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An endoscopic and immunopathological study of respiratory tract disorders in Thoroughbred racehorses

By

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Thesis Summary

AN ENDOSCOPIC AND IMMUNOPATHOLOGICAL STUDY OF RESPIRATORY TRACT DISORDERS IN THOROUGHBRED RACEHORSES

By

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Much of the impetus for this research can be attributed to Kenneth W. Hinchcliff, who has studied exercise-induced pulmonary haemorrhage (EIPH) extensively. This thesis focused on EIPH in Thoroughbred racehorses competing in South Africa. Using tracheobronchoscopy, the prevalence and severity of EIPH and the association with racing performance was determined. Thereafter, the prevalence of other respiratory tract disorders and their association with racing performance is reported. This is followed by a

study assessing interobserver variability using grading systems in the detection of respiratory tract disorders. Finally, there is a report on the immunopathogenesis of EIPH.

Using tracheobronchoscopy after racing, the prevalence and severity of EIPH was assessed in 1,005 racehorses competing at high altitude (> 1,400 meters above sea level) and at sea level in a racing jurisdiction that does not allow the use of furosemide and nasal dilator strips. The prevalence and severity of EIPH was affected by altitude as racing at sea level was associated with a higher prevalence and greater severity of EIPH. Results also suggested that EIPH was associated with superior performance in South African Thoroughbred racehorses.

Upper and lower respiratory tract disorders identified following tracheobronchoscopic examination included left arytenoid asymmetry, left laryngeal hemiplegia, epiglottic deformity, epiglottic entrapment, subepiglottic cysts, dorsal displacement of the soft palate, pharyngeal lymphoid hyperplasia (PLH), laryngeal and tracheal dirt, tracheal mucous (TM), tracheal stenosis and tracheal cartilage ring spikes in Thoroughbred racehorses after racing. Overall, there was a low prevalence of grade 2 and 3 arytenoid cartilage asymmetry, left laryngeal hemiplegia, epiglottic entrapment, subepiglottic cysts and epiglottic deformity, while more severe grades of PLH, laryngeal debris, tracheal debris, TM and tracheal cartilage ring spikes had a higher prevalence. An association with sex was identified as tracheal cartilage ring spikes occurred more often in male racehorses. Superior racing performance was identified in racehorses with grade 3 tracheal mucous and tracheal cartilage ring spikes.

Endoscopic grading of EIPH, PLH, arytenoid cartilage movement (ACM), and TM was performed by 3 observers that were blinded to each racehorse's identity and race day performance using previously established grading criteria. Excellent interobserver reliability was seen using the EIPH grading system, while the weighted kappa for PLH, ACM and TM was lower. The study demonstrated sufficient reliability for the use of the EIPH, PLH, ACM and TM grading systems in racehorses competing in South Africa. The study concluded that tracheobronchoscopy seemed to be a practical screening technique that may have prognosticative validity and clinical dependability and that would allow safe and quick assessment of the respiratory tract of a large number of racehorses in field conditions.

Venous blood was collected from 10 horses in each EIPH grade classification (grade 0 to 4) following tracheobronchoscopic examinations for the determination of the presence and severity of EIPH. Following RNA isolation and cDNA synthesis, real-time PCR was used to detect equine cytokine-specific mRNA for interleukin (IL) -1, -6, -10, interferon (INF) -γ, and tumor necrosis factor (TNF) -α. Results of this study indicated that increased IL-6, and -10 mRNA production was associated with more severe forms of EIPH. Also, there was greater expression of IL-6 mRNA at sea level and TNF-α mRNA at high altitude. This study concluded that although it was unclear whether the inflammatory response observed in the study was due to pre-existing pulmonary inflammation or as a direct consequence of pulmonary bleeding, the study demonstrated a systemic correlation to pulmonary inflammation.

The research reported in this thesis has contributed substantially to the determination of the prevalence, severity and affect on racing performance of respiratory tract disorders in Thoroughbred racehorses competing in South Africa. Also, determination of an association between EIPH and inflammation at a molecular level may assist future researchers in anti-cytokine therapies which may help reduce the prevalence and severity of EIPH.

Key words: altitude, arytenoid cartilage asymmetry, epiglottic deformity, epiglottic entrapment, exercise-induced pulmonary haemorrhage, grade scale, interleukin (IL)-1, IL-6, IL-10, interferon-γ, interobserver reliability, laryngeal debris, mRNA, pharyngeal lymphoid hyperplasia, race performance, real-time polymerase chain reaction, sea level, subepiglottic cysts, tracheal cartilage ring spikes, tracheal debris, tracheal mucous, tracheobronchoscopy, tumor necrosis factor-α.

Chapter 1 Exercise-induced pulmonary haemorrhage: An introduction

Exercise-induced pulmonary haemorrhage (EIPH) is a worldwide phenomenon in horses undergoing intense exercise, negatively affecting their health and performance. Most strenuously-exercised horses may be affected, although few will die as a direct result of EIPH. Exercise-induced pulmonary haemorrhage seems to be a physiological response to exercise in racehorses.

"Many horses, especially young horses, are oft the subject to this bleeding at the nose…it proceedeth from much abundance of blood, or that vein which endeth in that place [referring to the head] is either broken, fretted or opened"[83]

1.1 EPIDEMIOLOGY

"But broke a blood vessel and was beaten off"[83]

This was one of the first accounts of epistaxis recorded of Herod, a famous English Thoroughbred stallion, while racing in the 1766 Great Subscription Purse at York, England. For centuries, reports documenting the presence of blood in the nostrils of horses following intense exercise existed.[18] Using epistaxis (Figure 1.1) as the sole

criteria to identify horses with EIPH, the true prevalence of EIPH in the Thoroughbred racing population was repeatedly underestimated with a reported range of 0.15 to 2.41%.[13,18,42,80,81,98,103]

With the advent of modern technology and the availability of flexible fiberoptic endoscopes to examine the lower respiratory tract (Figure 1.2), estimates of the prevalence of EIPH using tracheobronchoscopy in the post race period have been reported in Thoroughbred racehorses (42 to 75.4%),[10,33,55,75,76,82,88] in Standardbred racehorses (26 to 87%),[10,47,50,75] in racing Quarter Horses (62.3%),[31] and in the racing Appaloosa horse (52.1%).[32] In addition, the prevalence of EIPH has been documented in Chilean Criollo horses (60.8%),[2] draft horses (0%),[14] polo ponies (11.1%),[102] cross-country ponies and mixed-breed horses (10%),[94] mixed-breed endurance horses (0%)[94] and Thoroughbreds competing in steeplechase (66.7%).[94] EIPH is also present in racing camels[3] and greyhounds.[44]

EIPH has also been described in man with reports documenting two athletes that developed shortness of breath, haemoptysis, and pulmonary oedema;[36] six elite athletes had increased red cells and protein concentration in bronchoalveolar lavage (BAL) fluid after extreme exertion;[106] in an apparent healthy man after running a marathon[28] and post-swimming.[104]

It is important to note that the reported prevalence and severity of EIPH may depend on the specific diagnostic modality (presence of epistaxis, use of tracheobronchoscopy,

cytological analysis of a tracheal aspirate or bronchoalveolar lavage), the timing and frequency of the diagnostic intervention (pre- or post-race and how often), intensity of exercise (training *versus* race day), and breed difference. Pertinent epidemiological studies reporting on tracheobronchoscopically-detected EIPH or EIPH-related epistaxis have been summarized in Table 1.1.

1.2 PATHOPHYSIOLOGY

Although EIPH is a ubiquitous condition, the precise aetiopathogenesis is unclear as can be seen from the many hypotheses which have been developed over the past years.

"This condition in racehorses remains an enigma"[49]

1.2.1 Chronic pulmonary disease

Chronic obstructive pulmonary disease (COPD) and emphysema was proposed as an underlying lesion of EIPH,[18] however no association was found between the presence of mucous and mucopurulent exudates in the trachea and EIPH.[16,92] Histopathological data was also dissimilar between EIPH and COPD-affected lung.[38,55]

1.2.2 Inflammatory airway disease

Inflammatory airway disease (IAD) is common in young racehorses, and may be characterized by exercise intolerance, coughing, and mucous in the airway[20,50] with a neutrophilic and lymphocytic BAL cytology.[19,87] Other reports on BAL cytology found a neutrophilic, eosinophilic or mastocytic inflammatory profile.[87,101] IAD and EIPH are frequently diagnosed in racehorses and may be a cause of impaired performance.[50] A report previously found an association between IAD and EIPH in young Thoroughbred racehorses.[64]

1.2.3 Upper airway obstruction

Upper airway obstruction (UAO) may include idiopathic laryngeal hemiplegia (ILH), dorsal displacement of the soft palate (DDSP), epiglottic entrapment, epiglottic hypoplasia, subepiglottic cysts, dorsal pharyngeal collapse, arytenoid chondritis, nasopharyngeal masses and vocal chord collapse. Alveolar transmural pressure is greater in horses with airway obstruction while racing[23,30,37] resulting in greater negative intrapleural and alveolar pressures leading to higher transmural pressure.

1.2.4 Pulmonary hypertension

Pulmonary vascular hypertension[48] and arterial hypertension[38] was reported in horses which suffered epistaxis, however these studies failed to record blood pressure prior to

epistaxis and therefore it was uncertain whether the rise in blood pressure was a precursor to epistaxis.

1.2.5 Parasites and thromboembolism

A parasitic or thomboembolic etiology was suggested as a cause of the lesions in the dorsocaudal lung lobes seen in EIPH-affected horses,[66] however no parasitic lesions or thromboemboli could be identified using histopathology.[55]

1.2.6 Haemostasis

Haemostasis was thought to be abnormal in horses with EIPH; however no defects in the intrinsic and extrinsic coagulation pathways or enhanced fibrinolysis were found in exercising horses or horses with EIPH.[5,38,39] Thrombocytopaenia and decreased clot retraction time was reported in horses with epistaxis[38] while exercise decreased adenosine diphosphate induced platelet aggregation.[6] In horses with EIPH, platelets are less responsive to agonists of platelet aggregation (adenosine diphosphate, collagen, platelet activating factor). Furosemide blocked the reduction in platelet aggregation,[4] enhanced platelet function and eliminated platelet trapping in the lung;[15] however how this relates to EIPH is not known.

1.2.7 Haemorrheologic variables

A survey of 49 racehorses before and after racing showed an increase in post-race red blood cell concentration, haemoglobin concentration and haematocrit, leading the authors to conclude hyperviscosity due to exercise-induced splenic contraction may play a causal role in EIPH.[56] Hyperviscosity and an increase in red blood cell deformability may increase shear rate and contribute to capillary wall failure. However, *in vitro*, the viscosity of equine blood decreases with increasing microvascular shear rate.[24]. Since furosemide (150 to 250 mg) had no appreciable affect on the relationship between haematocrit, shear rate, and blood viscosity,[91] haemoconcentration with associated splenic contraction increased cardiac output and mean blood flow velocity may result in increased shear rate and decrease in blood viscosity.[24] Increases in pulmonary vascular pressures during exercise are unlikely to be related to blood viscosity alterations.[79] The presences of echinocytes (speculated erythrocytes) were thought to cause EIPH.[12] It was suggested that as echinocytes are less deformable than erythrocytes and because echinocytosis occurred after endurance exercise,[11] that echinocytes may aggregate, form a thrombus and increase blood viscosity. No difference in the percentage of circulating echinocytes were reported in EIPH-affected and non-affected horses.[56] In contrast to echinocytes made *in vitro*, chronic furosemide induction of echinocytes *in vivo* (1 mg/kg, intramuscular, q12h, for 4 days) were less rigid, aggregated less, and did not alter blood viscosity at high shear rates.[105]

1.2.8 Capillary wall stress

Increased transmural capillary pressures (difference in pressure between the alveolar and capillary lumen) are generated during exercise which cause stress failure of the pulmonary capillaries.[107] Capillaries may then rupture should the transmural stress exceed the tensile strength of the capillary.[108] The stress failure is associated with reversible disruption of the capillary and alveolar epithelium resulting in haemorrhage into the interstitial and alveolar spaces due to the increased permeability (Figure 1.3).[107]

1.2.8.1 Role of the pulmonary circulation

Labeled microspheres injected into the pulmonary circulation and not into the systemic circulation recovered in BAL fluid of treadmill-exercised horses suggested that the pulmonary circulation may be the source of bleeding.[40] In horses, dramatic increases in pulmonary arterial pressure are associated with strenuous exercise.[9,52] Mean pulmonary arterial pressure is 20 to 25 mmHg at rest and may increase to greater than 90 mmHg during intense exercise due to the large cardiac output. In exercise, pulmonary capillary pressure may increase due to increased left atrial and pulmonary arterial pressure while pleural and alveolar pressures decrease resulting in severe stress to the alveolar wall.[8]

1.2.8.2 Role of the bronchial circulation

The bronchial circulation is associated with haemoptysis in humans.[17] Bronchiolitis, haemosiderophage sequestration, interstitial fibrosis and bronchial arterial neovascularization suggesting the involvement of the bronchial circulation in the aetiology of EIPH was reported,[73] however a direct contribution is lacking.

1.2.9 Small airway disease

Small airway disease may impair respiratory mechanics by decreasing dynamic compliance and increasing respiratory resistance, and may cause airway hyper-responsiveness.[22] Concurrent bronchiolitis[85] is common in EIPH-affected horses[55] and may predispose to bronchoconstriction resulting in a decrease in alveolar pressure during inspiration and capillary rupture.[84] Small airway disease may precede the onset of EIPH through previous studies that showed marked pulmonary bronchial circulation development[67] as confirmed by gross examination[70] and computerized tomography of affected lungs.[69] The small airway disease may be further exacerbated by concurrent viral infection, allergy or air pollution.[21]

1.2.10 Temperature and humidity

During exercise-induced bronchoconstriction, increased airflow may cause greater heat-loss from injured mucosa (in humans, the mucosa is hyperreactive to bronchoconstricting

stimuli) resulting in further bronchoconstriction. Temperature and humidity of inhaled air may be critical in the development of exercise-induced bronchoconstriction.[57] The association of EIPH with ambient temperature and humidity has been made previously.[18,82]

1.2.11 Pre-existing pulmonary inflammation

Pulmonary histopathological studies have demonstrated bronchiolitis in EIPH-affected lung sections[70] indicating that EIPH may be due to pre-existing airway inflammation.[73] Autologous intrapulmonary blood inoculation in horses caused a prolonged local inflammatory reaction (21 days) as assessed by bronchoalveolar leucocyte concentration.[60] This caused decreased dynamic compliance and increased respiratory resistance;[1] and resulted in bronchiolitis, alveolitis and increased vascularised interlobular tissue with increased number of haemosiderophages in air spaces and tissues.[99]

1.2.12 Reactive oxygen species

Reactive oxygen species (ROS) and oxidant injury may be responsible for the following: pulmonary structural damage, surfactant damage, leucocyte influx, release of vasoconstrictive and inflammatory mediators, and disrupt the synthesis and action of nitric oxide, a potent vasodilator.[63] A causal link between ROS and EIPH has yet to be proven.

1.2.13 Ventilation/perfusion

Despite intravascular pressures greater in the lower lung, there is evidence that preferential redistribution of flow to the dorsocaudal lung lobes during exercise.[7] However, pulmonary vascular scintigrams in horses with EIPH have shown a perfusion deficit in the dorsocaudal lung lobes.[72]

1.2.14 Impact-induced trauma

Through loading of the chest by the forelimbs which produce shear forces within the lung that can either cause or worsen EIPH, locomotory impact-induced trauma was proposed as the underlying cause of EIPH.[89,90] This theory proposed that following locomotory impact of the forelimb, a pressure wave is generated which passes from the scapula, through the body wall, to the dorsocaudal lung lobes causing tissue disruption which may lead to EIPH.[90] Interestingly, a recent report found that horses undertaking steeplechase races were at increased risk of epistaxis compared to horses competing in flat racing further suggesting impact-induced trauma may play a role in EIPH.[65] However, there is no evidence to support a locomotor-induced intrapulmonary or intrapleural pressure wave.[41]

1.3 PATHOLOGY

Bilaterally symmetrical discolouration of the dorsocaudal regions of the caudal lung lobes with associated partial small airway obstruction, increased tissue compliance and direct involvement of the bronchial arterial circulation were reported in a study of 26 Thoroughbred horses with confirmed EIPH.[67] Previous autopsy reports on horses suffering EIPH have included: pleural tears with thoracic haemorrhage and epistaxis,[93] massive pulmonary tissue haemorrhage,[80,83,100] and haemosiderophages.[100]

1.4 CLINICAL SIGNS

1.4.1 History

Frequently, racehorses with EIPH may suffer poor performance, or less commonly epistaxis. Depending on the volume of blood present in the trachea, unilateral or bilateral epistaxis may be noticed at the end of the race, in the parade ring, or on return to the paddock when the head is lowered. Epistaxis may be repeatable event following strenuous exercise.

1.4.2 Physical examination

No definitive set of clinical signs exists which can readily be used to diagnose EIPH. Affected horses may be anxious, cough and swallow frequently.[75,76] Coughing and

tracheal crackles may be non-specific for blood in the airway; however swallowing may be a more consistent sign. Swallowing may be initiated by blood pooling in the larynx and pharynx due to the mucociliary escalator clearing blood from the lower respiratory tract. Although infrequent, dyspnoea may be seen in the most severe forms of EIPH due to hypoxia caused by massive bleeding into the airway. Dyspnoea may also occur due to pulmonary abscess rupture, haemothorax, pneumonia or pneumothorax. Epistaxis may occur in a minority of racehorses following strenuous exercise.

1.5 DIAGNOSIS

1.5.1 Epistaxis

In the past, horses were classified positive for EIPH if blood was seen from one or both nostrils. Such horses are frequently referred to as "bleeders". A diagnosis of EIPH based solely 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. Therefore, the identification of horses with EIPH by the presence of epistaxis will grossly underestimate the prevalence of EIPH by allowing for inclusion into a study of only the most severely affected horses. Obviously, horses affected to lesser degrees, are not included in such analyses, thereby providing limited information regarding the association of EIPH and performance.

1.5.2 Tracheobronchoscopy

EIPH is definitively diagnosed by post exercise (within 120 minutes of racing) endoscopic examination of the upper respiratory tract and detection of the presence and severity of blood in the trachea. Tracheobronchoscopic assessment of EIPH is a quick, minimally-invasive technique that allows immediate classification of racehorses with EIPH according to a previously established grading system[34] without laborious, time-consuming laboratory processing of samples.

1.5.3 Cytological examination of airway secretions

EIPH may be detected in tracheal aspirates or broncho-alveolar lavage fluid (BALF) that contains red blood cells or macrophages with red blood cells or haemoglobin pigment known as haemosiderophages (Figures 1.4, 1.5 and 1.6).[26,58] Reports exist on BALF findings in normal horses.[25,26] Predominant cell types are macrophages and lymphocytes, and neutrophils (< 10% of cells).[25,26] BAL is a common technique and can be used to detect EIPH by measuring the concentration of erythrocytes and haemosiderophages.[58] A correlation between the amount of haemorrhage (erythrocyte concentration) and high mean pulmonary arterial pressure was demonstrated[62] while an association between EIPH and exercise intensity was suggested as horses with the highest maximal oxygen consumption have the most erythrocytes in the BALF.[59] Studies have reported using red blood cell counts to quantify EIPH,[27,43,46,62] however its association with the severity of EIPH has not been studied nor has its relationship with tracheobronchoscopic results.

It must be remembered that EIPH does not uniformly affect the lung, so the BALF cytology only evaluates a regional portion of the lung, and regional pulmonary differences may exist in red blood cell or haemosiderophage percentage.

1.5.4 Diagnostic imaging

Thoracic radiography may reveal distinctive changes to the dorsocaudal lung lobe (Figure 1.7 and 1.8). Radiographic changes may include diffuse but localized increase in density which is time-dependant, varying from an alveolar pattern to an interstitial or bronchial pattern.[66] Radiographically discernible increases in interstitial opacity may be related to lesion severity.[71] Lung abscesses may also be identified too.[77] However, many EIPH-affected horses may only have mild or no radiographic signs. Radiolabled red blood cells and scintigraphy have been used unsuccessfully in an attempt to localize and quantify pulmonary haemorrhage. Trans-thoracic ultrasonography may reveal structural changes in the dorsocaudal lung lobe (Figure 1.9).

1.5.5 Autopsy

Typically, post mortem findings may show epistaxis (Figure 1.10) and include petechiation and haemorrhage (acute) (Figure 1.11) to blue/grey or blue/brown discolouration of the visceral pleural surface of the dorsocaudal lung lobes in horses with chronic EIPH.[73] Typically, horses may have died from another cause, and the EIPH-induced lesions are incidentally noticed. Histopathological examinations of affected areas

reveal bronchiolitis, haemosiderophages, peribronchilolar and perivascular fibrosis, as well as fibrosis of the interlobular septa and pleura.[70]

1.6 THERAPEUTIC OPTIONS

1.6.1 High transmural pulmonary capillary pressure

Furosemide (frusemide) is currently used by racing jurisdictions (USA, Canada, Mexico, UAE, and parts of South America) as prophylaxis for EIPH in Thoroughbred, Standardbred and Quarter Horse racing. Although widespread use of furosemide exists, the clinical efficacy of furosemide in horses with EIPH has yet to be determined under natural field conditions. During exercise, high transmural capillary pressures cause stress failure of the pulmonary capillaries and subsequent haemorrhage.[106] Dose-dependant decreases in right atrial, pulmonary arterial/wedge/capillary pressures have been reported using furosemide in exercising horses.[54] Therefore, by limiting the increase in pulmonary artery and pulmonary capillary pressure of exercising horses, furosemide may reduce the frequency and severity of pulmonary capillary rupture.[52,53] Furosemide decreases the severity of EIPH in Thoroughbred horses exercised on a treadmill,[27,43] however did not have an affect on prevalence of EIPH under natural field conditions.[10,97] Conflicting reports exist regarding the use of furosemide in natural field conditions and its efficacy as evaluated by tracheobronchoscopic examination.[10,78]

A variety of drugs have been used to promote pulmonary vasodilatation in horses with EIPH and have failed to reduce the prevalence of EIPH or have an affect on pulmonary artery pressure: antihypertensive drugs (guanabenz and clonidine), angiotensin-converting enzyme inhibitors (enalopril), phophodiesterase inhibitors (sildenafil, aminophylline and pentoxifylline), and nitric oxide donors/analogs (nitroglycerin, nitroprusside and L-arginine).

1.6.2 Upper airway obstruction

UAO may increase alveolar transmural pressure in race horses resulting in greater negative intrapleural and alveolar pressures leading to higher transmural capillary pressure.[23,30,37] Although unproven, there may be an association between UAO and severity of EIPH. The use of nasal dilator bands (Flair® strips) reduced red blood cell counts in the BALF of intensely-exercised horses[27,43] by dilating the nasal valve and causing a reduction in nasal resistance.[35]

1.6.3 Lower airway obstruction

Horses with EIPH may have concurrent bronchiolitis[85] predisposing to bronchoconstriction and resulting in a decrease in alveolar pressure during inspiration and capillary rupture.[84] Although the exact role of small airway disease in EIPH is unclear, therapy is aimed at reliving inflammation and bronchoconstriction. The efficacy of beta-adrenergic bronchodilatory drugs (clenbuterol and albuterol) is still unclear in

preventing EIPH. An inhaled parasympatholytic drug (ipratropium) helped prevent EIPH in two horses.[96] Also the use of corticosteroids (dexamathasone, fluticasone and beclamathasone) and cromolyn sodium have not been demonstrated to prevent EIPH. Other therapies that have been unsuccessful in treatment of EIPH include inhaled water vapor and low allergenic stall bedding.

1.6.4 Interstitial inflammation and bronchial angiogenesis

Intrapulmonary blood accumulation results in alveolar fibrosis and bronchial artery angiogenesis.[60,61,68] Therefore treatments (such as corticosteroids) have been proposed to stop inflammation and decrease fibrotic damage. Certain racing jurisdictions maintain and enforce rest periods for horses displaying epistaxis, however how rest affects EIPH in the short or long term is unknown.

1.6.5 Haemostatic dysfunction

Neither defective intrinsic and extrinsic coagulation pathways nor enhanced fibrinolysis was found in horses with EIPH.[5,38,39] Despite an intact coagulation cascade, aminocaproic acid (inhibitor of fibrin degradation), estrogens and vitamin K have been used to prevent EIPH unsuccessfully.

The use of aspirin has been advocated due to increased platelet aggregation possibly contributing to EIPH;[51] however, aspirin inhibits platelet aggregation and prolongs coagulation time.[45]

Bioflavinoids have been used to decrease capillary fragility and stop bleeding; however there was no demonstrable efficacy in the use of hesperidin and citrus bioflavinoids.[95] Similarly, vitamin C has no proven benefit in EIPH.

1.7 RISK FACTORS

The prevalence of EIPH is affected by age, and speed. EIPH occurs more often in older horses[55,76,80,82] and with increasing speed.[74,82] Racing rather than breezing in Thoroughbred racehorses was associated with a higher prevalence of EIPH, while EIPH lesions were not seen in young Thoroughbred racehorses that were trained at speeds less than 7 meters per second.[74,82] The effect of sex on the prevalence of EIPH has not been consistently reported.[55,76,80,82,92]

1.8 EFFECT ON RACE PERFORMANCE

"[Herod's] form at times was unaccountably bad and it is significant that the first and only time he met a number of runners he showed the weakness [bleeding]"[83]

This was one of the earliest reports linking bleeding episodes with poor performance.[83] EIPH commonly occurs in racehorses throughout the world, and should only be considered the sole reason for poor performance if severe haemorrhage is present. Cytological examination of tracheobronchial aspirates and BALF may show evidence of EIPH or blood may be detected during tracheobronchoscopy in many under-performing racehorses. Epistaxis is associated with more severe forms of EIPH which cause poor performance and can be fatal.[29,42] Following tracheobronchoscopic evaluation, no relationship between EIPH and finish position was found in a group of 191 Thoroughbred racehorses that finished in first, second and third place,[76] nor in a another group of 98 racehorses.[76] Also, no relationship was proven between EIPH and finish position in 191 racehorses,[82] and between EIPH and performance in 258 Thoroughbred and 296 Standardbred race horses that finished in the top three places.[10] In contrast to the above mentioned reports, 43.9% of Thoroughbred racehorses that finished in the first 3 places had less severe tracheobronchoscopic evidence of EIPH than 55.9% that finished in fourth to fourteenth place.[55] Also, a cross-sectional study of Thoroughbred race horses in Victoria, Australia, showed a strong association between the presence and severity of EIPH and poor performance.[33] This study showed that horses with ≤ grade 1 EIPH were 4.03 times more likely to win and 1.78 times more likely to finish in the top three places

than horses with ≥ grade 2 EIPH.[33] Moreover, horses with higher grades of EIPH finished significantly ($P = 0.025$) farther behind the winner.[33]

Reports on a group of 29 and 92 Standardbred race horses show no relationship between EIPH and finish position.[47,92] However, post race tracheobronchoscopic detection of EIPH in a group of 965 Standardbred racehorses revealed that evidence of EIPH was 1.4 times more likely to occur in those racehorses finishing first or second than those race horses finishing in seventh or eighth place.[86]

As previous studies have either reported a positive,[86] negative[29,33,42,83] or no association[10,47,76,82,92] between EIPH and racing performance, it is still unclear what the relationship is between EIPH and racing performance. Moreover, since EIPH occurs commonly in racehorses throughout the world and due to the multifactorial causes of reduced racing performance in Thoroughbred racehorses, caution should exist when attempting to determine an association between EIPH and racing performance.

1.9 FIGURES AND TABLES

Figure 1.1 Epistaxis in a Thoroughbred racehorse.

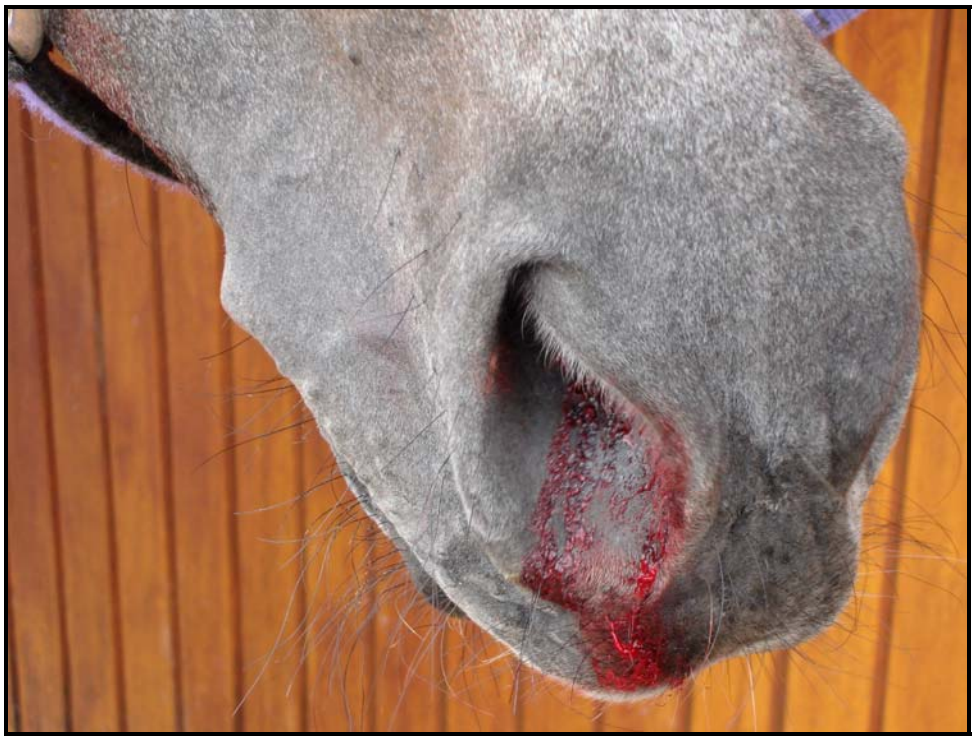


Figure 1.2 Tracheobronchoscopy in a Thoroughbred racehorse.

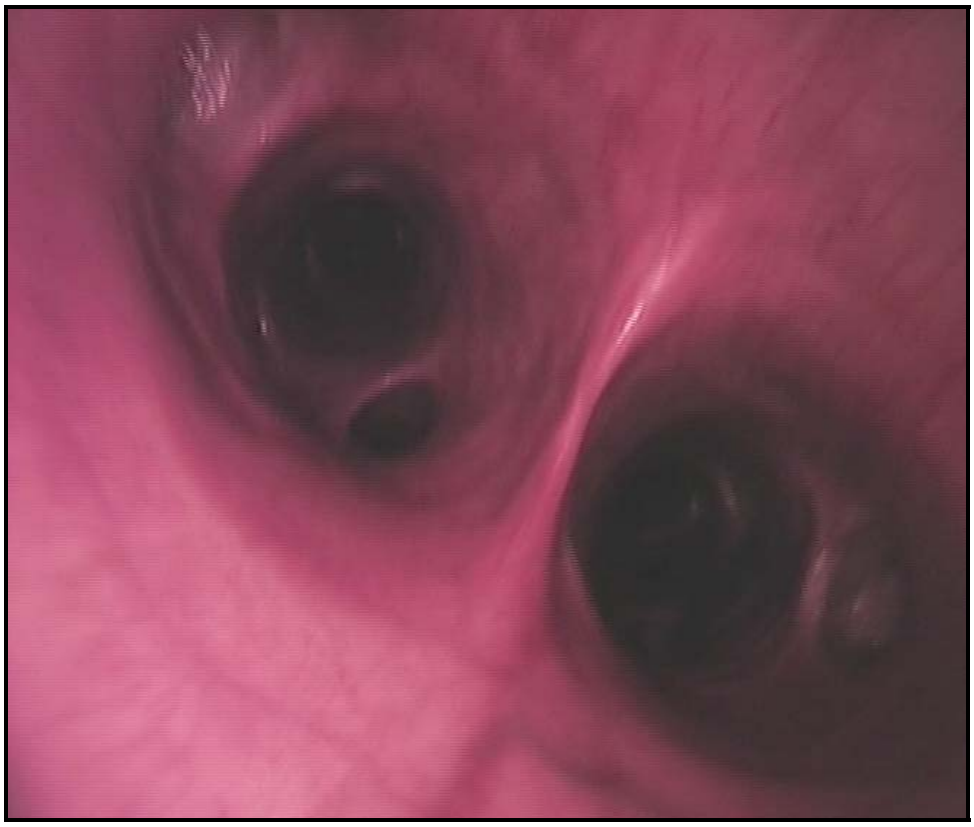


Figure 1.3 Schematic of the proposed mechanism of exercise-induced pulmonary haemorrhage with varying exercise intensity.

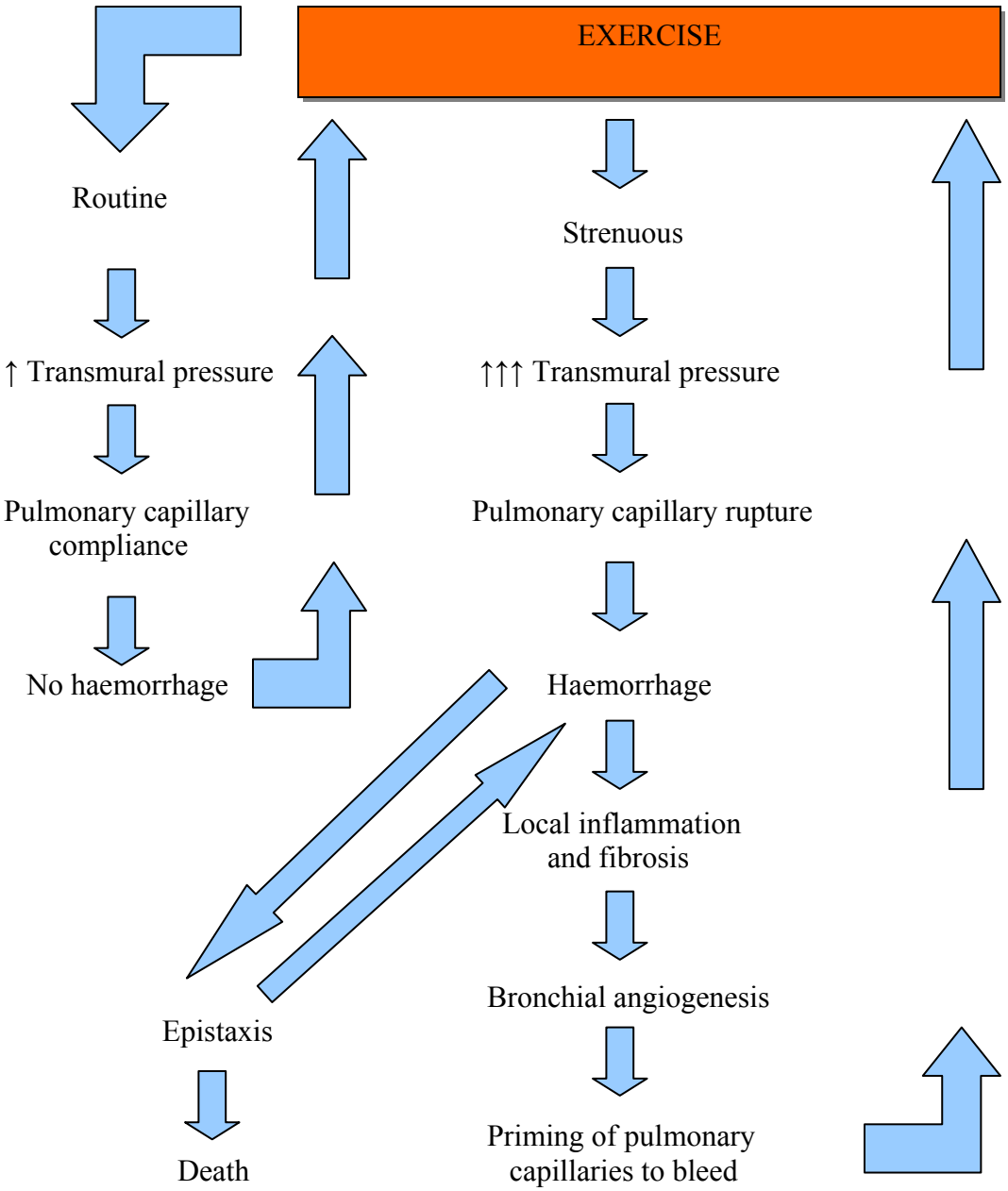


Figure 1.4 Collection of bronchoalveolar lavage fluid from a racehorse.

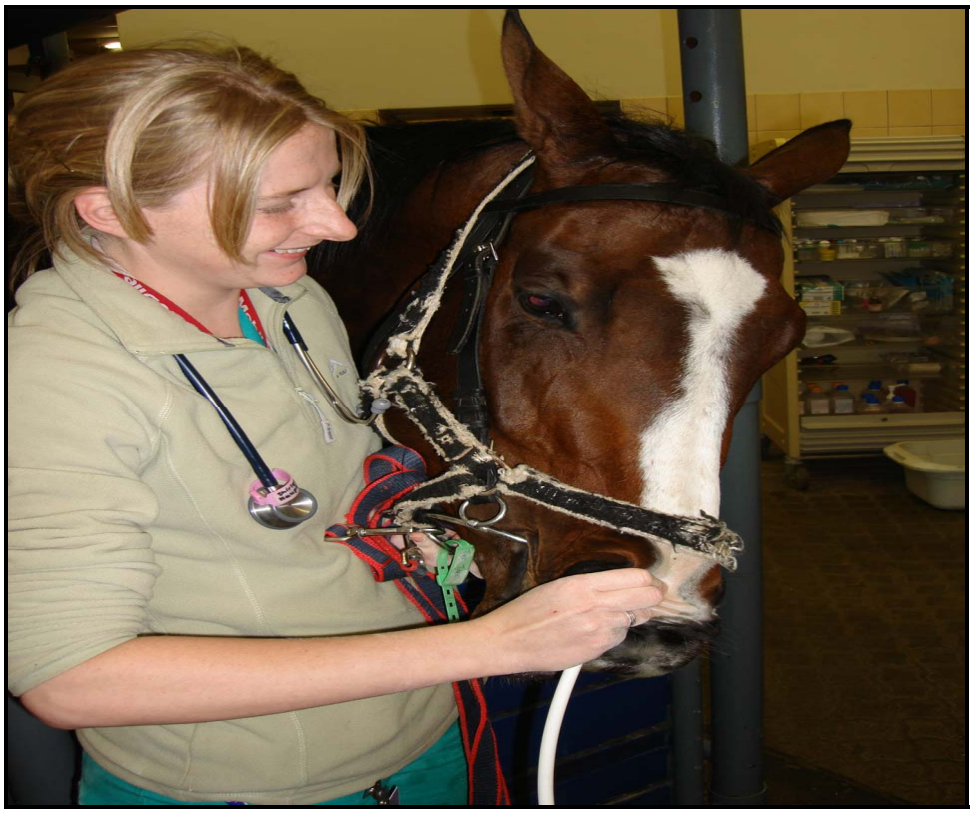


Figure 1.5 Red-tinged bronchoalveolar lavage fluid collected from a racehorse with exercise-induced pulmonary haemorrhage.

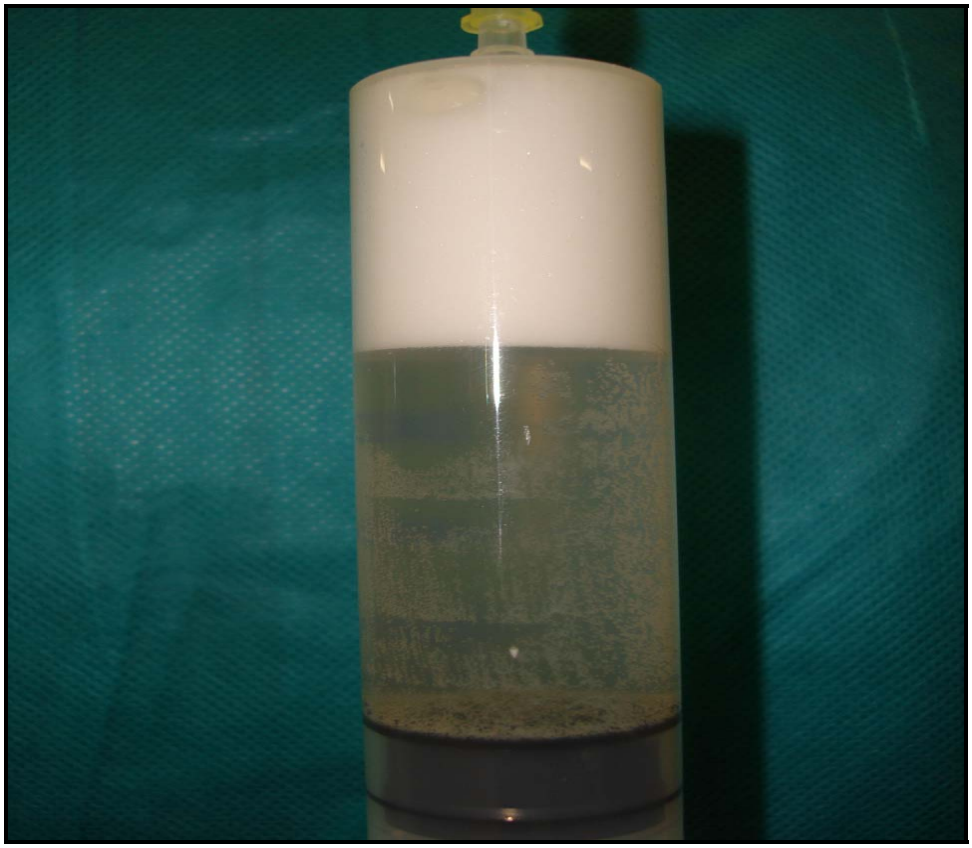


Figure 1.6 Cytological analysis of bronchoalveolar lavage fluid from a racehorse with exercise-induced pulmonary haemorrhage reveals numerous haemosiderophages.

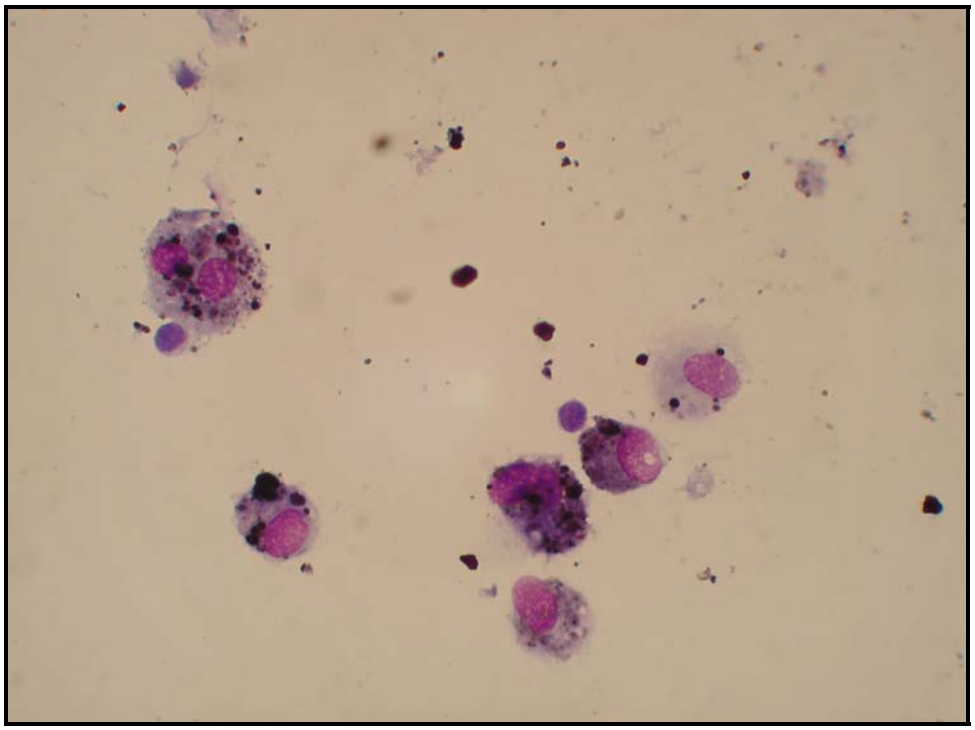


Figure 1.7 A three-year old Thoroughbred filly with a history of epistaxis after strenuous exercise. There is a mixed interstitial and alveolar infiltrate in the caudodorsal lung lobe suggestive of pulmonary haemorrhage.

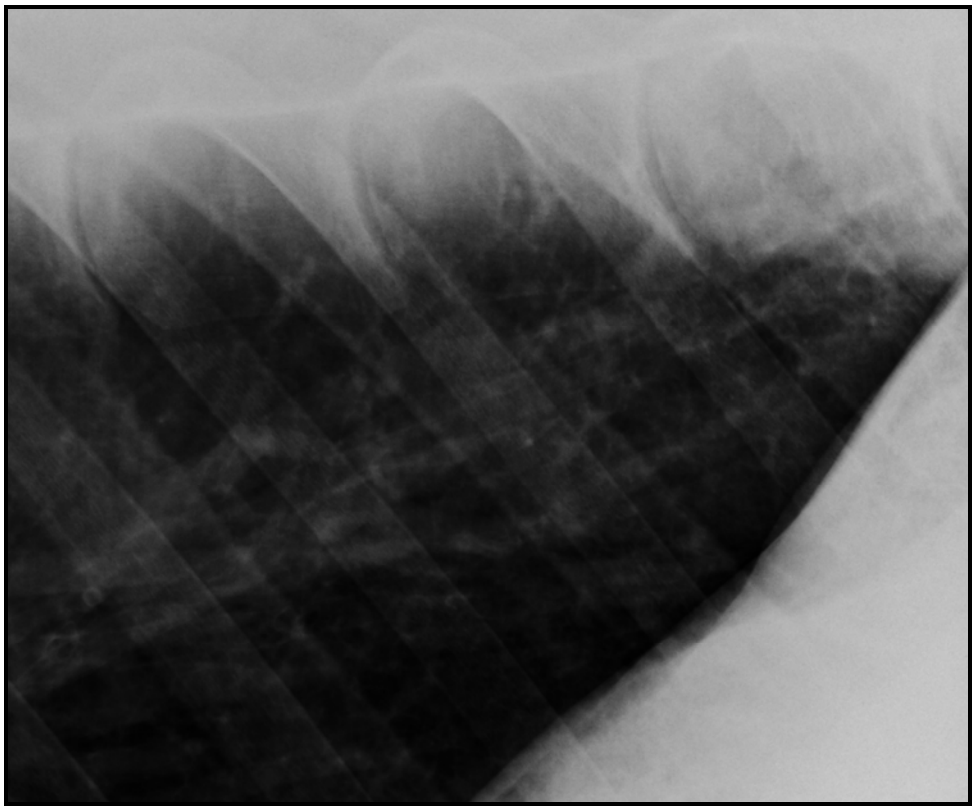
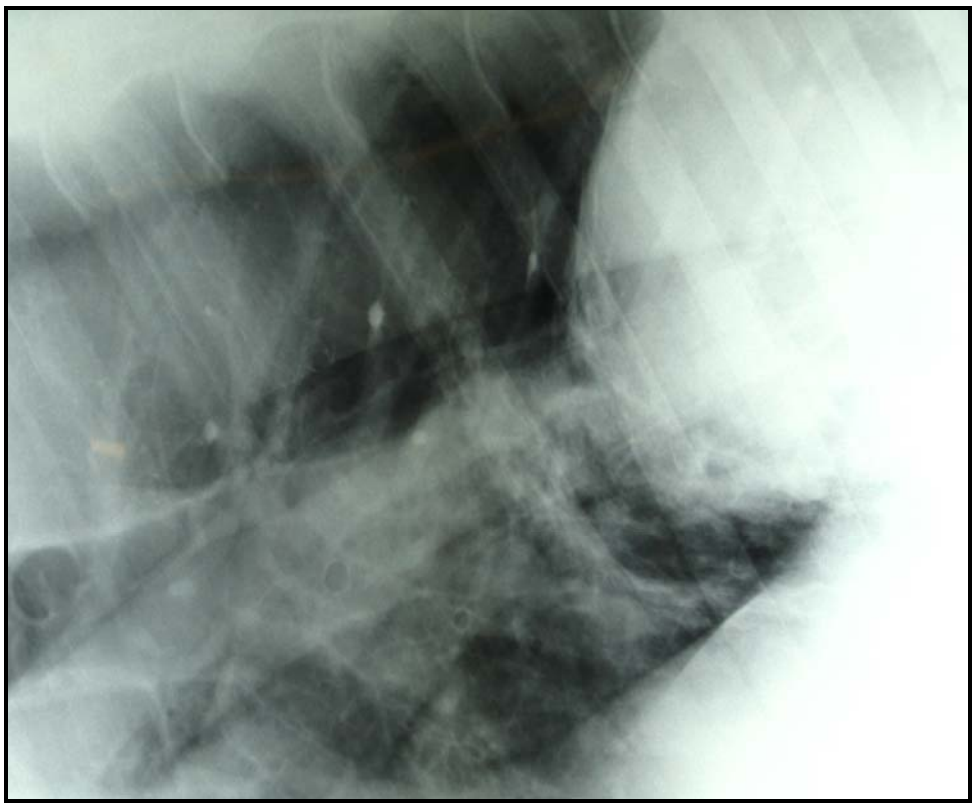


Figure 1.8 Dorsocaudal view of the thorax of a seven year-old Standardbred pacer with a history of poor performance, frequent coughing and pyrexia. Note the well demarcated, round mass consistent with a pulmonary abscess in the dorsocaudal lung lobe.



Courtesy of Dr. Alexa Burton, Large Animal Internal Medicine, Cornell University, USA.

Figure 1.9 Sonogram of the right, dorsocaudal hemithorax in the 17th intercostal space obtained from a three year old Thoroughbred filly with a history of epistaxis after strenuous exercise. Note the dimpling of the visceral pleural surface and comet-tail artifacts originating from the lung.

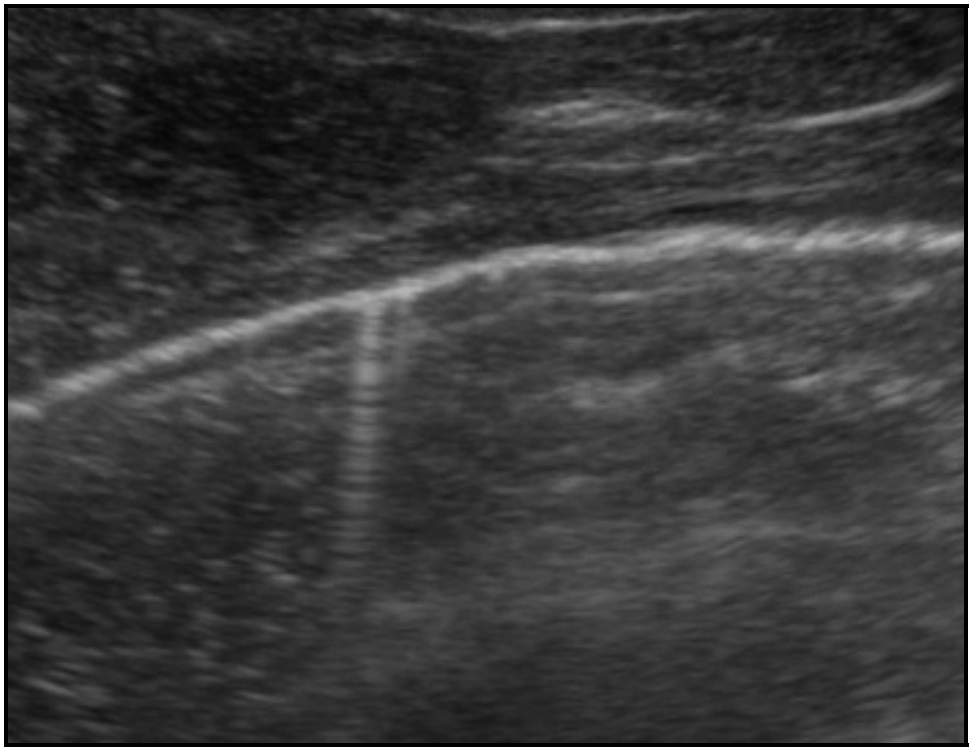


Figure 1.10 Acute death due to exercise-induced pulmonary haemorrhage in a Thoroughbred racehorse: note the mucous and blood present at both nares.

Courtesy of Dr. June Williams, Section of Pathology, Onderstepoort, University of Pretoria.

Figure 1.11 Acute death due to exercise-induced pulmonary haemorrhage in a Thoroughbred racehorse: note the massive pulmonary haemorrhage and blood pooling in the trachea.

Courtesy of Dr. June Williams, Section of Pathology, Onderstepoort, University of Pretoria.

Table 1.1 Epidemiological studies of exercise-induced pulmonary haemorrhage.

Study and Reference Number	Year	Study Size	Breed	Study Location	EIPH Prevalence	Epistaxis Prevalence	Diagnostic Modality
Pfaff, G. (80)	1950	4015	TB	South Africa	NR	1.2%	Epistaxis
Bourke, J.M. (13)	1973	NR	TB	Australia	NR	0.8%	Epistaxis
Cook, W.R. (18)	1974	50	TB	England	NR	0.5 to 2%	Epistaxis
Pfaff, G. (81)	1976	5292	TB	South Africa	NR	2.41%	Epistaxis
Pascoe, J.R. *et al* (75)	1980	1180	TB	USA	42%	3%	Endoscopy
Pascoe, J.R. *et al.* (75)	1980	249	STB	USA	26.5%	12%	Endoscopy
Pascoe, J.R. *et al* (76)	1981	235	TB	USA	43%	0.8%	Endoscopy (100cm)
Raphel, C.F. *et al* (82)	1982	191	TB	USA	75.4	9%	Endoscopy (140cm)
Mason, D.K. *et al* (55)	1983	485	TB	Hong Kong	62.5%	3.9%	Endoscopy
Hillidge, C.J. *et al* (31)	1984	231	QH	USA	62.3%	8.3%	Endoscopy (110cm)
Hillidge, C.J. *et al* (32)	1985	94	APP	USA	52.1%	4%	Endoscopy (180cm)
MacNamara, B. *et al* (50)	1990	965	STB	USA	26%	0%	Endoscopy
Lapointe, J.M. *et al* (47)	1994	60	STB	Canada	62%	0%	Endoscopy (100cm)
Kim, B. *et al* (42)	1998	61,181	TB	Korea	NR	0.84%	Epistaxis
Takahashi, T. *et al* (98)	2001	247,564	TB	Japan	NR	0.15%	Epistaxis
Williams, R.B. *et al* (109)	2001	222,993	TB	England	NR	0.83%	Epistaxis
Weideman, H. *et al* (103)	2003	51,465	TB	South Africa	NR	0.165%	Epistaxis
Hinchcliff, K.W. *et al* (33)	2005	744	TB	Australia	55.3%	0.8%	Endoscopy (170cm)
Saulez, M.N. *et al* (88)	2006	1014	TB	South Africa	54.5%	0.8%	Endoscopy (160cm)

NR: not recorded
TB: Thoroughbred
STB: Standardbred
QH: Quarter horse
APP: Appaloosa

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Research focus of this thesis

Disorders of the equine respiratory tract occur worldwide with varying frequency. Currently, a lack of reports exists on such disorders within South Africa, despite the large, active population of racehorses. In South Africa, the National Horse Racing Authority strictly enforces drug testing in Thoroughbred racehorses, thereby allowing research to be more accurately conducted on racehorses that have not been administered pharmaceutical agents which may modify athletic performance. By the creation of such an environment, we were able to determine the prevalence and severity of airway disorders and their relationship with athletic performance. The impetus for this research originated with Kenneth W. Hinchcliff, a world-renowned researcher on exercise-induced pulmonary haemorrhage (EIPH) in Thoroughbred racehorses.

Following the introduction (Chapter 1), this thesis reports original research in Thoroughbred racehorses competing in South Africa, detailing the prevalence and severity of EIPH and its relationship with racing performance (Chapter 2), the prevalence of pharyngeal, laryngeal and tracheal disorders and their relationship with performance (Chapter 3), and the inter-observer reliability in the use of endoscopic grading criteria for certain respiratory tract disorders (Chapter 4). This thesis also provides exciting new information on an association between EIPH and inflammation at a molecular level (Chapter 5) and concludes with a general discussion (Chapter 6).

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 (< $^{1}/_{4}$ 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 ≥ 1 (yes) and severity grade ≤ 1 (no) vs. severity grade ≥ 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 (1^{st}, 2^{nd} or 3^{rd}) (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 (2^{nd},3^{rd} or 4^{th} 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.

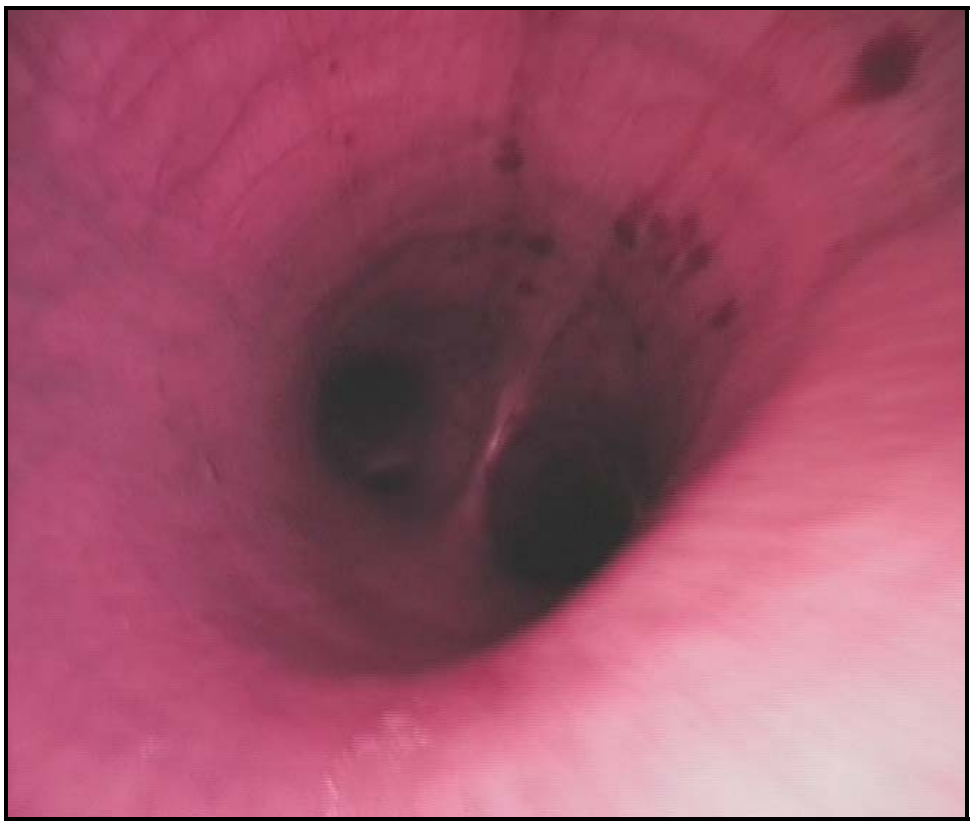

Figure 2.3 A racehorse with grade 3 exercise-induced pulmonary haemorrhage as detected by tracheobronchoscopy.

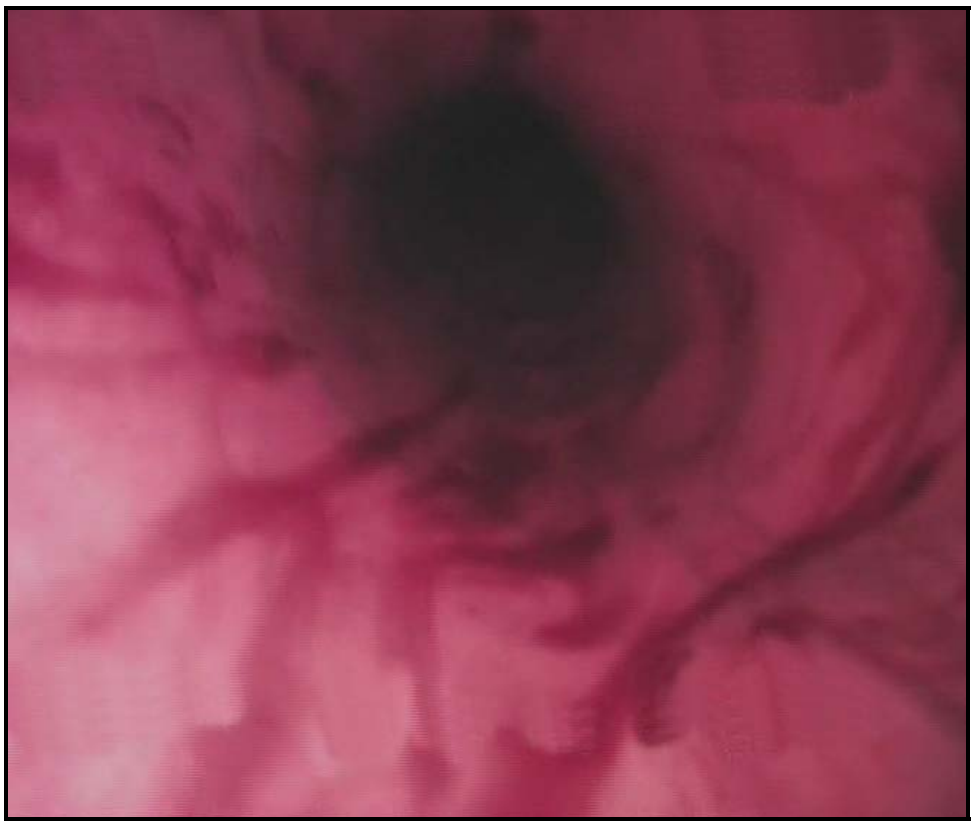

Figure 2.4 A racehorse with grade 4 exercise-induced pulmonary haemorrhage as detected by tracheobronchoscopy.

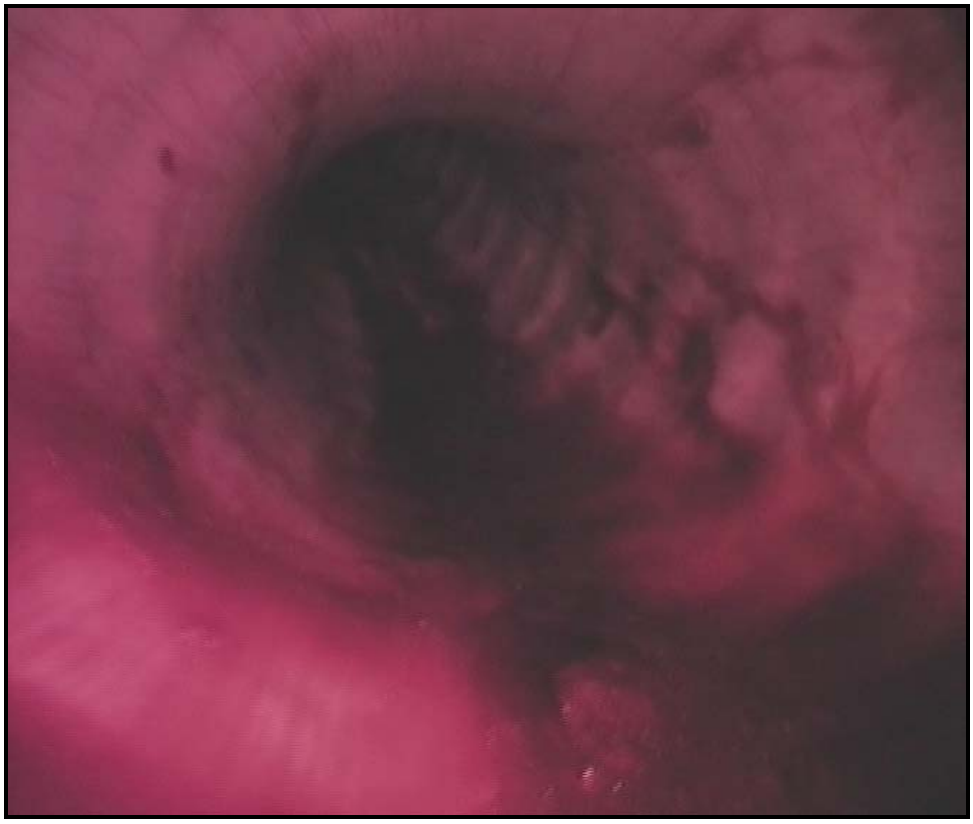

Figure 2.5 Exercise-induced pulmonary hemorrhage (EIPH) in South African Thoroughbred racehorses (n=1005) examined from August 4 to November 19, 2005 post race: overall tracheobronchoscopic assessment of the severity of EIPH using a 0 to 4 EIPH grade scale.

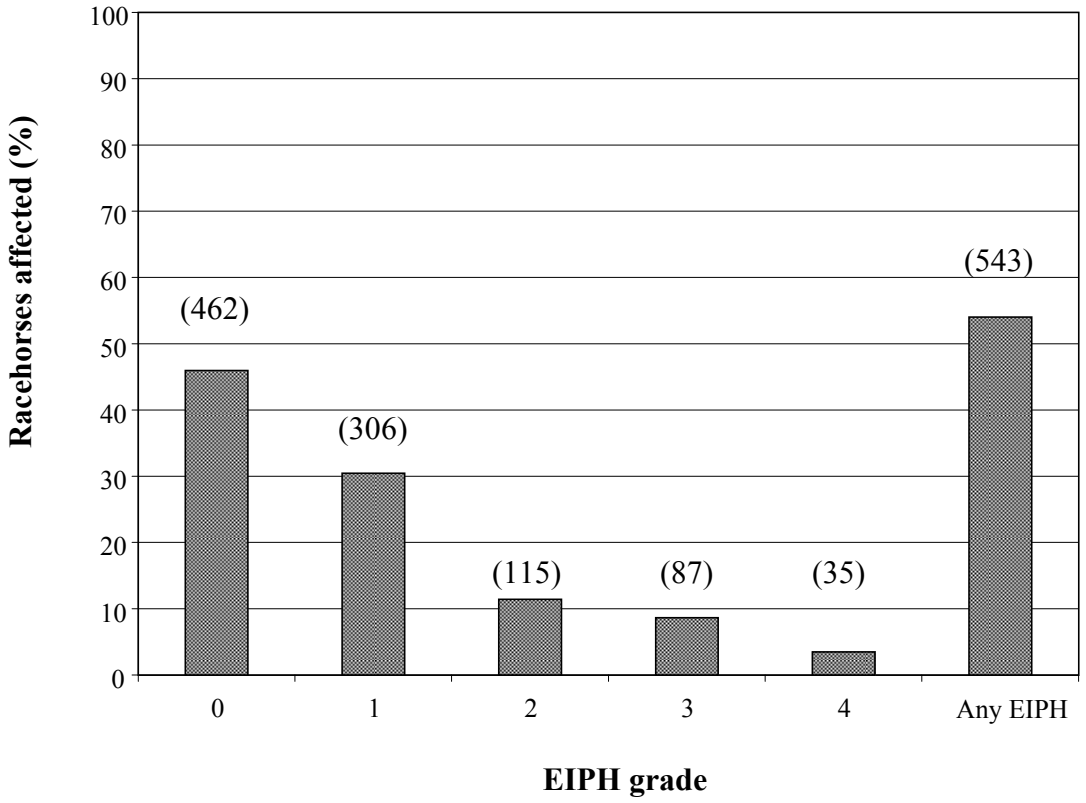

Figure 2.6 Exercise-induced pulmonary hemorrhage (EIPH) in South African Thoroughbred racehorses (n=1005) examined from August 4 to November 19, 2005 post race: tracheobronchoscopic assessment of the severity of EIPH using a 0 to 4 EIPH grade scale at high altitude (1,450 meters above sea level) and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

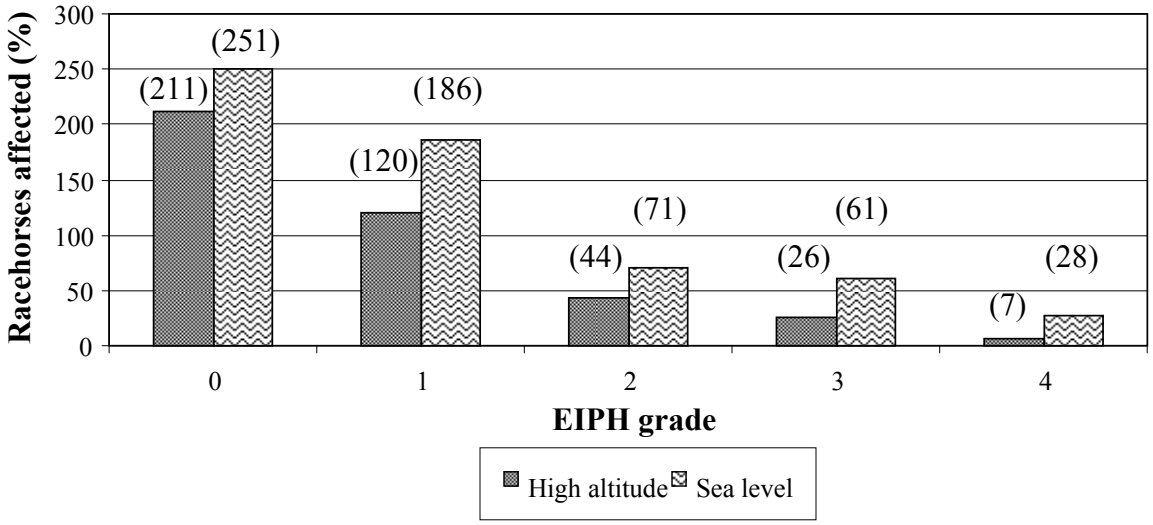

Figure 2.7 Exercise-induced pulmonary hemorrhage (EIPH) in South African Thoroughbred racehorses (n=1005) examined from August 4 to November 19, 2005 post race: finishing position as a function of severity of EIPH using a 0 to 4 EIPH grade scale.

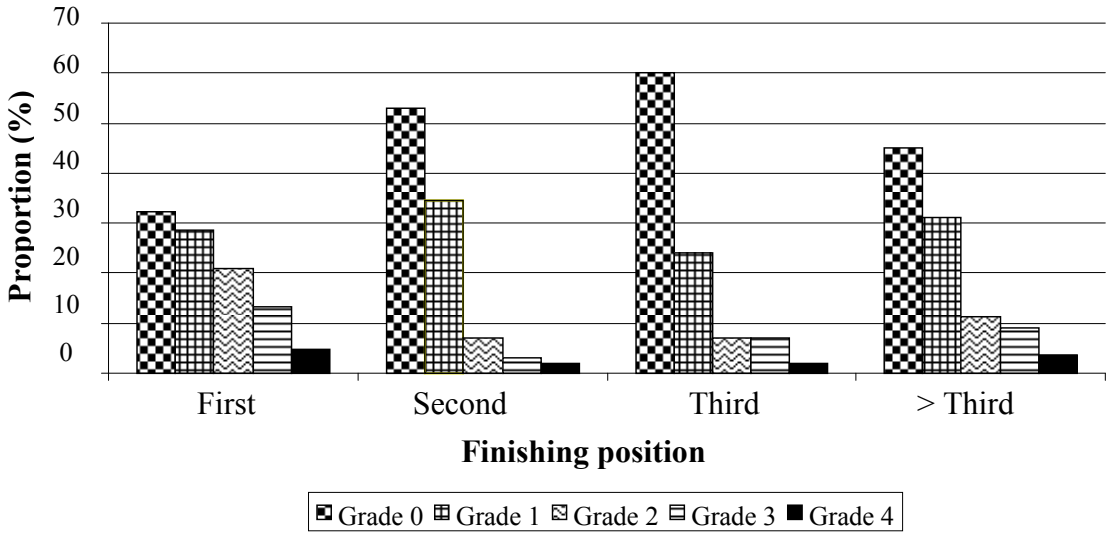

Table 2.1 Tracheobronchoscopic scoring of horses with exercise-induced pulmonary haemorrhage.

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 ≤ 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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Chapter 3 Pharyngeal, laryngeal and tracheal disorders in South African Thoroughbred racehorses: prevalence and relationship with performance

3.1 ABSTRACT

We sought to determine the prevalence of pharyngeal, laryngeal and tracheal disorders in Thoroughbred racehorses in South Africa and to determine their relationship with racing performance. A prospective cross-sectional study was conducted over 3.5 months on 1,005 Thoroughbred racehorses. After racing, videoendoscopic examination of the pharynx, larynx and trachea was performed on unsedated, pre-enrolled racehorses. The presence and characteristics of respiratory tract disorders were evaluated and recorded onto digital video disc. Thereafter, the prevalence of the observed disorders was compared to race career performance records.

Over a 3.5 month period, a single videoendoscopic examination was performed mean ± standard deviation (SD) 24 ± 12.3 min after racing on 1,005 racehorses. The prevalence of arytenoid cartilage asymmetry (grade 2 and 3) in racehorses examined was 2.22% (95% confidence interval [CI]: 1.29 to 3.14%). The median number of lifetime starts was higher in affected horses, with this being significantly so in racehorses with grade 2

arytenoid cartilage asymmetry and idiopathic laryngeal hemiplegia (ILH) ($P < 0.05$). No difference could be shown in median lifetime places between racehorses with grade 1 vs. 2 and 3 arytenoid cartilage asymmetry ($P > 0.05$), but racehorses with grade 2 arytenoid cartilage asymmetry had more lifetime wins ($P = 0.02$) and racehorses with ILH had a greater number of lifetime wins ($P = 0.03$) and lifetime places ($P = 0.01$). Both racehorses with grade 2 arytenoid cartilage asymmetry and ILH had higher lifetime stake earnings ($P < 0.05$). The apparent superior racing performance seen in racehorses affected by various grades of arytenoid cartilage asymmetry was most likely caused by more lifetime starts, which was acting as a confounder in these groups.

Compared to racehorses with grade 1 pharyngeal lymphoid hyperplasia (PLH), racehorses with grade 3 PLH were younger ($P < 0.01$), while racehorses with grade 2 and 3 PLH had fewer lifetime starts, lifetime wins, lifetime places and less lifetime stake earnings ($P < 0.01$). Racehorses with grade 2 PLH had more lifetime places ($P < 0.01$) and lifetime stake earnings ($P = 0.03$) than horses with grade 3 PLH. The apparent impaired racing performance was most likely caused by less lifetime starts, which was acting as a confounder in these groups.

Epiglottic deformity was detected in 0.6% of racehorses (95% CI: 0.12 to 1.08%). Affected racehorses were older ($P = 0.01$) and had more median lifetime starts, lifetime places, lifetime wins and higher lifetime stake earnings than unaffected racehorses ($P = 0.01$). The apparent superior performance was most likely caused by more lifetime starts was again found to be a confounder that probably accounted for the increased life time

wins and places. Epiglottic entrapment was detected in 1.29 % racehorses (95% CI: 0.59 to 2%). There was no difference between affected and unaffected racehorses for median age, sex, lifetime starts, lifetime wins and lifetime places ($P > 0.05$).

Racehorses with grade 3 tracheal mucous had more median lifetime wins, lifetime places and higher lifetime stake earnings compared to horses with grade 2 tracheal mucous ($P = 0.01$). The sixty eight racehorses (6.77%, 95% CI: 5.16 to 8.37%) affected with tracheal cartilage ring spikes were 3.6 times more likely to be male (relative risk = 3.63, 95% CI: 2.07 to 6.35; $P < 0.01$) and had more lifetime wins and lifetime places ($P = 0.03$). The presence of debris was detected in the larynx of 437 racehorses (43.53%, 95% CI: 39.44 to 47.61) and trachea of 220 racehorses (21.89%, 95% CI: 19.02 to 24.81) and did not affect median lifetime wins and lifetime places ($P > 0.05$).

In Thoroughbred racehorses competing in South Africa, the prevalence of pharyngeal, laryngeal and tracheal disorders is varied. Racing performance which was apparently superior in horses affected by arytenoid cartilage asymmetry (grade 2 and 4) and epiglottic deformity; and apparently impaired in horses affected by PLH (grade 2 and 3), was most likely as a result of more lifetime starts which acted as a confounder in these groups of racehorses. Racing performance was not impaired by the presence of epiglottic entrapment, and debris in the larynx and trachea. Superior racing performance was seen in horses with tracheal cartilage ring spikes and grade 3 tracheal mucous. This study also highlights the multifactorial nature of these conditions and the caution that needs to be taken before inferring causality to a particular factor.

3.2 INTRODUCTION

Three North American[21,23,27] and one Australian study[7] have reported on the prevalence of upper respiratory tract abnormalities in Thoroughbred racehorses. Excluding pharyngeal lymphoid hyperplasia, the reported prevalence of upper respiratory abnormalities were 3.4 to 8.1%[21,23,27] and 6.3%.[7]

Using flexible endoscopy, abnormalities identified by these studies included asymmetry of the left arytenoid cartilage,[7,21] left laryngeal hemiplegia,[21,23,27] prosthetic laryngoplasty,[21,23] ventriculectomy,[7] right arytenoid paralysis,[21,23] arytenoid chondropathy,[7,21] ulceration of the arytenoid mucosa,[7] aryepiglottic entrapment,[7,21,23,27] pharyngeal polyps,[21] dorsal displacement of the soft palate,[7,23,27] guttural pouch discharge,[23] nasal stenosis,[23] subepiglottic cyst,[7,23] epiglottic hypoplasia,[7] epiglottic deformity[7] and tracheal mucous.[21,23]

In Thoroughbred racehorses, although certain disorders of the upper respiratory tract such as left laryngeal hemiplegia[8,16] have been associated with reduced exercise capability while others not,[7] there still exists a paucity of objective data to fully evaluate the importance of these and other conditions which are less severe or occur infrequently. Historically, studies presenting data on horses with upper respiratory tract abnormalities have suffered from a selection bias, as horses have been examined only when respiratory strider or poor performance was noted. Although providing useful information, these studies were not an accurate representation of overall health status in a Thoroughbred

racehorse population, and functional consequences of upper respiratory tract abnormalities could not be assessed since racing performance was not evaluated.

A paucity of reports exists on the association between performance and respiratory tract disorders in Thoroughbred racehorses while the prevalence of these abnormalities is largely unknown outside North America[21,23,27] and Australia.[7] The purpose of this study was to firstly document the prevalence of pharyngeal, laryngeal and tracheal disorders in Thoroughbred racehorses competing in South Africa and secondly, to determine their relationship with racing performance.

3.3 MATERIALS AND METHODS

3.3.1 Thoroughbred racehorses

The study was a prospective cross-sectional study of the prevalence of pharyngeal, laryngeal and tracheal disorders and their association with racing performance in Thoroughbred racehorses. Racehorses of either sex, running on turf or sand, competing in flat races between 800 and 3,200 meters at five racetracks in South Africa (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]) were enrolled into this study between 4th August and 19th November 2005. These five racecourses are considered representative of the best racing in South Africa. 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. 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 horses (24 to 72 hours prior to race day) were entered into the study to avoid a potential enrollment bias. Any racehorse that was refractory to restraint that could comprise the safety of personnel or equipment, and any horse examined after racing that was not pre-enrolled prior to the race, was excluded from this study.

3.3.2 Endoscopic examination

Following racing, each pre-enrolled racehorse was identified and tagged during parading. Thereafter, tagged racehorses were brought into a dedicated stable for videoendoscopic examinations. Unsedated racehorses were restrained by a handler with a halter and nose twitch in a dedicated examination stable. Videoendoscopic evaluation of one nostril, ipsilateral nasal turbinate, nasopharynx, larynx and trachea was performed and all examinations were recorded onto digital video disc. At least one veterinarian always evaluated the image on the screen at all times. Following insertion of the videoendoscope (Pentax Corporation, Tokyo, Japan: endoscope model number EC3830FK, 1.5 m in length, 38 French in diameter, processor number EPK700) into either nostril, it was passed along the ventral meatus caudally to the nasopharynx. The nasopharynx and

larynx were carefully examined. Following confirmation of the position of the epiglottis and soft palate, the larynx was assessed for symmetry, synchronous movement and debris. Thereafter, the trachea was assessed for tracheal ring symmetry and the presence of mucous or debris up to and including the tracheal bifurcation at the level of the carina. For the purpose of this study, exercise-induced pulmonary hemorrhage was not evaluated.

3.3.3 Grading of pharyngeal, laryngeal and tracheal disorders

Arytenoid cartilage asymmetry was graded 1 to 4.[26] Grade 1 indicated symmetrical synchronous abduction and adduction of the left and right arytenoid cartilages (Figure 3.1); grade 2 indicated some asynchronous movement (hesitation, flutter or abductor weakness) of the left arytenoid cartilage during any phase of respiration and full abduction of the left arytenoid cartilage which could be maintained by swallowing; grade 3 indicated asynchronous movement (hesitation, flutter or abductor weakness) of the left arytenoid cartilage during any phase of respiration and full abduction of the left arytenoid cartilage could not be induced or maintained by swallowing; and grade 4 indicated no substantial movement of the left arytenoid cartilage during any phase of respiration and were subsequently classified as having idiopathic laryngeal hemiplegia (ILH) (Figure 3.2). Those horses with a fixed and immobile, abducted left arytenoid with or without a ventriculectomy were identified as having a prosthetic laryngoplasty and this was confirmed with the trainer or owner.

The severity of pharyngeal lymphoid hyperplasia (PLH) was graded on a scale from 1 to 4.[5] Grade 1 indicated lymphoid hyperplasia limited to < 180° of the dorsal pharyngeal recess (Figure 3.3); grade 2 indicated lymphoid hyperplasia extending to circumference of the dorsal pharyngeal recess (Figure 3.4); grade 3 indicated lymphoid hyperplasia made midline contact of the dorsal pharyngeal recess (Figure 3.5); and grade 4 indicated small masses (which may be abscesses) arising from either the dorsal pharyngeal recess or the pharyngeal walls (Figure 3.6).

Dorsal displacement of the soft palate (DDSP) was defined conservatively by our study and no nasal occlusion was performed as part of the examination (Figure 3.7). DDSP was clinically relevant only if it was present before and after rhinolaryngoscopy was performed, and if repositioning of the soft palate did not occur after 2 swallowing attempts. The length, width and shape of the epiglottis were subjectively evaluated. Epiglottic entrapment was diagnosed if the epiglottis was enveloped by the aryepiglottic fold. Endoscopic evaluations of the guttural pouches were not performed.

Mucous within the trachea was graded 0 to 5.[11] Grade 0 indicated the absence of mucous; grade 1 indicated singular droplets of mucous (Figure 3.8); grade 2 indicated multiple droplets of mucous that is partly confluent (Figure 3.9); grade 3 indicated mucous that is ventrally confluent (Figure 3.10); grade 4 indicated a large ventral pool of mucous (Figure 3.11); and grade 5 indicated profuse amounts of mucous covering > 25% of the tracheal lumen (Figure 3.12).

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. The data included lifetime starts, lifetime wins, lifetime places (2^{nd}, 3^{rd}, or 4^{th}) and lifetime stake earnings (South African Rand, ZAR).

3.3.4 Data analysis

Data for all racehorses was collated in Microsoft Excel (version 2003) and variables for racehorses that were diagnosed with epiglottic deformity, epiglottic entrapment, arytenoid cartilage asymmetry, prosthetic laryngoplasty, pharyngeal lymphoid hyperplasia, tracheal mucous, tracheal cartilage ring spike, laryngeal dirt and tracheal dirt were compared with variables in unaffected racehorses using the statistical software package NCSS 2006 (Hintze J. NCSS and PASS number cruncher statistical systems, Kaysville, Utah, 2006). Statistical analysis was not performed for other conditions due to the small number of affected racehorses. A Mann-Whitney *U*-Test was used to compare median age, lifetime runs, lifetime wins, lifetime places and lifetime earnings. A chi-squared analysis with Yates correction was used to analyze the sex distribution (male, gelding and female). For all comparisons, a value of $P < 0.05$ was considered significant. Where confounding was suspected, the relationship of the confounding was studied using multiple least squares regression.

3.4 RESULTS

Of the 2,684 eligible racehorses that competed in 230 flat races (194 turf races, 36 sand races) at 28 race meetings, 1,005 horses (37.4%) were endoscopically examined. Endoscopic examinations took place mean ± standard deviation (SD) 24 ± 12.3 minutes after racing. There were 509 females, 491 geldings, and 5 intact males with a mean age of 4 years (95% confidence interval [CI]: 2 to 9 years). Ninety seven trainers participated in this study. This study evaluated and identified PLH, arytenoid cartilage asymmetry, prosthetic laryngoplasty, laryngeal dirt, epiglottic deformity epiglottic entrapment, subepiglottic cyst, DDSP, tracheal stenosis, tracheal cartilage ring spike, tracheal mucous and tracheal dirt.

Following assessment of arytenoid cartilage asymmetry, racehorses were graded 1 (n = 970), 2 (n = 18), 3 (n = 4) and 4 (n = 6), while no grade was allocated to horses with laryngoplasty (n = 7). Results of the Mann-Whitney U-test showed that when compared to grade 1 arytenoid cartilage asymmetry, median values for racehorses with grade 2 arytenoid cartilage asymmetry were older (4 *vs*. 5 years; $P = 0.01$), had more lifetime starts (13 *vs*. 8; $P = 0.047$), more lifetime wins (2 *vs*. 1; $P = 0.02$), and had higher lifetime stake earnings (ZAR 79,567.50 *vs*. 35,287.50; $P = 0.04$) while those with grade 4 arytenoid cartilage asymmetry (ILH) were older (5 *vs*. 4 years; $P = 0.01$), had more lifetime starts (19.5 *vs*. 8; $P = 0.01$), more lifetime wins (3 *vs*. 1; $P = 0.03$), more lifetime places (6.5 *vs*. 2; $P = 0.01$), and higher lifetime stake earnings (ZAR 112,297,50 *vs*. 35,287,50; $P = 0.01$). ILH was detected in a total of 6 racehorses (0.6%, 95% CI: 0.12 to

1.07) consisting of 3 geldings; and 1 gelding, 1 male and 1 female due to a failed prosthetic laryngoplasty. One racehorse each finished in 1st, 4th, 8th and 10th place and two horses each in 3rd place in the race preceding endoscopic examination. Because of the significant difference in lifetime starts it was suspected that this may be confounding the lifetime wins and lifetime places. Multiple least squares regression was therefore performed using lifetime wins or lifetime places as a Y-dependent variable and lifetime starts and arytenoid asymmetry grades as independent variables. The relationship between lifetime wins or lifetime places and the various grades of arytenoid asymmetry was then shown to be insignificant ($P > 0.05$), while there was still a significant relationship ($P < 0.01$) between lifetime starts and lifetime wins or lifetime places. The increased lifetime wins and lifetime places in horses with arytenoid asymmetry are probably therefore due to more lifetime starts and not the arytenoid asymmetry itself.

Successful prosthetic laryngoplasty was detected in 7 racehorses (0.7%, 95% CI 0.18-1.21%) consisting of 1 male, 5 geldings and 1 female. No significant differences were observed between horses with laryngoplasty and those without for age ($P = 0.18$), sex ($P = 0.42$), lifetime starts ($P = 0.42$), lifetime wins ($P = 0.69$), lifetime places ($P = 0.14$) and lifetime stake earnings ($P = 0.75$). One racehorse each finished in 1st, 5th, 8th, 10th and 17th place, and two in 4th place each.

Racehorses with PLH were graded 1 (n = 372), 2 (n = 534), 3 (n = 83) and 4 (n = 4) while no grade could be allocated to 12 horses due to poor endoscopic imaging. Compared to grade 1 PLH, racehorses with grade 2 PLH, had less lifetime starts (6 *vs*. 12; $P < 0.01$),

less lifetime wins (0 vs. 1; $P < 0.01$), less lifetime places (2 *vs*. 4; $P < 0.01$), and had lower lifetime stake earnings (ZAR 29,720 *vs*. 53,050; $P < 0.01$); while racehorses with grade 3 PLH were also younger (3 *vs*. 4 years; $P < 0.01$), had less lifetime starts (4 *vs*. 12; $P < 0.01$), less lifetime wins (0 *vs*. 1; $P < 0.01$), less lifetime places (1 *vs*. 4; $P < 0.01$) and had lower lifetime stake earnings (ZAR 5,000 *vs*. 53,030; $P < 0.01$). Those racehorses with grade 2 PLH had more lifetime places (2 *vs*. 1; $P < 0.01$) and higher lifetime stake earnings (ZAR 29,720 *vs*. 5,000; $P = 0.03$) compared to racehorses with grade 3 PLH. Because of the significant difference in lifetime starts it was suspected that this may be confounding lifetime wins and lifetime places. Multiple least squares regression was therefore performed using lifetime wins or lifetime places as a Y-dependent variable and lifetime starts and PLH grades as independent variables. The relationship between lifetime wins or lifetime places and the various grades of PLH was then shown to be insignificant ($P > 0.05$), while there was still a significant relationship ($P < 0.01$) between lifetime starts and lifetime wins or lifetime places. The decreased lifetime wins and places in racehorses with PLH are probably therefore due to less lifetime starts.

Epiglottic entrapment was detected in 13 racehorses (1.29%, 95% CI: 0.59 to 2%) consisting of 3 geldings, 4 males and 6 females (Figure 3.13). No significant differences were observed between racehorses with epiglottic entrapment and horses without for age ($P = 0.62\%$), sex ($P = 0.1$), lifetime starts ($P = 0.54$), lifetime wins ($P = 0.6$) and lifetime places ($P = 0.38$). However, horses with epiglottic entrapment had higher lifetime stake earnings compared to those unaffected (ZAR 78,650 *vs*. 35,885; $P = 0.046$). Two of the thirteen racehorses (males) had epiglottic entrapment (of which 1 was intermittent) and a

subepiglottic cyst concurrently (Figure 3.14), while one racehorse (gelding) had an epiglottis that was entrapped intermittently and malformed. One racehorse each finished in 5th, 7th, 8th, 9th and 11th place, while two horses finished in 2nd, 3rd, 6th and 10th place each in the race preceding endoscopic examination.

Epiglottic deformity was detected in 6 racehorses (0.6%, 95% CI: 0.12 to 1.08%) consisting of 4 geldings, 1 male and 1 female and had a sex distribution that was not significantly different to the other racehorses examined (P = 0.66) (Figure 3.15). Racehorses with epiglottic deformity were older (5 *vs*. 4 years; P = 0.01), had higher lifetime stake earnings (ZAR 87,650 *vs*. 36,000; $P < 0.01$), more lifetime starts (20 *vs*. 8; $P < 0.01$), more lifetime wins (2.5 *vs*. 1; $P = 0.009$) and more lifetime places (6 *vs*. 2; $P = 0.03$). One racehorse finished in 2nd and 8th, and two in 4th and 6th place each in the race prior to endoscopic examination. Because of the significant difference in lifetime starts it was suspected that this may be confounding lifetime wins and lifetime places. Multiple least squares regression was therefore performed using lifetime wins or lifetime places as a Y-dependent variable and starts and epiglottic deformity as independent variables. The relationship between lifetime wins or lifetime places and epiglottic deformity was then shown to be insignificant ($P > 0.05$), while there was still a significant relationship ($P < 0.01$) between starts and wins or places. The increased lifetime wins and places in horses with epiglottic deformity are probably therefore due to more lifetime starts and not the epiglottic deformity itself.

Racehorses with tracheal mucous were graded 0 (n = 5), 1 (n = 412), 2 (n = 291), 3 (n = 164), 4 (n = 71) and 5 (n = 58), while no grade could be allocated in 4 racehorses due to the large volume of blood present in the trachea. Compared to grade 2 tracheal mucous, racehorses with grade 3 tracheal mucous had more lifetime wins (1 *vs*. 1; $P = 0.02$), more lifetime places (3 *vs*. 2; $P = 0.01$) and higher lifetime stake earnings (ZAR 47,880 *vs*. 33,225; $P = 0.01$) while horses with grade 4 tracheal mucous had more lifetime starts (10 *vs*. 7; $P = 0.046$).

Tracheal debris was detected in 220 racehorses (21.91%, 95%CI: 19.02 to 24.81%) consisting of 117 geldings, 38 males and 65 females with a sex distribution that was not significantly different to unaffected racehorses ($P = 0.11$). There were no significant differences between affected and unaffected racehorses for median age ($P = 0.91$), lifetime stake earnings ($P = 0.84$), lifetime starts ($P = 0.73$), lifetime wins ($P = 0.76$) and lifetime places ($P = 0.74$).

Laryngeal debris was detected in 437 racehorses (43.53%, 95% CI: 39.44 to 47.61%) consisting of 223 geldings, 59 males and 155 females with a sex distribution that was not significantly different to unaffected racehorses ($P = 0.14$). There were no significant differences for racehorses with laryngeal debris and those without for median age ($P = 0.28$), lifetime stake earnings ($P = 0.43$), lifetime starts ($P = 0.57$), lifetime wins ($P = 0.87$) and lifetime places ($P = 0.35$).

Tracheal cartilage spikes were detected in 68 racehorses (6.77%, 95% CI: 5.16 to 8.37%) consisting of 10 geldings, 53 males and 5 females with a sex distribution that was significantly different to unaffected racehorses ($P < 0.01$) (Figure 3.16). Affected racehorses were nearly 4 times more likely to be males (relative risk = 3.63, 95% CI: 2.07 to 6.35); and had more lifetime wins (1 *vs*. 1; $P = 0.03$) and more lifetime places (4 *vs*. 2; $P = 0.03$). There was no significant differences for racehorses with tracheal cartilage spikes and those without for median age ($P = 0.82$), lifetime stake earnings ($P = 0.06$) and lifetime starts ($P = 0.13$).

One racehorse (gelding) had tracheal stenosis and finished in 4th place, two racehorses had subepiglottic cysts consisting of 2 males that finished in 2nd and 8th place; while DDSP was identified in two racehorses consisting of 1 male and 1 female that finished in 5th and 7th place. Statistical analysis for performance could not be performed for these conditions as the number of affected racehorses was too low.

3.5 DISCUSSION

This study is the first to report the prevalence of pharyngeal, laryngeal and tracheal disorders in a natural population of high quality Thoroughbred racehorses in South Africa. Overall, there was a low prevalence of grade 2 and 3 arytenoid cartilage asymmetry, ILH, epiglottic entrapment, subepiglottic cyst and epiglottic deformity; while more severe grades of PLH, laryngeal debris, tracheal debris, tracheal mucous and tracheal cartilage ring spikes had a higher prevalence. Arytenoid cartilage asymmetry (grade 2 and 3), ILH and epiglottic deformity did not result in impaired racing performance but these horses had significantly more lifetime starts. Racehorses with grade 3 PLH were more likely to be younger and have impaired lifetime racing performance. The presence of tracheal and laryngeal dirt and epiglottic entrapment did not modify racing performance. Furthermore, racehorses with tracheal cartilage ring spikes (which were more prevalent in males) and tracheal mucous (grade 3) had better lifetime racing performance.

3.5.1 The prevalence of pharyngeal, laryngeal and tracheal disorders

Thoroughbred racehorses may have an endoscopic examination performed of the upper respiratory tract at different stages of life. Disorders may be identified during pre- or post sale endoscopic examination as a yearling,[26] or later in life when presenting for respiratory disease. Although studies have identified upper respiratory tract disorders in foals[17] and yearlings,[1,2,18] extrapolation of such results to more mature horses may be

inaccurate as was reported with laryngeal asymmetry detected in foals[17] and yearlings[1] versus older horses.[1,17] While these reports[1,2,17,18] are useful, data may not be applicable to racehorses. Poor performance, stridor or a combination of the aforementioned may be reasons for endoscopic examination in racehorses.[14,19,20] Such surveys of horses suffer from a selection bias and may not accurately reflect the true prevalence of upper respiratory tract disorders in a natural population of racehorses. Also, no association was made between upper respiratory tract disorders and athletic performance. Reports do exist on the prevalence of respiratory tract disorders in horses,[7,21,23,27] however only one study has made an association between athletic performance and upper respiratory tract disorders[7] but excluded PLH and tracheal disorders.

Grade 2 and 3 arytenoid asymmetry was detected in 2.2% of racehorses in this study as compared with 3.8% of Thoroughbred racehorses competing in North America,[21] 1.4% of Thoroughbred racehorses in Australia,[7] and 47% Thoroughbred racehorses New Zealand.[1] This study compares favorably with the Australian report that used a similar grading scale for arytenoid asymmetry, also evaluated higher quality racehorses soon after racing (on average 32 minutes post-race).[7] Other studies have reported much higher prevalence of arytenoid asymmetry using similar grading criteria[1] while another did not define their criteria for arytenoid asymmetry.[4] Plausible reasons may exist for the wide variation in reported prevalence of arytenoid asymmetry and include variation in criteria for grading, experience of the grader, selection criteria for endoscopic examination, quality of the racehorses examined, timing of endoscopic examination and affect of locality.

ILH was detected in 0.6% of racehorses in this study as compared to 1.3% to 3.3% of Thoroughbred racehorses competing in North America,[21,23,27] 0.3% of Thoroughbred racehorses competing in Australia,[7] and 1% of Thoroughbred racehorses competing in New Zealand.[1] The low prevalence reported by this study and another,[7] may be due to the higher quality of racehorses examined and the fact that all the horses were pre-enrolled and not selected for endoscopic evaluation based on abnormal stridor or poor racing performance.

Epiglottic deformity was detected in 0.6% of racehorses in this study compared to 0.1% of Australian Thoroughbred racehorses.[7] A subepiglottic cyst was detected in 0.2% of racehorses in this study compared to 0.2% of North American[23] and 0.1% of Australian Thoroughbred racehorses.[7] Similarly, there was a low prevalence of epiglottic entrapment (1.3%) in this study compared to 0.74 to 2.1% of racehorses competing in North America[21,23,27] and 0.9% of racehorses competing in Australia.[7]

DDSP was detected in 0.2% of racehorses in this study compared to 0.74 to 5.2% of North American racehorses[15,23,27] and 0.5% of Australian racehorses.[7] The low prevalence of DDSP may have resulted form the conservative definition employed by this study and the fact that nasal occlusion was not performed as part of the endoscopic evaluation.

Together, grade 2 to 4 PLH was detected in 63% of racehorses competing in this study compared to an overall prevalence of 29.5% and 34.2 % of Thoroughbred racehorses

competing in North America.[23,27] The prevalence of PLH reported in this study is difficult to compare with previous studies due to the use of different grading criteria.[15,23,27] However, similar to previous studies that found an association between age and PLH,[15,23,27] this study also confirmed that younger racehorses were affected by more severe grades of PLH (grade 3).

Laryngeal debris was detected in 43.5% of racehorses in this study compared to 1.3% of racehorses competing in North America,[21] while tracheal debris was present in 21.9% of South African racehorses compared to 0.4 to 0.9% of North American racehorses.[21,23] Debris may be caused either by inhaled sand or dirt during racing and their presence within the respiratory tract may be dependant on degree of mucociliary clearance. The higher prevalence of debris as reported in this study may be due to endoscopic examinations that were performed soon after racing before effective mucociliary clearance occurred and by the use of more advanced videoendoscopy equipment that aided detection of debris.

Although tracheal cartilage ring spikes have been noted to occur,[24] their prevalence in a natural population of Thoroughbred racehorses has not been previously reported to the author's best knowledge. These spikes are epithelium-covered cartilages which protrude into the tracheal lumen from the tracheal ventrum.[24] Tracheal cartilage spikes were detected in 6.8% of racehorses in this study and occurred more often in male racehorses. The clinical significance of this condition and why it occurs more commonly in males is still unclear.

Tracheal mucous was detected in 99.5% of racehorses in this study compared to 6 to 6.8% of Thoroughbred racehorses competing in North America.[21,23] Another study performed 1,900 endoscopic examinations on racehorses ≥ 24 hours post-race and detected tracheal mucous in 41.3% of racehorses.[15] This study examined horses on average 24 minutes after racing compared to evaluations performed much later in racehorses at rest.[15] The observed differences in prevalence of tracheal mucous may be due to a close temporal relationship between endoscopic examination and racing.

3.5.2 Association with athletic performance

Upper respiratory tract disorders may affect the athletic performance of horses. Surveys of horses with upper airway disorders have demonstrated that DDSP[12] and ILH[8,10,16] is associated with impaired performance, mucosal erosions had no effect on performance,[7] while epiglottic entrapment and grade 2 arytenoid cartilage asymmetry was associated with enhanced performance.[7] Plausible reasons exist why DDSP and ILH may impair athletic performance such as reduced oxygen consumption,[16] while it is unclear what the reason may be for enhanced performance seen in epiglottic entrapment.

In this study, the apparent superior performance with grade 2 arytenoid cartilage asymmetry and ILH could be partially explained by its association with the number of lifetime starts, which was acting as a confounder. So while it appeared that horses with grade 2 arytenoid cartilage asymmetry and ILH had better racing performance due to more lifetime wins and/or lifetime places, and higher lifetime stake earnings, this was

probably a function of the lifetime starts. This raises the question of whether presence of arytenoid asymmetry and ILH is related to the frequency of racing, with horses with more lifetime starts being at higher risk. Our findings agree with a previous report that found grade 2 arytenoid cartilage asymmetry did not impair performance[7] as this degree of asymmetry still allows maximal arytenoid abduction[22] and is not associated with hypoxaemia.[8] Numerous studies have reported a negative association between ILH and racing performance based on impaired gaseous exchange in horses exercised on a treadmill[8,16] and track.[6] However, these studies suggest that race performance may not be adversely affected since no effect of ILH on run time was reported.[6,16] This study is in agreement with previous studies that ILH was not associated with impaired race performance[6,16] but the reason for this in light of the confounding effect of lifetime starts is now debatable.

Racehorses with PLH (grade 2 and 3) had apparent impaired racing performance. Affected racehorses had fewer lifetime starts, fewer lifetime wins, fewer lifetime places, lower lifetime stake earnings and were younger. Within the different grades of PLH, superior racing performance was seen in racehorses with grade 2 compared to grade 3 PLH, due to more lifetime places and higher lifetime stake earnings. However, similar to arytenoid asymmetry, lifetime starts was having a confounding effect and the apparent effect on performance was probably as a result in differences in the number of lifetime starts rather than the presence of the disorder. This would be more consistent with previous studies that have reported no association between PLH and racing performance.[3,13,15]

Epiglottic deformity also appeared to be associated with superior racing performance as these horses were older, had higher lifetime stake earnings, more lifetime starts, more lifetime wins, and more lifetime places. However, once again lifetime starts was acting as a confounder making the result difficult to interpret. Causes of epiglottic deformity are varied and may have congenital, inflammatory or traumatic origins, or develop secondary to chronic entrapment by the aryepiglottic fold or soft palate. Previous studies have reported epiglottic deformity but were unable to make an association with performance due to inadequate statistical power[7] and selection criteria.[28]

Superior lifetime racing performance was seen in racehorses with tracheal cartilage spikes as affected racehorses had more lifetime wins and more lifetime places. In this case, lifetime starts did not appear to be a confounder. It is uncertain as to how enhanced racing performance is related to the presence of tracheal cartilage spikes.

Epiglottic entrapment was not associated with impaired racing performance. Although lifetime starts, lifetime wins, and lifetime places were similar between affected and unaffected racehorses, horses with epiglottic entrapment had higher lifetime earnings. This study is in agreement with another study that suggested epiglottic entrapment does not impair performance.[7] This is perhaps not surprising since previous reports could not document impaired gaseous exchange[16] nor changes in upper airway pressures (despite medical or surgical intervention) in horses with epiglottic entrapment.[29]

Tracheal mucous (grade 3) was associated with superior performance as affected racehorses had more lifetime wins, more lifetime places and higher lifetime earnings. Moreover, horses with grade 4 tracheal mucous had more lifetime starts. This is surprising since increased tracheal mucous is associated with exercise-induced arterial hypoxemia following a standardized treadmill test[9,25] indicating that tracheal mucous may be associated with reduced performance. However, the presence of tracheal mucous was not found to affect treadmill performance in racehorses after assessment of total run time, peak running speed, speed at a heart rate of 200 beats/minute and distance to fatigue.[9] Another study has reported that more severe accumulations of mucous in racehorses was associated with a higher race finish position and thus poor racing performance, however did not include detailed racing performance results and evaluated racehorses ≥ 24 hours post race.[15] The reason for the superior performance reported by this study may again be related to lifetime starts although this is less clear in this case. Our results may also have been affected by examining higher quality racehorses with improved fitness levels and the examination of racehorses soon after racing.

Due to the low prevalence of DDSP, tracheal stenosis and subepiglottic cysts, the relationship between the disorder and racing performance could not be made. It should be noted that although resting endoscopic examination as performed by this study may have only detected structural abnormalities within the respiratory tract, while dynamic or functional obstructive abnormalities can only be observed using high-speed treadmill examinations. Therefore, although the following abnormalities may have been present in the study population, this study could not identify disorders such as progressive laryngeal

hemiplegia, intermittent DDSP, pharyngeal collapse, tracheal collapse, epiglottic entrapment, epiglottic retroversion, axial deviation of the aryepiglottic folds and axial deviation of the vocal folds.

In Thoroughbred racehorses competing in South Africa, the prevalence of pharyngeal, laryngeal and tracheal disorders is varied. Racing performance was not impaired by the presence of epiglottic entrapment, and debris in the larynx and trachea. Superior racing performance was seen in horses with tracheal cartilage ring spikes and grade 3 tracheal mucous. This study also highlights the multifactorial nature of these conditions and that clinicians should be cautious to infer causality to a particular disorder.

3.6 CONCLUSIONS

This study identified left arytenoid asymmetry, ILH, epiglottic deformity, epiglottic entrapment, DDSP, PLH, laryngeal and tracheal dirt, tracheal mucous, and tracheal cartilage ring spikes in Thoroughbred racehorses post-race. An association with sex was identified as tracheal cartilage ring spikes occurred more often in male racehorses. Superior racing performance was identified in racehorses with grade 3 tracheal mucous and tracheal cartilage ring spikes.

3.7 FIGURES AND TABLES

Figure 3.1 Symmetrical abduction of the arytenoid cartilages in a Thoroughbred racehorse.

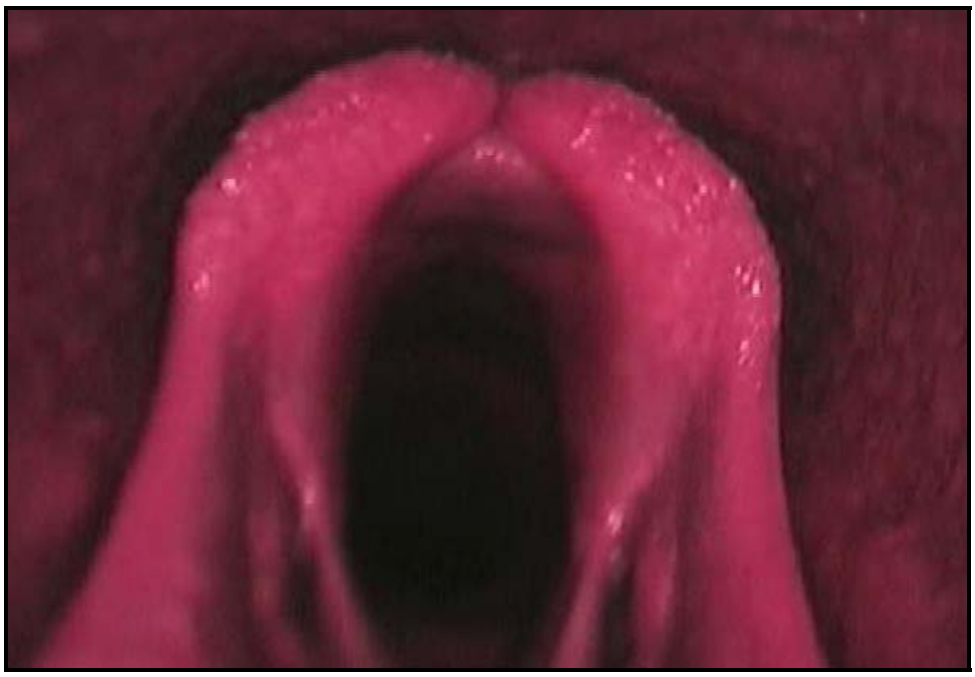


Figure 3.2 Idiopathic laryngeal hemiplegia in a Thoroughbred racehorse.

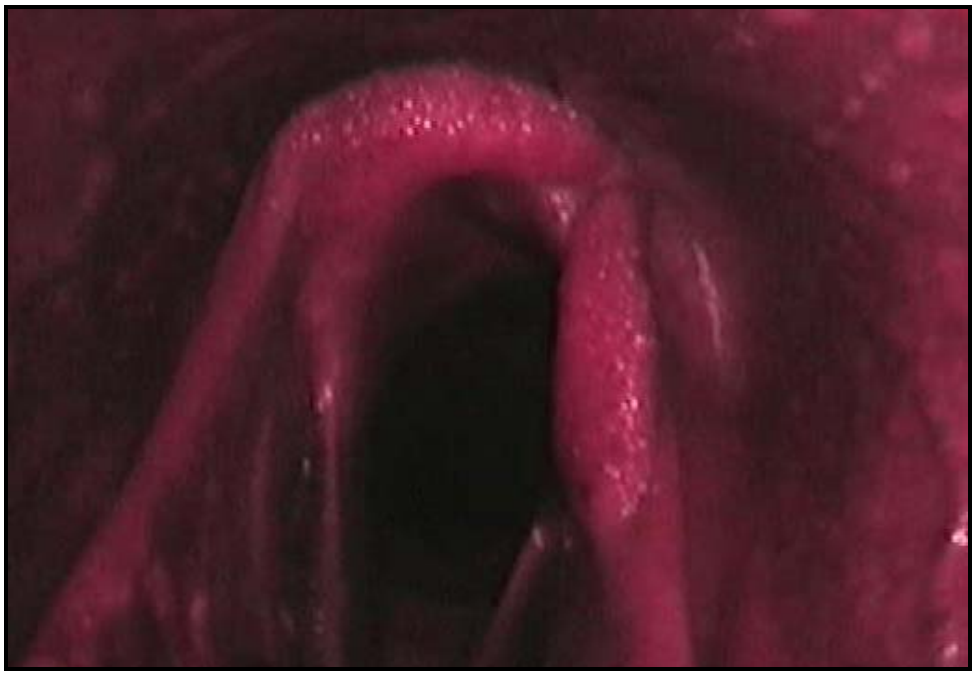


Figure 3.3 A Thoroughbred racehorse with grade 1 pharyngeal lymphoid hyperplasia.

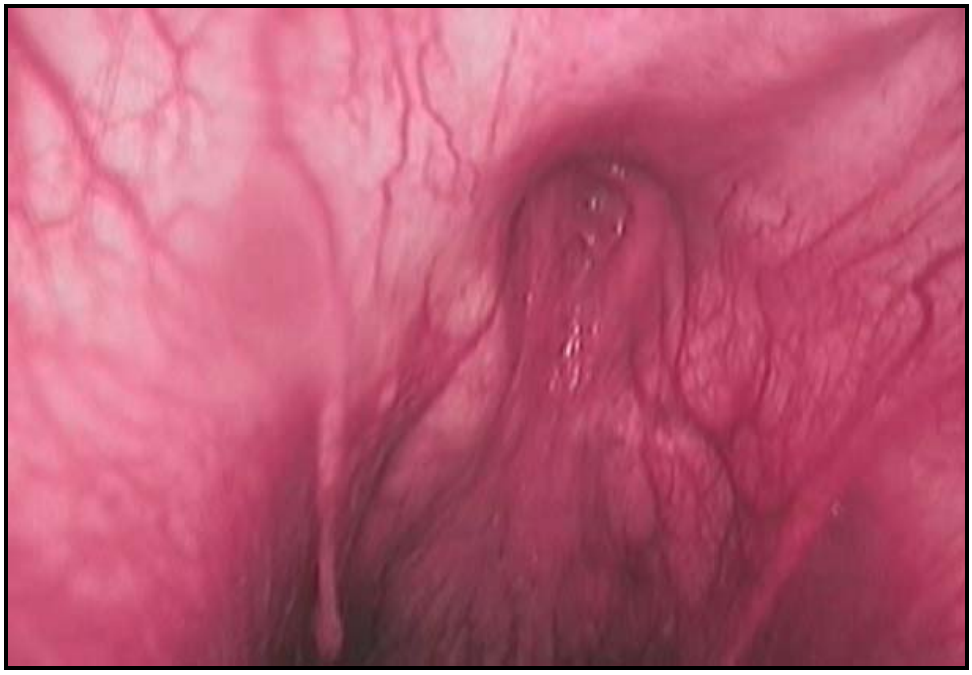


Figure 3.4 A Thoroughbred racehorse with grade 2 pharyngeal lymphoid hyperplasia.

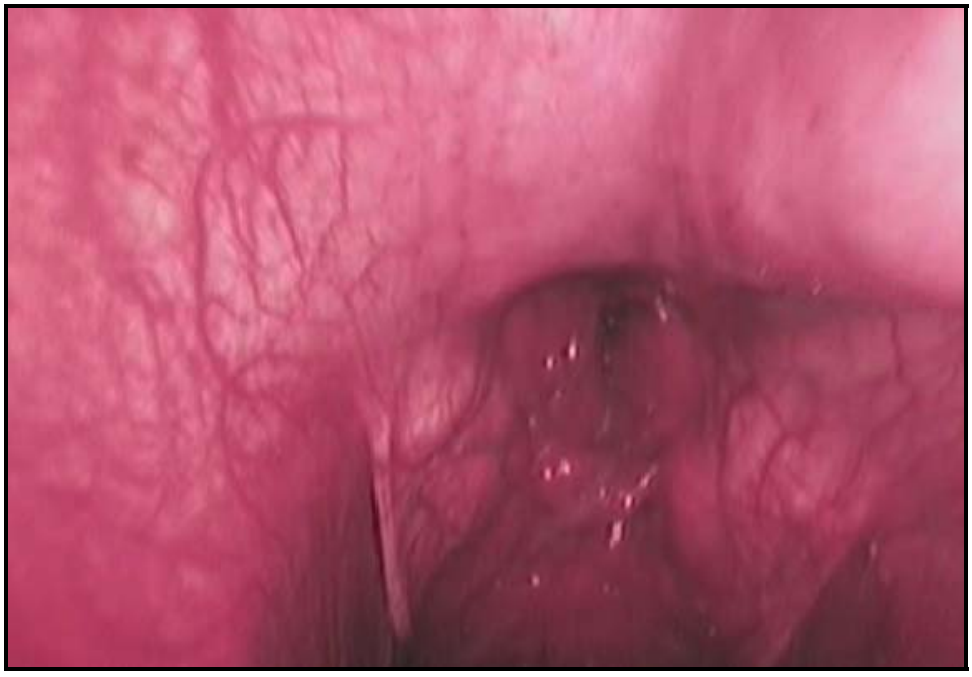


Figure 3.5 A Thoroughbred racehorse with grade 3 pharyngeal lymphoid hyperplasia.

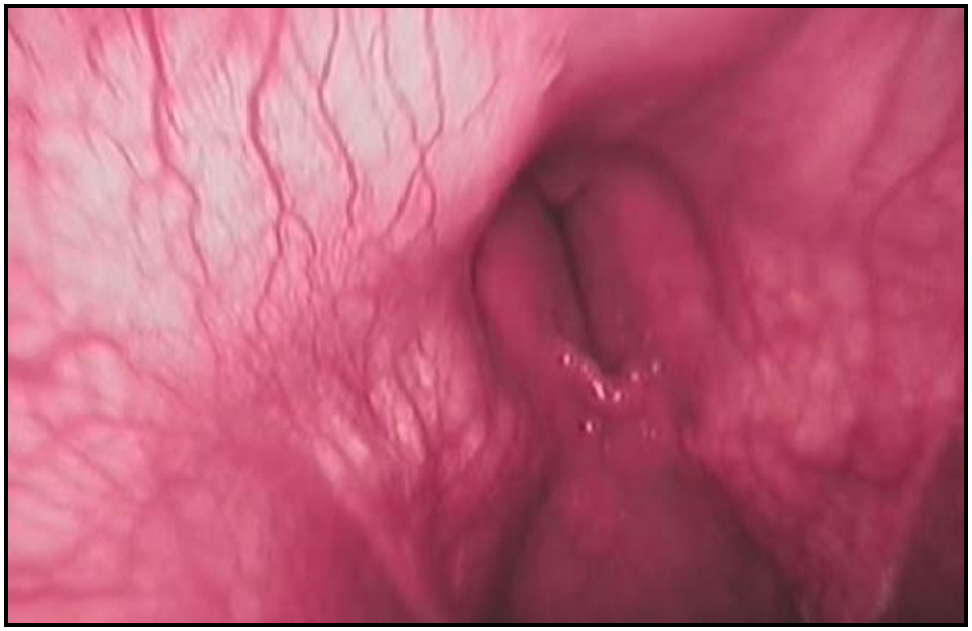


Figure 3.6 A Thoroughbred racehorse with grade 4 pharyngeal lymphoid hyperplasia.

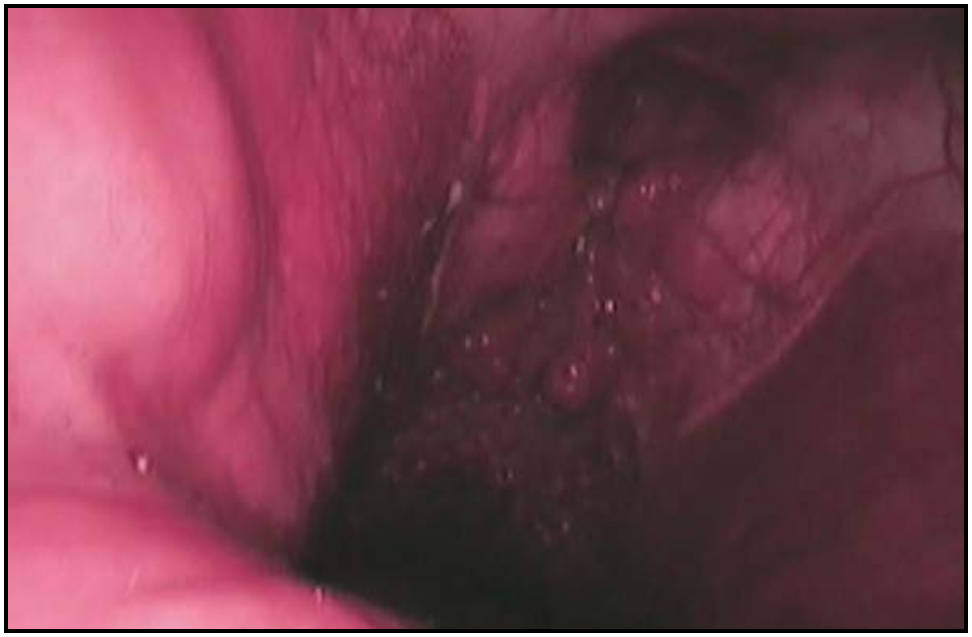


Figure 3.7 A Thoroughbred racehorse with dorsal displacement of the soft palate.

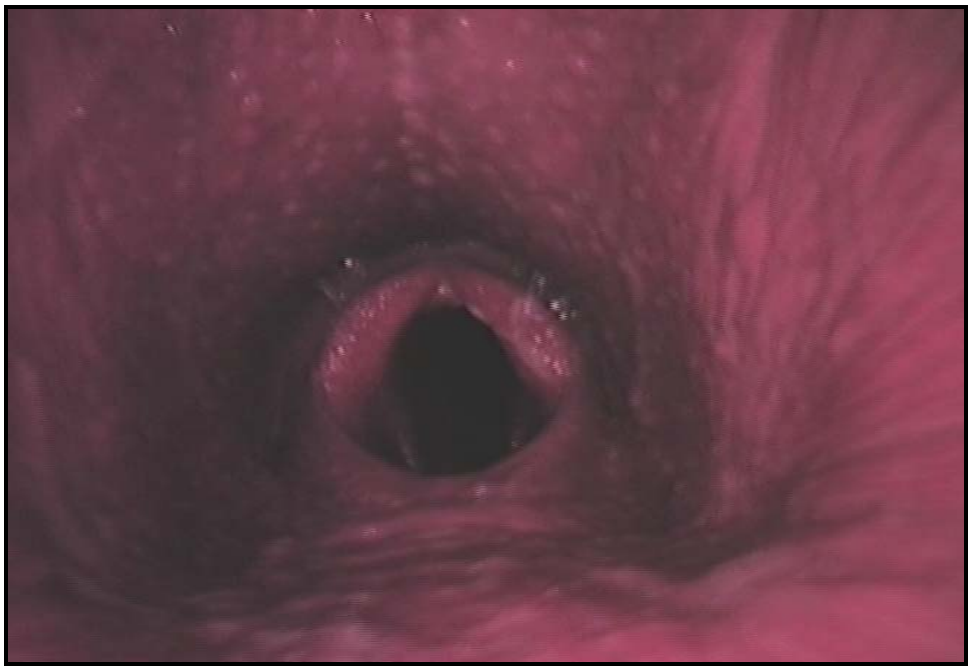


Figure 3.8 A Thoroughbred racehorse with grade 1 tracheal mucous detected by tracheobronchoscopy.

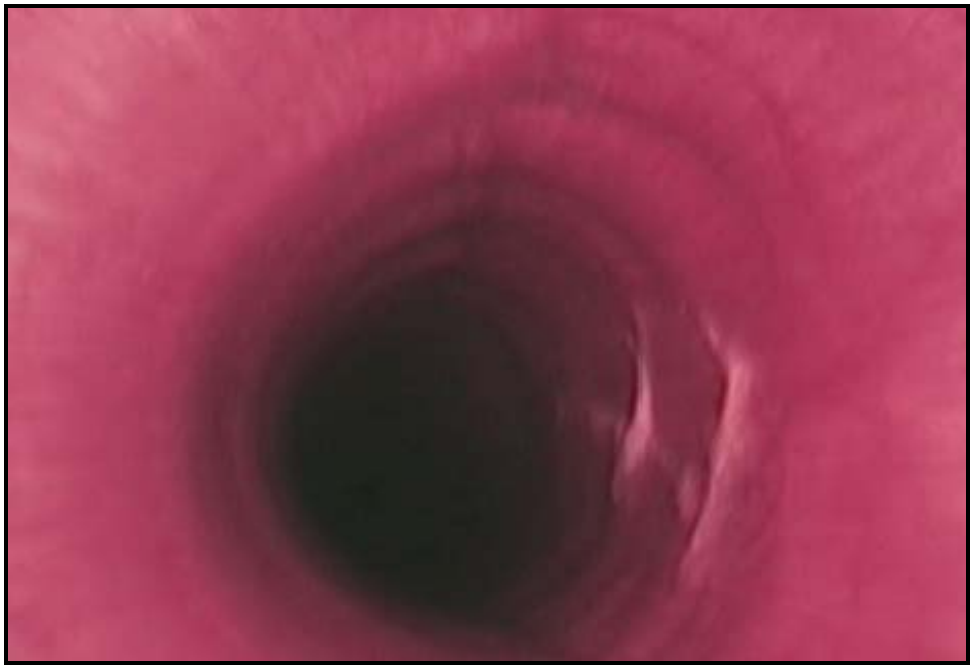


Figure 3.9 A Thoroughbred racehorse with grade 2 tracheal mucous as detected by tracheobronchoscopy.

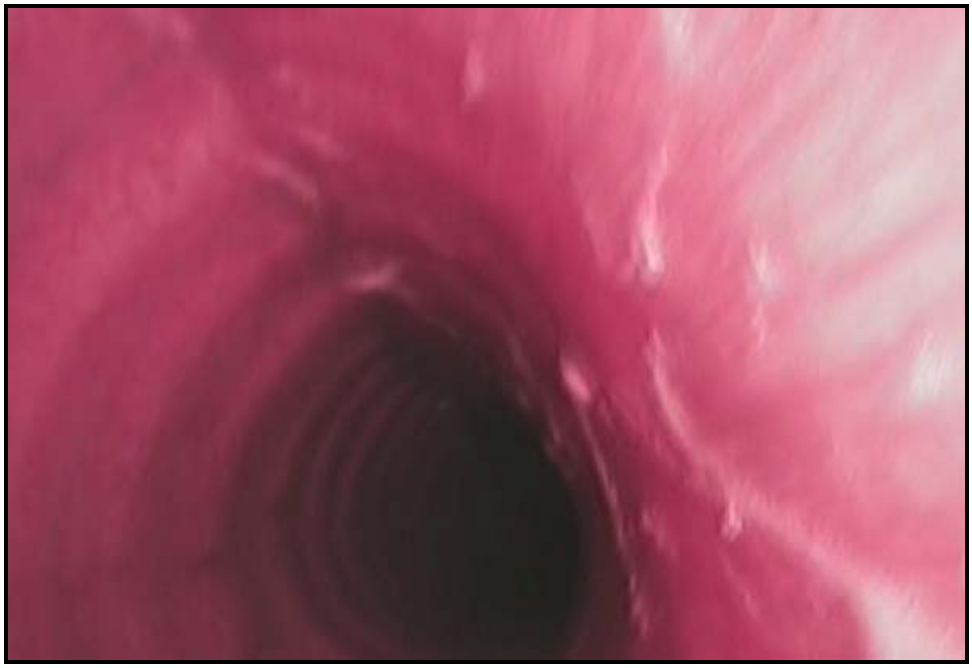


Figure 3.10 A Thoroughbred racehorse with grade 3 tracheal mucous as detected by tracheobronchoscopy.

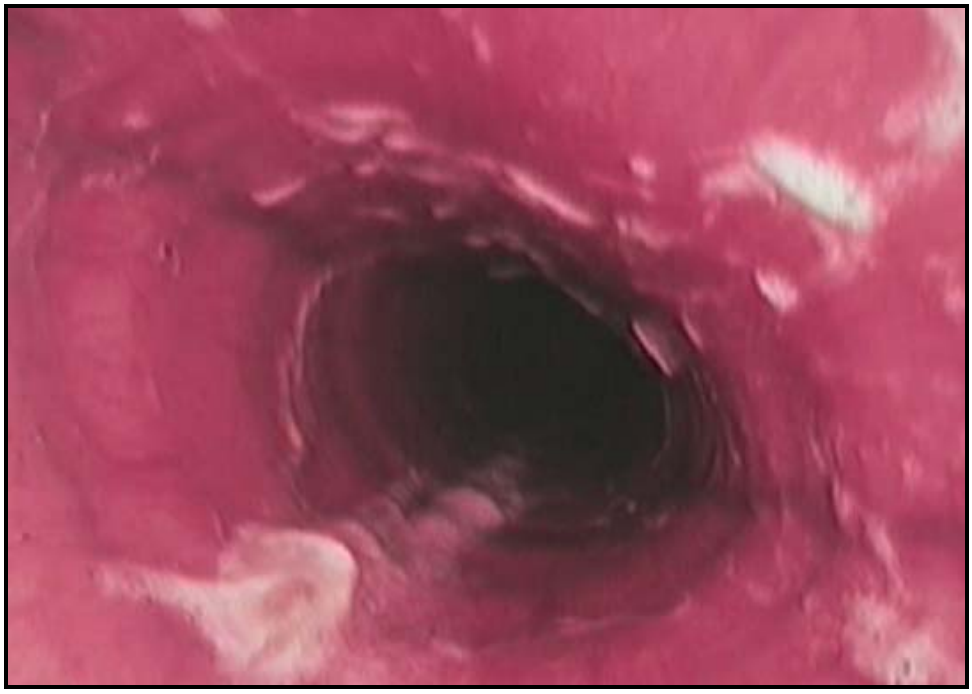


Figure 3.11 A Thoroughbred racehorse with grade 4 tracheal mucous as detected by tracheobronchoscopy.

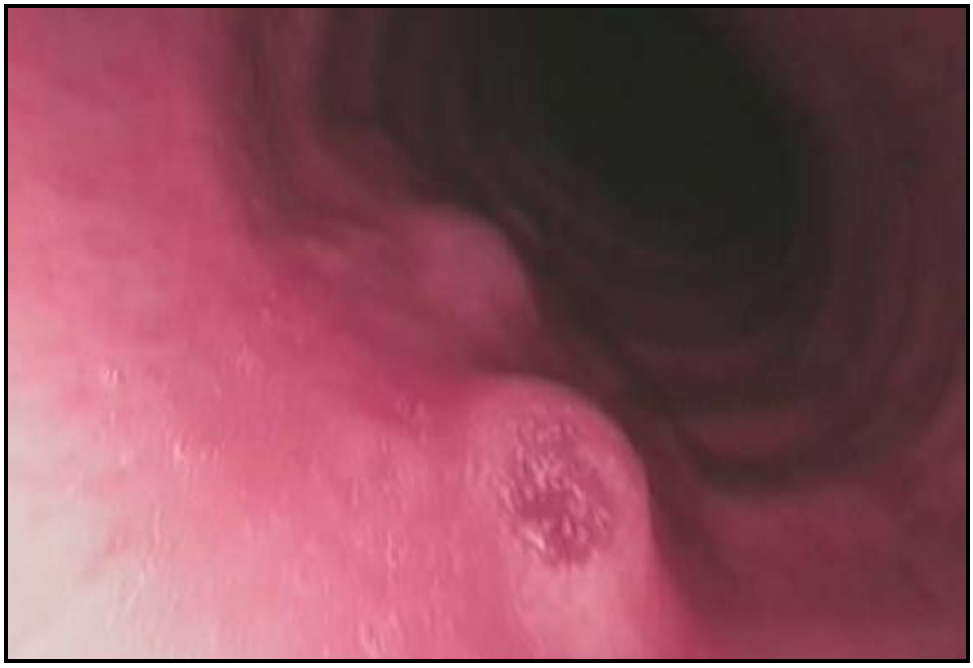


Figure 3.12 A Thoroughbred racehorse with grade 5 tracheal mucous as detected by tracheobronchoscopy.

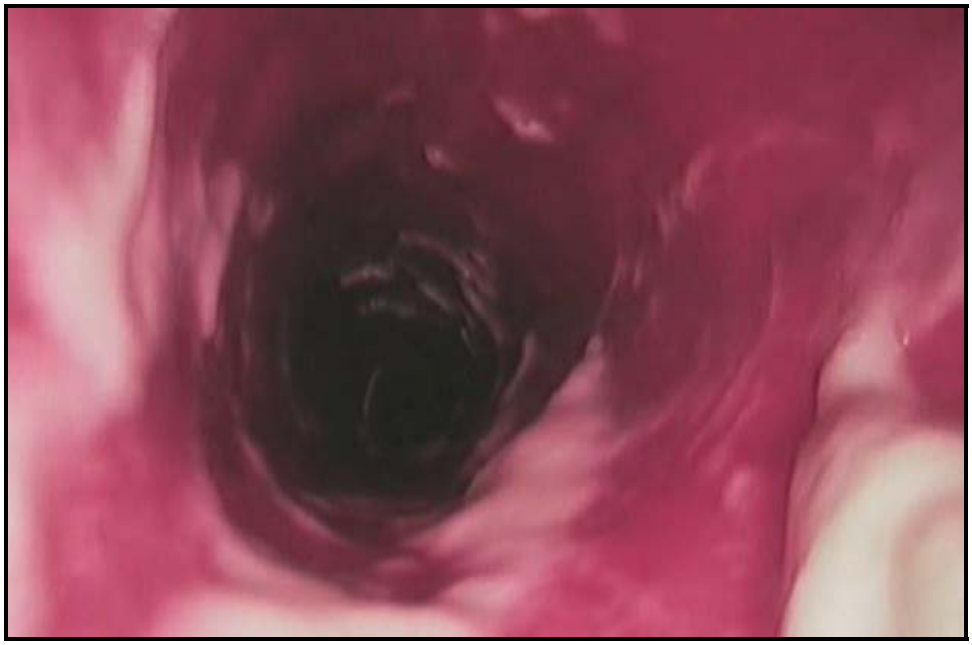


Figure 3.13 A Thoroughbred racehorse with epiglottic entrapment.

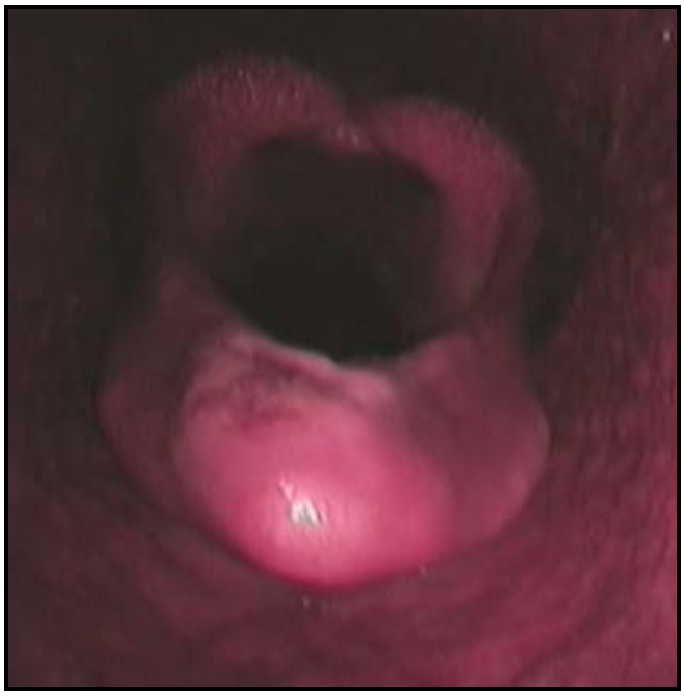


Figure 3.14 A Thoroughbred racehorse with epiglottic entrapment and a sub-epiglottic cyst.

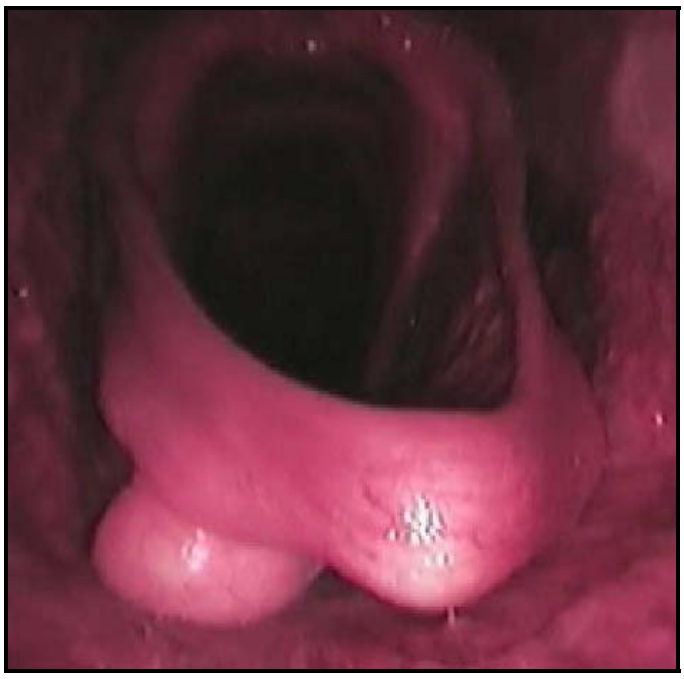


Figure 3.15 A Thoroughbred racehorse with epiglottic deformity.

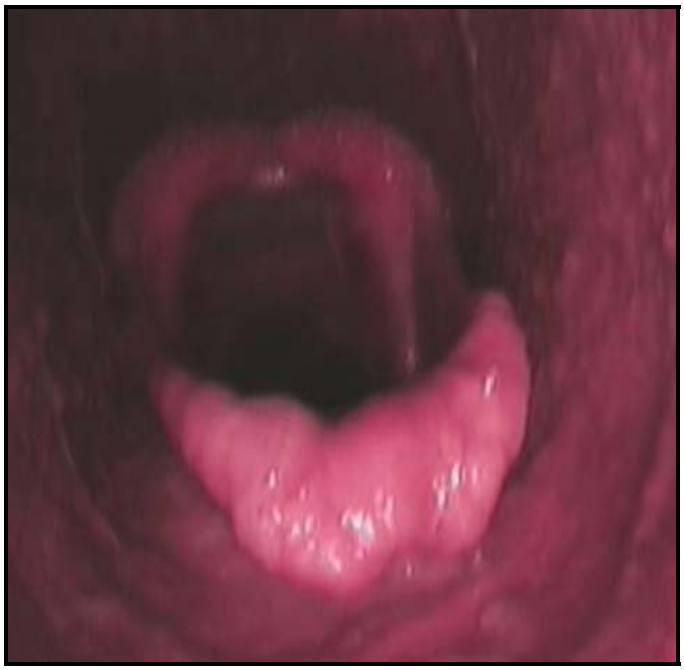
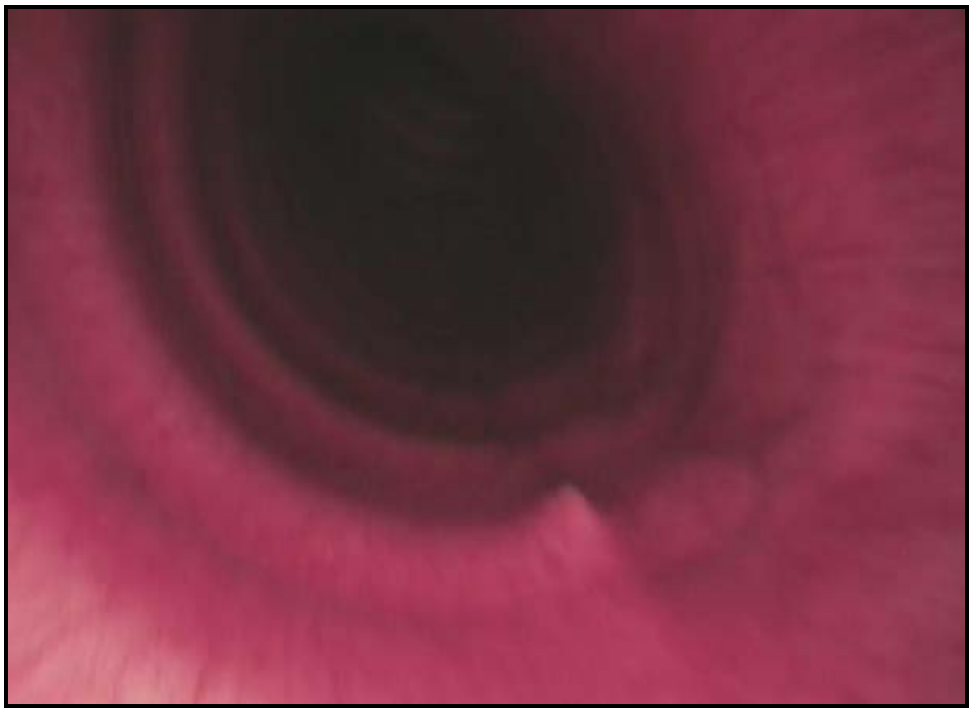


Figure 3.16 A Thoroughbred racehorse with a tracheal cartilage ring spike.



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Chapter 4 Reproducibility of endoscopic grading using tracheobronchoscopy in racehorses

4.1 ABSTRACT

We determined the interobserver reliability for the assessment of exercise-induced pulmonary haemorrhage (EIPH), pharyngeal lymphoid hyperplasia (PLH), arytenoid cartilage movement (ACM) and tracheal mucous (TM) following tracheobronchoscopic examination in 1,011 Thoroughbred racehorses. Tracheobronchoscopic examinations were performed on racehorses < 2 hours after racing and recorded onto digital video disc. Three veterinarians then assessed all recordings independently for the presence and severity of EIPH, PLH, ACM, and TM. All scores were tabulated and concordance was measured using the weighted κ statistic (κ_w).

The interobserver agreement was the highest for EIPH (κ_w = 0.78 to 0.84) and moderate for PLH (κ_w = 0.43 to 0.52), ACM (κ_w = 0.43 to 0.56) and TM (κ_w = 0.43 to 0.57). All observers agreed or 2 of 3 agreed and the third differed by ≤ 1 grade in 99.6% of observations for EIPH, 98.29% of observations for PLH, 100% of observations for ACM, and 91.67% of observations for TM.

Although the interobserver reliability of tracheobronchoscopic evaluation of EIPH was the highest as compared with PLH, ACM and TM; all four classifications are sufficiently reproducible when used by veterinarians performing tracheobronchoscopic examinations on horses.

4.2 INTRODUCTION

Risk factors for poor performance in Thoroughbred racehorses may include exercise-induced pulmonary haemorrhage (EIPH),[5] tracheal mucous (TM),[7] and idiopathic laryngeal hemiplegia (ILH).[8] Antigenic stimulation of the nasopharynx may cause localized inflammation of the nasopharynx resulting in pharyngeal lymphoid hyperplasia (PLH). Although PLH is not associated with impaired racing performance,[7] it may predispose to upper airway obstruction,[9] thereby negatively affecting performance.

Health and athleticism of racehorses may be affected by EIPH, TM, ILH and PLH and therefore reliable, repeatable methods of assessment are needed. Such methods should be able to accurately and quickly assess the severity of the condition and be able to monitor efficacy of treatment once therapy has started. Tracheobronchoscopy is a quick, minimally-invasive technique without laborious, time-consuming laboratory processing of samples that allows immediate classification of racehorses according to previously established grading systems for EIPH,[6] TM,[3] arytenoid cartilage movement (ACM)[12] and PLH.[1] Although the repeatability of interobserver reliability of tracheobronchoscopic

assessment of EIPH in Thoroughbred racehorses has been reported before,[6] no reports on the interobserver reliability for the detection of TM, ACM and PLH exist.

We sought to determine the interobserver reliability of tracheobronchoscopic assessment of the presence and severity of EIPH, PLH, ACM, and TM in Thoroughbred racehorses in South Africa.

4.3 MATERIALS AND METHODS

4.3.1 Thoroughbred racehorses

Tracheobronchoscopic examinations were performed on 1,011 Thoroughbred racehorses < 2 hours after racing at 5 race venues and in 28 race meets. Races were 800 to 3,200 meter flat races run on turf or sand at Turffontein (Gauteng Province) and Vaal (Free State Province) Race Courses; and at sea level at Clairwood and Greyville Turf Clubs (Kwazulu-Natal Province) and Kenilworth Race Course (Western Cape Province) in South Africa from August 4 to December 19, 2005.

4.3.2 Endoscopic examination

Following racing, unsedated racehorses were restrained by the use of a halter and nose twitch in a dedicated examination stable. Tracheobronchoscopic (Pentax Corporation, Tokyo, Japan) evaluation of the nasopharynx, larynx and trachea to the level of the carina

took place and all examinations were recorded onto digital video disc. All recordings were then independently reviewed by 3 veterinarians.

4.3.3 Grading of EIPH, ACM, PLH and TM

Racehorses were graded 0 to 4 for EIPH.[6] 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 (< $^1/_4$ 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).

Mucous within the trachea was graded 0 to 5.[3] Grade 0 indicated the absence of mucous; grade 1 indicated singular droplets of mucous (Figure 3.8); grade 2 indicated multiple droplets of mucous that is partly confluent (Figure 3.9); grade 3 indicated mucous that is ventrally confluent (Figure 3.10); grade 4 indicated a large ventral pool of mucous (Figure 3.11); and grade 5 indicated profuse amounts of mucous covering > 25% of the tracheal lumen (Figure 3.12).

The presence of ILH was assessed through severity of ACM and was graded 1 to 4.[12] Grade 1 indicated symmetrical synchronous abduction and adduction of the left and right arytenoid cartilages (Figure 3.1); grade 2 indicated some asynchronous movement (hesitation, flutter or abductor weakness) of the left arytenoid cartilage during any phase of respiration and full abduction of the left arytenoid cartilage which could be maintained by swallowing or nasal occlusion; grade 3 indicated asynchronous movement (hesitation, flutter or abductor weakness) of the left arytenoid cartilage during any phase of respiration and full abduction of the left arytenoid cartilage could not be induced or maintained by swallowing or nasal occlusion, and grade 4 indicated no substantial movement of the left arytenoid cartilage during any phase of respiration and were subsequently classified as having ILH (Figure 3.2).

The severity of PLH was graded on a scale from 1 to 4.[1] Grade 1 indicated lymphoid hyperplasia limited to < 180° of the dorsal pharyngeal recess (Figure 3.3); grade 2 indicated lymphoid hyperplasia extending to circumference of the dorsal pharyngeal recess (Figure 3.4); grade 3 indicated lymphoid hyperplasia made midline contact of the dorsal pharyngeal recess (Figure 3.5); and grade 4 indicated small masses (which may be abscesses) arising from either the dorsal pharyngeal recess or the pharyngeal walls (Figure 3.6).

4.3.4 Data analysis

Weighted kappa statistics (κ_w) were calculated for each combination of observers for the grading of EIPH, PLH, ACM, and TM. Partial agreement can be taken into account using a weighted kappa in which the pairs of test results that are close are considered to be in partial agreement through the use of a weight matrix. The weighted matrix used for the kappa statistic was one of the prerecorded matrixes (w) used by STATA (STATA Statistical Software [release 8]: STATA Corporation, College Station, Texas, USA).The weights are given by $1-|i-j| / (k-1)$, where i and j index the rows of columns of the ratings by the two raters and k is the maximum number of possible ratings. The weightings (agreement) used for EIPH and ACM if there was one rating apart was 0.75, two ratings apart was 0.5, three ratings apart was 0.25 and > three was 0. The weightings for PLH were 0.6667, 0.3333 and 0 for 1, 2 and 3 ratings apart respectively. The weightings for TM were 0.8, 0.6, 0.4, 0.2 and 0 for 1 to 5 ratings apart respectively. The strength of agreement was considered poor ($\kappa_w < 0.20$), fair ($\kappa_w = 0.21$ to 0.40), moderate ($\kappa_w = 0.41$ to 0.60), good ($\kappa_w = 0.61$ to 0.80) and very good ($\kappa_w = 0.81$ to 1.00).[9] Mean results with upper and lower 95% confidence interval (CI) are reported.

4.4 RESULTS

Good to very good interobserver reliability was reported for EIPH ($\kappa_w = 0.78$, [95% CI: 0.74 to 0.82]; $\kappa_w = 0.83$ [95% CI: 0.79 to 0.88]; and $\kappa_w = 0.84$ [95% CI: 0.80 to 0.88]). Agreement between the 3 reviewers was observed for 386 examinations as grade 0, 222

as grade 1, 50 as grade 2, 41 as grade 3, and 20 as grade 4. Complete agreement between the 3 observers was present in 71.1% of all examinations. Scores of 2 of 3 observers agreed and that of the third observer differed by ≥ 1 grade in 28% of examinations. All three observers disagreed in 0.4% (n = 4) of observations. All observers agreed or 2 of 3 agreed and the third observer differed by ≤ 1 grade in 99.6% of observations.

Moderate inter-observer reliability was reported for PLH (κ_w = 0.43 [95% CI: 0.37 to 0.48]; κ_w = 0.46 [95% CI: 0.41 to 0.51]; and κ_w = 0.52 [95% CI: 0.47 to 0.57]). Agreement between the 3 reviewers was observed for 233 examinations as grade 1, 310 examinations as grade 2, 6 examinations as grade 3 and 0 examinations as grade 4. Complete agreement between the 3 observers was present in 55.3% of all examinations. Scores of 2 of 3 reviewers agreed and that of the third reviewer differed by ≥ 1 grade in 42.9% of examinations. All three observers disagreed in 2.3% (n = 23) of observations. All observers agreed or 2 of 3 agreed and the third observer differed by ≤ 1 grade in 98.3% of examinations.

Inter-observer reliability for ACM was moderate (κ_w = 0.43, [95% CI: 0.38 to 0.48]; κ_w = 0.46 [95% CI: 0.41 to 0.51]); and κ_w = 0.56 [95% CI: 0.51 to 0.61]). Agreement between the 3 observers was observed for 952 examinations as grade 1, 1 examination as grade 2, 1 examination as grade 3, and 2 examinations as grade 4. Complete agreement between the 3 observers was observed for 95.5% of examinations. Scores of 2 of 3 observers agreed and that of the third observer differed by ≥ 1 grade in 4.5% of examinations. All

three observers disagreed in 0.2% (n = 2) of observations. All observers agreed or 2 of 3 agreed and the third observer differed by ≤ 1 grade in 100% of examinations.

Inter-observer reliability for TM was moderate (κ_w = 0.43, [95% CI: 0.39 to 0.47]; κ_w = 0.46 [95% CI: 0.42 to 0.50]; and κ_w = 0.57 [95% CI: 0.53 to 0.62]). Agreement between the 3 observers was observed for 187 examinations as grade 1, 66 examinations as grade 2, 70 examinations as grade 3, 12 examinations as grade 4 and 1 examination as grade 5. Complete agreement between the 3 observers was observed for 34.2% of examinations. Scores of 2 of 3 observers agreed and that of the third observer differed by ≥ 1 grade in 57.5% of examinations. All three observers disagreed in 9.2% (n = 90) of observations. All three observers agreed or 2 of 3 agreed and the third observer differed by ≤ 1 grade in 91.7% of examinations.

4.5 DISCUSSION

Tracheobronchoscopy offers the ability to quickly and accurately assess the upper and lower respiratory tract for EIPH, PLH, ACM and TM. Although previous investigators have utilized this technique, only one study reported on interobserver variability for assessment of the presence and severity of EIPH.[6] Interobserver variability may be affected by poor agreement between observers or lack of consistency within an individual observer. A highly reproducible and repeatable grading system would have great clinical and research applications. This would allow for more precise determination of the condition and be able to more accurately evaluate response to treatment.

Interobserver agreement was highest for classification of EIPH ($\kappa_w > 0.77$) as compared to a previous report that used a similar EIPH grade scale ($\kappa_w > 0.74$).[6] Lower interobserver agreements were seen with the PLH ($\kappa_w > 0.42$), ACM ($\kappa_w > 0.42$) and TM ($\kappa_w > 0.42$) grade scales. The magnitude of kappa is influenced by the extent of the agreement as well as by the prevalence of the condition. When the prevalence is very high or very low (outside the range of 0.2 to 0.8), the κ statistic becomes unstable and is difficult to interpret .[4] Since the prevalence of EIPH, PLH, ACM and TM was high in this study, especially at low grades, the κ statistic needs to be interpreted with this in mind. In addition the weighting matrix used will influence the final kappa statistic and this has not always been clearly specified in previous publications, which may explain slight differences between papers.

The observed proportion (OP) of agreement between 2 or more observers (that is the proportion of observations that the observers agree upon) in this study, differed by 1 grade or less in > 99%, > 98%, 100% and > 91% of examinations for EIPH, PLH, ACM and TM respectively, indicating good concordance using these grading systems. Despite the OP of agreement, between 2 or more observers being high for PLH, ACM and TM, weighted kappa was moderate and this may have been due to the high prevalence of the conditions and an over-representation of categories within each grade scale.

To fully evaluate association with performance, potential risk factors, and therapeutic interventions for EIPH, PLH, ACM and TM, grading systems which are reliable and repeatable are required. Quantification of EIPH has occurred in the past by

tracheobronchoscopic assessment and grading,[11] although the severity of EIPH may not be reflected by the grade allocation. Also, although no association has been proven, red cell counts in bronchoalveolar lavage fluid have been used to assess EIPH severity.[10]

Tracheobronchoscopy is a quick, safe, minimally invasive technique that may be performed on unsedated racehorses. It is a practical screening technique that may have prognosticative validity and clinical dependability and that would allow assessment of upper and lower respiratory tract of a large number of racehorses in field conditions. In this study, despite two of 3 reviewers being less experienced, excellent interobserver reliability was seen using the EIPH grading system[6] similar to a previous report that used three experienced observers.[6] Although the weighted kappa was lower for PLH, ACM and TM, this study demonstrated sufficient reliability to allow the use of the EIPH, PLH, ACM and TM grading system by veterinarians with limited experience and still achieve satisfactory clinical assessments.

4.6 CONCLUSIONS

Endoscopic grading of respiratory tract disorders is a relatively quick procedure which is easy to perform and eliminates the use of expensive time-consuming laboratory diagnostics. Moreover, it is a relatively safe diagnostic technique for both staff and racehorse. Using previously established grading criteria,[1,3,6,12] we demonstrated their reliability in the classification of EIPH, PLH, ACM and TM in racehorses competing in South Africa.

4.7 FIGURES AND TABLES

Figure 4.1 The portable flexible videoendoscopy system used in the grading of respiratory tract disorders in South African Thoroughbred racehorses.

4.8 REFERENCES

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Chapter 5 Proinflammatory mRNA response in racehorses with exercise-induced pulmonary haemorrhage

5.1 ABSTRACT

Exercise-induced pulmonary haemorrhage (EIPH) affects racehorses worldwide and may be due to stress failure of pulmonary capillaries. EIPH causes pulmonary neutrophilia and may be similar to acute lung injury in humans where neutrophil-mediated injury causes proinflammatory cytokine production. In an attempt to better understand the immunopathogenesis of EIPH, we designed a prospective, cross-sectional study of pre-enrolled Thoroughbred racehorses competing in flat races at high altitude (> 1,400 meters 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 hours after racing, the presence and severity of EIPH was graded 0 to 4 and venous blood was collected from 10 horses in each grade classification. Following RNA isolation and cDNA synthesis, real-time PCR was used to detect equine cytokine-specific mRNA for interleukin (IL) -1, -6, -10, interferon (INF) -γ, and tumor necrosis factor (TNF) -α. Overall, there was significantly greater expression of IL-6 and -10 within the different grades of EIPH ($P < 0.05$). Racehorses with a grade 4 versus 0, 1 and 2 EIPH expressed

increased IL-6 mRNA ($P < 0.05$), while greater IL-10 mRNA expression was present in horses with grade 3 versus 0 and 2 EIPH ($P < 0.05$). Overall, there was greater expression of IL-6 mRNA at sea level ($P = 0.009$) and TNF-α mRNA at altitude ($P = 0.005$). Although, it is unclear whether the inflammatory response observed in the study was due to pre-existing pulmonary inflammation or as a direct consequence of pulmonary bleeding, this study demonstrates a systemic correlation to pulmonary inflammation.

5.2 INTRODUCTION

Exercise-induced pulmonary hemorrhage (EIPH) is a worldwide phenomenon in Thoroughbred racehorses undergoing strenuous exercise with a reported prevalence of 43% to 75.4%.[38,39,42] No precise mechanism has been identified that can account for the site of occurrence and progression of EIPH within the lung; however pulmonary hypertension with secondary stress failure of pulmonary capillaries has been implicated.[48] EIPH is definitively diagnosed by post-exercise endoscopic examination of the upper respiratory tract and detection of blood in the trachea. Tracheal aspirates may reveal red cells and haemosiderophages while bronchoalveolar lavage may be used to quantify EIPH by measurement of red cell concentration.

Pulmonary inflammation is often seen in racehorses with EIPH.[33] This may be due to either pre-existing small airway disease[31] or due to the presence of blood in the airways.[30] Inflammatory chemical mediators may be intimately involved in airway inflammation.[40] We hypothesized that EIPH may be similar to acute lung injury in humans where

following post-traumatic haemorrhage, neutrophil-mediated injury may lead to local up-regulation of proinflammatory cytokines within the lungs. The parechymal lung inflammation may damage the alveolocapillary barrier leading to a systemic inflammatory response.

Because the determination of an association between EIPH and inflammation at a molecular level may assist in the development of preventative strategies aimed at reducing the prevalence and severity of this condition, we sought to measure interleukin (IL) -1, -6, -10, interferon (INF) -γ, and tumor necrosis factor (TNF) -α gene expression in a natural population of racehorses with EIPH immediately after racing in a racing jurisdiction that does not permit race day administration of furosemide nor nasal dilator strips, and in which horses race at both sea level and at high altitude (> 1,400 meters above sea level).

5.3 MATERIALS AND METHODS

5.3.1 Thoroughbred racehorses

The study was a cross-sectional study of a sample of Thoroughbred racehorses competing at Turffontein Race Course (Gauteng Province), Vaal Race Course (Free State Province), Clairwood and Greyville Turf Club (Kwazulu-Natal Province) and Kenilworth Race Course (Western Cape Province), in South Africa. Thoroughbred racehorses of either sex, running on turf or sand, competing in flat races were enrolled into the study between

August 4 and December 19, 2005. 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. Lists of available horses that were accepted to race were obtained from the NHRA. Eligible racehorses were then identified, trainers contacted individually and permission sought to examine the horse and draw blood. Only pre-enrolled horses (that is prior to race day) were entered into the study to avoid a potential enrollment bias.

5.3.2 Tracheobronchoscopy and sample collection

Tracheobronchoscopic evaluation was performed within 2 hours after racing on unsedated racehorses for evidence of EIPH using an endoscope (Pentax Corporation, Tokyo, Japan) that was passed through one of the nares, nasopharynx, larynx, to the level of the tracheal bifurcation. The severity of EIPH was immediately graded by one examiner according to a previously established grading system from 0 to 4[15] with grade 0 indicating the absence of blood in the pharynx, larynx, trachea, or mainstem bronchi; grade 1 indicating the presence of 1 or more flecks of blood or ≤ 2 short (< $^1/_4$ 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 indicating a 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 indicating 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

indicating multiple, coalescing streams of blood covering > 90% of the tracheal surface with blood pooling at the thoracic inlet (Figure 2.4).

Following allocation of a specific EIPH grade to each horse, 2.5 ml of venous blood was collected by routine jugular venipuncture from 10 horses in each EIPH grade classification (grade 0 to 4) directly into the Paxgene® RNA collection tubes (Qiagen, Valencia, CA) (Figure 5.1) within 2 hours after racing. Immediately following collection, the tubes were inverted 10 times to prevent coagulation that would hinder future extraction. The tubes were kept at room temperature overnight, and then stored at -20 °C until RNA extraction was performed.

5.3.3 RNA extraction and cDNA synthesis

Following thawing, cell pellets were isolated by centrifugation at 2,500 x *g* and RNA isolation carried out according to a modified manufacturer's protocol (Qiagen, Valencia, CA); after addition of Proteinase K, a 5 minute incubation period was added at room temperature before heating the samples to 55°C and the subsequent centrifugation was for 10 minutes at 16,000 x *g*. Total RNA was eluted in 40 ul RNase-free water (Figures 5.2 and 5.3) and then stored at -80 °C. Complementary DNA was synthesized according to the manufacturer's protocol (Qiagen, Valencia, CA).

5.3.4 Real-time polymerase chain reaction (real-time PCR)

Real-time PCR was performed on a 7500 Sequence Detection System machine (Applied Biosystems, Foster City, CA) (Figure 5.4). The five target genes of interest in this study were IL-1, -6, -10, TNF-α and IFN-γ. Applied Biosystems (Applied Biosystems, Foster City, CA) designed the primer and probe sequences for the cytokines and provided an Assay-by-Design (Applied Biosystems, Foster City, CA) kit containing both the designed primer and probe in solution (Table 5.1). In order to allow for potential variability in sample processing, the expression of the genes of interest were initially compared to β-glucuronidase (β-GUS). This control gene has been proven to have the lowest variability.[2] Additionally, relative quantiation (RQ) of gene expression was performed according to the method of Livak and Schmittgen[26] where the internal calibrator used was the average of grade 0 EIPH samples. Each cDNA sample was amplified in duplicate and all reaction solutions and samples were added to the plate using a robotic pipetting machine (EpMotion 5070, Eppendorf, Westbury, NY) (Figure 5.5 and 5.6) thereby allowing the study's samples to have the best pipetting accuracy and reproducibility. Also, a positive (LPS-stimulated lymphocytes) and a negative control (water) was included in each plate. The real-time PCR reaction mixtures had a final volume of 25 ul consisting of 10 ul of cDNA and 15 ul of the master mix. Amplification conditions were kept constant for all samples: 10 minutes at 95°C, 15 seconds at 95 °C, and 1 minute at 60 °C. The endpoint C_T was defined as the PCR cycle number that crosses signal threshold and ranged from 0 (no product) to 40.

5.3.5 *Data analysis*

Non-parametric tests were used to compare overall differences in target gene expression within the different grades of EIPH (Spearman's Rank-order correlation and Holm-Sidak t-test for multiple comparisons); and between location (altitude *versus* sea level) and EIPH grade (linear regression). Significance was set at $P < 0.05$. Statistical tests were conducted using commercially available computer software (SYSTAT®, Chicago, IL).

5.4 RESULTS

Mean expression of IL-1, -6, -10, INF- γ and TNF-α mRNA is depicted in Figures 5.7 to 5.11 respectively. While there was no statistically significant difference for mRNA expression of IL-1 ($P = 0.104$), TNF-α ($P = 0.06$), and INF- γ ($P = 0.36$) within the different grades of EIPH, significant difference was noted for IL-6 ($P = 0.046$) and IL-10 ($P = 0.02$) mRNA expression. Racehorses with a grade 4 EIPH expressed more IL-6 mRNA as compared to those horses with grade 0, 1 and 2 EIPH ($P < 0.05$), while racehorses with a grade 3 EIPH expressed more IL-10 mRNA compared to those horses with a grade 0 and 2 EIPH ($P < 0.05$). There was greater overall expression of IL-6 mRNA at sea level ($P = 0.009$), and TNF-α mRNA at altitude ($P = 0.005$). No significant difference was seen with the expression of IL-1 ($P = 0.82$), IL-10 ($P = 0.274$) and INF-γ mRNA ($P = 0.634$) between sea level and altitude.

5.5 DISCUSSION

Pulmonary inflammation in horses with more severe forms of EIPH is associated with histopathological evidence of small airway disease[33,34] and inflammation in bronchoalveolar lavage fluid and tracheal aspirates.[32] Whether the inflammation is a direct consequence of EIPH or if it predisposes to EIPH, is still not known. Autologous intrapulmonary blood inoculation in horses also causes prolonged airway inflammation.[30] Neutrophil-mediated injury may lead to intrapulmonary up-regulation of pro-inflammatory cytokines, damaging the alveolocapillary barrier causing a systemic inflammatory response.

In this study, we investigated mRNA IL-1, -6, -10, INF-γ, and TNF-α expression in a natural population of Thoroughbred racehorses with varying grades of EIPH competing at different altitudes. Although equine-specific monoclonal antibodies are not commercially available for IL-1, -6, -10, INF-γ, and TNF-α; and direct comparison can not be made between mRNA expression and protein levels; we assumed that mRNA expression reflected those of the biologically active cytokine. Furthermore, several studies have demonstrated a good correlation between inflammatory cytokine gene expression and disease conditions in the horse.[12,24,47]

We chose to study proinflammatory cytokines IL-1, -6 and TNF-α as these cytokines are responsible for induction of fever, neutrophil recruitment, tissue remodeling and immune activation[8] and INF-γ which is a pleotropic cytokine with proinflammatory properties that

augments TNF activity.[8] Interleukin-10 was studied for its potent anti-inflammatory activity as it may suppress proinflammatory cytokines such as IL-1 and TNF-α.

We have previously reported on the effect of altitude on the prevalence and severity of EIPH in Thoroughbred racehorses in South Africa using tracheobronchoscopy and concluded that EIPH is more prevalent ($P = 0.002$) and more severe ($P < 0.001$) at sea level.[42] EIPH may be assessed quickly and easily using tracheobronchoscopic examination, as this technique is minimally-invasive and allows immediate grading of racehorses with EIPH without laborious, time-consuming processing of samples in a laboratory. Although the repeatability of this tracheobronchoscopic grading system has been established[17] the relationship between the volume of blood in the airways and actual haemorrhage is not known. In this study, we assumed that horses with higher grades of EIPH were more severely affected and therefore suffered more haemorrhage.

Although pro-inflammatory responses have not been documented before in horses with EIPH, reports exist on increased mRNA expression of IL-1β, -8 and TNF-α in the bronchoalveolar lavage fluid of horses with recurrent airway obstruction,[14] increased mucosal IL-4 and -10 associated with the presence of Cyathostominae larvae in the equine large colon wall,[7] and increased IL-1β, -8 and TNF-α in blood leukocytes of horses following infection with *Anaplasma phagocytophilia*.[22]

Although a previous report found no significant effect of exercise on IL-4, -12 and IFN- γ mRNA expression,[3] the present study is, to the author's best knowledge, the first to report

an association between mRNA expression and EIPH. Racehorses with a higher grade EIPH and therefore more blood loss had greater pro-inflammatory IL-6 mRNA expression which was counter-regulated by a corresponding increase in anti-inflammatory IL-10 mRNA expression compared to lower grades of EIPH. Previous reports have also indicated that IL-6 expression may increase dramatically[19,44] with highest concentrations correlating with the volume of blood lost[31] and may remain elevated between 3[19] to 21[10] days. Expression of IL-6 can also increase in response to higher concentrations of TNF-α and IL-1, and is regarded as a pro-inflammatory cytokine which has anti-inflammatory properties.[4,35] Also, infiltrating neutrophils express after post-traumatic haemorrhage increased TNF-α mRNA in humans,[1] and there is up-regulation of this cytokine within 30 minutes after haemorrhage.[46] TNF-α is inhibited by IL-10 through stabilization of IκBα, preventing translocation of NF-κB.[25,49] In humans, IL-10 is the most important anti-inflammatory cytokine within the pulmonary innate immune response,[35] with anti-inflammatory properties[13,20,29] and is also up-regulated in the lung after haemorrhage[43] as was reported in this study.

In this study, altitude seemed to affect mRNA expression, as more IL-6 was expressed at sea level, while greater TNF-α expression was seen at altitude. Stressors (hypoxia, exercise) may initiate an immune and inflammatory response[27] characterized by increased IL-6 and TNF-α. In humans, exercise following acute exposure to high altitude was associated with increased IL-6 and not TNF-α expression,[15,23] while TNF-α is elevated after prolonged and intense exercise at sea level.[36] This study differs from previous reports[15,23,36] since IL-6 was increased at sea level and TNF-α greater at altitude. As

horses raced over shorter distances at sea level (as reported in Chapter 2), it is possible that overexertion over shorter race distance may have caused a more profound increase in IL-6 expression. Moreover, at altitude, racehorses competing over longer distances may have expressed more TNF-α as was found in human athletes.[36] Other plausible reasons exist for differences in cytokine expression and may include the use of fully-acclimatized horses that did not suffer hypoxaemia while racing at altitude (oxygen saturation was however not tested in this study), differences in actual elevation above sea level between the various studies, and that EIPH may elicit a different immune and inflammatory cytokine response.

Altitude and EIPH grade had no effect on venous IL-1 or INF-γ mRNA expression. In humans following trauma, IL-1 is undetectable within the first few hours[19] and can remain low for up to 5 days.[41] Interferon-gamma assists in immunomodulation, lymphocyte recruitment and activation and has anti-pathogen activity.[5] Through enhanced cell-mediated immunity, INF-γ causes a Type 1 response which results in destruction of virus-infected cells and recovery from infection. In the horse, production of INF-γ by $CD4^+$ and $CD8^+$ T cells in the lung of adult horses was associated with clearance of virulent *Rhodoccocus equi,*[18] equine infectious anaemia virus stimulated peripheral blood mononuclear cells to produce INF-γ,[11] infection with equine influenza virus or the use of a recombinant vaccinia Ankara viral vector resulted in increased expression of INF-γ mRNA,[6,45] and infection with equine herpes virus-1 resulted in age-related increased INF-γ production by peripheral blood mononuclear cells.[37] Since an infectious etiology has not been implicated in the pathogenesis of EIPH, it is not surprising that INF-γ which

affects cell-mediated cytotoxicity was consistently expressed at low levels in the racehorses irrespective of grade or location.

Although this study did not report the origin nor the cell type involved, it has been previously shown that intrapulmonary blood inoculation initially causes a local neutrophilic infiltration, followed by macrophages and to lesser degree lymphocytes.[30] Equine neutrophils have been demonstrated to produce proinflammatory IL-1, -6, -8, and TNF-α and not IL-4, -5, and INF-γ mRNA which is mainly produced by lymphocytes.[21] All together this suggests that following EIPH-induced pulmonary neutrophilia, the neutrophils may be actively involved in the observed systemic inflammatory response as reported in this study.

The mRNA expression of cytokine profiles in a natural population of racing Thoroughbreds presented in this report may assist in the understanding of the immunopathogenesis of EIPH. In future, gene linkage studies may prove useful in determining the susceptibility to EIPH by studying the balance of expression of proinflammatory and anti-inflammatory cytokines. Further research on therapeutic strategies which may include neutralizing antibodies, receptor antagonists, soluble receptors and inhibitors of proteases may be warranted.[9] This may interrupt the proinflammatory cytokine cascade and reduce the prevalence and severity of EIPH.

5.6 CONCLUSIONS

Results of this study indicate that increased IL-6, and -10 mRNA production is associated with more severe forms of EIPH. Also, there was greater expression of IL-6 mRNA at sea level and TNF-α mRNA at altitude. Although, it is unclear whether the inflammatory response observed in the study was due to pre-existing pulmonary inflammation or as a direct consequence of pulmonary bleeding, this study demonstrates a systemic correlation to pulmonary inflammation. Further studies are warranted to understand the relationship between cytokine expression and EIPH.

5.7 FIGURES AND TABLES

Figure 5.1 A PAXgene® Blood RNA Tube containing venous blood.

Figure 5.2 Pipetting the sample onto the PAXgene® RNA spin column during the RNA extraction procedure.

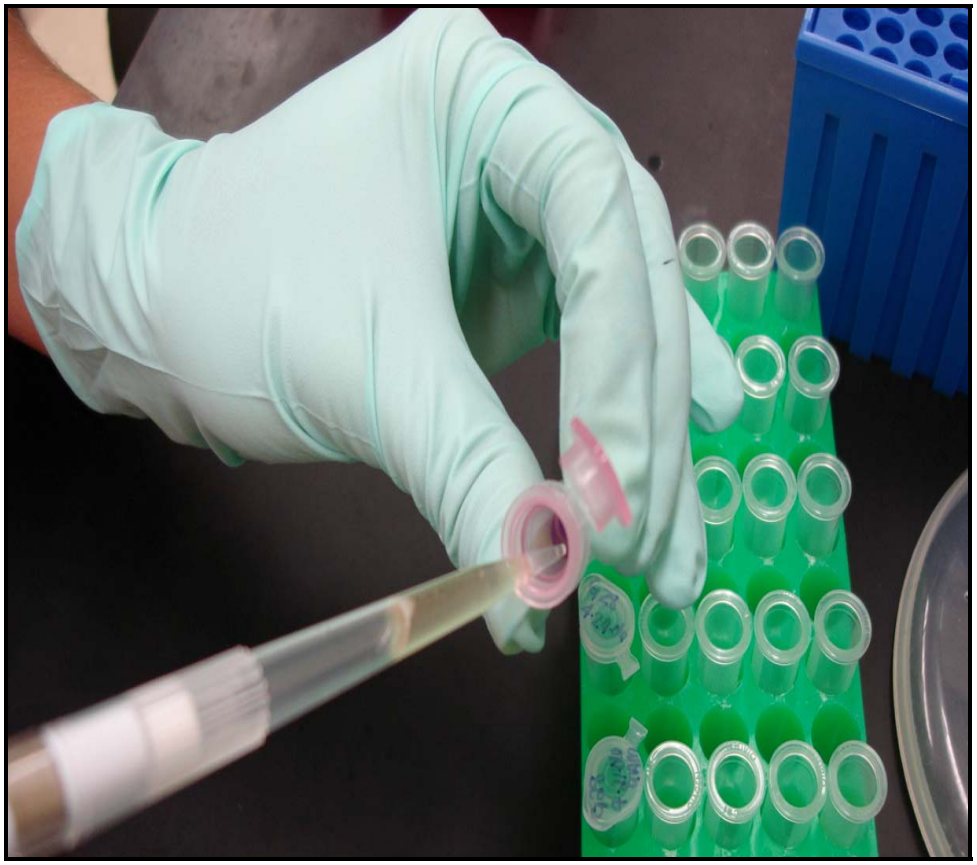


Figure 5.3 Preparing to perform RNA elution following centrifugation of the PAXgene® RNA spin column.

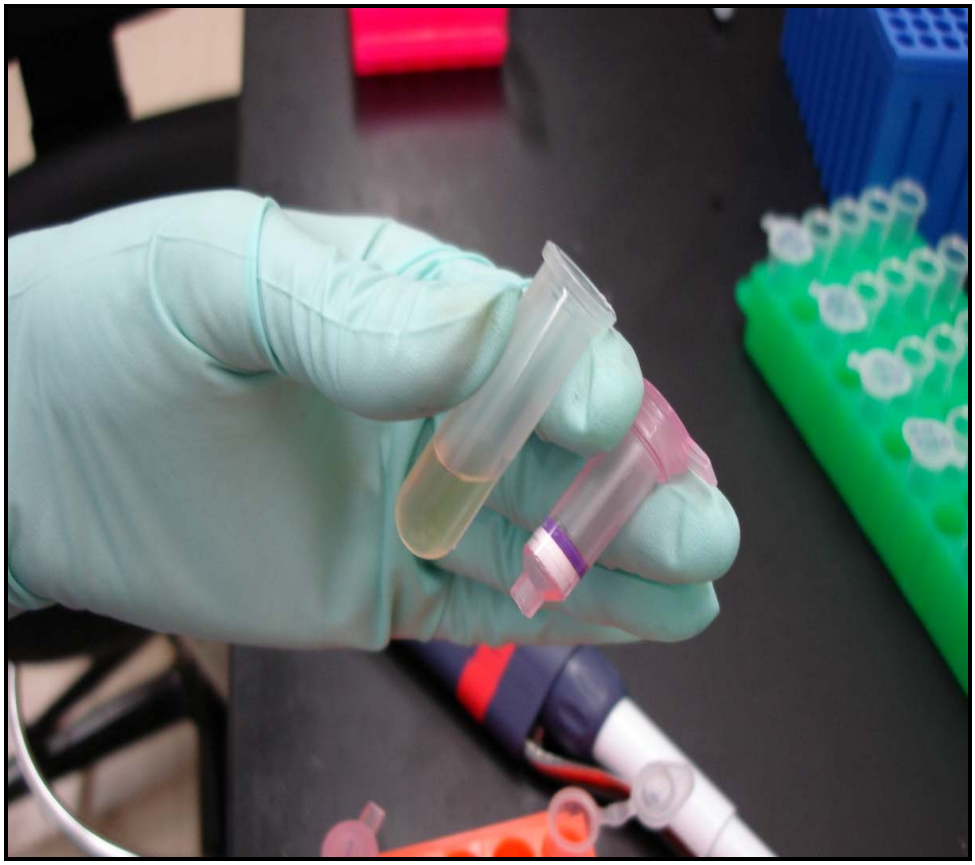


Figure 5.4 Preparing to perform real-time polymerase chain detection on the Applied Biosystems 7500 sequence detection system machine.

Figure 5.5 The epMotion 5070 robotic pipetting machine.

Figure 5.6 Primers and probes ready to be added to each cDNA sample by the epMotion 5070 robotic pipetting machine.

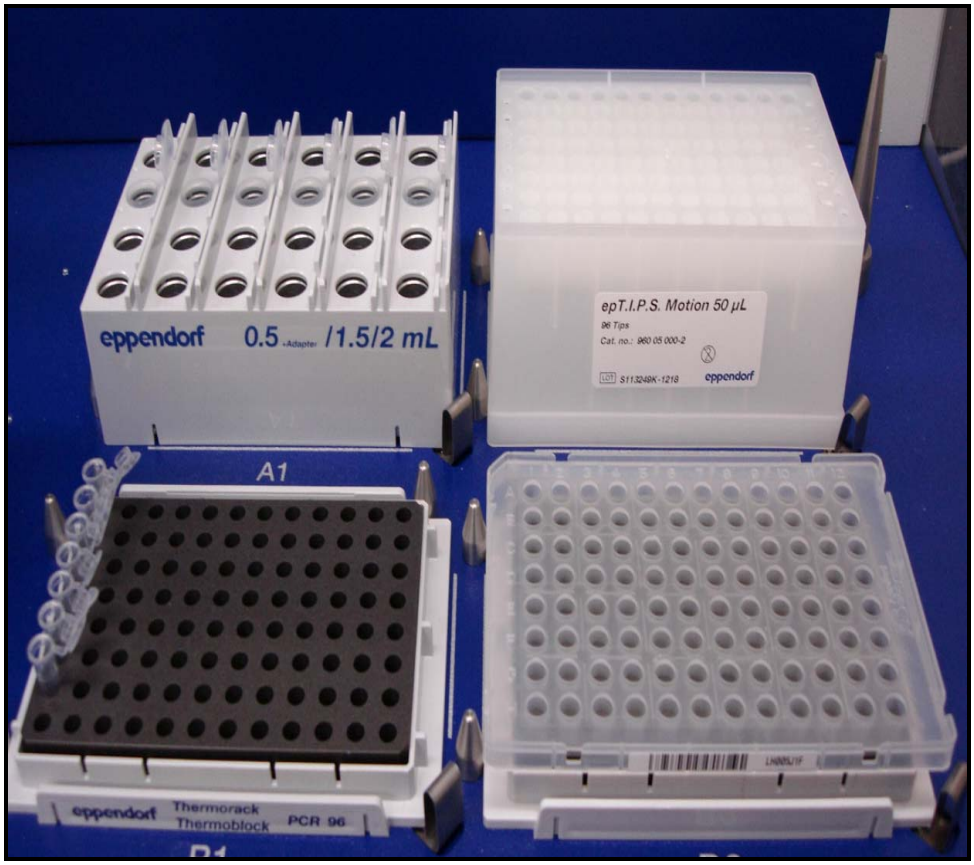
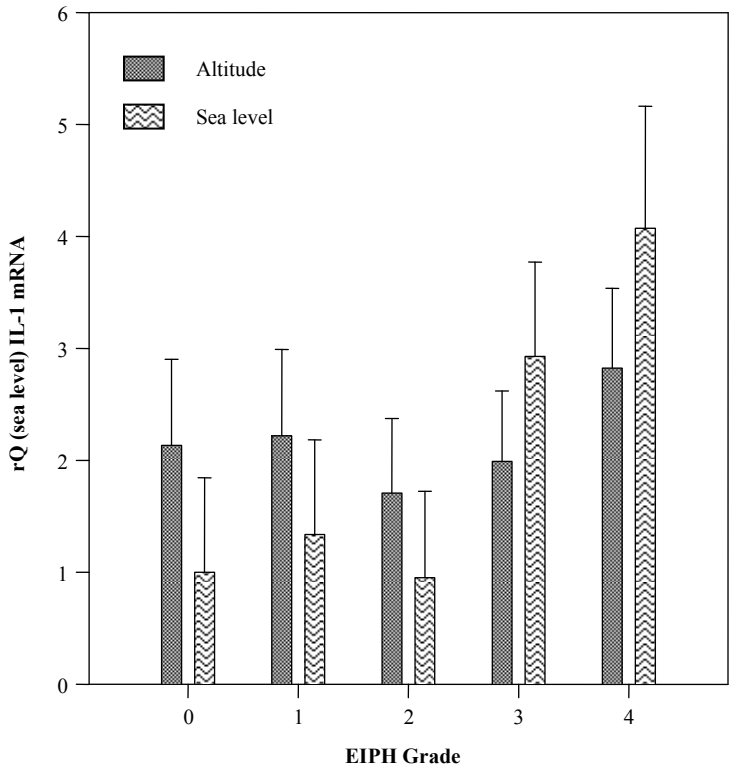
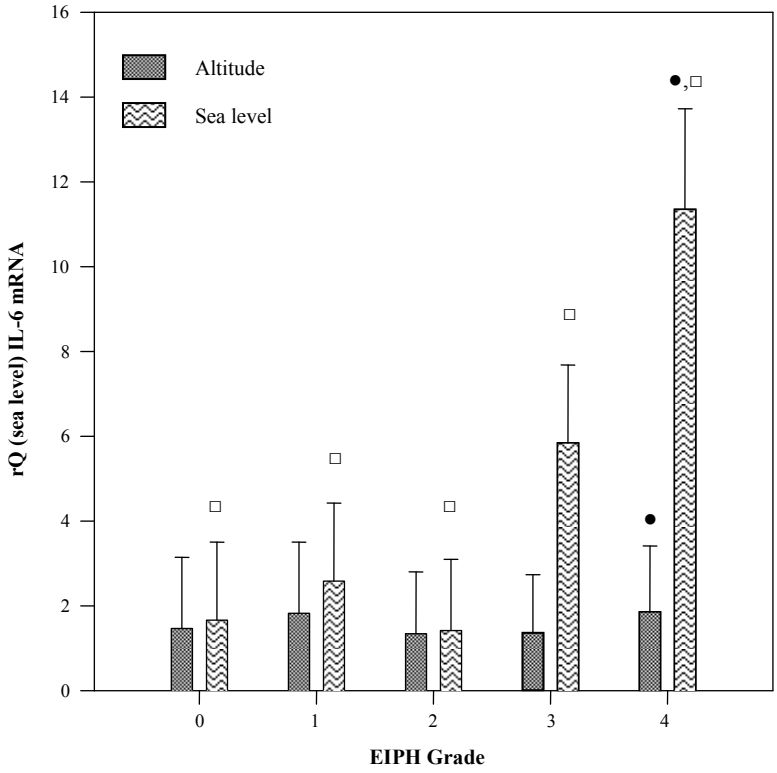


Figure 5.7 Expression of IL-1 mRNA in Thoroughbred racehorses with grade 0 to 4 exercise-induced pulmonary haemorrhage after racing at high altitude and at sea level.



No significant statistical differences existed between IL-1 mRNA expression and EIPH grade or location.

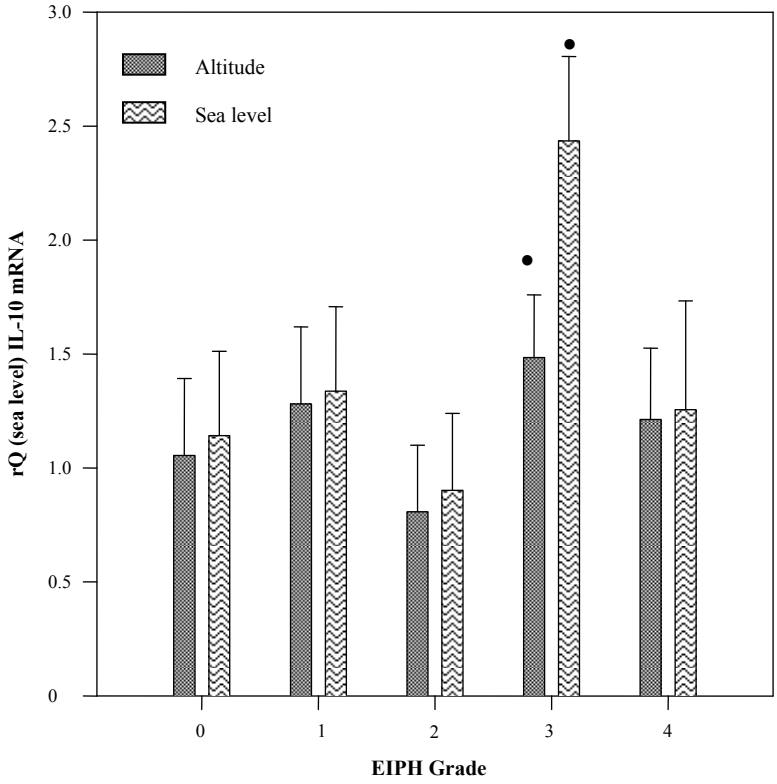
Figure 5.8 Expression of IL-6 mRNA in Thoroughbred racehorses with grade 0 to 4 exercise-induced pulmonary haemorrhage after racing at high altitude and at sea level.



• Significant differences ($P < 0.05$) existed between expression of IL-6 mRNA in racehorses with grade 4 *vs*. 0, 1 and 2 EIPH.

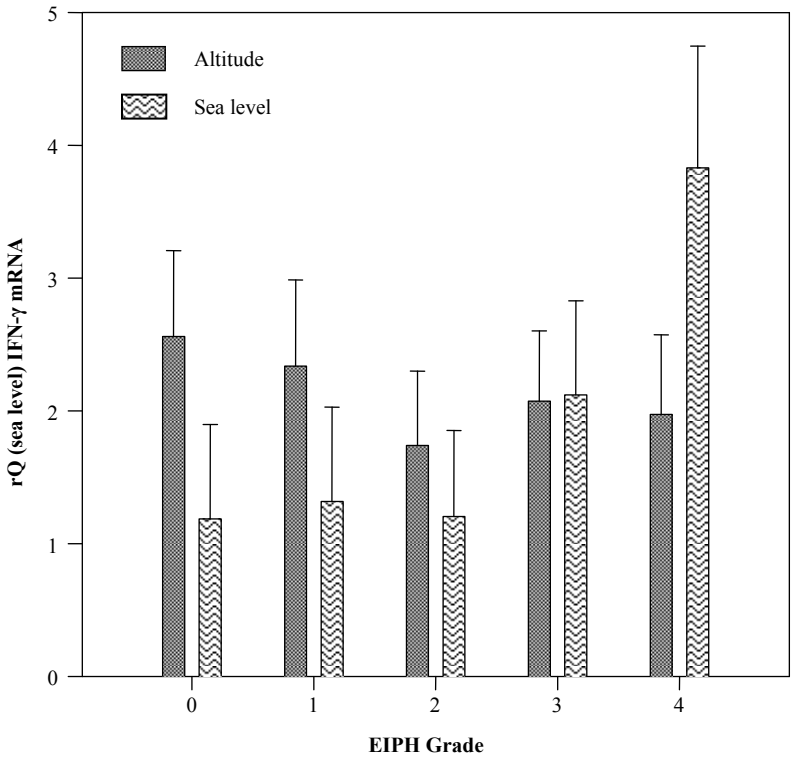
□ Significant differences ($P < 0.05$) in expression of IL-6 mRNA in racehorses at sea level compared to altitude.

Figure 5.9 Expression of IL-10 mRNA in Thoroughbred racehorses with grade 0 to 4 exercise-induced pulmonary haemorrhage after racing at high altitude and at sea level.



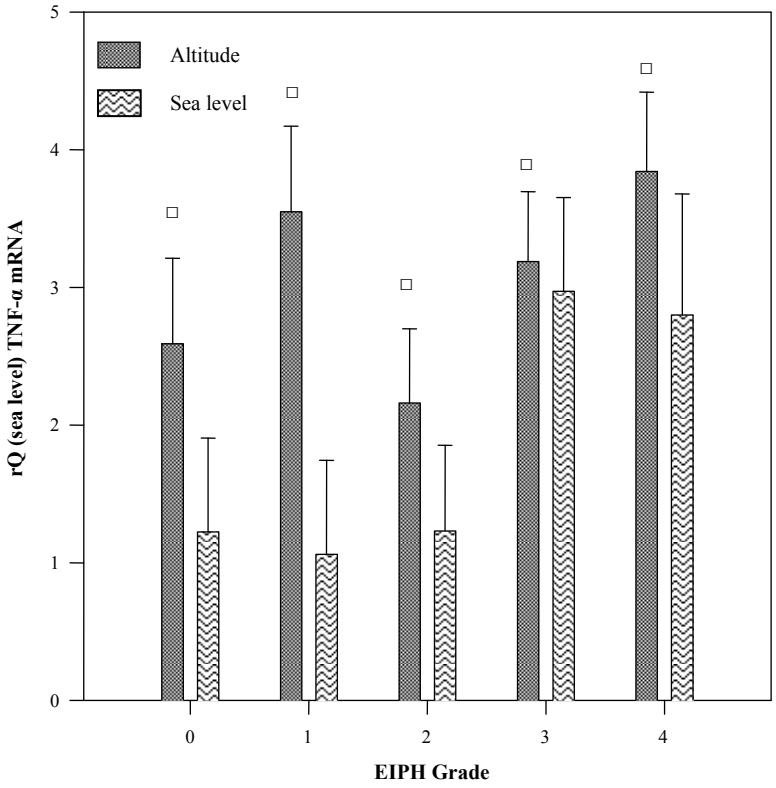
• Significant differences ($P < 0.05$) existed between expression of IL-10 mRNA in racehorses with grade 3 *vs*. 0 and 2 EIPH.
No significant differences existed between expression of IL-10 mRNA in racehorses and location.

Figure 5.10 Expression of IFN-γ mRNA in Thoroughbred racehorses with grade 0 to 4 exercise-induced pulmonary haemorrhage after racing at high altitude and at sea level.



No significant statistical differences existed between IL-1 mRNA expression and EIPH grade or location.

Figure 5.11 Expression of TNF-α mRNA in Thoroughbred racehorses with grade 0 to 4 exercise-induced pulmonary haemorrhage after racing at high altitude and at sea level.



□ Significant differences ($P < 0.05$) existed between the expression of TNF-α mRNA at altitude *vs*. sea level.

Table 5.1 Accession name and order number of target gene studied.

Gene	GeneBank Number	ABI Order Name
IL-1	U92480	EQIL-1B-JN2
IFN-γ	U04050	EQIFNGIS-JN3
IL-6	U64794	EQIL-6
IL-10	U38200	EQIL-10IS-JN2
TNF-α	M64087	EQTNFAIS-JN2
β-GUS	Not available	GUS

IL: interleukin
IFN: interferon
TNF: tumor necrosis factor
GUS: glucuronidase

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Chapter 6 General discussion

6.1 EIPH: A SOUTH AFRICAN PERSPECTIVE

Exercise-induced pulmonary haemorrhage (EIPH) was first described in South Africa in 1950.[23] At that stage, flexible videoendoscopes did not exist, therefore the study had to rely on the presence or absence of epistaxis from one or both nostrils in order to detect EIPH.[23] Also, the precise aetiology was unclear, and suggestions were made that the prevalence of EIPH may be affected by genetic factors, feeding of hay, bedding on straw, or nasogastric intubation.[23] However, already in 1950, that study suggested that there may be an association between altitude and EIPH as racehorses competing at sea level had more EIPH-related epistaxis.[23] Later, more reports confirmed that a greater number of racehorses had EIPH related epistaxis at sea level.[24,32]

In South Africa, horse racing occurs at both high altitude and at sea level and horses are not allowed to race on furosemide nor use nasal dilator strips. The National Horse Racing Authority strictly controls racing, and routinely performs drug screening on urine to test for the use of prohibited medications on race day. Through creation of such an environment, this research project was able to determine the prevalence and severity of EIPH, and its association with racing performance.

The results reported here (Chapter 2) indicate that the prevalence and severity of EIPH was greater at sea level than at high altitude agreeing with previous reports.[23,24,32] However, plausible reasons do exist why EIPH may be more prevalent and more severe at altitude as racehorses often suffer from exercise-induced arterial hypoxaemia (EIAH),[3,31] develop pulmonary hypertension,[20] causing pulmonary capillary stress failure[4] and that hypoxic vasoconstriction occurs at high altitude[27] which may worsen the degree of EIAH, thereby directly causing EIPH or exacerbating pre-existing EIPH. Moreover, the overall prevalence of EIPH was 54% which indicated that the prevalence of EIPH as detected by tracheobronchoscopy was high in racehorses competing in South Africa.

In South Africa, the presence of EIPH was associated with superior racing performance in Thoroughbred racehorses not medicated with furosemide and not using nasal dilator strips. The association between racing performance and EIPH is more difficult to evaluate as numerous factors may affect a racehorse's performance on race day. Such factors may include the environment, climate (ambient temperature and humidity), genetics, training techniques, and evaluation technique. Also, factors which may be identified to affect performance at a specific geographical location may not necessarily act in a similar manner at another location. Moreover, are the criteria used to evaluate performance (distance finished behind winner, race earnings and finishing position) suitable enough to allow for accurate evaluation of racing performance?

Although not allowed on race day in South Africa, numerous racing jurisdictions (USA, Canada, Mexico, UAE, and parts of South America) currently use furosemide as prophylaxis for EIPH in the racing industry. Although the clinical efficacy of furosemide in horses with EIPH has yet to be determined under natural field conditions, extensive use of furosemide exists. As annual costs to the Thoroughbred racing industry associated with furosemide administration may be as high as $28 000 000,[14] the use of furosemide in horses with EIPH needs to be re-evaluated to determine the clinical efficacy.

6.2 RESPIRATORY TRACT DISORDERS: PREVALENCE AND ASSOCIATION WITH RACING PERFORMANCE

6.2.1 Prevalence of respiratory tract disorders

Studies reporting respiratory tract disorders in Thoroughbred racehorses have been performed in North America[22,25,30] and Australia.[5] These studies have identified asymmetry of the left arytenoid cartilage,[5,22] idiopathic laryngeal hemiplegia (ILH),[22,25,30] prosthetic laryngoplasty,[22,25] ventriculectomy,[5] right arytenoid paralysis,[22,25] arytenoid chondropathy,[5,22] ulceration of the arytenoid mucosa,[5] aryepiglottic entrapment,[5,22,25,30] pharyngeal polyps,[22] dorsal displacement of the soft palate,[5,25,30] guttural pouch discharge,[25] nasal stenosis,[25] subepiglottic cyst,[5,25] epiglottic hypoplasia,[5] epiglottic deformity[5] and tracheal mucous.[22,30]

Despite having a large population of racehorses in South Africa, the prevalence of respiratory tract disorders was historically unknown. In South African Thoroughbred racehorses, this research (Chapter 3) detected a low prevalence of left arytenoid asymmetry, idiopathic laryngeal hemiplegia (ILH), epiglottic entrapment and deformity, and dorsal displacement of the soft palate; while the prevalence of pharyngeal lymphoid hyperplasia (PLH), laryngeal and tracheal dirt, and tracheal mucous was higher. The prevalence of tracheal ring cartilage spikes was reported for the first time, and an association with sex identified as more male racehorses were affected. An association between age and PLH was found as younger racehorses had higher grades of PLH.

Considering all the respiratory tract disorders identified and reported in this research (Chapter 3), the more important disorders such as left arytenoid asymmetry, left laryngeal hemiplegia and epiglottic deformity had similar prevalence compared to elsewhere.[5,22,25,30] It seems therefore that the South African racing Thoroughbred population is comparable to other Thoroughbred racehorses competing elsewhere in terms of health. Although this research reported on other disorders too, the higher prevalence of laryngeal and tracheal dirt, and PLH should not be interpreted as a sign of ill-health.

The high prevalence of tracheal mucous is more concerning and may indicate that South African Thoroughbred racehorses suffer greater subclinical tracheobronchial inflammation. The exact cause is unclear but neutrophilic inflammation, bacteria, viruses

and environmental allergens may contribute directly or indirectly to the production of tracheal mucous.[5,6]

6.2.2 Relationship with racing performance

Results of this research (Chapter 3) indicate that racing performance in South African Thoroughbred racehorses was not impaired by grade 2 arytenoid cartilage asymmetry, ILH, PLH (grade 2 and 3), epiglottic deformity and epiglottic entrapment; while superior racing performance was seen in racehorses with tracheal cartilage ring spikes and grade 3 tracheal mucous.

Similar to the results of this research (Chapter 3), previous studies have reported that racing performance was not affected by grade 2 arytenoid cartilage asymmetry, epiglottic entrapment and PLH.[5,16] However, contrary to previous reports that ILH was associated with impaired performance,[6,9,18] this report found no evidence of impaired racing performance. This finding was unexpected as studies have reported impaired gaseous exchange in affected horses.[2,6,18] This certainly does not mean that surgical correction of ILH should no longer be employed!

Although tracheal cartilage ring spikes have been reported before,[27] their significance is currently unknown. It is unclear to why this disorder may be associated with superior racing performance and occur more frequently in make racehorses. It was also surprising that racehorses with greater amounts of tracheal mucous (grade 3) had superior racing

performance since tracheal mucous is associated with exercised-induced arterial hypoxaemia during a standardized treadmill test.[7,28]

Although this research reported on a wide variety of upper respiratory tract disorders, the low prevalence of certain disorders did not allow statistical analysis to determine their relationship with racing performance. Furthermore, since certain respiratory tract disorders are dynamic, such disorders could not be identified, nor could their relationship with racing performance be determined.

It should be noted that previous reports have largely been conducted on horses using high-speed treadmills[6,7,18,28] and that these studies may have either lacked adequate statistical power, random selection, and did not examine a natural population of racehorses under field conditions.

This research highlights the need for further objective research in racehorses under field conditions, as disorders that are found to impair performance in racehorses during high-speed treadmill exercise may not act in a similar manner under field conditions. Also, since reasons for impaired racing performance may be multifactorial, respiratory tract disorders should cautiously be interpreted as the sole reason for impaired racing performance.

6.3 DETECTION AND GRADING OF RESPIRATORY TRACT DISORDERS

Until endoscopy was introduced into equine medicine, horses with respiratory tract disorders could not be thoroughly examined. Although historically, rigid endoscopes provided a wealth of knowledge for internists studying equine upper airway disorders at first, these instruments were cumbersome to use, traumatized tissues easily, generated excess heat and had limited power reserves. Many of the aforementioned problems were alleviated with the advent of flexible endoscopy. Today, equine internists can evaluate the upper and lower respiratory airway and identify structural disorders at rest, while dynamic or functional disorders can be diagnosed during high-speed treadmill exercise.

Under natural field conditions, examining a large population of horses requires a diagnostic technique that can be performed quickly and safely for both personnel and horse, while yielding the maximum amount of information possible. Moreover, it should not interfere with training schedule nor be performance modifying causing disqualification of a horse participating in athletic events due to the detection of drug residues.

Following flexible videoendoscopic examinations on Thoroughbred racehorses, we used previously described grading critera[1,11,15,29] to access the presence and severity of EIPH, PLH, arytenoid cartilage movement (ACM) and tracheal mucous (TM) and established good interobserver reliability for EIPH, while moderate interobserver reliability was observed for PLH, ACM and TM. This study (Chapter 4) is in agreement with another

study that also demonstrated high interobserver reliability for EIPH; however that study unfortunately did not access other disorders.[15]

Since the equine respiratory tract is an important cause of poor performance and may be examined on numerous occasions throughout the horse's lifetime, and that respiratory disorders occur worldwide, it is important to employ similar grading criteria that are reliable and reproducible by internists of varying experience.

Currently, there exists a paucity of studies documenting the prevalence of respiratory tract disorders in natural populations of Thoroughbred racehorses, making collation of data using similar grading criteria even more important. Researchers may then have the opportunity to conduct multi-centered studies at different locations throughout the world that could have a significant impact on the importance of performance-limiting disorders of the equine respiratory tract.

6.4 SYSTEMIC INFLAMMATION AND THERAPEUTIC INTERVENTIONS

Cytokines are soluble, regulatory polypeptides produced by a variety of nucleated cells of heamatopoietic and non-heamatopoietic origin. Cytokines can act locally to initiate autocrine, paracrine or endocrine effects and are critical for normal immune function.
They are involved in the regulation of growth, development and activation of the immune system, and mediation of the inflammatory response. These substances exert their effects by influencing gene activation that results in cellular activation, growth, differentiation,

functional cell molecular expression, and cellular effector function. Cytokines may therefore have a dramatic effect on immune response regulation and pathogenesis of diseases.

Pulmonary inflammation has been previously reported in racehorses with EIPH and may be due to pre-existing small airway disease[19] or intrapulmonary accumulation of blood.[21] Following autologous blood installation within the lung, there is a pulmonary neutrophilia,[21] and since neutrophils are a major source of proinflammatory cytokines,[17] it is therefore not surprising that a systemic inflammatory response was detected by this study (Chapter 5). However, as previously reported, it is still uncertain whether this inflammatory response is pre-existing or if it develops as a consequence of EIPH.

It is possible, that racehorses may have mild, undetectable episodes of EIPH repeatedly during strenuous training periods. This is exacerbated on race day, resulting in tracheobronchoscopic detection of blood. The ability to identify horses that have subclinical EIPH may be of benefit to trainers and allow earlier therapeutic intervention. Although therapeutic options for horses with EIPH have included lowering transmural pulmonary capillary pressure, relieving upper and lower airway obstruction, decreasing interstitial inflammation and bronchial angiogenesis, and treating for haemostatic dysfunction,[8] no strategies exist for combating this disorder at a molecular level. Anti-cytokine therapies exist in human medicine and include neutralizing antibodies, receptor antagonists, soluble receptors and inhibitors of proteases.[10] For instance, in models of

inflammation, neutralizing antibodies to IL-8 reduces neutrophil infiltration in the lung, joint, kidney, skin, and myocardium.[13]

These results are novel and certainly interesting, and provide insight into the immunopathogenesis of EIPH at a molecular level. Further research is needed to identify whether the use of anticytokine therapy may help reduce the prevalence and severity of EIPH.

6.5 CONCLUSIONS

The research reported in this thesis has contributed substantially to the determination of the prevalence, severity and affect on racing performance of respiratory tract disorders in Thoroughbred racehorses competing in South Africa. Also, determination of an association between EIPH and systemic inflammation at a molecular level may assist future researchers in anti-cytokine therapies which may help reduce the prevalence and severity of EIPH.

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