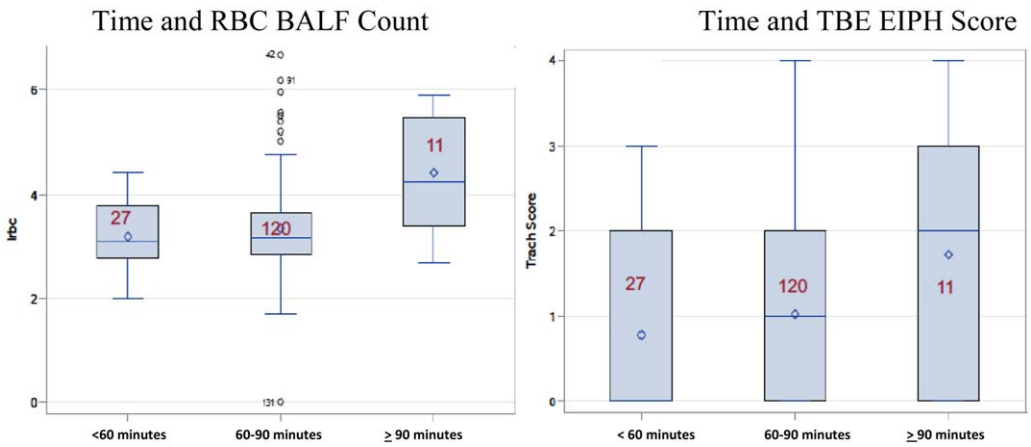

FIGURE 3 Relationship of time to perform BAL and TBE with TBE EIPH score and BALF RBC numbers/μL, respectively. The BALF RBC count is expressed on a $\log_{10}$ scale. A significant relationship was seen between post-exercise time of performing the BAL and BALF RBC count ($P = .004$), but the correlation was poor ($r^2 = .09$). There was no significant relationship between post-exercise time of TBE and TBE EIPH score ($P = .07$) and the correlation was very weak ($r^2 = .00$)

17.99 ± 2.05 ft/s to 19.15 ± 1.23 ft/s depending upon length of race),[3] barrel racing clearly constitutes strenuous exercise, with horses reaching maximal heart rate and rapidly developing high pulmonary vascular pressure while competing.[10–16] In keeping with the belief that all strenuously exercising horses experience EIPH to some extent,[1,2,6,16] the prevalence findings in our study are not surprising.

Pre-race administration of furosemide was not associated with differences in the severity of EIPH based on TBE EIPH score or BALF RBC count. This finding appeared contrary to evidence from several studies of Thoroughbred and Standardbred racehorses indicating that pre-exercise administration of furosemide decreases frequency of EIPH and mitigates the post-exercise TBE EIPH score.[1,9,10,18–21] However, we did not perform a controlled cross-over study and examined no horses that competed with and without receiving furosemide before racing. Therefore, whether furosemide affects the frequency or severity or both of EIPH in barrel racing horses has yet to be determined.

Administration of clenbuterol before racing was associated with a higher TBE EIPH score when compared to scores for horses that did not receive the drug. Why clenbuterol was associated with increased TBE EIPH score in some of the barrel racing horses in our study is unclear. We could find no data regarding clenbuterol and EIPH although it has been shown to have no effect on ventilatory mechanics, pulmonary vascular pressures or maximal oxygen consumption in horses galloping on a treadmill.[15,16] Factors not taken into account in our study, such as the concomitant administration of other drugs, humidity, altitude, and depth of arena surface, could have contributed to the more severe EIPH in horse receiving clenbuterol.

Our study did not find any correlation between performance and TBE EIPH score ($r^2 = .01$), BALF RBC count > 1,000 cells/μL ($r^2 = .02$), or pre-exercise treatment with medications ($P = .31$) for BALF RBC count > 1,000 cells/μL and TBE EIPH score ($P = .14$). The apparent lack of effect of these variables on performance may a consequence of the very short distance barrel racing horses must race when compared to that for Thoroughbreds and Standardbreds for which EIPH can be performance limiting.[7,15,22]

The prevalence of coughing ranged from 33% at rest to 49% during exercise. This finding was compatible with reports that 16%-50% of racehorses[27,28] with IAD and EIPH or both cough while at rest or during exercise, with coughing occurring most frequently at the beginning of exercise.[20,21,23] No evidence however was found that a history of coughing was associated with EIPH or its severity in our study. Similarly, no correlation was found between BALF cytology and evidence of IAD or correlation with EIPH either by TBE EIPH score or BALF RBC count > 1,000 cells/μL. Therefore, based on these findings, it does not appear that a relationship existed between IAD and EIPH in this study population. More information is needed however regarding the relationship among non-specific inflammation, EIPH and IAD.

A limitation to our study was that horses were enrolled voluntarily, some with a known EIPH history, and others with unknown history. It also relied solely on information that was disclosed by the owners relating to previous history of respiratory illness, other illness, cough with work or at rest, and medications administered before running the barrels. This approach could have resulted in bias toward a higher prevalence of EIPH, with owners enrolling in the study if their horse had any respiratory signs or previous bouts of EIPH although not every horse enrolled in the study had a history of EIPH.

Another potential limitation was the time post-exercise when the TBE and BAL were performed. In the majority of horses, these procedures were performed close to or at 60 minutes post-exercise, but the exact time varied. The owners were told to report with their horses no sooner than 45 minutes post-race, but not all complied. In some instances, the need to obtain owner consent or having multiple horses waiting at the same time for TBE and BAL or both may have caused variation in the actual timing of the TBE. Two of the horses had TBE and BAL performed 12 hours after running and both were EIPH positive with both methods. The most appropriate time for performing TBE or BAL to determine EIPH has not been established and the time the diagnostics tests were performed could have affected determination of the prevalence of EIPH because not every horse that had a TBE EIPH score ≥ 1 had an BALF RBC count > 1,000 cell/μL, and not every horse that had an BALF RBC count >1,000 cells/μL had a TBE EIPH score ≥ 1.

We found a high prevalence of EIPH in barrel racing horses in the Pacific Northwest during indoor and outdoor races using TBE EIPH scoring and BALF RBC count. The post-exercise performance of BAL and TBE played a role in determining EIPH in horses, but the most appropriate post-exercise time at which to undertake these evaluations has yet to be determined. Further studies are needed to determine if other factors such as footing, humidity, hauling, and IAD influence the prevalence and severity of EIPH in barrel racing horses.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

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