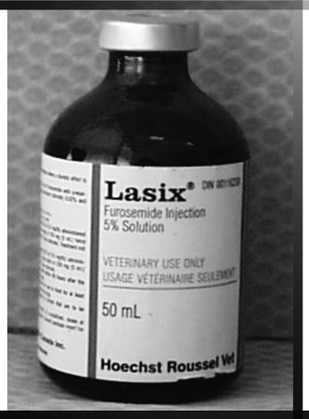

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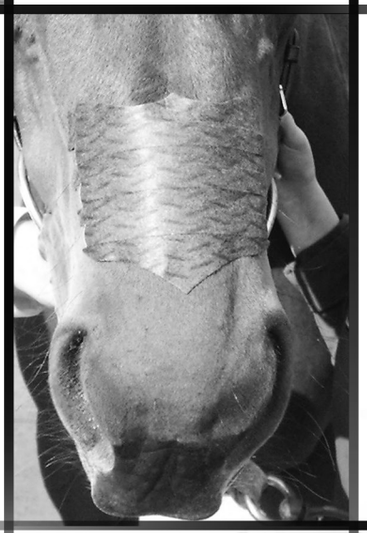

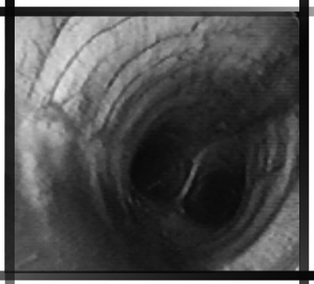

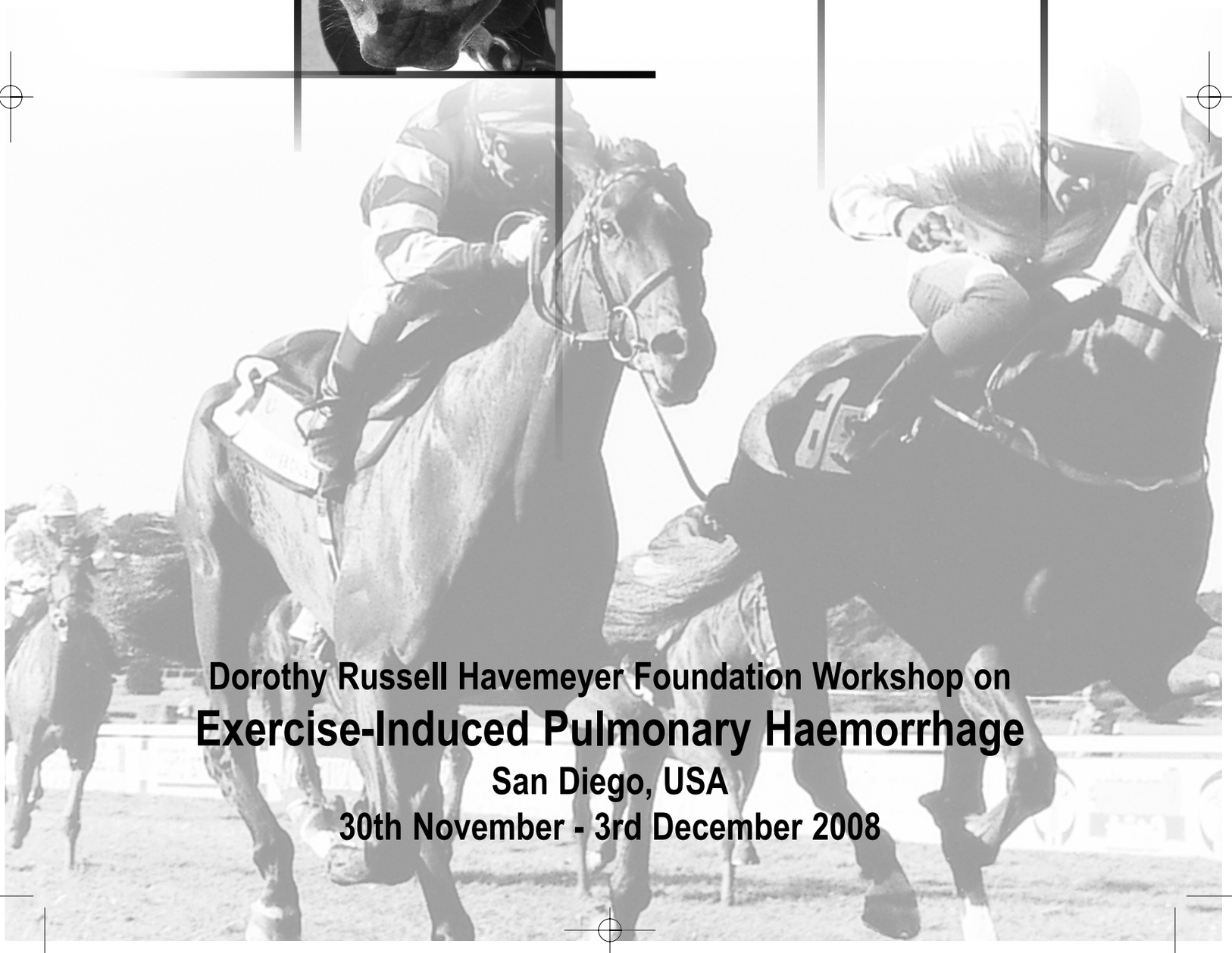

Dorothy Russell Havemeyer Foundation Workshop on

Exercise-Induced Pulmonary Haemorrhage

San Diego, USA

30th November - 3rd December 2008

Contents

by presenting author

Preface

The first Havemeyer Foundation Workshop on EIPH was held between 9th–12th of March 2006 in Vancouver, Canada and was organised by myself and Professor Ken Hinchcliff. There were 20 participants representing the main horse regions of the world. Topics covered in this first workshop included diagnosis, pathology, epidemiology, the role of airway inflammation, the role of cardiac disease, effects on performance, treatment, regulatory issues and overviews of EIPH in the USA, Hong Kong, Australia and South Africa. At the conclusion of the Workshop a list of high priority research areas for EIPH were identified by the group. A number of researchers have subsequently used the priority list to support applications for funding.

Up until around 30 years ago, before the development and widespread application of endoscopy, our knowledge of EIPH was limited. Horses were classified as 'bleeders' if they exhibited epistaxsis, which was relatively uncommon; of the order of 0.1% of race starts for example. The introduction of endoscopy indicated a much higher prevalence of EIPH in racehorses, with around 40–70% of horses having blood in the trachea after galloping or racing. Other studies provided evidence that all racehorses in hard training bled on at least one occasion, as evidenced by the universal presence of haemosiderophages in tracheal wash samples (Whitwell and Greet 1984). And more recently, studies by Rik Birks have demonstrated that if horses are endoscoped on 3 separate occasions after racing, then blood will be seen in the trachea on at least one occasion. Furthermore, if we use the more sensitive technique of bronchoalveolar lavage to look for elevations in red blood cell numbers in lung lavage fluid from the lower airways, we see that all horses experience some haemorrhage even after light exercise. Thus, whilst the threshold for haemorrhage and the severity varies between horses, it is safe to conclude that all horses experience some degree of EIPH at exercise intensities above a trot. And of course we now know that EIPH is not limited to racehorses, but a ubiquitous consequence of exercise in all horses in almost all disciplines. These considerations may justify EIPH being considered as the most common exercise-related injury that occurs in horses.

This second workshop includes around half the participants of the first workshop. The second workshop will build on the progress of the first Workshop rather than simply go over ground already covered. The aim will be to review new publications on EIPH that have arisen in the last 2 years and to explore novel aspects of EIPH. EIPH is without doubt a complex condition and many factors interact to explain the nature and level of haemorrhage in individual horses under different circumstances. Because of its prevalence, potential effects on health and performance, impact on the economic value of horses and the fact that the manifestation of extreme EIPH (ie epistaxis) is distressing to the lay public, EIPH continues to be a condition that is both challenging and of concern to the global horse industry.

David Marlin
Workshop Chairman

Programme

SUNDAY 30TH NOVEMBER 2008

19.30 Welcome Reception (Grand Hyatt Hotel)

MONDAY 1ST DECEMBER 2008

EPIDEMIOLOGY

9.00-9.30 Introduction and overview of new publications since first workshop
Gene Pranzo and Dave Marlin

9.30-10.15 Risk factors for EIPH in National Hunt racehorses
Jackie Cardwell

10.15-11.00 Prevalence of EIPH in a population of Standardbred trotters: poor vs good performers
Anne Couroucé-Malblanc

11.00-11.30 **Coffee**

AIRWAYS

11.30-12.15 EIPH and upper airway disorders
Sam Franklin

12.15-1.00 Clinical data on EIPH in horses from all equestrian disciplines and the relationship between EIPH, BALF cytology and respiratory functional tests in these horses
Emmanuelle Vanerck

1.00-2.00 **Lunch**

2.00-2.45 Cardiac contributions to EIPH
Mary Durando

2.45-3.30 *Discussion*
Are treadmill and field studies on EIPH consistent?
Do we have the necessary tools to investigate EIPH?

3.30-4.00 **Tea**

4.00-4.45 Discussion

TUESDAY 2ND DECEMBER 2008

NEW CONCEPTS IN EIPH

9.00-9.45	EIPH: is it really haemorrhage? *David Marlin*
9.45-10.30	Pulmonary venous remodeling in EIPH *Fred Derksen*
10.30-11.00	**Coffee**
11.00-11.45	Contribution of pro-inflammatory enzymes in EIPH: could the neutrophils play a role in the pathogenesis of the condition? *Dominique Votion*
11.45-12.30	Potential role of pulmonary inflammation in predisposing the equine lung to EIPH *Shaun McKane*
12.30-1.30	**Lunch**
1.30-2.15	Platelet-activating factor is involved in the pulmonary inflammation of exercised young racing Thoroughbred colts *Pedro Michelotto*
2.15-3.00	Biomarkers for EIPH in blood *Montague Saulez*
3.00-3.30	**Tea**
3.30-4.15	Which therapeutics mitigate EIPH and how should their efficacy be determined? *Tammi Epp*
4.15-5.00	*Discussion* Impact of EIPH in training vs racing: Are they the same? Where are the gaps in our knowledge concerning EIPH?
7.30	Meet in hotel lobby
8.00	Workshop Dinner

WEDNESDAY 3RD DECEMBER 2008

TREATMENT OF EIPH

8.00-8.45 EIPH combination medications in North America: observations from a racetrack study
Rik Birks

8.45-9.30 Efficacy of first time furosemide use in Thoroughbred racehorses in Brazil
Fernanda Costa

9.30-10.15 Effect of furosemide and furosemide-carbazochrome combination on EIPH in Standardbred racehorses
Laurent Couëtil

10.15-10.35 **Coffee**

10.35-11.20 *Discussion*
Future priorities for EIPH research?

11.20 Close of meeting followed by lunch

Risk factors for EIPH in National Hunt racehorses

J. Cardwell

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Endoscopic and cytological measures of exercise-induced pulmonary haemorrhage (EIPH) were recorded during a prospective longitudinal study of Inflammatory Airway Disease (IAD) in National Hunt (NH) horses in the United Kingdom. The study was conducted in 5 NH training yards over 2 racing seasons. Horses were endoscopically examined and sampled monthly, in a consistent manner after exercise. The intensity of exercise undertaken prior to endoscopy was recorded. The presence and amount of blood visible in the trachea was noted and tracheal wash samples were collected for quantitative bacteriological and cytological evaluation. Multiple tracheal wash samples (n=1184) were obtained from 177 horses during the study. There were 85 episodes (7.2%; 95% CI: 5.7–8.6) of endoscopically visible tracheal blood and 426 episodes (36.0%; 95% CI: 33.2–38.7) of increased (moderate to high) proportions of siderophages in tracheal wash samples. Risk factors for both measures of EIPH were identified using multivariable mixed-effects logistic regression, to account for multiple factors and repeated measures from individuals. The odds of both endoscopically visible tracheal blood and increased siderophages varied significantly between trainers and increased with increasing time in training, season of sampling from 'autumn' (August to October) to 'spring' (February to April) and with different indicators of airway inflammation (airway neutrophilia and IAD). Airway neutrophilia was more strongly and significantly associated with both measures of EIPH than IAD (a combination of visible tracheal mucus and airway neutrophilia) and visible tracheal mucus alone was not significantly associated with either measure. Increasing age was not a significant risk factor having accounted for increasing time in training. The odds of endoscopically visible tracheal blood, but not of increased siderophages, increased with increasing exercise intensity and were higher in mares than in stallions and geldings. There was no evidence of an association between either measure of EIPH and *Streptococcus zooepidemicus* - the only bacterial species significantly associated with IAD in multivariable analysis in this population. There were no common risk factors identified for EIPH and airway inflammation, suggesting that there is either a direct causal link between the two, or unmeasured risk factors common to both. This study complements a previous epidemiological study in young racehorses by accounting for the effects of exercise intensity and time in training. The association between inflammation and EIPH is consistent with a number of previous studies. Further work is still required to eludicate this relationship and to identify modifiable risk factors for EIPH.

Prevalence of EIPH in a population of Standardbred trotters: poor vs good performers

Anne Couroucé-Malblanc

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One hundred and seventeen horses aged 2 to 9 years, ready to race or actively racing were involved in this study. Twenty five horses were good racing performers and represented the control group. Ninety two horses had poor racing performance. The standardised exercise test utilised a 10 min warm-up step followed by 3 steps each of 3 min. Then, the horses were stopped on the treadmill for 1 min, the endoscope was placed and the horse performed a fourth step at maximal speed. Heart rate (HR) was measured throughout the test and blood was collected via a transverse facial artery catheter during the 10 last seconds of each step. Then, 1 h after exercise, a broncho-alveolar lavage (BAL) was realised.

Endoscopy during exercise and post-exercise BAL helped to divide poor performers into 4 groups : Group 1 - upper respiratory airway (URA) disease, Group 2 - lower respiratory airway (LRA) disease, Group 3 - URA and LRA disease and Group 4 - other disease and also to evaluate the metabolic response to exercise according to these different problems. For lower respiratory diseases, horses were considered with IAD when neutrophils percentage was over 12% in the post exercise BAL. Also, they were considered with EIPH when they had hemosiderophages over 5% and irritative disease when they had epithelial cells over 8%.

For post exercise BAL results, neutrophils, epithelial cells and hemosiderophages were significantly higher in poor performers compared to control population (Table 1). However, all the horses showed free erythrocytes in the BAL one hour after exercise. Also, the results of this study suggest that there was a significantly different metabolic response (HR, blood lactate, PaO_2) to exercise in poor performers compared to good performers.

In the reference population, three horses showed EIPH (12%). In the poor performer population, 27 horses over the 92 showed EIPH (29.3%). The percentage was respectively: 45.9% in group 2 and 34.6% in group 3.

For Group 2, 17 horses showed EIPH. Nine showed EIPH only; 7 showed EIPH associated with IAD and one showed EIPH associated with an irritative disease. Considering the rest of the group, 11 horses showed IAD, 6 horses showed an irritative disease; 3 horses showed IAD associated with an irritative disease and 3 horses showed hypersensibility (mastocytes over 2%). For Group 3, 9 horses showed EIPH associated with DDSP. Three of these horses had EIPH only and 6 had EIPH associated with IAD.

In this population of French Standardbred Trotters, nearly 30% of poor performers showed EIPH. This was associated with other lower respiratory diseases such as IAD and/or with upper respiratory diseases such as DDSP.

TABLE 1: Results of cytologic analysis of BAL (expressed in %) in the control study and the poor performers (mean ± sd)

	Cell count/ mm^3	Neutrophiles	Lymphocytes	Mastocytes	Epithelial cells	Macrophages	Hemosiderophages
Control (n = 23)	566^a ± 297	11.9^a ± 6.3	25.6^a ± 6.3	1.5^a ± 1.3	2.0^a ± 4.4	56.3^a ± 12.5	0.9^a ± 2.0
Poor performer (n = 92)	498^a ± 414	18.3^b ± 13.3	21.8^b ± 14.1	1.2^b ± 1.6	7.8^b ± 16.5	42.0^b ± 19.0	6.1^b ± 11.4

Different superscript show significantly different results ($P<0.05$).

EIPH and upper airway disorders

S. Franklin and K. Allen

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It has been suggested that the airways may play an important role in the development of exercise-induced pulmonary haemorrhage (EIPH). During inspiration, the negative alveolar pressures are likely to enhance pulmonary capillary transmural pressure. Therefore any disorder of the airways that exacerbate alveolar pressures may increase the risk of EIPH. In horses with upper respiratory tract (URT) obstructions, inspiratory pressures become more negative and hence it is expected that transmural pressures will greater in these horses, leading to an increased risk of EIPH. Despite this, there is currently no evidence to support this hypothesis. There is some evidence to suggest an association between EIPH and inflammatory airway disease. However, a causal effect has not been proven, and there is evidence that the presence of RBC's in the airway may stimulate an inflammatory response.

We examined 300 horses (255 racehorses and 45 horses involved in other athletic disciplines) that were referred to the University of Bristol's Equine Sports Medicine Centre for investigation of poor athletic performance. All horses underwent endoscopy of the URT during high-speed treadmill exercise. Endoscopy of the trachea was subsequently performed 1–2 h post exercise and tracheal wash (TW) and broncho-alveolar lavage (BAL) samples taken for analysis.

A history of epistaxis was reported in 24 (8%) horses. However, no horses were observed to have epistaxis following the treadmill exercise test. Blood was visible in the trachea of 38 (13 %) horses following treadmill exercise. A further 164 (55%) had evidence of blood in the TW or BAL samples (53 had visible discolouration of the washes and a further 111 had microscopic evidence of red blood cells in their washes) and 151 (50%) horses had evidence haemosiderin in alveolar macrophages. Upper respiratory tract obstructions were diagnosed in 264 horses whilst examination of the lower airways revealed 106 had excessive mucus and 175 had neutrophillic inflammation in TW and / or BAL.

Univariable analysis revealed that horses with a history of EIPH were significantly more likely to have visible blood in the trachea compared with horses with no history of EIPH. Racehorses were significantly more likely to have EIPH than non-racehorses. An increased risk of EIPH was associated with increases in both Stepmax and HRpeak. No significant associations were found between EIPH and presence or type of URT obstruction. However, significant associations were identified between EIPH and inflammatory airway disease. There was a significant association between EIPH and the presence of excessive tracheal mucus. There was no association between EIPH and TW inflammation but an association between EIPH and BAL inflammation approached significance and remained significant in the final multivariate model, together with HRpeak and the presence of excessive mucus.

In conclusion, we have been unable to show any association between EIPH and URT obstructions suggesting that other factors are more important in the development of EIPH. Our data supports the finding that there may be an association between EIPH and lower airway. However further work is requires to determine whether EIPH occurs subsequent to IAD or vice versa.

Clinical data on EIPH in horses from all equestrian disciplines and the relationship between EIPH, BALF cytology and respiratory functional tests in these horses

E. Vanerck and T. Art

Equine Sports Medicine Centre, Equine Pole, Faculty of Veterinary Medicine, University of Liege, Belgium

The literature currently published on exercise-induced pulmonary haemorrhage (EIPH) almost exclusively concerns racehorses and there is only anecdotal information available on the occurrence of EIPH in sport horses performing in other equestrian disciplines. We reviewed 620 cases of sport horses referred between 2002 and 2008 for poor performance or athletic evaluation. We examined the owner complaints, clinical symptoms and the occurrence of other diseases, and evaluated the prevalence of EIPH in that population. The following exams were systematically performed in all horses: upper airway endoscopy without sedation, trachea-bronchial endoscopy under sedation, tracheal wash cytology and bacteriology and broncho-alveolar lavage cytology. Additional information was obtained in some horses from standardised treadmill or track exercise tests, respiratory function tests (arterial blood gas analysis and impulse oscillometry) and/or upper airway endoscopy during exercise. The following equestrian disciplines were represented: showjumping (international and national levels), eventing, dressage, endurance racing, driving and leisure riding. Data from a group of Standardbred and Thoroughbred racehorses was also included for comparison.

The diagnosis of EIPH was based on the combination of at least 2 of the following criteria: a history of post-effort epistaxis, evidence of blood in the trachea after exercise, haemorrhagic aspect of TW or BALF, presence of red blood cells and/or erythrophages and/or haemosiderophages on the TW and/ or BALF cytological slide.

Evidence of EIPH was found in all groups, with large variations in prevalence according to the discipline practiced and level of performance. It was always associated with poor performance in all sport horses. The large majority of horses had other simultaneous affections, mostly touching the lower respiratory tract. Prevalence will be presented and qualified according to clinical and ancillary findings. The pertinence of exercise tests in eliciting EIPH will also be discussed.

The fact that this study was performed on referred cases raises several issues. Firstly, there was always a variable delay between the initial episode of EIPH and the referral examination of the horse, which could have lead to an evolution in the horse's symptoms and a higher incidence of concomitant or secondary respiratory diseases. Secondly, the circumstances leading to EIPH and the possible treatments previously administered sometimes lacked in precision and could have concealed the initial condition. Lastly, this study probably does not accurately reflect the true prevalence of EPIH in sport horses, which could be currently underestimated, as definitive diagnosis was generally based on realisation of TW and BALF samples which are less commonly performed in practise.

Cardiac contributions to EIPH

M. M. Durando

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It is generally accepted that stress failure of pulmonary vessels occurring secondary to very high exercising trans-pulmonary pressures is the major initiating cause of EIPH. While other factors undoubtedly modify the extent or frequency of detectable bleeding, extreme elevations in pulmonary vascular pressures play a central role. Because of the importance of cardiac function in determining pulmonary vascular pressures, it is logical to conclude that cardiac function can affect the occurrence of EIPH.

Cardiac outputs in fit TB horses undergoing maximal treadmill tests approximate 800 ml/kg/min; such extremely large volumes coincide with increased pulmonary artery (PA) pressures in horses with normal cardiac function. Abnormal cardiac function can exert additional effects on pulmonary vascular pressures that may increase the severity of EIPH. Atrial fibrillation has been associated with more severe bleeding, through increases in left atrial pressure. Other dysrhythmias also have the potential to elevate pulmonary vascular pressures resulting in more severe EIPH. In addition, abnormal left ventricular function could possibly contribute to the severity of EIPH, through poor systolic or reduced diastolic function.

We evaluated the records of horses that had been admitted for a complete high speed treadmill exam with a complaint of poor performance, to determine if an association exists between documented cardiac disease and either EIPH induced by the treadmill exam or severity of prior EIPH as indicated by degree of hemosiderosis. All horses had echocardiography, exercising ECG, ABG and videoendoscopy, and post exercise TW or BAL performed.

Two hundred and seventeen horses were evaluated. Of these, 73 had cardiac disease (exercising dysrhythmias or poor LV function post-exercise). Eight of the cardiac cases and 12 of the non-cardiac cases had severe prior EIPH. Only 18 horses had RBCs in the BALF; 6 of these also had cardiac disease. No differences in distribution of EIPH between cardiac and non-cardiac cases were seen.

Three horses had repeat treadmill exams that supported cardiac influences. Increased severity of hemosiderosis coincided with a new diagnosis of cardiac disease on sequential exams in 2 horses, and one horse had documented elevations in exercising PA pressures, significant cardiac disease, and severe hemosiderosis in both exams.

Despite associations in some individuals, there was no consistent relationship between cardiac disease and evidence of hemorrhage or prior EIPH. However, many uncontrolled variables may have obscured a relationship. These included: timing and intensity of last previous workout or race relative to the treadmill exam, treadmill test intensity, and other clinical abnormalities. Additional large population studies are needed to clarify these issues.

EIPH: is it really haemorrhage

D. J. Marlin and R. J. Schroter*

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The assumption until now has been that in EIPH, frank blood is present in the airways. Electron microscopy studies of lungs after exercise showing alveolar-capillary membrane disruption and a knowledge of pulmonary mechanics both suggest that protein exudation should precede, or occur to a greater extent than, the release of RBC into the airways. We set out to test the hypothesis that the ratio of RBC to total protein (TP) in bronchoalveolar lavage (BAL) following intense treadmill exercise is different to the ratio of RBC to TP in blood from the systemic circulation.

MATERIALS AND METHODS

Eight Thoroughbred horses (mean[±SD] age 6.0±2.6; 5 mares, 3 geldings; weight 478±35 kg) free of obvious signs or history of cardiac or respiratory disease were studied after being trained and acclimated to exercise on a treadmill for a minimum of 16 weeks. Each horse performed 3 runs to fatigue in an incremental exercise protocol. Left and right lung BAL and scoring of visible blood in the trachea was performed after exercise.

RESULTS

RBC counts relative to TP levels in mg per unit volume of BAL ranged from constant ratios of 5000:1 to 30,000:1 in different horses compared with the anticipated ratio of approx. 106:1 for frank blood. Furthermore, all horses had significantly greater RBC counts ($P<0.001$), and all but one, greater TP levels in the left lung BALs.

CONCLUSIONS

EIPH in this group of horses following intense treadmill exercise, some of which showed visible blood in trachea, does not appear to represent frank haemorrhage, but rather a plasma or protein rich filtrate of blood, which includes some RBC. These results cannot be explained by differences between systemic and pulmonary capillary haematocrits.

Pulmonary venous remodeling in equine EIPH

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The pathogenesis of exercise-induced pulmonary hemorrhage (EIPH), and the cause of the caudodorsal distribution of the hemorrhage are poorly understood. This paper describes the regional distribution and co-localisation of pulmonary veno-occlusive remodeling with fibrosis, hemosiderin accumulation, and angiogenesis within the lungs of EIPH horses. In 7 horses with EIPH, 6 sections were collected from both the right and left lung, representing the cranial, middle, and caudal region of the dorsal and ventral lung. Histologic scores were assigned to each region, based upon the presence and severity of interstitial fibrosis, hemosiderin accumulation, pleural/interlobular septal thickness, arterial and venous wall thickness and evidence of angiogenesis. 46% of the sections were histologically normal, 39% were moderately affected, while 14%, primarily from the dorso-caudal lung, had severe lesions. In the latter, veno-occlusive remodeling of the intra-lobular veins was colocalised with hemosiderosis, fibrosis, hypertrophy of vessels within the pleura and interlobular septa, and bronchial neovascularisation. We also utilised morphometric methods to analyse the distribution and accumulation of pulmonary collagen, hemosiderin, and pleural vasculature in the lungs of 5 of these EIPH horses and 2 control horses. The data were analysed based upon lung region, as well as upon the severity of the histopathology. In EIPH affected horses, vein wall collagen thickness was greatest in the dorsocaudal lung, and significantly correlated with hemosiderin accumulation. When data were analysed based on lesion severity, increased venous, interstitial, pleural, and septal collagen, lung hemosiderin and pleural vascular profiles occurred together, and were most common in the dorsocaudal lung. Further, hemosiderin accumulation co-localised with decreased pulmonary vein lumen size. When data from all regions were evaluated, vein wall thickening, hemosiderin accumulation, and histological score were highly correlated and these changes occurred only in the dorsal part of the lung. No regional differences in vein wall thickness or hemosiderin content were observed in the lungs of control horses. On average, vein wall thickness was less in these horses, and in caudodorsal regions, was half that in EIPH-affected animals.

The data presented here suggest that regional pulmonary venous remodeling is central to the pathogenesis of EIPH. We propose the following hypothesis: exercise-induced venous hypertension, primarily within the caudodorsal lung, leads to venous remodeling and collagen accumulation. This leads to local increases in pulmonary capillary pressure, capillary stress failure, bleeding, hemosiderin accumulation, and subsequently fibrosis in the lung.

Contribution of pro-inflammatory enzymes in EIPH: could the neutrophils play a role in the pathogensis of the condition?

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Intense exercise in horses has been shown to induce changes in white blood cell count and to stimulate neutrophils. Over stimulation of neutrophils results in their degranulation with release of their granules' content. A previous study showed that after a competition (eg 3-day event competition, endurance race), the plasmatic levels of myeloperoxidase (MPO), an oxidant enzyme of the azurophilic granules of neutrophils, increased till values reported in some inflammatory pathologic conditions. Degranulation of azurophilic granules from blood neutrophils results not only in MPO release but also in the discharge of other enzymes such as elastase. Enzymes of neutrophils play a key role in the host defence mechanisms but they are also implicated in tissue-damaging inflammation. The MPO and elastase contribute to tissue destruction by 2 different pathways: MPO through its contribution to the production of reactive oxygen species and elastase through its proteinase property enable to degrade and solubilize fibrous elastin and some extracellular matrix proteins.

The lungs play an important role in the fate of activated neutrophils by trapping dehiscent circulating cells. The pulmonary retention of activated and/or degranulated neutrophils might favour accumulation of pro-inflammatory enzymes. Furthermore, in a recent *in vitro* study, a MPO capture by endothelial cells, in particular arterial cells, was demonstrated. This study suggests that tissue infiltration by MPO might arise not only from infiltrated and degranulated neutrophils but also from endothelial transcytosis of circulating MPO occurring after its release by activated neutrophils. In the hypothesis of a MPO-mediated tissue inflammation, an intense exercise could be the initial trigger of the tissue-damaging process.

Based on the mentioned-above observations, the question of a potential role of neutrophils in the pathogenesis of exercise-induced pulmonary haemorrhage (EIPH) was raised. Is there an association between exercise intensity, stimulation of neutrophils in blood, accumulation of neutrophils granular proteins in lung tissue and EIPH? To challenge this hypothesis, further investigations should be pursued.

To the authors' knowledge, the association between the neutrophils degranulation in blood, the level of marker neutrophils enzymes in bronchoalveolar lavage (BAL) and exercise-induced pulmonary haemorrhage (EIPH) has not already been investigated. As well, the kinetic of release and clearance of neutrophils marker enzymes in plasma has not been determined in exercising horses. And ultimately, it is wondered if blood markers of neutrophils activation released during exercise might be used in the detection and/ or management of bleeders.

Recently, enzyme-like immunosorbent assay (ELISA) kits for equine MPO and elastase in plasma have been validated. These ELISA kits might help to define the contribution of neutrophils enzymes in EIPH.

Potential role of pulmonary inflammation in predisposing the equine lung to EIPH

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Previous studies have shown that intrapulmonary blood is capable of producing prolonged alveolar inflammation leading to pulmonary fibrosis. Alternatively the ability of pulmonary inflammation to predispose the equine lung to exercise-induced pulmonary hemorrhage (EIPH) has long been suspected, but never proven. Theories regarding the cause of EIPH have centered on reasons other than pulmonary inflammation in recent years. This study aimed to use bronchoalveolar lavage (BAL) cytology to evaluate the affect of intense exercise on both normal and inflamed regions of equine lung, to determine whether pulmonary inflammation predisposed the lung to hemorrhage during exercise. Segments of equine lungs were inoculated with a dilute (0.01%) acetic acid solution to provoke the development of mild neutrophil dominated pulmonary inflammatory changes. Following intense exercise, 24 h after inoculation, these sites were lavaged and the results compared with those of segments within the lungs of the same horses that had not been inoculated with acetic acid. Erythrocytes were obtained from only 12.5% (1/8) control lung sites compared with 75% (6/8) inoculated sites following intense exercise, indicating a significant increase ($p=0.04$) in the relative risk of EIPH following the development of pulmonary inflammation. BAL samples from inoculated sites had a significantly higher percentage and number of neutrophils (12.1±1.0% and 601±98 cells/µl) than control samples (4.3±0.3% and 214±52 cells/µl). Similar significant differences were observed in erythrocyte numbers (14304±6862 cells/µl) compared with control sample value of 3.5±3. cells/µl. The results of this study suggest that pre-existing pulmonary inflammation increased the risk of developing pulmonary hemorrhage during exercise. These findings do not conflict with current theories as to the common causes of EIPH in racehorses but suggest that extra care need be taken when recommending exercise in horses suspected to be suffering from pulmonary inflammatory disease. That all cases of EIPH result from previous pulmonary inflammation is unlikely, however the fact that some cases of EIPH may well be predisposed to or made more severe by strenuously exercising horses that are suffering from pulmonary inflammatory disease in any form has important implications for the training of racehorses.

Platelet-activating factor is involved in the pulmonary inflammation of exercised young racing Thoroughbred colts

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The present study aimed to investigate the occurrence of PAF, oxidative stress, and the alveolar macrophage function in the airways of young thoroughbred colts challenged by the environment and exercise. The colts were divided into three groups: the Natural Condition (NC) group (yearlings in natural farm condition), the Environmental Challenge (EC) group (colts stabled for thirty days), and the Exercise Challenge (EX) group (colts that had their first 800–1,000 m workout, around 15–6 meters/s, in a racetrack, 24 h before the evaluation). This study was approved by the Committee on Animal Experimentation of the Pontifícia Universidade Católica of Paraná. The colts of the EX group had the BALF percentual neutrophils count 57.1% and 83.3% increased in comparison to the NC and EC groups, respectively, as well as the percentual count of eosinophils was significantly higher (vs. EC). Also, the mean neutrophils and eosinophils percentual counts for the exercised colts were higher than the upper limits for normal horses. Moreover, a significantly higher total nucleated cell count in the BALF of the EX group (22.6%) in comparison to the stabled colts, pointed out to an inflammatory profile. Hemosiderophages were 2.2% of the total BALF cells and were present in the BALF of 64.5% of the exercised colts, evidencing exercise-induced pulmonary haemorrhage (EIPH) in young Thoroughbred colts in race training. Bioactivity of the pro-inflammatory mediator PAF was identified in the BALF of the exercised colts, increased in 93.3% (vs. NC) and 91.8% (vs. EC). The alveolar-capillary permeability, which is increased by PAF, was investigated by measuring the BALF total protein concentration. In the exercised group, this parameter was significantly increased in 90.2% (vs. NC) and 56.3% (vs. EC). Exercise resulted in a significantly decreased production of nitric oxide in the BALF although there was an increased antioxidant activity (catalase). Moreover, the phagocytic capacity and the respiratory burst of the alveolar macrophages (production of anion superoxide and hydrogen peroxide) were significantly decreased in the EX group. Finally, BALF lipid peroxidation resulted in an increment of 81.3% vs. the NC group and a significantly decrease of 66.3% compared to EC group.

In conclusion, the present study has shown that exercised young thoroughbred colts evidence inflammatory airway disease and EIPH, and that higher PAF bioactivity is involved in this process, as well as there is a reduced innate pulmonary defense in this advanced phase of the race training.

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Biomarkers for EIPH in blood

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INTRODUCTION

Pulmonary inflammation has been previously reported in racehorses with exercise-induced pulmonary hemorrhage (EIPH) and may be due to pre-existing small airway disease or intrapulmonary accumulation of blood. Following autologous blood installation within the lung, there is a pulmonary neutrophilia. Neutrophil-mediated injury may lead to intrapulmonary up-regulation of pro-inflammatory cytokines, damaging the alveolocapillary barrier causing a systemic inflammatory response. In order to better understand the immunopathogenesis of EIPH, we investigated mRNA IL-1, -6, -10, INF-γ, and TNF-α expression in a population of Thoroughbred racehorses with varying grades of EIPH competing at different altitudes

MATERIAL AND METHODS

The study design was a prospective, cross-sectional study of pre-enrolled Thoroughbred racehorses competing in flat races at altitude (>1,400 m above sea level) and at sea level in a racing jurisdiction that does not permit the use of furosemide nor nasal dilator strips. After tracheobronchoscopy was performed <2 h after racing, the presence and severity of EIPH was graded 0–4 and venous blood was collected from a maximum of 10 horses in each grade classification. Following RNA isolation and cDNA synthesis, real-time PCR was used to detect equine cytokine-specific mRNA for IL-1, -6, -10, INF-γ, and TNF-α

RESULTS

Neither location nor grade of EIPH affected the expression of IL-1 and INF-γ. There was greater overall expression of IL-6 mRNA at sea level ($P=0.009$) with more IL-6 expressed in racehorses with Grade 4 EIPH compared to horses with Grade 0, 1 and 2 EIPH ($P<0.05$). At altitude, no difference between the various grades of EIPH was seen for IL-6. There was greater overall expression of TNF-α mRNA ($P =0.005$) at altitude however there was no difference within the various grades of EIPH ($P=0.06$). Expression of IL-10 was affected by grade of EIPH ($P=0.02$) as horses with Grade 3 EIPH expressed more IL-10 mRNA compared to Grade 0 and 2 EIPH ($P<0.05$); but was not affected by location ($P=0.27$).

DISCUSSION

Results of this study indicate that increased IL-6 production is associated with more severe forms of EIPH and that altitude may affect proinflammatory mRNA cytokine production particularly IL-6 and TNF-α. The pathophysiological significance of these findings remains to be explained. Further research is required to determine whether the reported inflammatory response is due to pre-existing pulmonary inflammation or is a direct consequence of pulmonary bleeding.

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Which therapeutics mitigate EIPH and how should their efficacy be determined?

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Effective therapeutic amelioration of EIPH must be based on understanding the complex etiology of EIPH and the mechanistic interactions as a bases for treatment, as successful treatments developed with this rationale have reduced but not abolished EIPH. Selective breeding for winning racehorses and EIPH have evolved simultaneously over millennia: Consequently, the probability of abolishing EIPH with vascular or airway manipulation alone is unlikely to overcome the unique physiology of the racehorse that causes EIPH (ie, high cardiac output and pulmonary vascular pressures, increased blood viscosity, airways resistance, and prodigious gas exchange). Combination and integration of interventions targeted towards a series of specific EIPH mediators may maximise EIPH reduction and minimise its consequences (fibrosis, chronic inflammation, increased severity). Importantly, agreements need to be reached within the scientific community as regards testing conditions (treadmill laboratory versus field testing) and acceptable diagnostic methods for determining efficacy of these treatments (eg, graded endoscopy, [RBC] in bronchoalveolar lavage (BAL), or by other to-be-developed methods). Though endoscopy is regarded by many as 'the gold standard' for evaluating EIPH, limitations include the potential for iatrogenic bleeding and most importantly, the subjective and semiquantitative nature of the technique resulting in the inability to discriminate between improvement or worsening of EIPH severity under different exercise conditions and therapeutics. For example, 5 studies from 3 independent laboratories including both treadmill and field protocols and utilising BAL demonstrated a substantial EIPH reduction with the nasal stip (33–65%). One endoscopic study refuted this claim and the disparity likely resulted from the insensitivity of endoscopy and its subjectivity. In the same regard, at least 4 treadmill studies from 2 independent laboratories have conclusively shown EIPH reductions with BAL (58–90% dependent on exercise intensity) after furosemide administration whereas numerous earlier endoscopic and epistaxis investigations were inconclusive. BAL has also revealed that 95–100% of exercising horses bleed to some degree, Concentrated Equine Serum reduces EIPH by 53%, and omega-3 fatty acids attenuate the increased EIPH observed in time-matched controls by 76–86% when supplemented over a 5 month period. In addition, BAL has demonstrated that NO, L-NAME, endothelin antagonists, herbal formulations, aminocaproic acid, and conjugated estrogens all fail to decrease EIPH. The significant increases of EIPH observed during sub-maximal exercise (750% over resting) and inclined running (35% over flat) would have been undetectable with endoscopy. Despite limitations, blind BAL is quantitative, repeatable (CV 5%), correlates well with histopathology, is not time sensitive, samples the dorsocaudal region of the lung (primary site of EIPH), and is the most sensitive method available for diagnosing EIPH and its severity. It is possible that modification and combination of endoscopy and BAL could make field testing with BAL more acceptable and feasible. An endoscopically guided bronchoalveolar lavage with simultaneous grading could be performed bilaterally at multiple sites, and quantification of bleeding improved by a modified cell counter or spectrophotometric techniques. Future regulations regarding adjunct bleeder therapeutics and performance enhancing criteria must also be considered in selection of pharmacological versus natural and non-pharmacological treatments for EIPH.

EIPH combination medications in North America: observations from a racetrack study

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Although a number of treatments have been utilised in an attempt to minimise EIPH, surprisingly few controlled studies have evaluated the efficacy of such treatments. Presently in North America, furosemide (Salix) can be administered prior to competitions to reduce the severity of EIPH. Because of a perceived ineffectiveness of furosemide, the anti-fibrinolytic drug aminocaproic acid (Amicar) has been used in combination with furosemide to limit EIPH. This study was conducted to examine the possibility that race-day administration of Amicar in combination with furosemide could reduce the incidence/severity of EIPH in racehorses.

Trainers or owners voluntarily enrolled their horses in this study. To be included in the analyses, each horse was required to compete in 2 races: one race with administration of furosemide alone (F; 250 mg furosemide iv 4 h prior to racing), and one race with administration of furosemide plus Amicar (F+A; 250 mg furosemide iv 4 h prior to racing and 5 gm Amicar iv 2 h prior to racing). Only those horses previously certified to have EIPH were entered in the study. Treatment order was assigned in a randomised, cross-over design, and was 'blinded' to the trainer, owner and jockey.

Forty-three horses completed the study: 22 received F+A and 21 received F as the initial treatment. Videoendoscopic examination and a bilateral bronchoalveolar lavage (BAL) were completed on all horses 12–15 h post race. 200 ml sterile saline was used per lung side for BAL and veterinary clinical pathologists unaware of the study evaluated recovered fluid for RBC and nucleated cell counts, and WBC differential analysis. Repeated measures ANOVA was used to analyse data to examine possible effects of treatment or race order on these parameters. Significance was $P<0.05$.

Treatment or race order had no significant effect on total RBC numbers (mean±SEM, F 1271±530 vs F+A 1550±442 cells/ml, $P=0.62$). However, it was observed that the right lung had significantly greater RBC numbers than the left lung after both treatments (mean±SEM right vs left; F 1833±999 vs 410±124 cells/ml and F+A 2330±703 vs 415±119 cells/ml, $P<0.01$). WBC numbers were also not affected by treatment or race order. An effect of lung side on WBC numbers was not seen.

Although this study evaluated only a limited number of Thoroughbred racehorses, it appears that the pre-race administration of Amicar in addition to furosemide has no additional influence on the incidence and/or severity of EIPH compared with furosemide alone. It is also of importance to note that WBC enumeration and distributions were unaffected by this combination treatment. What is of interest is the fact that significantly more RBCs were recovered from the right than the left lung. Influences on these observations will require further study, including whether this is related to direction of travel, predominant lead, or track surface. Additional studies are presently planned to examine possible influences of race direction/course configuration and racing surface on EIPH.

Efficacy of first time furosemide use in Thoroughbred racechorses in Brazil

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Introduction

Treatment of exercise-induced pulmonary haemorrhage (EIPH) with furosemide (FUR) has become widespread, despite the fact that its results are questionable in efficiently and consistently controlling haemorrhage. In Brazil, Thoroughbred racehorses are entitled to the use of FUR once they are endoscopically diagnosed as EIPH positive by authorised veterinarians. Our objective is to evaluate the efficacy of FUR use for the first time under competition circumstances, by comparing endoscopies after 2 consecutive races.

Materials and methods

Thoroughbreds racing at Gavea Hippodrome (Rio de Janeiro, Brazil) from August–December 2006, that displayed endoscopic signs of EIPH for the first time after a race (Group FUR, R1), were included in the study. EIPH grading (0–4) was according to the literature. Horses in group FUR were then medicated with 4 ml of Furosemide (50 mg/ml) 4 h prior to their next competition. A second endoscopy (Group FUR, R2) was performed up to 30 min after the end of this second race. The control group (Group CON) consisted of animals with endoscopies after 2 consecutive races but that were not medicated with furosemide. The study included intact males, females and geldings between 2.5 and 6 years of age. There were 147 horses that met the selection criteria, however to eliminate confounding due to initial EIPH grade and to have both FUR and CON horses in each initial EIPH grade, 10 controls without initial EIPH were omitted. Fifteen FUR horses with an initial grade of 4 were also deleted due to lack of matched controls. The Mantel-Haenszel estimate of the risk ratio of a decrease in EIPH grade when comparing FUR and CON horses was calculated.

Results

A total of 96 animals were included in Group FUR and 26 were included in Group CON. Horses that received FUR had a higher chance (risk ratio 2.1, 95%CI 1.2 to 3.8, P=0.0013) of presenting a subsequent lower degree of hemorrhage than horses not medicated. In Group FUR, 62 horses (64.6%)) displayed a decrease in EIPH grade after FUR; 29 (30.2%) did not show any difference and 5 (5.2%) presented an increase between R1 and R2. In Group CON, 8 horses (30.8%) displayed a decreased grade of EIPH in the second endoscopy, while 9 (34.6%) did not show any difference and 9 (34.6%) presented an increase between the 2 endoscopies.

Conclusions

FUR reduced at least one grade of EIPH in 64.6% of the Thoroughbreds using the drug for the first time in competition compared with 30.8% of control horses. Horses affected with EIPH had a significantly higher chance of subsequent reduced bleeding if medicated with furosemide than controls.

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Effect of furosemide and furosemide-carbazochrome combination on EIPH in Standardbred racehorses

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Background

The majority of racehorses exhibit some degree of exercise-induced pulmonary hemorrhage (EIPH) during racing. Pre-race administration of furosemide to racehorses with EIPH is an approved therapy in most American racing jurisdictions. Experimental studies suggest that furosemide help decrease severity of lung bleeding. Several adjunct bleeder medications such as carbazochrome are permitted in a handful of racing jurisdictions when used in combination with furosemide. However, to date, no scientific studies have examined the safety and efficacy profile of these medications. Therefore, the objective of this study was to quantify the effect of furosemide and carbazochrome on EIPH in Standardbred horses using red blood cell (RBC) count and hemoglobin concentration in bronchoalveolar lavage fluid (BALF).

Method

Six healthy Standardbred horses with prior evidence of EIPH performed a standardised treadmill test 4 h after administration of placebo, furosemide or furosemide – carbazochrome combination using a randomised, cross-over design. Endoscopy and BAL were performed one hour after completion of the treadmill test. Red blood cell count was performed using a hemocytometer and hemoglobin concentration was determined by a modified benzidine assay on BAL fluid.

Results

RBC count in BALF ranges were [2903 – 26025 cells/µl], [445 – 24060] and [905 – 3045] for placebo, furosemide and furosemide – carbazochrome, respectively. Hemoglobin concentration ranges were [0.03 – 0.59 mg/ml], [0.01 – 0.55] and [0.007 – 0.16] for placebo, furosemide and furosemide – carbazochrome groups, respectively. No significant differences were detected among treatments.

Conclusion

Furosemide or furosemide – carbazochrome combination did not reduce EIPH severity following treadmill exercise to fatigue. However, variability in EIPH severity between horses was very large; suggesting that a larger sample size or better selection of horses is needed to be able to more definitely assess the effect of bleeder medications on EIPH severity.

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