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Chapter 2 Altitude affects the prevalence and severity of exercise-induced pulmonary haemorrhage in South African Thoroughbred racehorses

2.1 ABSTRACT

We sought to determine the prevalence and severity of exercise-induced pulmonary haemorrhage (EIPH) in South African Thoroughbred racehorses in a racing jurisdiction that did not allow furosemide and nasal dilator strips, racing at different altitudes and to determine the relationship between EIPH and racing performance. The study was an observational cross-sectional study conducted in 1,005 Thoroughbred racehorses competing in Gauteng, The Free State, Kwazulu-Natal and Western Cape Province, South Africa.

Tracheobronchoscopic examination was performed once on pre-enrolled racehorses < 2 hours after racing. Following recording of the examinations onto digital video disc, the presence and severity of EIPH (grade 0 to 4) was determined by a single observer blinded to the identity and performance of the racehorses. Race records were extracted from a race cards for all horses examined.

Overall, 37% of eligible racehorses aged of 4.03 ± 1.11 years (mean ± standard deviation [SD]) were examined for EIPH 24.06 ± 12.36 minutes over a race distance of 1,486 ± 415 meters at high altitude (> 1,400 meters above sea level) and 1,419 ± 345 meters at sea level. The severity of EIPH for 408 horses examined at high altitude and 597 horses examined at sea level was: 51.17 *vs*. 42.04% (grade 0), 29.41 *vs*. 31.16% (grade 1), 10.78 *vs*. 11.89% (grade 2), 6.37 *vs*. 10.22% (grade 3) and 1.72 *vs*. 4.69% (grade 4) respectively. Racehorses competing at sea level were 1.56 times more likely to have EIPH severity grade ≥ 1 and 1.68 times more likely to have an EIPH severity grade ≥ 2 than horses racing at high altitude. Horses with EIPH severity grade ≥ 1 were 1.75 times more likely to win while horses with EIPH severity grade ≥ 2 were 2.01 times more likely to win. EIPH did not appear to have any significant effect on finishing in the first 3 positions nor on the distance finished behind the winning horse.

In South Africa, racing at sea level is associated with a greater prevalence of EIPH which is more severe. Moreover results suggest that EIPH is associated with superior performance in Thoroughbred racehorses competing in a racing jurisdiction that neither allows furosemide nor nasal dilator strips.

2.2   INTRODUCTION

EIPH is a worldwide phenomenon in horses undergoing intense exercise. In Thoroughbred racehorses, the prevalence of EIPH using tracheobronchoscopy has been reported in Australia (55.3%),[11] Hong Kong (62.5%),[17] and North America (42 to 75.4%),[20,24] while reports of EIPH-related epistaxis exist in Australia (0.8%),[4] England (0.5 to 2%),[5] Korea (0.84%),[14] Japan (0.15%),[26] and South Africa (0.16 to 2.41%).[22,23,29] Numerous risk factors such as age,[5,20,26] sex,[20,26] type of race, racing distance,[24,26] racing speed[24] and environmental factors[22,23] are reported to effect the prevalence of EIPH, however objective data on the affect of altitude has not been reported.

Strenuously exercised Thoroughbred racehorses commonly develop exercise-induced arterial-hypoxaemia (EIAH)[1,27] and this may impair athletic performance.[6,13,28] Furthermore, these horses also develop pulmonary arterial hypertension[16] which leads to higher transmural pulmonary capillary pressures and stress failure of the pulmonary capillaries,[2] resulting in EIPH. The relative hypoxaemia experienced by a racehorse may be exacerbated by high altitude as oxygen partial pressure decreases proportionally to increases in barometric pressure. Hypoxic vasoconstriction further increases pulmonary arterial pressure[30] and may directly cause or exacerbate EIPH. Although reports exist of Thoroughbred racehorses competing at sea level experiencing EIPH as detected by the presence of epistaxis in South Africa[22,23,29] or tracheobronchoscopy in the mid-Atlantic states,[24] the effect of racing at different altitudes on the prevalence and severity of EIPH as detected by tracheobronchoscopy is unknown.

The purpose of this study was to determine the prevalence and severity of EIPH and the relationship with racing performance in Thoroughbred racehorses competing in South Africa, a racing jurisdiction that does not allow the use of furosemide and nasal dilator strips, and in which racing is conducted at both sea level and high altitude.

2.3   MATERIALS AND METHODS

2.3.1   *Thoroughbred racehorses*

The study was a prospective cross-sectional study of a sample of racehorses competing at 5 racetracks in South Africa in 2005. Informed consent was obtained from the owners and trainers of each participating racehorse 24 to 72 hours before race day. Using tracheobronchoscopy, the prevalence and severity of EIPH was determined within 2 hours after racing. Each racehorse was endoscopically examined once. Data on each racehorse's performance on race day as well as previous race performance was then collected and analyzed to determine the association between race performance and EIPH.

Thoroughbred racehorses of either sex competing in flat racing at Turffontein Race Course (Gauteng Province), Vaal Race Course (Free State Province), Clairwood and Greyville Turf Club (Kwazulu-Natal Province) and Kenilworth Race Course (Cape Town Province), South Africa, were enrolled in this study from August 4 to November 19, 2005. These five racecourses are considered representative of the best racing in South Africa. Of these 5 racecourses, Turffontein and Vaal Race Course are located at high

altitude (1,713 and 1,438 meters above sea level respectively) while the other three (Clairwood and Greyville Turf Club, and Kenilworth Race Course) are at sea level (elevation less than 100 meters). Apart from the Vaal Race course where races were held on turf and sand, all other races were held on turf and occurred between 12h00 and 21h00. Race day administration of medications such as furosemide is not allowed in South Africa and drug testing is strictly enforced by the National Horse Racing Authority (NHRA) through screening of urine and blood for prohibited and therapeutic substances, therefore it is highly unlikely that either racing performance or severity of EIPH could have been affected by therapeutic substances.

2.3.2 *Dissemination of project information*

Prior to commencement of the study, project information was disseminated among registered trainers, owners and local veterinarians in all the provinces using facsimile transmission, articles published in newsletters and local newspapers, a live national broadcast, live interviews at racetracks and private venues, and personal communication with all registered trainers. Lists of available horses that were accepted to race were obtained from the NHRA. Eligible racehorses were then identified, trainers contacted individually and permission obtained to examine the horse. Not all trainers allowed their racehorses to participate in this study. Only pre-enrolled racehorses (that is 24 to 72 hours prior to race day) were entered into the study to prevent a post race selection bias.

2.3.3   Tracheobronchoscopic examination

Following racing, handlers lead the racehorses to a parade ring where each pre-enrolled racehorse was visually identified and tagged by the study personnel. Thereafter, tracheobronchoscopy was performed on racehorses restrained in a dedicated examination stall within 2 hours after racing. Any racehorse that was refractory to restraint that could comprise the safety of personnel or equipment, and any horse examined after the race that was not pre-enrolled prior to the race, was excluded from this study.

Tracheobronchoscopy was performed on all pre-enrolled, unsedated racehorses for the presence and severity of EIPH within 2 hours of racing. Following restraint with a halter and nose twitch in a dedicated examination stall, an endoscope (Pentax Corporation, Tokyo, Japan: model number EC3830FK, 1.5 m in length, 38 French in diameter, processor number EPK700) was passed through the nostril, along the ventral meatus caudally to the nasopharynx, larynx, and trachea up to the level of the carina. All examinations were recorded onto digital video disc. Thereafter, the presence and severity of EIPH was evaluated by a single observer who was blinded to the identity and race day performance of the racehorses assessed.

Racehorses were graded 0 to 4 for EIPH (Table 2.1).[9] Briefly, grade 0 indicated the absence of blood in the pharynx, larynx, trachea, or mainstem bronchi; grade 1 indicated the presence of 1 or more flecks of blood or ≤ 2 short ($< \frac{1}{4}$ length of the trachea), narrow ($< 10\%$ of the tracheal surface area) streams of blood in the trachea or mainstem bronchi

(Figure 2.1); grade 2 indicated long stream of blood (> $^1/_2$ length of the trachea) or > 2 short streams covering < $^1/_3$ of the tracheal circumference (Figure 2.2); grade 3 indicated multiple, distinct streams of blood covering > $^1/_3$ of the tracheal circumference without blood pooling at the thoracic inlet (Figure 2.3); and grade 4 indicated multiple, coalescing streams of blood covering > 90% of the tracheal surface with blood pooling at the thoracic inlet (Figure 2.4).

2.3.4 *Data analysis*

The racehorses' age and sex as well as racing career performance record immediately preceding the endoscopic examination on race day was extracted from race cards and included lifetime starts, lifetime wins, lifetime places (2nd, 3rd, or 4th) and lifetime stake earnings (South African Rand, ZAR). Further variables recorded on the day of endoscopic evaluation included horse name, microchip number, date of race, race venue, racing surface (turf or sand), trainer, jockey, locality (altitude or sea level), race reference number, number of starters, race time (day or night), race start time, race purse, time of endoscopic evaluation, elapsed time between race completion and endoscopic evaluation, weight carried, merit rating, draw number, starting bet, finishing position, horse length behind the winner, winner's margin, finishing time of winner, finishing time of horse, race distance, track condition, falserail position and whether a horse was suspended due to epistaxis. Meteorological data were extracted from a commercial database (Pretoria Central Forecasting Office, South African Weather Service) and included penetrometer reading (an objective measure of track surface), ambient temperature, humidity,

barometer, wind direction, wind speed, cumulative rainfall over the past 7 days, cumulative rainfall for the last 24 hours.

Data was analyzed using Microsoft Excel (version 2003) and the statistical package NCSS (Hintze J. NCSS, PASS and GESS number cruncher statistical systems, Kaysville, Utah, 2004). Descriptive statistical analyses were initially carried out to summarize trends in the data. The relationship between EIPH and performance was evaluated by means of logistic regression. The presence of EIPH was defined as a dichotomous variable (yes vs. no) in 2 ways: severity grade 0 (no) vs. severity grade $\geq$ 1 (yes) and severity grade $\leq$ 1 (no) vs. severity grade $\geq$ 2 (yes).

Included in the regression model were variables that may have affected the racing performance or which may have acted as confounders. The variables were similar to those included by a previous study.[11] Numeric independent variables included were race purse, elapsed time between race completion and endoscopic evaluation, weight carried, number of starters, race distance, penetrometer reading, age, lifetime starts.

Dichotomized categorical independent variables included locality (sea level/altitude), sex (male/female) and EIPH (yes/no). The response variable for measuring performance was if a horse won (yes/no), or placed (1st, 2nd or 3rd) (yes/no) on the day of endoscopic examination. The Wald test was used to test the significance of the regression coefficient (β) and an estimated odds ratio (OR) with 95% confidence interval (CI) was calculated for each associated regression coefficient.

Multiple regression analysis was performed to assess the relationship between EIPH and the distance behind the winner. The same independent variables as used in the logistic regression were included in the model. However because the distance behind the winner was not normally distributed, logarithmic transformation was performed prior to analysis. Least squares regression coefficient estimates were used to evaluate the relationship between the independent variables and distance behind the winner and a t-test was carried out to determine the significance of the coefficients. For all analyses, significance was set at $P < 0.05$. A total of 921 horses were included in the regression analyses as 84 horses had missing penetrometer readings and were excluded.

Because of the possibility of interactions and collinearity, the data was examined for pairwise correlations as a first step to determine if principle component analysis was necessary. In addition variance inflation and Eigenvalues of centered correlations were also generated during the multiple regression to test for multicollinearity.

To see if there was any relationship between race day EIPH status and performance history, racehorses and EIPH (yes vs. no) were compared for lifetime starts, lifetime wins, lifetime places (2nd, 3rd or 4th place) and lifetime earnings using the Mann-Whitney U or Wilcoxon Rank-Sum Test for the difference in medians.

2.4 RESULTS

One thousand and five (37.4%) out of 2,684 eligible racehorses, competing in 230 flat races at 28 race meetings were evaluated using tracheobronchoscopy. Endoscopic examinations took place mean ± standard deviation (SD) 24.06 ± 12.36 minutes after racing. Ninety seven trainers participated in this study with each having contributed a median 7 horses (range 1 to 46 horses). There were mean (± SD) 11.56 ± 2.65 racehorses in each race. Race distance ranged from 800 to 3,200 m with a mean (±SD) of 1,446 ± 376 meters. Mean (± SD) race distance was greater at high altitude (> 1,400 meters above sea level) than at sea level (1,486 ± 415 meters *vs*. 1,419 ± 345 meters, $P = 0.03$).

Racehorses examined included 509 females, 491 geldings, and 5 intact males with a mean (± SD) age of 4.03 ± 1.11 years. Racing performance of the 1,005 horses that were evaluated included 105 wins (10.45%, 95% confidence interval [CI]: 8.35 to 12.45%) and 303 placed positions (30.15%, 95% CI: 26.75 to 33.54%). Mean (± SD) age of unaffected horses (grade 0 EIPH) was not significantly different to affected horses (3.88 ± 1.12 years *vs*. 4.08 ± 1.11 years, $P = 0.18$).

Overall, blood was detected in the airway of 543/1005 (54.03%, 95% CI: 49.49 to 58.57%) racehorses with grade 1 EIPH detected in 306/1005 (30.45%) horses, grade 2 EIPH detected in 115/1005 (11.44%) horses, grade 3 EIPH detected in 87/1005 (8.66%) horses and grade 4 EIPH detected in 35/1005 (3.48%) horses (Figure 2.5). Of the 1005 horses that were evaluated, 408 horses were examined at high altitude and 597 horses

were examined at sea level. At high altitude, 197/408 (48.28%) horses were affected, with grade 1 EIPH detected in 120/408 (29.41%) horses, grade 2 EIPH detected in 44/408 (10.78%) horses, grade 3 EIPH detected in 26/408 (6.37%) horses and grade 4 EIPH detected in 7/408 (1.72%) horses (Figure 2.6). At sea level, 346/597 (57.96%) horses were affected, with grade 1 EIPH detected in 186/597 (31.16%) horses, grade 2 EIPH detected in 71/597 (11.89%) horses, grade 3 EIPH detected in 61/597 (10.22%) horses and grade 4 EIPH detected in 28/597 (4.69%) horses (Figure 2). Racing at sea level increased the odds of having EIPH severity grade ≥ 1 by 1.56 (95% CI: 1.18 to 2.07, $P <$ 0.01) and EIPH severity grade ≥ 2 by 1.68 (95% CI: 1.20 to 2.35, $P < 0.01$).

Epistaxis as defined by the presence of blood at one or both nostrils was identified overall in 8 racehorses (0.8%), six horses had grade 4 EIPH, one horse had grade 3 EIPH, while one horse had no evidence of blood within the trachea. Only two of the 8 horses with epistaxis were identified by the stipendiary stewards immediately following racing resulting in a mandatory 3 month racing suspension each. Of the 8 horses with epistaxis, four horses each were identified at high altitude and at sea level. Blood was also detected in the larynx of 85/1005 horses (8.46%, 95% CI: 6.66 to 10.26%).

The results of the tests for multicollinearity showed no strong evidence for pair wise correlations between variables (Table 2.2, 2.3 and 2.3). Pearson correlations are given for all variables in the Table 2.2. Outliers, nonnormality, nonconstant variance, and nonlinearities can all impact these correlations. These correlation coefficients show which independent variables are highly correlated with the dependent variable and with

each other. Independent variables that are highly correlated with one another may cause multicollinearity problems. Table 2.2 showed that with the exception of lifetime runs, there was very little correlation between variables and thus little evidence of multicollinearity using this test.

The variance inflation factor (VIF) is a measure of multicollinearity and given in Table 2.3. It is the reciprocal of $1-Rx^2$, where Rx^2 is the R^2 obtained when this variable is regressed on the remaining independent variables. A VIF of 10 or more for large data sets indicates a multicollinearity problem since the Rx^2 with the remaining X's is 90 percent. For small data sets, even VIFs of 5 or more can signify multicollinearity. In this study, there was a large data set allowing a less conservative appraisal of multicollinearity, even so VIFs were well below 5, meaning that this test also showed there is little evidence of multicollinearity in this data set. The fact that this study had a larger dataset than a previous study,[11] adds further strength to this study. Rx^2 is the R^2 obtained when this variable is regressed on the remaining independent variables. A high Rx^2 indicates a lot of overlap in explaining the variation among the remaining independent variables. This was not the case with this data set.

The sums of the Eigenvalues are equal to the number of independent variables and are given in Table 2.4. Eigenvalues near zero indicate a multicollinearity problem in the data. Few of the Eigenvalues in the data set were close to zero. They were however not very high and it may be interpreted that there is a small amount of collinearity in the study. But this should be expected in a study of this nature and together with the correlation results and VIF results don't present sufficient evidence to warrant removal of variables

from the model. Incremental percent is the percent this Eigenvalue is of the total. In an ideal situation, these percentages would be equal. Percents near zero indicate a multicollinearity problem in data. There are no percentages near zero and there is a fairly even distribution of incremental percentages again giving little evidence for collinearity in this data set. The condition number is the largest Eigenvalue divided by each corresponding Eigenvalue. Since the Eigenvalues are really variances, the condition number is a ratio of variances. Condition numbers greater than 1,000 indicate a severe multicollinearity problem while condition numbers between 100 and 1,000 indicate a mild multicollinearity problem. All values in this study were well under 100 and thus support the other evidence that multicollinearity is playing only a minor role, if any in this set of data

Results of the logistic regression analyses as outlined previously[7,8,12] for both EIPH severity grade 0 (no) *vs.* severity grade ≥ 1 (yes) and severity grade ≤ 1 (no) vs. severity grade ≥ 2 (yes) showed significant positive regressions for horses with EIPH ($\beta = 0.56$, *P* = 0.02 and $\beta = 0.7$, *P* < 0.01), lifetime starts ($\beta = 0.04$, *P* = 0.03 and $\beta = 0.04$, *P* = 0.04) and elapsed time between race completion and endoscopic evaluation ($\beta = 0.03$, *P* < 0.01 and $\beta = 0.03$, *P* < 0.01) and a negative regression for number of starters ($\beta = -0.15$, *P* < 0.01 and $\beta = -0.15$, *P* < 0.01). Only the regression coefficients for EIPH were of any practical importance, the others having odds ratios close to 1.

Horses with EIPH severity grade ≥ 1 were 1.75 times more likely to win (95% CI: 1.10 to 2.79) while horses with EIPH severity grade ≥ 2 were 2.01 times more likely to win (95%

CI: 1.27 to 3.21). EIPH could not be shown to have any significant effect on whether a horse finished in the first 3 places for EIPH severity grade ≥ 1 nor ≥ 2 ($P = 0.35$ and $P = 0.7$ respectively) (Figure 2.7). Similarly, EIPH could not be shown to have any significant linear relationship to the distance finished behind the winner for EIPH severity grade ≥ 1 nor ≥ 2 ($\beta = 0.05$, $P = 0.27$ and $\beta = 0.02$, $P = 0.73$ respectively).

Horses with an EIPH severity grade ≥ 1 had no significant difference in median lifetime wins ($P = 0.09$) but horses that had an EIPH severity grade ≥ 2 had more median lifetime wins than horses with EIPH scores < 2 ($P = 0.03$). Both horses with EIPH severity grades of ≥ 1 and ≥ 2 had more median lifetime places (3 *vs*. 2) compared to horses with grade 0 EIPH ($P = 0.02$ and $P < 0.01$ respectively). Horses with an EIPH severity grade ≥ 1 had no significant difference in number of lifetime starts between affected and non-affected EIPH horses ($P = 0.06$) but horses with EIPH severity grade ≥ 2 did have significantly more median lifetime starts (11 *vs*. 7, $P < 0.01$). No significant difference in median lifetime earnings could be shown between EIPH severity grade ≥ 1 and horses with grade 0 EIPH ($P = 0.06$), however horses with EIPH severity grade ≥ 2 had greater median lifetime earnings than horses without EIPH (ZAR 45,380 *vs*. 33,585; $P < 0.01$ respectively).

2.5   DISCUSSION

Similar to previous studies conducted abroad, this study found using tracheobronchoscopy a high overall prevalence of EIPH in Thoroughbred racehorses competing in South Africa.[11,17,20,24] Moreover, a positive association was found between racing at sea level and the presence of EIPH severity grades ≥ 1 and ≥ 2. No relationship between the severity of EIPH and finishing in the first 3 positions nor distance behind the winner was found. In fact, a positive association between the presence of EIPH of severity grade ≥ 1 as well as ≥ 2 and higher odds of winning was identified. This study therefore concludes that in Thoroughbred racehorses competing in South Africa not medicated with furosemide nor using nasal dilator strips, the presence of EIPH is associated with superior performance and that racing at sea level is associated with an increased prevalence and severity of EIPH.

Historically, surveys of horse populations determining the prevalence and relationship with performance of EIPH have relied on the presence of epistaxis[4,5,14,22,23,26,29] or tracheobronchoscopically detected blood.[11,17,20,24] However, a diagnosis of EIPH based only on the presence or absence of epistaxis should be actively discouraged as its use as sole criteria for estimating the prevalence of EIPH is inaccurate. Epistaxis is an insensitive indicator of EIPH, occurring in only the most severely affected horse and may also be non-specific for pulmonary haemorrhage. As can be seen by the present study, although blood was detected in 54% of racehorses using tracheobronchoscopy, epistaxis was only present in 8/1005 (0.8%) of racehorses. Of these eight horses, 7 were affected

by more severe grades of EIPH (6 horses had grade 4 EIPH and 1 horse had grade 3 EIPH), while one horse had no evidence of blood in the trachea despite having profuse epistaxis. Studies have used finishing first or in the first three positions as indicators of impaired racing performance and have found that epistaxis negatively impacts racing performance.[17,26] However, these studies may have underestimated the true prevalence of EIPH by only reporting on the most severely affected horses, while those horses with less severe grades of EIPH were not included and so information regarding the relationship between less severe grades of EIPH and racing performance could not be made.[17,26]

In this study, we used tracheobronchoscopy to detect and quantify the presence and severity of EIPH. Therefore, in order to detect a horse affected with EIPH, blood needed to be present within the trachea and major bronchi at the time of tracheobronchoscopic evaluation. As has been eluded by another study, it is not certain whether horses that suffer minimal hemorrhage in the peripheral lung parenchyma may actually show blood within the airways.[11] Moreover, as the movement of blood from the lung into the trachea may be time-dependant, and since this study endoscopically evaluated racehorses soon after racing, this study may have failed to identify such horses with the least severe grade of EIPH.

The relationship between altitude and the prevalence of EIPH-related epistaxis has been previously reported.[22,23,29] These studies have demonstrated a greater prevalence of EIPH-related epistaxis at sea level than at high altitude, however due to the methodology used, failed to report on less severe forms of EIPH.[22,23,29] Interestingly, should the present

study have also only used EIPH-related epistaxis as sole criteria for the detection of EIPH as previous studies have done,[22,23,29] the prevalence of EIPH at sea level *versus* high altitude would not have been dissimilar. The author is unaware of any previous studies reporting on an association between altitude and EIPH as detected by tracheobronchoscopy. Plausible reasons do exist as to why there may be a greater prevalence and increased severity of EIPH at high altitude. Strenuously exercised racehorses often have EIAH[1,27] and develop pulmonary arterial hypertension[16] leading to pulmonary capillary stress failure.[2] The degree of EIAH may be worsened by high altitude-induced hypoxic vasoconstriction[30] which may either directly cause EIPH or worsen pre-existing EIPH. Further research is clearly needed to establish why the prevalence and severity of EIPH is greater at sea level.

The relationship between racing performance and EIPH has been previously reported. Often poor racing performance is attributed to the presence of EIPH, however rarely does EIPH cause death of Thoroughbred racehorses.[10] Using tracheobronchoscopy to detect EIPH after racing, no relationship was seen between the presence of EIPH and finishing in the first, second, or third position in 191 Thoroughbred racehorses nor in 98 Thoroughbred racehorses that were placed in the first 3 or lower positions in California (USA);[21] and in 191Thoroughbred racehorses that finished in the first three positions or lower in Pennsylvania (USA).[24] Similar findings were reported in a study conducted on 258 Thoroughbred and 296 Standardbred racehorses again in Pennsylvania.[3] However, after tracheobronchoscopic examinations were performed on 452 racehorses in Hong Kong, the study concluded that horses that finished first, second or third had less severe

grades of EIPH.[17] That study reported that the prevalence of EIPH was 43.9% in horses that finished in the first three positions while in the horses that finished in the 4th to 14th position, the prevalence was 55.9%.[17] A more recent study conducted in Melbourne (Australia) has demonstrated the presence and severity of EIPH is associated with impaired racing performance.[11] Horses with an EIPH severity grade ≤ 1 were 4.03 times more likely to win and 1.78 times more likely to finish in the first three positions than were horses with an EIPH severity grade ≥ 2, and that horses with an EIPH severity grade ≥ 1 finished further behind the winner than unaffected horses.[11]

Unlike the present study and the Australian study[11] where the administration of furosemide was prohibited by the local racing jurisdiction, previous reports that assessed the relationship of racing performance and EIPH and found no association, may have been influenced by the use of furosemide.[3,21,24] Furosemide may attenuate pulmonary capillary pressure, thereby decreasing pulmonary capillary stress failure causing a reduction in the severity grade of EIPH.[15] Moreover, since the administration of furosemide is associated with superior performance,[9] it may have masked the effect of EIPH on racing performance. Therefore, since racehorses that were treated with furosemide may have had EIPH which was not detected, thereby decreasing sensitivity, the use of furosemide may have masked a positive association between EIPH and superior performance. However, although furosemide may reduce red blood cells collected by bronchoalveolar lavage in horses strenuously exercised on a treadmill,[18] there is no current evidence that the use of furosemide reduces the prevalence and

severity of EIPH in a natural population of Thoroughbred racehorses under field conditions.

Both the present study and the Australian study[11] used a large number of pre-selected racehorses that were only endoscopically examined once. Plausible reasons exist why previous studies may not have identified an association between EIPH and racing performance. The pre-selection of horses prior to racing is important as horses only selected after racing may show a post-race selection bias by selecting and evaluating only horses that have performed poorly. Low statistical power due to small study populations may have affected previous studies and prevented demonstration of an association between EIPH and racing performance. Also, multiple examinations of the same horses were not performed in the present study as this may have confounded statistical outcome.

When conducting studies with large data sets, multicollinearity may arise from two sources. Sample-based multicollinearity arises from the inclusion of correlated predictor variables, which is the most common source of multicollinearity in epidemiological studies.[25] In order to assess this, a thorough knowledge of the biological aspects of the study is required and statistical evaluation for this type of multicollinearity needs to be biologically plausible as well as statistically correct. The second source is structural multicollinearity, which arises from the creation of correlated variables by adding power terms (example: quadratic terms) or interaction terms to the regression model. Since this has not been the case in this study, structural multicollinearity is less likely, it can however be dealt with by centering the variables of interest. Identifying multicollinearity

is often more of an art than a science and hence several tests have been put forward to try and identify it. None of them on their own are fool proof and one has to take a holistic view of the study when interpreting the results, including biological plausibility.

Similar to another study, the present study could not demonstrate an association with impaired performance when the presence of EIPH was defined as horses with a severity grade ≥ 1.[11] In that study, an association with impaired performance was only seen once the presence of EIPH was defined as horses with a severity grade ≥ 2.[11] It may seem plausible that horses with a small volume of blood (that is grade 1 EIPH) may not suffer impaired racing performance. However since greater volumes of intrapulmonary installed blood (200 ml) is associated with impaired gaseous exchange in horses exercised on a treadmill,[19] horses with an EIPH severity grade ≥ 2 may show impaired racing performance as was suggested by the Australian study.[11] Although the present study was conducted in a similar way to the Australian study,[11] results suggest that horses with EIPH are more likely to win. It is important to realize that the association with performance of EIPH may be affected by many variables which include geographic differences, climatic conditions, race track surfaces, respiratory diseases, grading techniques and genetic factors. These factors may have accounted for differences reported between studies. Also, this highlights that the effect of EIPH on racing performance is still not clear and may be a reflection of our lack of current knowledge of the pathogenesis of EIPH as well as specific methods by which racing performance is measured.

2.6   CONCLUSIONS

In the study reported here, we have demonstrated in racehorses competing in South Africa not medicated with furosemide nor using nasal dilator strips, the prevalence and severity of EIPH is affected by altitude. Racing at sea level is associated with a higher prevalence and greater severity of EIPH. Moreover results suggest that EIPH is associated with superior performance in South African Thoroughbred racehorses.
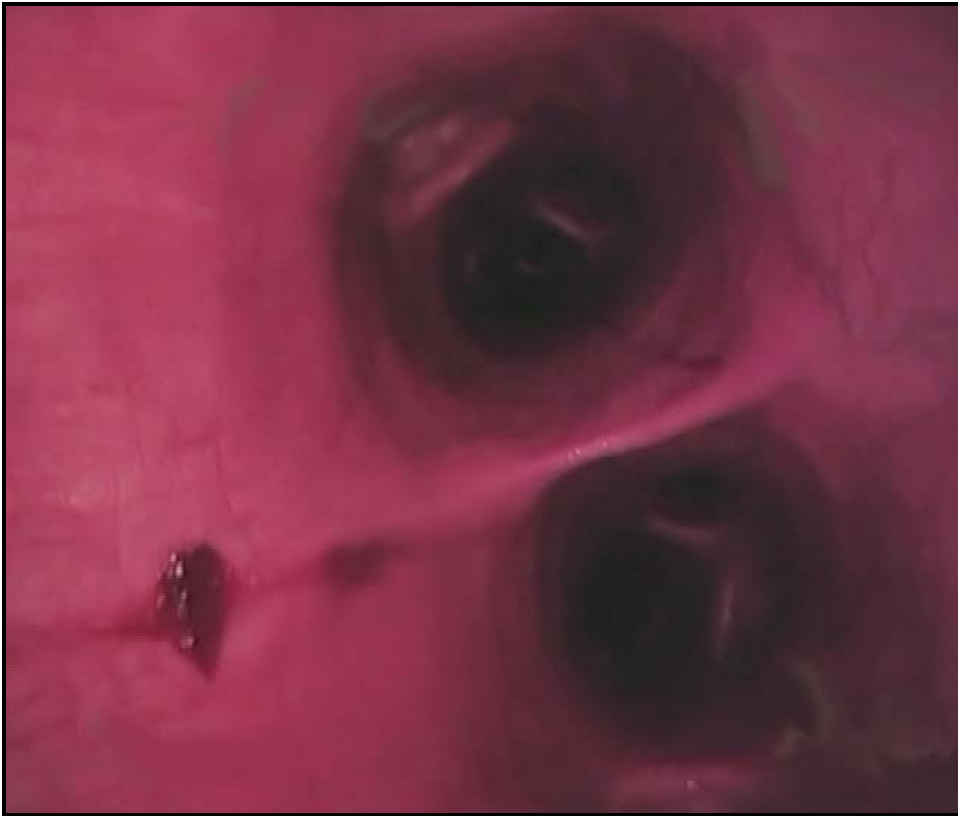
2.7   FIGURES AND TABLES

Figure 2.1   A racehorse with grade 1 exercise-induced pulmonary haemorrhage as detected by tracheobronchoscopy.
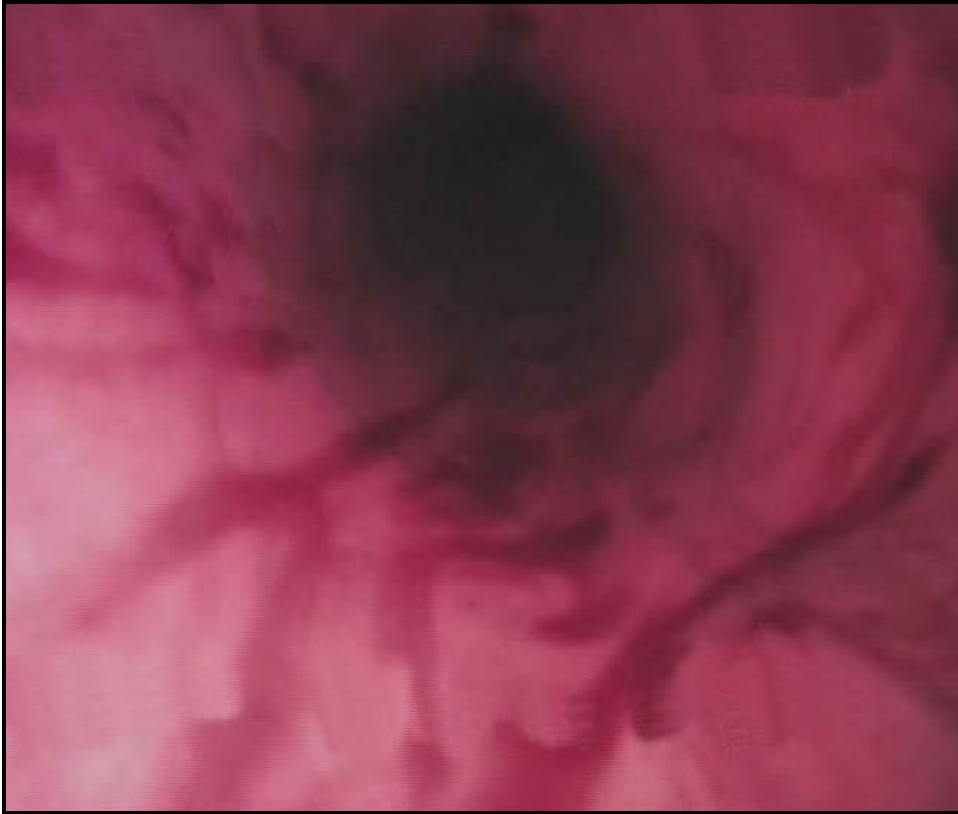
Figure 2.2 A racehorse with grade 2 exercise-induced pulmonary haemorrhage as detected by tracheobronchoscopy.
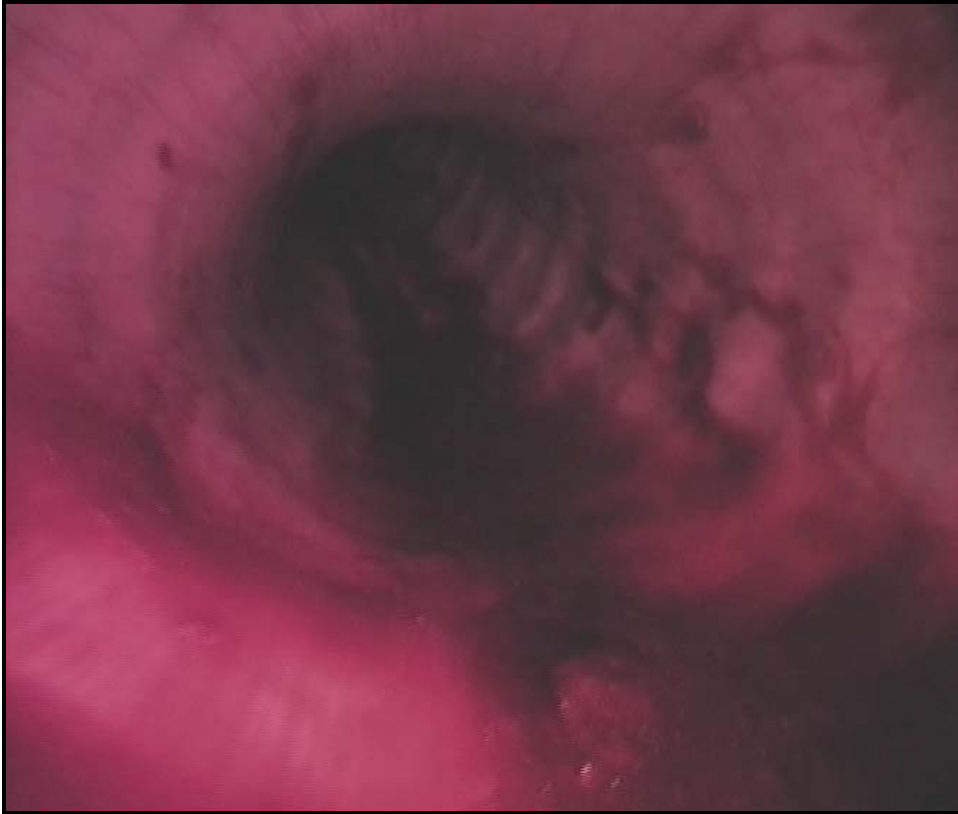
Figure 2.3   A racehorse with grade 3 exercise-induced pulmonary haemorrhage as detected by tracheobronchoscopy.
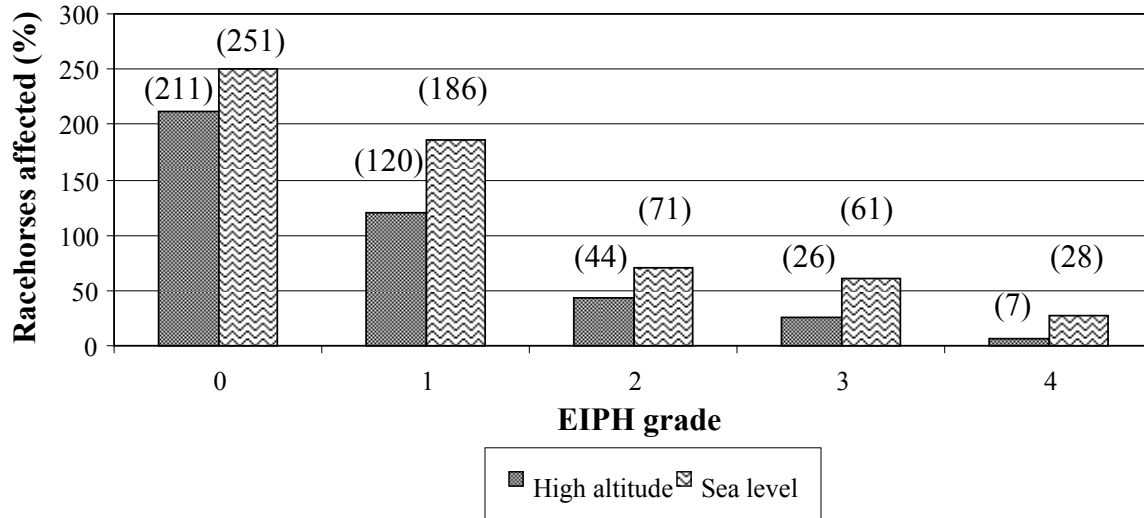
Figure 2.4   A racehorse with grade 4 exercise-induced pulmonary haemorrhage as detected by tracheobronchoscopy.

Figure 2.5   Exercise-induced pulmonary hemorrhage (EIPH) in South African Thoroughbred racehorses (n=1005) examined from August 4 to November 19, 2005 post race: overall tracheobronchoscopic assessment of the severity of EIPH using a 0 to 4 EIPH grade scale.
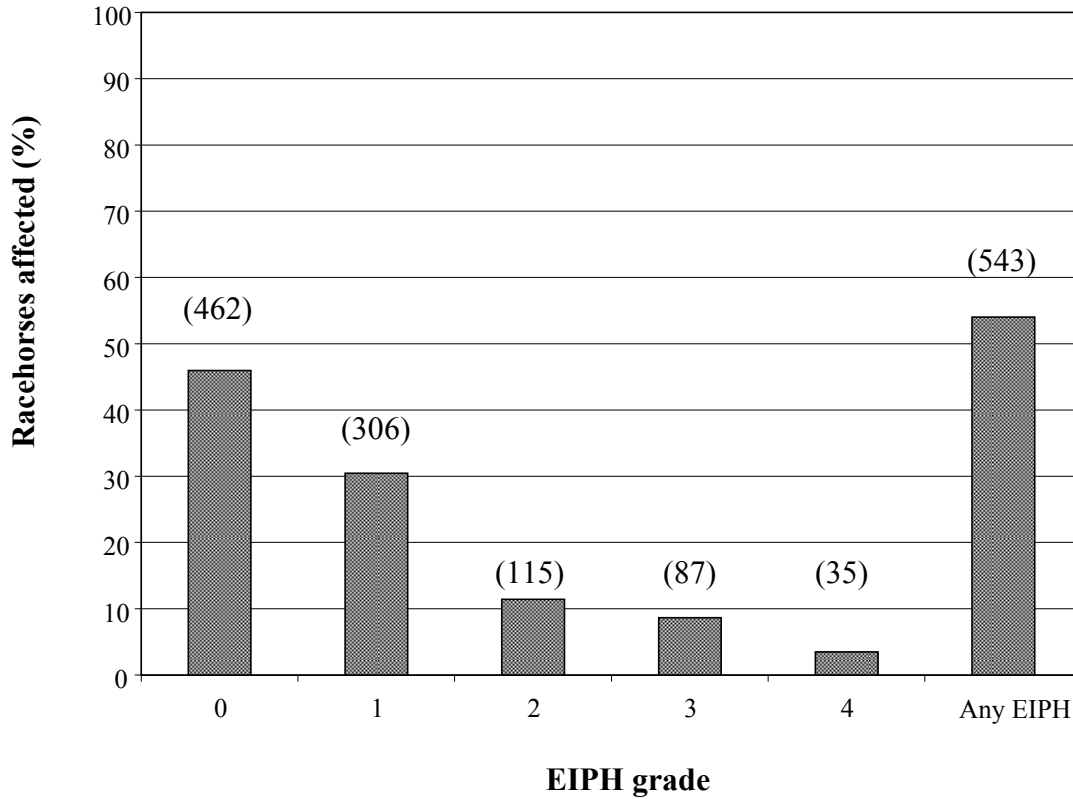
Figure 2.6   Exercise-induced pulmonary hemorrhage (EIPH) in South African Thoroughbred racehorses (n=1005) examined from August 4 to November 19, 2005 post race: tracheobronchoscopic assessment of the severity of EIPH using a 0 to 4 EIPH grade scale at high altitude (1,450 meters above sea level) and at sea level.
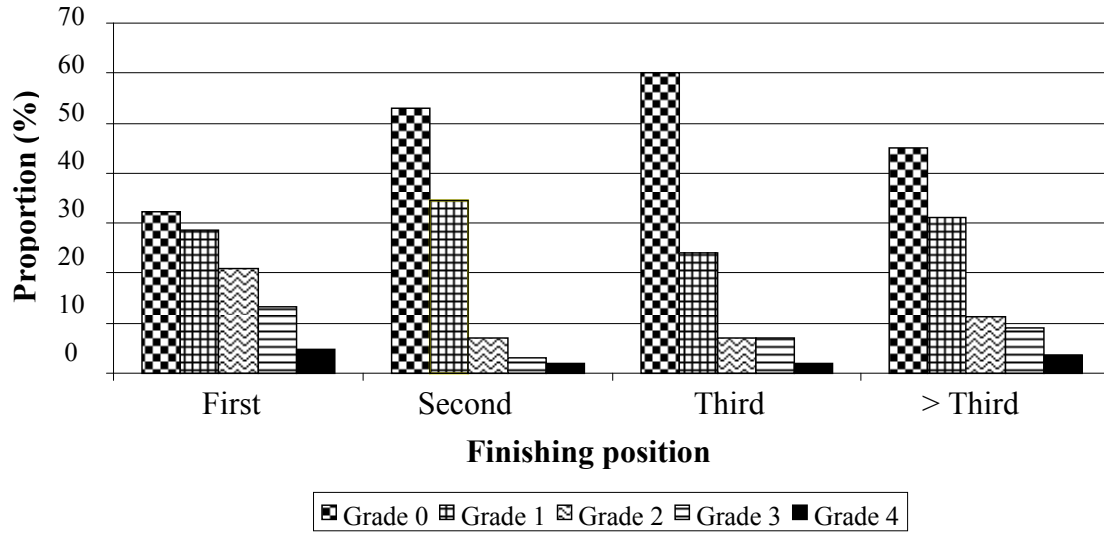
Figure 2.7   Exercise-induced pulmonary hemorrhage (EIPH) in South African Thoroughbred racehorses (n=1005) examined from August 4 to November 19, 2005 post race: finishing position as a function of severity of EIPH using a 0 to 4 EIPH grade scale.

Table 2.1   Tracheobronchoscopic scoring of horses with exercise-induced pulmonary haemorrhage.

EIPH Grade	Tracheobronchoscopic Findings
0	No blood present in pharynx, larynx, trachea, or main stem bronchi
1	Presence of one or more flecks of blood or $\leq$ 2 short [< ¼ the length of the trachea] narrow [<10% of the tracheal surface area] streams of blood in the trachea or main stem bronchi visible from the tracheal bifurcation
2	One long stream of blood (> $^1/_2$ length of the trachea) or > 2 short streams occupying less than $^1/_3$ of the tracheal circumference
3	Multiple, distinct streams of blood covering more than $^1/_3$ of the tracheal circumference without blood pooling at the thoracic inlet
4	Multiple, coalescing streams of blood covering > 90% of the tracheal surface with blood pooling at the thoracic inlet

Table 2.2   Correlation matrix: Pearson correlations for variables in the exercise-induced pulmonary haemorrhage study.

Independent Variable	Gross Stake for the Race	Time Difference	Weight carried	Number of Starters	Race distance	Penetrometer	Age	Lifetime Runs	Day Win
Gross stake for the race	1.00	0.12	0.02	0.14	-0.08	0.15	0.14	0.11	0.00
Time difference	0.12	1.00	0.00	0.18	-0.04	0.02	0.02	-0.03	0.11
Weight carried	0.02	0.00	1.00	0.02	-0.14	-0.01	-0.15	-0.23	0.01
Number of starters	0.14	0.18	0.02	1.00	-0.14	0.11	-0.06	-0.10	-0.08
Race distance	-0.08	-0.04	-0.14	-0.14	1.00	-0.03	0.26	0.26	-0.01
Penetrometer	0.15	0.02	-0.01	0.11	-0.03	1.00	-0.04	-0.01	-0.05
Age	0.14	0.02	-0.15	-0.06	0.26	-0.04	1.00	0.82	0.01
Lifetime runs	0.11	-0.03	-0.23	-0.10	0.26	-0.01	**0.82**	1.00	0.04
Day win	0.00	0.11	0.01	-0.08	-0.01	-0.05	0.01	0.04	1.00

Table 2.3   Least squares multicollinearity: variance inflation factors, Rx^2 and tolerance for variables in the exercise-induced pulmonary haemorrhage study.

Independent Variable	Variance Inflation Factor	R-Squared Vs Other X's	Tolerance
Gross stake for the race	1.0884	0.0812	0.9188
Time difference	1.0499	0.0475	0.9525
Weight carried	1.0722	0.0673	0.9327
Number of starters	1.0849	0.0782	0.9218
Race distance	1.1152	0.1033	0.8967
Penetrometer	1.0381	0.0367	0.9633
Age	3.1136	0.6788	0.3212
Lifetime runs	3.1772	0.6853	0.3147

Table 2.4   Eigenvalues of the correlation matrix for variables in the exercise-induced pulmonary haemorrhage study.

Independent Variable	Eigenvalue	Incremental Percent	Cumulative Percent	Condition Number
Gross stake for the race	2.077606	25.97	25.97	1
Time difference	1.409104	17.61	43.58	1.47
Weight carried	1.001929	12.52	56.11	2.07
Number of starters	0.964861	12.06	68.17	2.15
Race distance	0.85465	10.68	78.85	2.43
Penetrometer	0.787122	9.84	88.69	2.64
Age	0.729367	9.12	97.81	2.85
Lifetime runs	0.175362	2.19	100	11.85

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