



STANDARD ARTICLE

Effect of different protocols on the mitigation of exercise-induced pulmonary hemorrhage in horses when administered 24 hours before strenuous exercise

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Abstract

Background: Public pressure exists in the United States to eliminate race-day furosemide administration despite its efficacy in decreasing the severity of equine exercise pulmonary hemorrhage (EIPH). No effective alternative prophylaxis strategies have been identified.

Objective: To investigate alternative protocols to race-day furosemide that might mitigate EIPH.

Animals: Seven fit Thoroughbreds with recent EIPH.

Methods: Double-blinded placebo-controlled Latin square crossover using a treadmill followed by a blinded placebo-controlled crossover study at a racetrack. First, horses exercised supramaximally to fatigue 24 hours after initiating 5 EIPH prophylaxis protocols: 0.5 and 1.0 mg/kg furosemide IV 24 hours pre-exercise with and without controlled access to water, and 24 hour controlled access to water. Effects were compared to those measured after giving a placebo 24 hours pre-exercise, and 0.5 mg/kg furosemide IV 4 hours pre-exercise. Bronchoalveolar lavage (BAL) erythrocyte count was determined 45-60 minutes postexercise after endoscopy to assign an EIPH score. Data were analyzed using linear mixed effects models. The most promising protocol from the treadmill study was further evaluated in 6 horses using endoscopy and BAL after 1100 m simulated races.

Results: Intravenous furosemide (0.5 mg/kg) administered 24 hours pre-exercise combined with controlled access to water decreased the severity of EIPH on the treadmill and at the racetrack.

Conclusion and Clinical Importance: Administering 0.5 mg/kg furosemide 24 hours pre-racing combined with controlling water intake may be a strategy to replace race-day furosemide administration for the management of EIPH. A larger study is indicated to further evaluate whether this protocol significantly mitigates EIPH severity.

Abbreviations: BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; C, control; EIPH, exercise-induced pulmonary hemorrhage; HD, high dose of furosemide; HDW, high dose of furosemide plus controlled access to water; LD, low dose of furosemide; LDW, low dose of furosemide plus controlled access to water; P, placebo; RBC, red blood cells; TBE, tracheobronchoscopic endoscopy; VO₂max, maximal oxygen consumption; W, controlled access to water; ΔBAL RBC, the difference between pre-protocol and postexercise bronchoalveolar lavage red blood cell numbers.

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KEYWORDS

bleeding, bronchoalveolar lavage, endoscopy, furosemide, horseracing, racetrack, treadmill, water consumption

1 | INTRODUCTION

Exercise-induced pulmonary hemorrhage (EIPH) is defined as bleeding that occurs in the lung during exercise.¹ Scientific evidence indicates that EIPH occurs, to a variable extent, in most racehorses and in many other horses undergoing strenuous exercise, and that IV administration of furosemide 4 hours before racing attenuates its severity.^{1,2} Furosemide is the only pharmacologic agent that has been scientifically demonstrated to mitigate EIPH,^{2,3} but its mechanism of action is still unclear. Doses of up to 0.5 or 1.0 mg/kg IV can be given on race day at American racetracks, depending on the racing jurisdiction, but this treatment is not permitted in many other nations. Increasing national and international pressure exists to prohibit any race day medication to horses racing in the United States.

Because of public and racing industry concerns regarding race day medication, and the recognition that repeated bouts of EIPH can induce pathologic changes in the morphology of small pulmonary airways, alveoli, and surrounding tissues,^{4,5} there is increased interest in identifying effective approaches to mitigating EIPH that do not involve administration of furosemide on race day.

We aimed to provide some answers to the question, "If medication is prohibited in the 24 hours before racing, how can EIPH be best managed?" The overall goal was to determine whether a prophylaxis protocol could attenuate the severity of EIPH when implemented 24 hours before supramaximal exercise.

2 | MATERIALS AND METHODS

The investigation had 3 phases. First, a preliminary study was conducted to evaluate the effect of performing 2 bronchoalveolar lavage (BAL) procedures 24 hours apart on recovered BAL fluid erythrocyte (BALF RBC) number. We then conducted a double-blinded placebo-controlled Latin square crossover treadmill study that aimed to identify a prophylaxis protocol or protocols that decreased the severity of EIPH in racehorses performing a standard supramaximal exercise test.⁶ Finally, we performed a blinded placebo-controlled crossover study to determine whether the most effective of these protocols also attenuated the severity of EIPH in horses competing in simulated races on a racetrack.

2.1 | Preliminary study

Four mature sedentary horses were used. Three of them had once raced and may have experienced some degree of EIPH, but none had exercised for at least 6 months when this aspect of the study was

conducted. The BAL technique used has been described previously⁷ and involved instillation of 300-mL aliquots of phosphate-buffered saline into the lung through a BAL catheter that had an inflatable cuff near its tip (Bivona Inc, Gary, IN, USA) and had been wedged in a bronchus before inflation of the cuff. The bronchoalveolar lavage fluid (BALF) was immediately aspirated from the catheter until negative pressure on the syringe was observed, with the first 25 mL of recovered fluid being discarded because it represented the catheter's dead space. The BALF RBC number was determined by hemocytometry and expressed as RBC/ μ L of recovered fluid. The first BAL had no effect on the BALF RBC number observed after the second BAL (see results), and thus the study proceeded as designed.

2.2 | Treadmill study

Seven Thoroughbred horses (1 male, 2 females, 4 geldings; aged 6.7 ± 1.9 SD years) that had been racing regularly and had been diagnosed endoscopically by racetrack veterinarians as having a postrace EIPH⁸ score ≥ 2 were procured at the end of their racing season and enrolled in the study. All horses were housed in the same barn and fed the same diet. All horses were sound and healthy based on veterinary examination. Horses were monitored daily for food and water intake. They were weighed every 10-14 days and their soundness was evaluated on pavement at a walk and trot before all treadmill conditioning exercise and each supramaximal treadmill test. Within 3 weeks of its arrival at Washington State University, each was acclimated to exercising on a high-speed treadmill while wearing a face mask and had its maximal oxygen consumption (VO_{2max})^{9,10} measured 4-5 days before beginning the study.

2.2.1 | Treatment protocols

The effects of 5 potential prophylaxis protocols on the severity of EIPH were compared with the effects of giving 0.5 mg/kg furosemide IV 4 hours before a treadmill exercise test and the subsequent withholding of water until postexercise (positive control; F), and with a negative control condition (C) in which horses received an IV 5-mL saline placebo 24 hours pre-exercise and had free access to water until 4 hours before the exercise test (Table 1). The 5 protocols were: (1) controlled water consumption (W) in which horses had water removed 24 hours before exercise and were given 6 mL/kg (approximately 3.0 L) water at 4 hourly intervals (20, 16, 12, and 8 hours before exercise); (2) 0.5 mg/kg IV furosemide (regarded as a low dose; LD) 24 hours before exercise; (3) 1.0 mg/kg IV furosemide (considered a high dose; HD) 24 hours before exercise. For both LD and HD, horses had free access to water until 4 hours before exercise; (4) LD

with provision of 6 mL/kg water every 4 hours until 8 hours before exercise (LDW); and (5) HD with the same controlled access to water as for LDW (HDW). The order in which each protocol was administered to each horse was determined using a 7 × 7 Latin square with 2-3 weeks between protocols and the associated exercise test. Horses' fitness levels were maintained between exercise tests by exercising them at a trot and gallop on the treadmill every third day. No training occurred in the 72 hours before any treadmill exercise test. Horses were weighed immediately before starting any protocol and again immediately before the exercise test.

2.2.2 | Supramaximal treadmill test

The treadmill exercise test was conducted on a 10% slope and consisted of a warm-up for 3 minutes at 40% of each horse's VO_2 max after which the treadmill was accelerated to a speed that had been calculated from the regression equation for the linear portion of the individual horse's VO_2 -speed curve, so as to have an oxygen requirement that was 115% of its VO_2 max. Each horse galloped at this speed until fatigued, with fatigue being defined as the point at which the horse could not keep pace with the treadmill despite verbal encouragement.

2.3 | Assessing the severity of EIPH

The severity of EIPH was assessed in 2 ways. Tracheobronchial endoscopic (TBE) examination to the level of the carina was performed 45-60 minutes postexercise using a 100-cm, 7-mm diameter flexible fiber-optic endoscope. Each examination was videotaped and 3 observers who were not present for the TBE and were blinded to the horses' prophylaxis schedules, independently viewed the videorecordings, and assigned an EIPH score of 0-4 based on the

amount of blood visualized in the tracheobronchial tree using a previously described scoring system.⁸ Each observer had access to a written description of the criteria for each TBE EIPH score as well as pictorial examples of each score and viewed the videorecordings on similar screens. In the event that scores from the 3 observers differed, the score favored by the majority was used.

The severity of EIPH also was quantified by counting BALF RBC. To account for any EIPH that occurred during training, BAL was performed 24 hours before initiating a protocol (ie, 48 hours before exercise). The procedure was repeated immediately after the postexercise TBE. The difference between pre-exercise and postexercise BALF RBC number (Δ BALF RBC) was considered to be indicative of any EIPH associated with the specific exercise test that had just been performed. A BALF RBC number > 1000/ μ L was regarded as positive for EIPH.^{11,12}

2.4 | Simulated races

This phase of the study used a crossover design to further evaluate the efficacy of the protocol judged to most effectively mitigate EIPH, based on the results of the treadmill study (see the Results section). Six of the horses completed 2 simulated 1100 m races from the starting gate 2 weeks apart at Emerald Downs Racetrack in Auburn, Washington, after 8 weeks of training on the Washington State University Hitchcock Research Racetrack followed by 4 weeks with a professional thoroughbred racehorse trainer at the suburban racetrack. No medications were administered in the 5 days before the races.

Before the first race, horses were assigned to either the control group or the treatment group using a random number generator. Horses in the treatment group received 0.5 mg/kg furosemide IV 24 hours before the first simulated race and access to water was

TABLE 1 Five potential exercise-induced pulmonary hemorrhage (EIPH) prophylaxis protocols and positive and negative controls evaluated in 7 fit Thoroughbred horses exercising to fatigue on a treadmill at a speed with an oxygen demand that was 115% of the maximal oxygen consumption. Abbreviations used in the column headed "Experimental group label" are defined in the 3 columns to the right of the label

Experimental group label	Experimental group description	Intravenous treatment	Access to water
F	Positive control group, furosemide 4 hours	0.5 mg/kg 4 hours before exercise	Free choice until 4 hours before exercise
C	Control group, no furosemide and free choice water	5 mL saline as placebo 4 hours before exercise	Free choice until 4 hours before exercise
W	Controlled water access only	None	6 mL/kg water at 20, 16, 12, and 8 hours before exercise
LD	Low-dose furosemide with free choice water	0.5 mg/kg 24 hours before exercise	Free choice until 4 hours versus exercise
HD	High-dose furosemide with free choice water	1.0 mg/kg 24 hours before exercise	Free choice until 4 hours before exercise
LDW	Low-dose furosemide with controlled water access	0.5 mg/kg 24 hours before exercise	6 mL/kg water at 20, 16, 12, and 8 hours before exercise
HDW	High-dose furosemide with controlled water access	1.0 mg/kg 24 hours before exercise	6 mL/kg water at 20, 16, 12, and 8 hours before exercise

controlled as described above for the LDW experimental protocol. Horses in the control group received a placebo of 5 mL saline IV 24 hours before this race and had free access to water until 4 hours beforehand. The protocol was repeated in the second simulation with horses being assigned to the alternative group. Investigators responsible for postexercise evaluations were blinded to the identity of protocols assigned to each horse. The severity of EIPH was assessed using the same procedures as employed for the treadmill study.

2.5 | Statistical analysis

Data from the preliminary study to evaluate the effects of the BAL procedure on BALF RBC number were analyzed using a paired *t* test. Continuous outcomes such as BALF RBCs were analyzed using linear mixed models with random effects particular to each study design. When conditions for normality were not met, data were transformed to their \log_{10} values so as to meet the conditions for normal distribution. Endoscopic EIPH score was analyzed using ordinal logistic regression. In all cases, significance was set at $P < .05$.

3 | RESULTS

3.1 | Preliminary study

Results indicated that performing 2 BAL procedures 48 hours apart on sedentary horses had no effect on BALF RBC number ($P = .88$; Table 2). Red cell numbers ranged from 75 to 412 RBC/ μL in the first

TABLE 2 Bronchoalveolar (BAL) fluid red blood cell counts (RBC)/ μL from 4 sedentary horses that underwent BAL on 2 occasions 24 hours apart

Horse	1st BAL RBC/ μL	2nd BAL RBC/ μL
1	412	475
2	239	444
3	75	25
4	125	0

TABLE 3 Median and interquartile ranges for time to fatigue, maximum heart rate (HRmax), and maximum oxygen consumption (VO_2max) associated with 7 possible EIPH prophylaxis protocols, in 6 Thoroughbreds galloping to fatigue on a treadmill at speeds calculated to have an oxygen demand that was 115% of the VO_2max . See Table 1 for summaries of each protocol

Protocol	Time to fatigue (s)	HRmax (bpm)	VO_2max (mL/[kg-min])
C	95 (87-119)	208 (204-212)	157 (150-158)
HD	99 (92-146)	208 (203-212)	154 (149-158)
HDW	100 (78-148)	209 (206-213)	153 (145-171)
LD	97 (81-117)	207 (203-213)	154 (146-164)
LDW	105 (83-121)	209 (204-211)	153 (148-166)
W	87 (71-136)	207 (204-213)	156 (145-170)
F	107 (95-128)	207 (205-213)	156 (145-169)

sample and from 0 to 475 RBC/ μL for the second sample. The greatest change in BALF RBC number for an individual horse was an increase of 205 RBC/ μL . In 2 of the 4 horses, the BALF RBC number was lower after the second BAL.

3.2 | Treadmill study

Seven horses began the study, but only 6 completed all 7 protocols because of persistent lameness in 1 horse. Mean and median body weights did not change over the course of the treadmill study (491 ± 25 kg; 480 [475-505, interquartile range (IQR)] kg at the start of this phase, 482 ± 29 kg; 473 [461-492] kg at its conclusion). The VO_2max was not affected by the prophylaxis protocols ($P = .45$; Table 3) nor did the maximum heart rate or run time to fatigue change with any protocol ($P = .77$; Table 3).

None of the tested prophylaxis protocols had a significant effect on TBE EIPH score. Interobserver agreement was 0.63 with respect to assignment of EIPH scores. In no instance did the 3 observers give 3 different scores. Significant differences were found among horses with respect to severity of the EIPH grade ($P < .001$). Bronchoalveolar lavage fluid RBC counts from BALs performed 48 hours before exercise ranged from 100 to 13 472 RBC/ μL (median, 472; IQR, 350, 801) RBC/ μL . Of the 42 pre-exercise BALs performed, 1 BAL from each of 5 different horses had a BALF RBC count $>1000/\mu\text{L}$, suggesting recent EIPH. The BALF RBC number from all postexercise BALs, regardless of prophylaxis protocol, was $11\,079 \pm 2055$ RBC/ μL (median, 5642; IQR, 2580, 14 075) RBC/ μL .

Neither the ΔBAL RBC nor the absolute postexercise BALF RBC numbers were normally distributed, but their \log_{10} values met the requirements for normality. The $\log_{10}\Delta\text{BAL}$ RBC number for the LDW protocol was significantly lower than for C ($P = .04$; Figure 1), whereas the postexercise $\log_{10}\text{BALF}$ RBC numbers for the same protocols did not differ ($P = .05$). The $\log_{10}\Delta\text{BALF}$ RBC counts for the other protocols also were not different to that for C, and differences between results for LDW and F also were not different ($P = .67$; Figure 1). Controlled access to water had no effect on the severity of EIPH when compared to TBE, $\log_{10}\Delta\text{BAL}$ RBC and $\log_{10}\text{BALF}$ RBC results for C ($P = .92$).

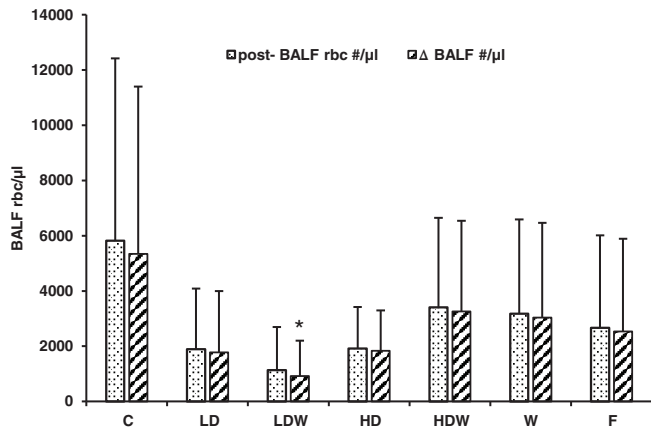


FIGURE 1 Mean ± SD postexercise bronchoalveolar lavage (BAL) fluid erythrocyte numbers (post-BALF RBC/μL) and the difference between preprotocol and postexercise BAL red blood cell numbers (ΔBALF RBC/μL). The asterisk denotes significant difference from C ($P < .05$) based on analysis of log10 transformations of the postexercise BAL RBC numbers and the ΔBALF RBC numbers for all protocols. The protocols are summarized in Table 1

Prophylaxis protocols had a significant effect on body weight ($P = .02$). Loss of body weight was highest with HDW and LDW, whereas that with W was higher than with HD or LD. The F protocol resulted in similar weight loss to W and less weight loss than for HDW or LDW (Figure 2).

3.3 | Racetrack study

Based on the results of the treadmill studies, LDW was selected for further evaluation in the simulated races. Three of the 12 pre-exercise BALF RBC counts from 3 different horses were $>1000/\mu\text{L}$. Neither postexercise BALF RBC number ($P = .10$) nor ΔBALF RBC ($P = .17$) was significantly affected by the LDW protocol (Figure 3A), but EIPH endoscopic score was decreased ($P = .03$; Figure 3B). The variable estimate for the control was -1.43 , meaning that the likelihood that the LDW protocol would result in a lower EIPH score was 4.19 greater than would be expected by chance. The same horse won each race, completing the first race in 67.2 seconds (LDW run) and the second (placebo) in 66.4 seconds.

FIGURE 2 Effect of 7 EIPH prophylaxis protocols on total and percent body weight (B wt) loss (mean ± SD) in the 24 hours prior to supramaximal treadmill exercise in 6 fit Thoroughbreds. The protocols are summarized in Table 1

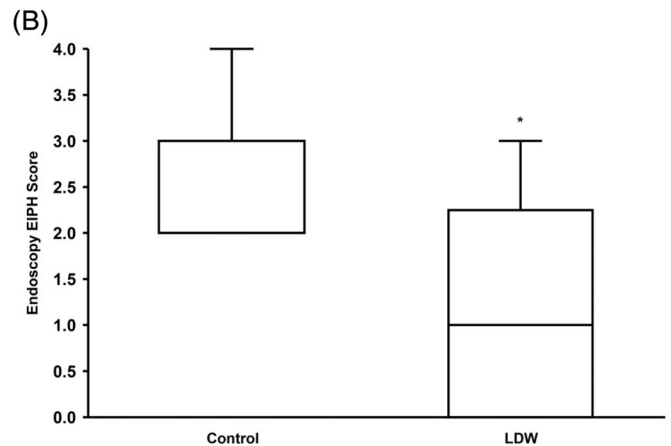
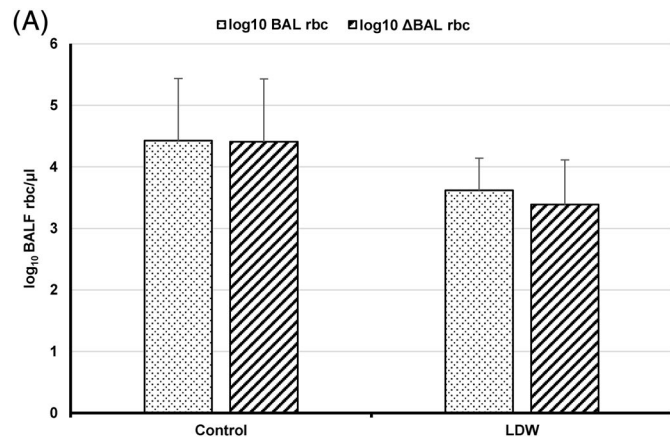
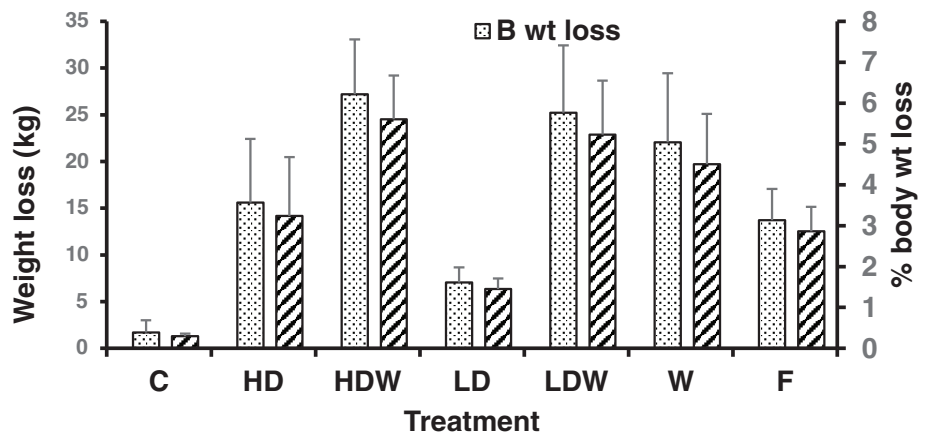


FIGURE 3 Effects of 0.5 mg/kg IV administration of furosemide 24 hours before simulated races of 1100 m combined with controlled access to water (6 mL/kg 20, 16, 12, and 8 hours before racing; LDW) for postexercise bronchoalveolar lavage red blood cell numbers (BAL RBC) and the difference between pre-protocol and postexercise bronchoalveolar lavage red blood cell numbers (ΔBAL RBC) expressed as mean ± SD log10 equivalents (A) and tracheobronchoscopic (TBE) EIPH scores (B) in 6 fit Thoroughbreds. The asterisk denotes a significant difference between LDW and control condition results ($P = .03$)

4 | DISCUSSION

Of the 6 treatment and/or management protocols evaluated on the treadmill, only LDW significantly reduced the severity of EIPH when compared with that observed in the control situation. This effect was evident in the results from the Δ BALF RBC counts after logarithmic transformation. Endoscopic EIPH score did not change with any treatment on the treadmill. One factor that possibly contributed to this outcome was the relatively small number of horses included in the study. Because of the ordinal nature of EIPH scores (only 5 possible scores), a large number of horses previously was required to definitively demonstrate the efficacy of furosemide in the attenuation of EIPH.² The likelihood of achieving a significant effect with the number of horses used in our study was further hindered by the lack of homogeneity in the study group. Although the majority of horses with EIPH have endoscopic scores of 1 or 2,^{9,13} considerable variability frequently occurs in severity among horses and from exercise bout to exercise bout in the same horse.¹¹

Because of the continuous nature of the BALF RBC count, it was more likely that treatment effects would be reflected in BALF RBC numbers. Previous studies have evaluated postsupramaximal exercise BALF RBC data, most of which were conducted on treadmills.^{7,14,15} Although those studies aimed to objectively assess the severity of EIPH associated with a single bout of exercise in at least a semiquantitative manner,¹⁴ they did not take into account the effects on BALF RBC number of other recent bouts of strenuous exercise that preceded the specific exercise test of interest. Because of the importance of knowing as accurately as possible the effects of each of the 6 prophylaxis protocols on the severity of EIPH, we elected also to determine the number of RBCs in BALF before initiating each prophylaxis and exercise test protocol. Because these horses were undergoing conditioning at a gallop on a regular basis, it was possible that these episodes also would induce some EIPH, especially because it is unknown if an EIPH “threshold” speed or exercise intensity exists in individual horses. The possibility that the BAL procedure itself could induce an increase in BALF RBC number was decreased by the results of repeated BALs 24 hours apart in the preliminary study with sedentary horses. Conducting this study was important because this possible effect of the BAL procedure had not been previously evaluated. It is well established that mild increases in postprocedural BALF leukocyte counts often occur¹⁶ but, until now, it was unknown whether concomitant changes in BAL RBC number also occurred. None of the horses involved in our preliminary evaluation had a BALF RBC count $>500/\mu\text{L}$ after the second BAL and the most marked increase was $205 \text{ RBC}/\mu\text{L}$. Because a previously published BALF RBC count for the diagnosis of EIPH was $>1000/\mu\text{L}$,^{11,12} and the majority of postexercise BALF RBC counts were much higher than this number, it is unlikely that performing BAL 48 hours before the treadmill test would have influenced results of the postexercise BAL. Conversely, performing the pre-exercise BAL identified 5 samples from 5 horses that reflected recent EIPH, and being able to take this into account by calculating the Δ BALF RBC enabled us to best assess the effects of the treadmill exercise test on the severity of EIPH and the associated effect, if any,

of the prophylaxis protocol being evaluated. The fact that the \log_{10} - Δ BALF RBC after LDW was significantly different from the control results provides further evidence for the value of determining Δ BALF RBC for research purposes.

Decreased access to or withholding of water for variable periods before racing has been practiced for many years at Standardbred and Thoroughbred racetracks in different parts of the world in the belief that doing so will decrease the severity of EIPH.¹⁷ The rationale for this approach and the many ways in which it is practiced are unclear, but presumed to be related to assumptions that doing so lowers pulmonary vascular pressure during exercise and thus decreases EIPH. However, the effectiveness of this management approach alone or in combination with the administration of any pharmacologic agent has not previously been the subject of a controlled study. In our study, there was no effect on the severity of EIPH of controlled access to water alone. Although the power of the treadmill study was low, the high *P* value (.98) made it unlikely that an effect of controlled access to water alone would have been discerned had a larger number of horses been used.

Results from the simulated races indicated that the LDW treatment protocol decreased the severity of EIPH based on TBE EIPH score. This finding was much stronger than that expected by chance despite the lack of a significant effect on \log_{10} BALF RBC or $\log_{10}\Delta$ BALF RBC numbers. The apparent discordance between the TBE and BALF findings was consistent with previous findings of a weak correlation between the 2 variables.^{11,12} Tracheobronchial endoscopy is the most widely used method of diagnosing EIPH and, from a statistical perspective, the ordinal nature of the scoring system makes it harder to identify statistically significant differences in results than is possible with a continuous variable such as BALF RBC number. Therefore, the significant effect of the LDW protocol on TBE score is of considerable clinical relevance. The lack of an effect of LDW on either BALF RBC variable may have been partly a consequence of the very large range of results associated with administration of the placebo. This wide range decreased the statistical power of the study and meant that the chance of demonstrating an effect of LDW on $\log_{10}\Delta$ BALF RBC was lowered. We previously conducted a pilot study of 4 different fit horses evaluated using the C, F, and LDW protocols. A power calculation based on that data indicated that a minimum of 6 horses was needed to ensure that our study would have power ≥ 0.8 . However, a retroactive power analysis using the results from our current study indicated that it lacked this power. Despite the lack of effect of LDW on Δ BALF RBC after the simulated races, the associated decrease in TBE EIPH score warrants further investigation of LDW using a larger number of horses.

The simulated races were run over a distance (1100 m) that is classified as a sprint for racehorses. The majority of horse races are conducted over longer distances and currently there is no way to know whether the positive effect of LDW on the severity of EIPH that we detected might be enhanced, diminished, or unchanged in association with races over longer distances. This possibility also warrants further investigation.

We are aware of only 1 report that studied any effects of furosemide for >8 hours after administration, and that study indicated that urinary clearance of calcium, phosphorus, sodium, and chloride still

were increased 24 hours after a single LD IV injection of the drug despite urine flow returning to normal in <4 hours.¹⁸ Consequently, because of this information (albeit limited) and the possibility that race day treatment with furosemide might be banned, we elected to investigate whether the initiation of different prophylaxis protocols involving furosemide 24 hours before exercise could have similar prolonged effects on the severity of EIPH. Some of these protocols included controlled access to water to determine whether superimposition of this practice on the LD and HD protocols had an additive effect to any effects that were detected with the LD or HD protocols alone.

If administration of furosemide IV on race day is prohibited, alternatives to the established use of furosemide will be sought. Based on our study, the LDW protocol appears to be an effective alternative. We cannot comment on how effective LDW would be when compared with furosemide given 4 hours prerace, because such a comparison was not a goal of our study. Rather, we aimed to evaluate prophylaxis protocols that might be reasonable alternatives to the established furosemide protocol in the event the latter was banned. One recently reported study compared the effects on TBE EIPH scores and postexercise BALF RBC numbers of IV administration of 250 mg furosemide 4 hours before 1000 m breezes by pairs of horses to those of the same dose of furosemide given 24 hours beforehand.¹⁹ The authors found that furosemide given 4 hours before exercise decreased the severity of EIPH whereas no difference was found between the results for the control and 24 hour pre-exercise protocols. However, there also was no difference in the TBE or BAL RBC results between the 4 and 24 hour groups, and the median TBE score for the placebo and both treatment groups was the same (0). The horses in that study were younger (2-4 years), had unknown histories of EIPH, and had free access to water until 4 hours before breezing (ie, they followed the LD protocol of our study). As well as having had few or no race starts, the horses in the previous study¹⁹ also had much lower postexercise BAL RBC numbers and likely ran slower than did our horses (59-64 seconds for 1000 m), given that they had moving starts rather than jumping from a starting gate. Our study may have been statistically stronger because of the larger possible differences in BAL RBC numbers when compared to the control situation and because the changes in BAL RBC number for each horse were evaluated.

We found significant differences between the results for the LD and LDW protocols in the treadmill phase of our study. What difference controlling access to water has on EIPH when combined with administration of furosemide has yet to be clarified. The effect that differences in speed have on EIPH also is unclear, although it appears that EIPH occurs beyond a certain threshold speed for each horse. Our horses were older, and multiple TBE examinations had confirmed that they frequently had EIPH scores ≥ 2 . Previous bouts of EIPH are likely to predispose horses to additional bouts, and the number of race starts and time spent in training are major determinants of the severity of bouts of EIPH.²⁰ Two-year-old Thoroughbreds generally bleed less than do older horses, based on TBE, because they usually have had fewer race starts.²⁰ Despite differences in history of EIPH severity, as well as differences in prerace access to water and the

conditions under which the simulated races were conducted, we do not know if our results would have differed from those previously reported¹⁹ had the horses in our study also been treated with furosemide 4 hours before the simulated races.

The results of both the treadmill and the racetrack studies suggest that furosemide 0.5 mg/kg IV along with controlled access to water (ie, LDW) has the potential to mitigate the severity of EIPH in racehorses when initiated 24 hours before strenuous exercise. When compared to the results when furosemide was given 4 hours before exercise (F), the effect of LDW on EIPH score and BALF RBC number was not different in the treadmill phase of our study. However, because of the small number of horses involved, a larger prospective clinical study is indicated before any definitive recommendations regarding the ability of LDW to reliably attenuate the severity of EIPH can be made.

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CONFLICT OF INTEREST DECLARATION

Bayly and Sellon are former members of the Board of Directors of the American Association of Equine Practitioners (AAEP) but were not at the time of funding. Bayly is currently a member of the Grayson-Jockey Club Research Advisory Committee, but was not at the time of funding. Sellon is currently a member of the AAEP Foundation Advisory Committee, but was not at the time of funding. The other authors have no conflicts 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

Approved by the IACUC at Washington State University.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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REFERENCES

- Hinchcliff KW, Couetil L, Knight P, et al. Exercise-induced pulmonary hemorrhage in horses: ACVIM consensus statement. *J Vet Intern Med.* 2015;29:743-758.
- Hinchcliff KW, Morley PS, Guthrie AJ. Efficacy of furosemide for prevention of exercise-induced pulmonary hemorrhage in Thoroughbred racehorses. *J Am Vet Med Assoc.* 2009;235:76-82.
- Sullivan SL, Whittem T, Morley PS, Hinchcliff KW. A systematic review and meta-analysis of furosemide for exercise-induced pulmonary hemorrhage in Thoroughbred and Standardbred racehorses. *Equine Vet J.* 2015;47:341-349.
- McKane SA, Slocombe RF. Alveolar fibrosis and changes in equine lung morphology in response to intrapulmonary blood. *Equine Vet J.* 2002;34(Suppl 34):451-458.
- Williams KJ, Derksen FJ, de Feijter-Rupp H, Pannirselvam RR, Steel CM, Robinson NE. Regional pulmonary veno-occlusion: a newly identified lesion of equine exercise-induced pulmonary hemorrhage. *Vet Pathol.* 2008;45:316-326.
- Kingston JK, Sampson SN, Beard LA, et al. The effect of supramaximal exercise on equine platelet aggregation responses. *Equine Vet J.* 1999; 31(Suppl 30):181-183.
- Buchholz BM, Murdock A, Bayly WM, et al. Effects of intravenous aminocaproic acid on exercise-induced pulmonary haemorrhage (EIPH). *Equine Vet J.* 2010;42(Suppl 38):256-260.
- Hinchcliff KW, Jackson MA, Brown JA, et al. Tracheo-bronchoscopic assessment of exercise-induced pulmonary hemorrhage in horses. *Am J Vet Res.* 2005;66:596-598.
- Rose RJ, Hodgson DR, Bayly WM, et al. Kinetics of VO₂ and VCO₂ in the horse and comparison of five methods for determination of maximal oxygen uptake. *Equine Vet J.* 1990;22(Suppl 9):39-42.
- Sides RH, Kirkpatrick R, Renner E, et al. Validation of masks for determination of VO₂max in horses exercising at high intensity. *Equine Vet J.* 2018;50:91-97.
- Gold JR, Knowles DP, Coffey T, Bayly WM. Exercise-induced pulmonary hemorrhage in barrel racing horses in the Pacific northwest region of the United States. *J Vet Intern Med.* 2018;32:839-845.
- Lopez C, Gold J, Sellon D, et al. Relationship between tracheobronchoscopic EIPH score and bronchoalveolar lavage erythrocyte count in horses. *J Vet Intern Med.* 2017;31:1349 [Abstr E33].
- Crispe EJ, Lester GD, Secombe CJ, Perera DI. The association between exercise-induced pulmonary haemorrhage and race-day performance in Thoroughbred racehorses. *Equine Vet J.* 2017;49:584-589.
- Meyer TS, Fedde MR, Gaughn EM, et al. Quantification of exercise induced pulmonary hemorrhage with bronchoalveolar lavage. *Equine Vet J.* 1998;30:284-288.
- Langetsmo I, Fedde MR, Meyer TS, et al. Relationship of pulmonary arterial pressure to pulmonary hemorrhage in exercising horses. *Equine Vet J.* 2000;32:379-384.
- Sweeney CR, Rossier Y, Ziemer EL, et al. Effect of prior lavage on bronchoalveolar lavage fluid cell population of lavaged and unlavaged lung segments in horses. *Am J Vet Res.* 1994;55:1501-1504.
- Marlin DJ. Injury to the equine respiratory system associated with exercise. *Havemeyer Foundation Monograph Series.* 2004;15: 63-68.
- Pagan J, Waldrige B, Whitehouse C, Fuchs S, Goff M. Furosemide administration affects mineral excretion in exercised Thoroughbreds. *Equine Vet J.* 2014;46(Suppl 46):4.
- Knych HK, Wilson WD, Vale A, et al. Effectiveness of furosemide in attenuating exercise-induced pulmonary haemorrhage in horses when administered 4- and 24-h prior to high speed training. *Equine Vet J.* 2017;50:350-355.
- Hinchcliff KW, Morley PS, Jackson MA, et al. Risk factors for exercise-induced pulmonary haemorrhage in Thoroughbred racehorses. *Equine Vet J.* 2010;42(Suppl 38):228-234.

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