Exercise-induced pulmonary haemorrhage impairs racing performance in Thoroughbred racehorses

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Summary

Reasons for performing study: Exercise-induced pulmonary haemorrhage (EIPH) occurs commonly in Thoroughbred racehorses worldwide. While EIPH is believed to be an important cause of impaired performance in these horses, there is limited evidence from sufficiently powered studies to evaluate this association.
Objectives: To evaluate whether EIPH is associated with finishing position, distance finished behind race winners and differences in race earning among Thoroughbred horses racing in South Africa.
Study design: Prospective cross-sectional study.
Methods: One thousand Thoroughbred horses racing in South Africa were enrolled prior to a single race and underwent tracheobronchoscopic examination within 2 h of racing. Three observers, blinded to the horses' identity and race performance, independently evaluated EIPH occurrence and severity using video recordings of the examination. Data were analysed using multivariable logistic and linear regression while controlling for important horse and race factors as potential confounding variables.
Results: Overall, 68% of horses had evidence of EIPH (*grade* ≥*1*). Horses without evidence of EIPH (severity *grade 0*), when compared with horses with any evidence of EIPH (*grade* ≥*1*), were >2 times more likely to win races (odds ratio = 2.3; 95% confidence interval 1.4–3.7; P = 0.001), finished an average of one length ahead of horses with EIPH (P = 0.03), and were 2.5 times more likely to be in the highest decile in race earnings (odds ratio = 2.5, 95% CI 1.5–4.1, P<0.001). However, no association was identified regarding finishing in the top 3 positions or earning money when analysed as a continuous variable or analysed as any winnings vs. none.
Conclusions: Exercise-induced pulmonary haemorrhage was associated with impaired performance in Thoroughbred racehorses not medicated with furosemide and not using nasal dilator strips. These findings provide strong corroboration of previous research indicating that the occurrence of EIPH has a major impact on the ability of Thoroughbred racehorses to compete successfully as elite athletes.

Keywords: horse; exercise; lung; haemorrhage; eiph; risk factor

Introduction

Exercise-induced pulmonary haemorrhage (EIPH) is frequently a concern in Thoroughbred racehorses and other horses performing strenuous exercise. Exercise-induced pulmonary haemorrhage is an important issue because of its high frequency of occurrence, the perceived importance of EIPH regarding its impact on performance and well-being of horses, the common use of prophylactic treatments such as furosemide in some jurisdictions, and concerns about whether these treatments provide an unfair competitive advantage. Estimates of prevalence vary widely and are highly dependent upon the detection method, but it has been estimated that EIPH can be detected in at least 80% of Thoroughbred horses during their racing careers [1–3], and that 35–60% of sudden deaths not related to catastrophic musculoskeletal failure that occur during racing can be attributed to severe pulmonary haemorrhage [3,4], although a recent study of horses examined endoscopically multiple times while racing in Hong Kong found a lower cumulative incidence of EIPH (63% in post racing examinations) [5]. While EIPH is a concern because of potential association with performance and welfare of racehorses, it is also important because of the potential harm to public perceptions of the sport, which in turn might influence the economic viability of the racing industry. Thus, further corroboration of the impact of EIPH occurrence on performance of racehorses would be useful in debates that must weigh the importance of EIPH with other considerations.

Several studies have examined the impact of EIPH on racing performance in Thoroughbred horses. Overall, results of smaller studies have been equivocal, especially those conducted on treadmills [1,6–8]. However, published evidence from the best documented large study performed under actual racing conditions provides strong evidence that EIPH can have a substantial effect on racing performance [2]. It is likely that the ability of smaller studies to detect an association between EIPH and performance was impaired by inadequate statistical power, nonrandom selection of subjects, and failure to control for confounding related to administration of furosemide, track and race factors, variability in innate performance abilities, and other factors. Because a successful race is often determined by very small performance margins, especially when considered relative to the overall length of races, studies examining the effect of EIPH on performance must include a large number of study subjects in order to detect differences in outcome measures that are relevant markers of successful performance in elite athletes. Furthermore, a large number of factors can affect the athletic performance of horses, and analysis of epidemiological information gathered from field investigations requires appropriate use of sophisticated statistical tools to fully account for these sources of extraneous variability that can impact race performance but are unrelated to EIPH occurrence.

The documented efficacy of furosemide along with its common use as a prophylactic treatment for EIPH in North America impairs the ability to investigate the relationship between EIPH and performance in those racing jurisdictions. However, the National Horseracing Authority (NHRA) of South Africa, which governs Thoroughbred racing in that country, imposes strict bans on the use of furosemide or nasal dilator strips on race day, making it an ideal population to assess the impact of EIPH. Furosemide and other interventions used to prevent EIPH are allowed during training. The primary goal of this study was to evaluate the putative association between EIPH occurrence and performance in a population of Thoroughbred racehorses untreated with furosemide. Secondarily, we evaluated potential risk factors for EIPH, including hardness of track surfaces and elevation.

Methods

Study overview

Horses were enrolled longitudinally prior to racing in this cross-sectional study. After racing, enrolled subjects underwent tracheobronchoscopic examinations that were video recorded. A panel of investigators that were blinded to horse identification, racing performance and observations of field personnel reviewed the video recordings and determined a consensus score for EIPH severity. Multivariable regression modelling was used to investigate the potential impact of EIPH on various performance outcomes while controlling for potential confounding variables. Secondarily, data were used to investigate factors that could have affected EIPH occurrence, such as track hardness and elevation.

Study design and setting

This study was purposefully designed to be similar to a previously published field investigation of EIPH and performance to allow direct comparison of results [2]. Study subjects for this cross-sectional study were 1000 Thoroughbred racehorses competing in flat races at 5 racecourses in South Africa (Clairwood, Greyville, Kenilworth, Turffontein and Vaal Racecourses) between 4 August and 19 November 2005. Pursuant to rules enforced by the NHRA, administration of furosemide and use of nasal dilator strips was not permitted on the day of racing.

Enrolment

In order to raise awareness and promote participation in this study, registered trainers, owners and local veterinarians participating in Thoroughbred racing in all provinces of South Africa were contacted via facsimile, articles published in newsletters and local newspapers, television broadcasts, live interviews at racetracks and private venues, and through personal communication. After identifying days when field studies would be conducted at a particular venue, lists of horses accepted to race were obtained from the NHRA, and their trainers were contacted by telephone 24–72 h prior to racing to request permission to examine horses. Lists of horses entered in races were randomised to determine the order in which representatives were approached for participation in the study. Only horses enrolled prior to racing were eligible to participate, and informed consent was obtained in writing from trainers prior to racing. A sample size of 1000 horses was chosen prior to initiating the study based on the design, sample size, effect magnitude and variability identified in a prior research project [2] after which this study was modelled. Additionally, to allow examination of the potential effect of elevation on EIPH occurrence, a goal was set to enrol approximately 500 horses that raced at sea level and 500 horses that raced at high elevation.

Detection and quantification of EIPH

All enrolled horses underwent a standardised examination to evaluate presence and severity of EIPH. Briefly, horses were restrained in a stall for examinations, but were not sedated. A veterinarian who was hired specifically for the conduct of this study performed all examinations (M.N.S.). A standardised protocol for tracheobronchoscopy was established prior to initiating the study, and all horses were to be examined within 2 h of racing. A 1.5 m fibreoptic endoscope was used to visualise the respiratory tract to the level of the tracheal bifurcation and examinations were recorded digitally to allow subsequent review. Each recording was time stamped and included a unique identification number that was assigned to study subjects at the time of enrolment. These video recordings were reviewed by 3 investigators (P.S.M., K.W.H., A.J.G.) who were blinded to the identity of horses and race performance markers at the time of evaluations. Reviewers watched recorded examinations at the same time without discussion of observations (slowing, stopping and rewinding recordings as necessary), and then independently recorded their severity scores. Scores were then compared; if there was any discordance among reviewers' initial scores, recordings were re-reviewed and observations discussed to obtain a mutually agreeable consensus score.

The occurrence of EIPH was graded on a scale of 0–4, as previously validated [9]. Briefly, *grade 0* indicated that no blood was detected in the pharynx, larynx, trachea or mainstem bronchi visible from the tracheal bifurcation; *grade 1* indicated the presence of one or more flecks of blood or ≤2 short (<¼ of the length of the trachea), narrow (<10% of the tracheal surface area) streams of blood in the trachea or mainstem bronchi visible from the tracheal bifurcation; *grade 2* indicated the presence of a long (>½ the length of the trachea) stream of blood or >2 short streams occupying <⅓ of the tracheal circumference; *grade 3* indicated detection of multiple distinct streams of blood covering >⅓ of the tracheal circumference but without evidence of blood pooling at the thoracic inlet; *grade 4* indicated the presence of multiple coalescing streams of blood covering >90% of the tracheal surface with pooling of blood at the thoracic inlet. If the reviewers' consensus was that the recorded examination did not permit conclusive evaluation of the presence or severity of EIPH (because of movement of the horse, limited duration of the examination or poor recording quality), the examination was scored as being 'nondiagnostic'.

Racing performance on study dates and during their entire racing careers

At the time of examination, investigators recorded the date, venue, race number, horse's name, horse's unique microchip number (which corresponds to their unique NHRA identification number), trainer's name, and the time that tracheobronchoscopic examinations were conducted. After completing all field investigations, this information was combined with racing performance data that are routinely compiled by the NHRA. Using horses' unique NHRA identification numbers to link records, information obtained from this database included the following data regarding racing on the day that horses were examined: age (years), sex (female, intact male or gelding), trainer's name, jockey's name, weight carried (kg), race distance (m), post time, track surface (sand or turf), track surface condition (good or other), track penetrometer measurement (mm) as an objective measure of surface hardness and moisture, purse (South African Rand, [ZAR]), number of starters, whether horses completed the race (yes vs. no), finishing position, stakes won (ZAR), lengths finished behind winner, and finishing time(s). Elevation for races was assigned based upon racing venue; Turffontein Racecourse and the Vaal Racecourse were classified as 'high elevation' (1713 m and 1438 m, respectively) while Clairewood, Greyville and Kenilworth Racecourses were classified as 'sea-level' (<100 m). Race records for all starts for horses enrolled in the study were obtained from the same database, and career racing statistics were summarised. These data included lifetime starts, wins, second place finishes, third place finishes, total stakes earned (ZAR), days elapsed since their previous race (one race prior to enrolment in the study), as well as the days elapsed since the next-to-last race (i.e. 2 races prior to the study).

Data analysis

After combining databases, data entries were validated, descriptive statistics were calculated and distributions of continuous variables were evaluated for normality. Adjusted confidence intervals for binomial proportions (adding 2 successes and 2 failures) were estimated as previously described [10]. Horses with nondiagnostic endoscopic evaluations were excluded from all inferential analyses. To evaluate the potential for enrolment bias, distributions for age, sex, weight carried, race earnings, finish position and lengths finished behind the winner were compared between horses enrolled in the study and those that were not.

Associations between EIPH and racing performance: The measures of racing performance that were used as outcomes when addressing the primary study goal (evaluate the association between EIPH occurrence and racing performance) included variables summarising finishing position, race earnings, and distance that horses finished behind the winning horse. Two categorisations were used in analyses regarding finishing position: whether the horse won (yes vs. no) and whether the horse finished in the first 3 positions (yes vs. no). Because race earnings were strongly right skewed with many zero values, one ZAR was added to all observations, and the resultant values were log-transformed. In addition, this variable was categorised as earning any money (yes vs. no, with no additional ZAR added) and having race earnings that were in the highest decile (i.e. ≥90th percentile for all horses in all races, which was ≥25,000 ZAR; yes vs. no). Distance finished behind the winner was also log-transformed due to non-normality, and horses that did not finish the race were assigned a last-place finish and arbitrarily said to have finished 50 lengths behind the winner. The primary exposure variable of interest for these analyses was the EIPH consensus score, dichotomised as yes vs. no (severity *grade 0* vs. *≥1*).

Additionally, variables that could have affected or predicted a horse's performance beyond the occurrence of EIPH were considered *a priori* to be potential confounders and included in all analyses as covariates. These included factors related to the race under investigation and career performance indicators and track characteristics. Specifically, variables regarding horses' sex, track condition and track surface were included in all analyses. However, because of collinearity among many of these potentially confounding variables, principal component analysis was used to create orthogonal scores for these additional covariates, and the principal components were included in analyses [2,11] (PROC PRINCOMP, SAS v9.3)[a]. Factors included in the principal components included elapsed race time for the winning horse, weight carried, number of horses in the race, distance, purse, horses' age, number of career starts, career wins, career seconds, career thirds, career earnings, days since last race and days since next-to-last race. In order to calculate principal component scores related to elapsed time since previous races, 75 horses that had not raced before the study were assigned the maximum value for the immediately previous race that was observed for other horses (510 days). Similarly, the 166 horses that had not raced twice prior to the study were assigned a value of 518 days for the next-to-last race which was the maximum value for other horses. In addition, because blood originating from the lower airways can accumulate over time in the upper respiratory tract after racing, the relationship between elapsed time from race start until tracheobronchoscopic examination was evaluated relative to the severity of EIPH.

Categorical outcomes (winning, finishing in the top 3 positions and being in the highest decile for race earnings) were analysed using multivariable logistic regression, using generalised estimating equations to account for lack of independence among observations for horses racing in the same race (using an independent correlation structure; PROC GENMOD, SAS v9.3)[a]. Odds ratios (ORs) and likelihood-ratio-based 95% confidence intervals (95% CIs) were derived from model results. Continuous outcomes (log of race earnings and log of distance finished behind the winner) were analysed using mixed-effects linear regression, using a random slope/random intercept model to account for lack of independence and differences in effects among different races (PROC MIXED, SAS v9.3)[a]. Least-square (LS) mean estimates and 95% CIs were obtained from models for outcome measures relative to EIPH severity score. The relationships between racing outcomes (winning and finishing in the top 3 positions) and the elapsed time from post time until tracheobronchoscopic examinations were also explored using logistic regression.

Associations with EIPH occurrence: To address secondary study objectives, potential associations with the occurrence of EIPH (*grade 0* vs. *≥1*) were analysed using logistic regression, using generalised estimating equations to account for lack of independence among observations for horses racing in the same race (using an independent correlation structure; PROC GENMOD, SAS v9.3)[a]. Independent variables that were analysed included elevation (high elevation vs. sea level), penetrometer reading (categorised as quartiles), time of race (day vs. night), going (good vs. other), race surface (grass vs. sand), age (years) and sex (female, male or gelding). Univariable models were analysed for independent variables of interest, and variables with P≤0.25 were included in multivariable model building using backward selection. Odds ratios and 95% CIs were obtained from regression results. The relationship between EIPH occurrence and the elapsed time from post time until tracheobronchoscopic examinations was also explored using logistic regression.

Sensitivity analyses were used to evaluate the potential impact of confounding by other variables by excluding these covariates from final models and evaluating the changes in parameter estimates for the exposure variables of interest in the different models. We also examined the impact of excluding horses with nondiagnostic tracheobronchoscopic evaluations from inferential modelling. First, baseline characteristics for horses (Table 1) were compared between those with tracheobronchoscopic examinations judged to be diagnostic vs. those considered to be nondiagnostic using the Wilcoxon–Mann–Whitney test for continuous variables, and the χ^2 test for categorical variables. Additionally, multivariable analyses related to performance outcomes were repeated while assuming that all nondiagnostic examinations were *grade 0*. It was assumed that this would provide a most-conservative estimate of the impact of EIPH by biasing results toward the null (i.e. no difference).

Results

During the period from 4 August to 19 November 2005, data were collected from horses racing on 28 days at the 5 racetracks. A total of 230 races were held on these days, and, 53% (1000/1896) of horses racing in 94% (216/230) of these races were enrolled in the study (minimum = one horse per race, Q1 = 3, median = 4, Q3 = 6, maximum = 13). Among the 2692 entries in these races, there were 1896 unique horses. These horses predominantly raced in only one of the 216 study races, but 31% (592/1896) were entered in >1 study race (max = 6). Among the 1000 horses enrolled in the study, 68% (680/1000) only raced in the race in which they were examined, while 32% (320/1000) raced in ≥1 additional race.

Among these horses, 37% (372/1000) raced at Kenilworth Racecourse, 21% (212/1000) at the Vaal Racecourse, 19% (194/1000) at Turffontein Racecourse, 17% (171/1000) at Clairwood Racecourse and 5% (51/1000) at Greyville Racecourse. All racing occurred on turf, with the exception of Vaal Racecourse, in which 100 horses sampled raced on a sand track. The study horses were managed by 98 trainers, contributing a median of 7 horses (range 1–46) to the study population. Horses racing in the study races that were not enrolled (n = 896) were managed by 56 additional trainers. The median distance raced was 1400 m (range 800–3200 m), and there was widely varying purses for these races (Table 1). There was a similarly wide variation in prior racing performances (Table 1). Horses enrolled and analysed as part of this study had similar distributions of age and sex when compared with horses that had nondiagnostic tracheobronchoscopic examinations. For all study races, horses enrolled in the study (n = 1000) had nearly identical distributions for age, sex, weight carried, race earnings, finish position and lengths finished behind the winner when compared with race records for horses that were not enrolled in the study (n = 869).

The consensus opinion of the blinded reviewers was that 11% (114/1000) of tracheobronchoscopic examinations were nondiagnostic because recordings did not allow accurate characterisation of the presence or severity of EIPH (i.e. because of movement of the horse, limited duration of the examination or poor recording quality). Of the remaining 886 horses, 68% (606/886; 95% CI 65.2–71.5%) showed some evidence of EIPH (*grade ≥1*; Fig 1). Of these horses, 64% were EIPH *grade 1* (388/606; 95% CI 60.1–67.9%), 21% were *grade 2* (128/606; 95% CI 17.9–24.6%), 12% were *grade 3* (71/606; 95% CI 9.3–14.6%), with only 3% having *grade 4* (19/606; 95% CI 1.9–4.9%). Independent scoring of EIPH severity by the blinded panel of expert reviewers was concordant for all examinations that were deemed to be nondiagnostic. For the remaining examinations, initial independent scoring was concordant for 99.4% (881/886) of examinations. For the 5 examinations with discordant results, in all instances only one of the evaluators was not in initial agreement (i.e. the other 2 evaluators scores were concordant) and the discordant score only differed by a single grade. Consensus was reached after review of all 5 of these examinations.

Finishing position

Considering horses with diagnostic tracheobronchoscopic examinations, adjusting for factors that could influence or predict race performance, presence of EIPH was significantly associated with poorer race performance. Horses with EIPH severity *grade 0* were 2.3 times more likely to win a race compared with horses with EIPH severity *grade ≥1* (OR = 2.3; 95% CI 1.4–3.7; P = 0.001; Fig 2). When assessing each grade of EIPH, there was a negative trend relative to increasing EIPH severity grade; horses without evidence of EIPH (severity *grade 0*) had 2 times greater odds of winning compared with horses with EIPH *grades 1–3* (OR = 2.3; 95% CI = 1.4–3.7; P = 0.001), and had >6 times greater odds of winning compared with horses with EIPH *grade 4* (OR = 6.1, 95% CI 2.1–17.6, P<0.001). However, there was not a statistically significant difference in likelihood of

TABLE 1: Characteristics of horses and races included in this study

Variable	Minimum	Q1	Median	Q3	Maximum
Race characteristics (n = 216)					
Distance (m)	800	1200	1400	1600	3200
Purse (ZAR)	29,000	48,000	50,000	53,000	200,000
Number of runners	6	9	11	13	19
Penetrometer (mm)	18	19	19	20	22
Horse characteristics[a] (n = 1000)					
Money earned in race (ZAR)					
Diagnostic[b] (n = 886)	0	0	0	5200	125,000
Nondiagnostic[c] (n = 114)	0	0	1200	2600	45,000
Lengths finished behind winner					
Diagnostic (n = 886)	0	2	5	10	50
Nondiagnostic (n = 114)	0	3	5	9	19
Lifetime races					
Diagnostic (n = 886)	1	15	25	38	111
Nondiagnostic (n = 114)	3	18	27	41	104
Lifetime earnings (ZAR)					
Diagnostic (n = 886)	0	45,715	100,675	192,375	3.0 Billion
Nondiagnostic (n = 114)	0	47,650	113,990	226,475	2.8 Billion
Lifetime wins					
Diagnostic (n = 886)	0	1	2	4	12
Nondiagnostic (n = 114)	0	1	2	4	20
Lifetime seconds					
Diagnostic (n = 886)	0	1	2	4	16
Nondiagnostic (n = 114)	0	1	2	4	11
Lifetime thirds					
Diagnostic (n = 886)	0	1	2	4	19
Nondiagnostic (n = 114)	0	1	2	4	13
Days since last race					
Diagnostic (n = 886)	4	19	28	49	365
Nondiagnostic (n = 114)	7	21	27	49	509
Merit rating					
Diagnostic (n = 886)	0	48	62	74	113
Nondiagnostic (n = 114)	0	50	64	73	103
Age (years)					
Diagnostic (n = 886)	2	3	4	5	9
Nondiagnostic (n = 114)	2	3	4	5	8

	Percent (n)	95% CI
Sex		
Diagnostic (n = 886)		
Female	42.7% (378)	39.4–45.9%
Gelding	53.0% (470)	49.8–56.3%
Stallion	4.3% (38)	3.1–5.9%
Nondiagnostic (n = 114)		
Female	38.6% (44)	30.2–47.8%
Gelding	58.8% (67)	49.6–67.4%
Stallion	2.6% (3)	0.6–7.9%
Finishing position		
Diagnostic (n = 886)		
1	11.5% (102)	9.6–13.8%
2	9.9% (88)	8.1–12.1%
3	10.0% (89)	8.2–12.2%
≥4	68.5% (607)	65.4–71.5%
Nondiagnostic (n = 114)		
1	2.6% (3)	0.6–7.9%
2	7.9% (9)	4.1–14.6%
3	10.5% (12)	6.0–17.7%
≥4	78.9% (90)	70.5–85.4%

[a]Lifetime statistics are for sanctioned races that occurred before and after the date of inclusion in the study. [b]Horses that had post racing tracheobronchoscopic examinations that were judged to be of sufficient diagnostic quality for inclusion in the study. [c]Horses that had tracheobronchoscopic examinations that were judged to be nondiagnostic.

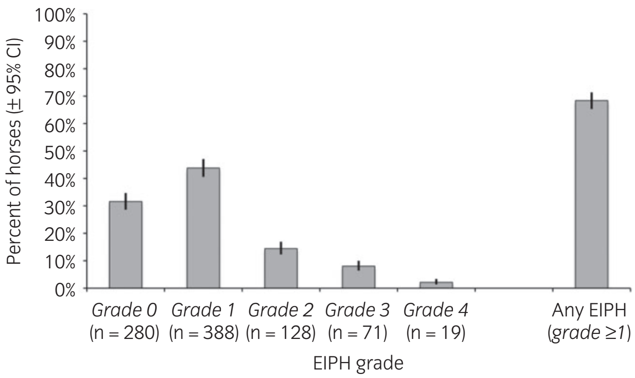

Fig 1: Severity of exercise-induced pulmonary haemorrhage (EIPH) among Thoroughbred horses racing in sanctioned races in South Africa, among horses with tracheobronchoscopy examinations classified as diagnostic (n = 886). Severity of EIPH was graded on a scale of 0–4 by a blinded panel of expert reviewers.

finishing in the first 3 positions when comparing horses with EIPH *grade 0* with those with EIPH *grade ≥1* (OR = 1.1; 95% CI 0.8–1.26; P = 0.6).

Distance behind the winner

On average, controlling for factors that could influence or predict race performance, horses with EIPH severity *grade ≥1* finished about one length further behind race winners when compared with horses without EIPH (*grade 0*; P = 0.03; Fig 3). When assessing each level of EIPH, there was a trend for horses to finish further behind the winner as EIPH severity grade increased, such that horses without evidence of EIPH (severity *grade 0*) finished an average of about 2 lengths behind the winner (LS means = 2.3, 95% CI 1.6–3.4), horses with EIPH *grades 1–3* finished an average of about 3 lengths behind the winner (LS means = 3.2, 95% CI 2.3–4.5), and horses with EIPH *grade 4* finished an average of about 5 lengths behind the winner (LS means = 5.0, 95% CI 1.9–12.9).

Earnings

Controlling for factors that could influence or predict race performance, horses without EIPH (i.e. EIPH *grade 0*) had about 2.5 times higher odds of earning in the highest decile (i.e. ≥90th percentile) for earnings in the study races compared with horses EIPH severity *grade ≥1* (OR = 2.5, 95% CI 1.5–4.1, P<0.001). However, when analysed as a continuous variable or dichotomised as any vs. no race earnings, no significant differences were identified between horses with EIPH severity *grade ≥1* compared with those with no EIPH (*grade 0*).

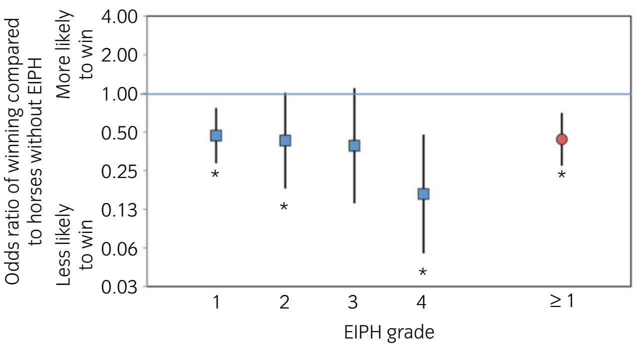

Fig 2: Forest plot depicting odds ratios and 95% confidence intervals for the likelihood of winning as a function of severity of exercise-induced pulmonary haemorrhage (EIPH) among Thoroughbred horses (n = 886) racing in South Africa. Horses without EIPH (severity *grade 0*) are the reference group. *Odds of winning were significantly different (P<0.05) from the reference group.

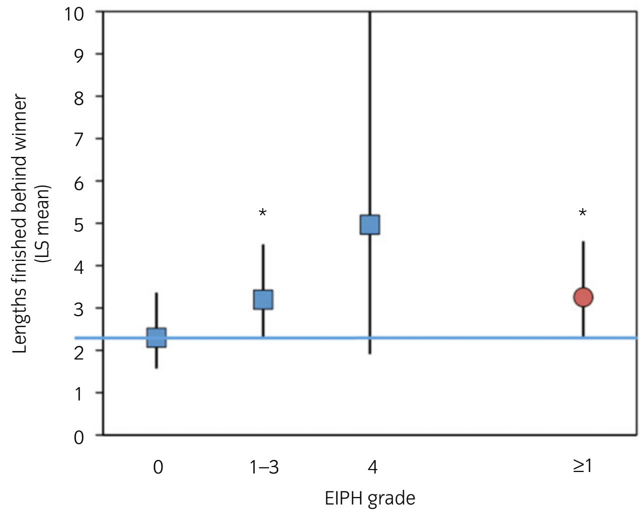

Fig 3: Least-square (LS) mean distance (±95% confidence intervals) that horses finished behind the race winner as a function of severity of exercise-induced pulmonary haemorrhage (EIPH) among Thoroughbred horses racing in South Africa (n = 886). Error bars represent 95% confidence intervals. *Margin was significantly different (P<0.05) from LS mean for horses with *grade 0* EIPH.

Risk factors for EIPH

There was no detectable association (P>0.25) between the occurrence of EIPH (0 vs. any EIPH *grade ≥1*) and any of the factors investigated using univariable logistic regression (age, sex, going, racetrack surface, penetrometer reading or elevation).

The distribution of elapsed time from race start to tracheobronchoscopic examination was strongly right skewed and 74% of horses (738/1000) were examined within <30 min of the starting time for races (for all horses [n = 1000]: minimum = 7 min, Q1 = 16 min, median = 22 min, Q3 = 30 min, maximum = 110 min). Elapsed time was not included as a covariate in models evaluating the relationship between EIPH and performance because it was deemed to be an intervening variable in this relationship rather than being a true confounding variable [12]. There was a significant association between EIPH severity and the elapsed time from the start of races until the time of examination (P = 0.03). As elapsed time increased, there was an increase in the proportion of *grade 0* scores, and a decrease in the proportion of *grade 1–3* scores (Fig 4); there was a consistent, low number of *grade 4* scores in all categories of elapsed time. There was also a significant difference in the distribution of elapsed times for horses that won races vs. those that did not (P<0.001). Enrolled horses that won races (n = 105) had a median elapsed time from race start to examination of 39 min (minimum = 19 min, Q1 = 28 min, Q3 = 48 min, maximum = 110 min) compared with horses that did not win (n = 895), which had a median elapsed time until examination of 21 min (minimum = 7 min, Q1 = 15 min, Q3 = 29 min, maximum = 104 min). Although the distributions of elapsed time until examination were more similar for horses that did (n = 303) and did not (n = 697) finish in the top 3 positions, the median elapsed time was significantly longer (P<0.001) for horses that finished in a top position (horses finishing in top 3 positions: median = 24 min, range = 7–110 min; horses finishing >third position: median = 22 min, range = 7–104 min). There was not a significant association between the elapsed time prior to examination and whether examinations were classified as nondiagnostic (P = 0.7).

Sensitivity analysis

For all analyses, removing factors that were considered to potentially influence or predict race performance (i.e. unadjusted analyses) did not alter results substantially, indicating that these factors were not actually confounding (data not shown). Simple comparisons were used to investigate the potential for bias created by excluding horses with nondiagnostic tracheobronchoscopic examinations. However, the only

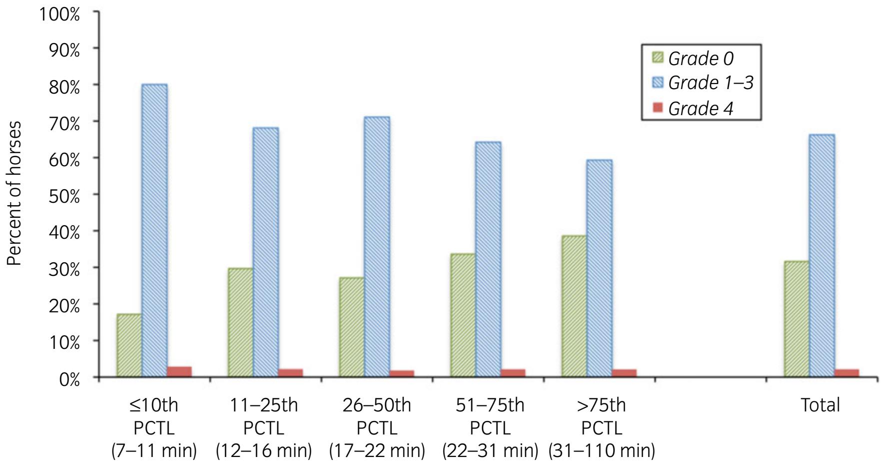

Fig 4: The elapsed time from start of racing until tracheobronchoscopy examinations were conducted, stratified by exercise-induced pulmonary haemorrhage severity grade, among Thoroughbred horses racing in sanctioned races in South Africa. Only includes horses with examinations that were classified as diagnostic (n = 886). Severity of exercise-induced pulmonary haemorrhage was graded on a scale from 0–4 following review by a blinded panel of expert reviewers. PCTL = percentile.

difference detected in baseline characteristics between horses judged to have diagnostic vs. nondiagnostic tracheobronchoscopic examinations was related to finishing in first position; there was a smaller proportion of horses with nondiagnostic examinations that finished first (2.6%), as compared with those with diagnostic examinations (11.5%, P = 0.02, Table 1). There was no difference between horses with diagnostic vs. nondiagnostic examination in other finishing positions (e.g. second, third, ≥fourth), or for other baseline characteristics that were compared (age, sex, merit rating, money earned in study race, lengths finished behind the winner, lifetime races, lifetime earnings, lifetime wins etc.). We also performed separate multivariable analyses in which the nondiagnostic examinations were all assigned a *grade 0* severity score; this was presumed to have the greatest potential for nullifying bias. When horses with nondiagnostic tracheobronchoscopic examinations (114/1000) were grouped with horses with *grade 0* EIPH (606/1000), differences were more conservative, but significant associations were still identified. Controlling for factors that could influence or predict race performance, the odds of winning a race and being in the highest decile for race earnings was about 1.6 and 1.8 times lower, respectively, when comparing horses with EIPH *grade ≥1* to horses with *grade 0* combined with those having nondiagnostic examinations (OR for winning = 0.62, 95% CI 0.13–0.98, P = 0.04; OR for earnings = 0.55, 95% CI 0.09–0.89, P = 0.02). Differences were no longer statistically significant for distance finished behind the winner (P = 0.3).

Discussion

This study provides further strong corroboration that EIPH frequently occurs in Thoroughbred racehorses and is associated with significant negative impacts on performance [2,6,13–16]. Horses with EIPH were less likely to win, finished an average of one length further behind winners when compared with horses without EIPH, and were less likely to earn above the 90th percentile in race earnings. Other study outcomes (finishing in the top 3 positions, amount of money earned [analysed as a continuous variable] and earning any money [dichotomised as yes/no]) were not associated with EIPH occurrence. These contrasting findings for related outcomes (e.g. likelihood of winning vs. finishing in the top 3 positions, and being in the highest decile in race earnings vs. earning any money [yes/no]) may indicate that EIPH had its greatest impact on the ability of horses to perform as elite athletes compared with having marked impact on all athletic performance. It is also notable that no association was identified between EIPH occurrence and the hardness of track surface or with the elevation of the racetrack, which contrasts findings from other studies that evaluated risk factors for epistaxis [17,18].

This study was intended to mimic the approach and design used in a previous investigation of EIPH that was conducted in Melbourne, Australia [2]. That previous study showed similar results: that horses with EIPH of severity *grade ≥2* were less likely to win or finish in the top 3 positions, they finished at a greater distance behind the winner, and were less likely to be in the highest decile in race earnings [2]. Key features of both studies were the enrolment of large numbers of Thoroughbred horses competing under actual racing conditions, blinded evaluation of standardised tracheobronchoscopic examinations by a panel of expert reviewers, and use of advanced statistical analyses to control for potential confounding by factors that could predict or affect the performance of study subjects. Pre-enrolment of horses prior to racing, and the high participation rate from horses that were representative of the referent populations decreased the potential for enrolment bias to have affected these studies. Further, race-day medications (e.g. furosemide) and nasal dilator strips are prohibited in sanctioned Thoroughbred racing in South Africa and Australia, helping to ensure that findings of these studies were not confounded by these prophylactic interventions that could have affected the occurrence of EIPH and performance ability.

While neither study conclusively proves that there is a causal relationship between EIPH occurrence and decreased performance, the strength of effect and similarity of findings from studies conducted in different populations of Thoroughbred horses on different continents provide substantive support for the theory that these findings are repeatable and not unusual. Further, there is an apparent dose effect for the relationship to the distance horses finished behind race winners and for the likelihood of horses winning, where horses tended to show poorer performance as EIPH severity grade increased (Figs 2, 3). A similar apparent ‘dose effect’ for length finished behind the winner was seen in the Australian study [2]. These findings strengthen the theory that EIPH is causally associated with impaired ability for Thoroughbred racehorses to perform as elite athletes.

Important factors to consider when evaluating the findings of this study are the relatively short elapsed times from racing until tracheobronchoscopic examinations were conducted in many horses, and the number of examinations that were considered nondiagnostic by a blinded panel of expert reviewers. Using tracheobronchoscopy to evaluate EIPH presumes that blood originating in the smallest peripheral airways has sufficient time to move rostrally so as to be present in the upper airways prior to examination. If bleeding associated with EIPH occurs during racing, it is reasonable to presume that the high tidal and minute volumes of ventilation seen during maximal exercise are sufficient to propel modest amounts of blood to the trachea during or within minutes of finishing a race, but it is possible that smaller amounts of blood may take longer to move from the peripheral lung tissues to accumulate in the trachea. Thus, it is frequently recommended to allow 30–120 min to elapse after racing before conducting tracheobronchoscopy so that EIPH severity grades are likely to be maximal at the time of examination [19]. It is for this reason that a related field trial [15] required a minimum of 30 min elapsed time from finish of races until examination (mean ± s.d. 41.6 ± 5.9 min). However, the study of EIPH and performance conducted in Australia did not require this minimum waiting period, and the mean elapsed time from racing until examination was 31 min (minimum = 13 min, Q1 = 25 min, median = 30 min, Q3 = 35 min and maximum = 175 min) [20]. This study also found that increasing the time period from racing until examination was associated with the likelihood of identifying any EIPH or EIPH *grade 2* or greater [20].

In comparison, 75% of horses in this study were examined within 7–30 min after racing and the distributions of elapsed times differed between horses diagnosed with EIPH and those that were assigned EIPH *grade 0*. In this study, as elapsed time from the start of races until examination increased, the proportion of horses assigned a severity *grade* of *0* increased, while the proportion of horses with EIPH *grades 1–3* decreased (Fig 4). These differences in distributions for elapsed time probably occurred because of constraints imposed by the need to examine several horses after each race on all study days, in combination with constraints regarding the availability of enrolled horses that were selected to be included in routine surveillance for drug exposures by racing authorities. Thus, there was pressure to examine a large number of horses from multiple races on days when the study was being conducted, but winners and other selected horses were not allowed to undergo tracheobronchoscopy until after sampling was completed for official drug testing. The minimum elapsed time from race start until tracheobronchoscopy was performed in any of the race winners (n = 105) was 19 min, whereas 45% (402/895) of the nonwinners had been examined between 7–19 min after post time. Considering the short time after racing when examinations were initiated, it is logical that horses assigned EIPH severity grade of 0 tended to be examined later because winning was associated with lower EIPH severity grades, and horses that performed worse were more likely to be available for examination in this early post racing period.

Despite this association between EIPH severity and the elapsed time prior to examination, it was not possible to include this variable in models that examined the association between EIPH severity and race performance outcomes. Elapsed time was identified as an intervening variable instead of being a true confounding variable, and it is inappropriate to include intervening variables (variables that lie between the exposure of interest and the outcome in a causal pathway) in multivariable models [12]. Instead, we accounted for the impact of these differences in elapsed time by dichotomising EIPH severity for analysis as *grade 0* vs. *grade ≥1* instead of as *grade ≤1* vs. ≥ *2* as was used in previous research [2,15].

It is unfortunate that approximately 10% of recorded tracheobronchoscopic examinations were deemed to be nondiagnostic by the expert review panel. However, the only difference detected in baseline characteristics between horses judged to have diagnostic vs. nondiagnostic tracheobronchoscopy examinations was related to finishing in first position; there were no differences between horses with diagnostic vs. nondiagnostic examination in other finishing positions (e.g. second, third, ≥ fourth), or for other baseline characteristics that were compared (age, sex, merit rating, money earned in study race, lengths finished behind the winner, lifetime races, lifetime earnings, lifetime wins etc.). Results of sensitivity analyses suggested that when using the most conservative interpretation possible (i.e. assuming that all nondiagnostic examinations were severity *grade 0*) we still found that EIPH was strongly associated with a decreased likelihood of winning races, but not with other performance outcomes. However, the likelihood of classifying examinations as nondiagnostic was not associated with whether horses won races, were in the top 3 finishing positions, had race earnings ≥90th percentile, or the elapsed time from race start until examination. In view of this lack of association with factors that were associated with EIPH occurrence, it is not apparent that classification of examinations as nondiagnostic was associated with the occurrence of EIPH. As such, we believe it is most reasonable that these nondiagnostic examinations were excluded from analyses as we have done.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

Protocols used in this investigation were approved by the University of Pretoria Institutional Animal Care and Use Committee prior to initiation. Owners of animals or the owners' agents provided explicit informed consent for inclusion in the study.

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Authorship

A.J. Guthrie led the design and execution of this study in consultation with K.W. Hinchcliff. M.N. Saulez performed all endoscopic examinations. A.J. Guthrie, P.S. Morley, and K.W. Hinchcliff conducted all grading of EIPH scores. P.S. Morley and J.L. Bromberek conducted the data analysis and led in data interpretation and preparation of the manuscript. All authors provided interpretation of findings and approved the final version.

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