Exercise-induced pulmonary haemorrhage in Thoroughbred racehorses: an investigation of risk factors, the association with race day performance and a longitudinal study.

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Author’s declaration

I declare that this thesis is my own account of my research and contains as its main content, work that has not previously been submitted for a degree at any tertiary education institution.

________________________
Eleanor Crispe
Statement of contribution

The three experimental chapters in this thesis have been submitted and published as peer reviewed publications with multiple co-authors. Eleanor Crispe was the first and corresponding author of these publications and was substantially involved in conceiving ideas, study design, data collection, data entry, data analysis and the preparation and submission of all manuscripts. All publication co-authors have consented to their work being included in this thesis and have accepted this statement of contribution.
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“I am still under the impression that there is nothing alive quite so beautiful as a Thoroughbred horse”.

- John Galsworthy
Abstract

Exercise-induced pulmonary haemorrhage (EIPH) is a highly prevalent disease of racehorses. Recognised for centuries, there has been advances in our knowledge and understanding of the disease, but the cause and prevention of EIPH remains elusive. The aim of this study was to contribute to the knowledge base of EIPH with a broader exploration of risk factors, the impact of race day EIPH on a range of novel and routine performance parameters, and to document the longitudinal nature of the disease.

Subject to timing, tracheobronchoscopy after racing is a reliable technique to detect pulmonary haemorrhage. Although the association between tracheobronchoscopic haemorrhage and pulmonary histological changes has not been substantiated, the grading of tracheal haemorrhage is an accepted marker of disease presence and severity. A prospective, observational and longitudinal study design was used to examine a group of Thoroughbred racehorses competing in Western Australia. Horses were examined with tracheobronchoscopy between 30 and 220 minutes after racing. The examinations were reviewed and graded (0-4) by experienced veterinarians that were blinded to the horses’ identity, date of examination and performance. In total, there were 3,794 observations from 1,567 horses collected over a 3-year period.

Using a subset of animals, the association between EIPH and a variety of horse, race and climatic factors were examined. Racing at lower ambient temperature and horses’ racing with bar shoes were associated with EIPH presence and increasing severity. Increasing race distance was associated with EIPH being detected and increasing the number of career race starts increased the risk of more severe EIPH.

Using the entire dataset horses with the most severe grades of EIPH (≥3) were significantly more likely to have a lower finishing position and finish further behind the winner, less likely to place in the first 3 positions and collect race earnings, collected less earnings per race start and were slower over the last 600m of the race than horses without EIPH (grade 0). Mild or moderate haemorrhage was not associated with inferior race day performance.

The longitudinal analysis of disease progression was conducted using 747 horses with a minimum of 2 observations. Tracheobronchoscopic EIPH mildly increased in severity over the first
thirty race starts. The preceding EIPH score was significantly associated with the current EIPH score. Significant factors associated with the current EIPH score included ambient temperature and the number of days in the current racing preparation. Ambient temperature, weight carried in races and the number of days since last racing was associated with variation in EIPH severity between races.
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**EJ Crispe, GD Lester, ID Robertson, CJ Secombe,** Bar shoes and ambient temperature are risk factors for exercise-induced pulmonary haemorrhage in Thoroughbred racehorses, (2016), Equine Vet J, 48(4), 438-41

**EJ Crispe, GD Lester, CJ Secombe, DI Perera,** The association between exercise-induced pulmonary haemorrhage and race-day performance in Thoroughbred racehorses (2017), Equine Vet J, 49(5) 584-9

**EJ Crispe, CJ Secombe, DI Perera, AA Manderson, BA Turlach, GD Lester,** Exercise-induced pulmonary haemorrhage in Thoroughbred Racehorses: a longitudinal study, Equine Vet J, Accepted manuscript 31-March-2018
Chapter One. Review of Literature

1.1. Definitions

Epistaxis (ep′istak′sis) is a medical term for a nosebleed. Epistaxis is derived from the ancient Greek word epistazein, ‘epi’ meaning to drop on, and ‘stazein’ meaning to fall in drops. Epistaxis after high intensity exercise is referred to as exercise-associated epistaxis (EAE). Exercise-induced pulmonary haemorrhage (EIPH) describes the presence of blood in the trachea or larynx after exercise.

1.2. Historical perspective

Horses returning from intense exercise with blood at the nostrils have been reported since the early eighteenth century. Often referred to as a ‘bleeder’, one of the first documented cases in the Thoroughbred was ‘Bartlett’s Childers’, foaled in 1716 [1]. Due to the frequency of EAE, the stallion was initially named ‘Bleeding Childers’ and subsequently never raced, but became a successful breeding stallion as the grandsire of ‘Marske’, and the great grandsire of the undefeated champion stallion ‘Eclipse’, foaled in 1764 [2]. In 1913, Robertson expressed concern that this lineage entered every Thoroughbred in the world and questioned if this trait had entered the pedigree of all future racing generations [1]. Current estimations suggest that up to 95% of Thoroughbred paternal lineages today can be traced back to Eclipse [3, 4].

Historically, the condition was confused with hemophilia due to similarities drawn with people suffering the same affliction. In 1913, it was documented that, unlike hemophiliacs, horses with nosebleeds could clot readily enough [1]. The blood was thought to originate from the nasal cavity or another location within the head. Often referred to as ‘breaking a blood vessel’, the condition was attributed to unusually weak blood vessel walls, which when ruptured, bled profusely. At the same time the seriousness of the condition was not overlooked; “When this happens, the horse may fall
suddenly. Very occasionally he never regains his feet and dies almost immediately from asphyxia” [1].

The condition was described as sporadic, reappearing or not, without a regular pattern, rendering the horse undesirable for racing [5]. The horse could be “perfectly normal for weeks, and then, quite unexpectedly be afflicted with epistaxis in a race” [5].

Mahaffey (1962) suggested that the origin of the haemorrhage could be the lung and, more specifically, rupture of the alveolar capillaries [5]. This was based on post mortem studies of lung tissue containing bright red, frothy blood. Although blood had been detected previously in the trachea at necropsy, it had been hypothesised that the blood was aspirated from the nasal passages during galloping [1]. Opinions regarding the origin of the epistaxis persisted until 1974 when rigid endoscopy became available to the veterinary profession. Endoscopic examination of the nasal passages of fifty horses with EAE was unable to locate the source of bleeding [6]. In the absence of evidence to the contrary the source of the bleeding was ascribed to the lung. Cook further reported pulmonary haemorrhage detected in three horses. One horse was galloped and then anaesthetised for the procedure. Despite not having evidence of epistaxis, the horse had a considerable quantity of blood present in the trachea and “this experience made the writer wonder how often pulmonary haemorrhage occurred without epistaxis” [6].

These findings were further supported in 1981, with the induction of flexible fiber-optic endoscopy. Endoscopic examination of 235 Thoroughbred horses within 2 hours of racing revealed that blood was present in the trachea in 43.8% of horses, despite only 2 (0.8%) horses having epistaxis [7]. A pattern of increasing amounts of haemorrhage in the caudal trachea was identified, and the authors argued that the mucociliary action of the tracheobronchial tree had a cranial direction; hence it was reasonable to assume the blood originated within the lung. In 1981 John Pascoe proposed the phrase ‘exercise induced pulmonary haemorrhage’ (EIPH) to describe not only horses with EAE, but also the recently discovered, significant proportion of population that had pulmonary haemorrhage without epistaxis.

The advent of flexible endoscopy led to several observational studies after Thoroughbred and Standardbred racing, “breezing” or track gallops [7–10]. This additional research substantiated
that a considerable proportion of the racehorse population experience EIPH. Increasing disease prevalence was demonstrated when horses were examined on multiple occasions, suggesting that most racehorses experience EIPH at some stage in their career [8]. There is some debate as to whether EIPH is a condition or a disease. For the purpose of this review, EIPH will be referred to as a disease.

1.3. **Anatomy, physiology and pathophysiology**

1.3.1. **Anatomy of the head, neck and thorax**

The respiratory system in any mammal can be divided into the upper and lower respiratory system [11]. The upper respiratory system consists of the nasal cavity, sinus and larynx. The lower respiratory system consists of the trachea, bronchi, bronchioles and alveoli.

The nostrils are comma-shaped; the medial border is supported by cartilage and the lateral border is soft and moveable allowing dilation during strenuous exercise permitting the nostril to become spherical. Dorsally, there is the alar fold, which separates the nasal cavity from a blind-ended nasal diverticulum dorsally, and ventrally from the entrance to the nasal cavity.

An osseous nasal septum separates the left and right nasal cavity and within each nasal cavity there is further division. The ventral concha projects rostrally to form the alar fold dorsally, and the basal fold ventrally. The dorsal nasal concha projects rostrally to form the straight fold (Fig.3.1-1). The caudal aspect of the nasal cavity contains the ethmoidal conchae. The dorsal, middle and ventral meatus (nasal passages) are lined with a highly vascular respiratory mucosa.

The pharynx consists of the oropharynx, laryngopharynx and the nasopharynx. The caudal aspect of the oral cavity extends into the oropharynx, extending from the base of the glossopalatine arch and continuing to the base of the epiglottis (Fig.1-3.2). The laryngopharynx continues from the oropharynx around the larynx to be continued by the oesophagus. The nasopharynx is dorsal to the soft palate and is a continuation of the ventral meatus. The free edge of the soft palate extends to the base of the epiglottis, which is why mouth breathing is difficult in the horse, and gastric reflux (vomit) is present at the nostrils, and not the mouth.
Figure 1.3-1. The nose, nasal and oral cavity of the horse. Taken from Budras et al, 2009 [14].

Figure 1.3-2. The pharynx of the horse. Taken from Budras et al, 2009 [14]
The larynx is composed of hard cartilages that form a tube. Positioned at the ventral junction between the head and the neck, the larynx functions to close the airway during swallowing, and permits vocalisation. The most cranial aspect of the larynx is the epiglottis, positioned to contact the soft palate during breathing or retroflex to safeguard the entrance to the larynx during swallowing. The thyroid cartilage is ventrally positioned in the larynx. The cricoid cartilage is located caudal to the thyroid cartilage and is shaped similar to a signet ring, with the larger expanded end positioned dorsally. The lamina of the cricoid cartilage overlaps the first tracheal cartilage. The paired, triangular arytenoid cartilages lie on the dorsal cranial aspect of the larynx. The vocal folds, responsible for vocalisation, attach to the vocal process of the arytenoid cartilages. The cricoarytenoideus dorsalis muscle is responsible for dilating the glottis.

The trachea is a cylindrical tube, a continuation of the glottis opening of the larynx. The trachea consists of a series of cartilaginous rings overlapping dorsally. The trachea commences at the caudal aspect of the larynx and terminates dorsal to the heart, bifurcating into the left and right bronchi. The airways divide, becoming narrower and shorter they move deeper into the lung. Each bronchus divides into lobar, segmental and sub-segmental bronchi before forming bronchioles [12], increasing the cross-sectional area of the conducting airways with each generation of bronchi. Terminal bronchioles are the smallest airway tubes without alveoli. Terminal bronchioles branch into respiratory bronchioles, which occasionally have alveoli, and then branch into alveolar ducts, which are lined entirely with alveoli [13]. The alveoli are the region where gas exchange occurs; the remaining lung is referred to as dead space and plays no role in gas exchange.

The left and right lung is divided into lobes, which in the horse, consist of the cranial, caudal lobes and accessory lobes on the right, and the cranial and caudal lobes on the left [14]. The right lung is larger than the left, due to the additional accessory lobe. In the horse lobulation of the lung is indistinct; rather than separation of lobes by inter and intra-lobular fissures as in other mammals, the caudal and cranial lobes are separated by a wide cardiac notch (Fig 3.1-3).
The lung is long and laterally compressed, mimicking the shape of the thoracic cavity [14]. The diaphragm borders the lung caudally separating the thoracic and abdominal cavity, cranially the muscles and skeleton of the foreleg cover the thorax, and dorsally the muscles of the back border the lung. The thorax is lined by two pleural sacs, which meet on the ventral median aspect to form the mediastinum.

The heart is located within the mediastinal space with the long axis of the heart orientated 10° to the vertical with the base laying dorsal and tilted cranial to the apex [15]. The heart rests between the cardiac notch of the lungs and contacts the 3rd to 5th intercostal space on the left side, and the 3rd to 4th space on the right [14]. The heart has four chambers, two upper chambers, the left and right atria; and two lower chambers, the left and right ventricles. Blood enters via the atria, and leaves via the ventricles. Oxygenated blood returns from the lungs to the left side of the heart, and deoxygenated blood returns from the systemic circulation to the right side of the heart. The cranial and caudal vena cava (draining structures from the head, neck and abdomen) empties into the right

Figure 1.3-3. The bronchial tree and lung of the horse. Taken from Budras et al 2009 [14].
atria, along with the azygous vein (draining the thorax) [15]. The right atrioventricular (AV) valve forms on the floor of the atria and the entrance to the right ventricle. The right ventricle is crescent shaped, wrapping around the cranial aspect of the heart. The right ventricular outflow tract leads to the pulmonary artery and the right semilunar valve. Multiple pulmonary veins enter the left atria. On the floor of the left atria is the left atrioventricular (AV) valve, which is the entrance to the left ventricle. The left ventricular free wall is three times thicker than the right ventricular wall and forms the bulk of the heart. The left ventricular outflow tract is positioned centrally in the heart and leads to the left semilunar valve and the aorta.

1.3.2. The blood gas barrier

The blood gas barrier (BGB) in the human lung has an estimated surface area of between 50-100m² yet remains incredibly thin, measuring 0.2-0.3μm [16]. The membrane partitions air and blood, providing the interface for gas exchange. The movement of respiratory gases occurs entirely by passive diffusion along partial pressure differences [13]. Membrane requirements for efficient gas exchange correlate inversely with the thickness of the barrier. Meanwhile, the BGB needs to maintain structural integrity, stability and tolerate torsion. This creates conflicting bioengineering requirements - extremely thin for efficient gas exchange, yet strong enough to maintain structural integrity. In the Thoroughbred, the BGB is neither thick enough to prevent EIPH, nor thin enough to prevent hypoxia during exercise [45]. Compared to systemic blood vessels that are anchored in connective tissue, pulmonary blood vessels are literally “suspended in air” by a diffuse fibro-skeletal framework allowing blood to flow through the capillaries as a sheet [17]. Because of this the alveoli are subject to collapse during positive alveolar pressure and can bulge into the alveolar space during negative alveolar pressure or positive luminal pressures.

The BGB is a three-ply design comprising of a thin epithelial cell that lines the alveolus, extracellular matrix and an endothelial cell that lines the capillaries (Fig 1.3-4). Pulmonary capillaries have a thin and a thick side. On the thin side of the capillary is the blood gas barrier, covered by a thin layer of surfactant [17]. The thin side is designed to facilitate efficient gas diffusion and is thought to gain strength from the basement membrane of the extracellular matrix between the alveolar
epithelial cell and the endothelial cell which is strengthened by type IV collagen [17]. The thicker side of the capillary is where the interstitium is wider, containing type 1 collagen fibers. This side facilitates fluid movement between the pulmonary capillary and the interstitium when capillary pressure rises [17]. It is assumed that failure of the BGB occurs on the weaker thin side, however it is unknown what stresses are present on the thicker side of the BGB. There is evidence that the basement membrane on the thin side provides the ultimate strength of the BGB [18].

Figure 1.3-4. Electron micrograph of a pulmonary capillary in the alveolar wall demonstrating the extremely thin BGB, and the thicker side of the capillary containing an endothelial cell (EN) and fibrils of type 1 collagen (COL). RBC- red blood cell; PL- plasma; EP- epithelial cell; EN- capillary endothelial cell, FB- fibroblast. Taken from West 2008 [13]

1.3.3. Pulmonary blood flow during exercise and rest

The lung has two blood supplies: the bronchial circulation, which is a high pressure system, arising from left ventricular outflow and supplying the bronchi and connective tissue of the lung; and the pulmonary circulation, a low pressure, low resistance system facilitating gas and heat exchange at the alveolar level [12].

Considerable inequality of pulmonary regional blood flow exists, and in people the blood flow decreases linearly from the bottom to the top of the lung [13]. The uneven distribution of blood flow at rest was initially thought associated with hydrostatic pressure differences and explained by a proposed ‘zonal’ model. In quadrupeds, gravity and the ‘zonal’ model has minimal influence of blood
flow distribution [19, 20]. In resting horses, despite a large vertical lung height, blood flow is increased to the caudodorsal region [20] suggesting factors other than hydrostatic pressure control perfusion. The heterogeneity of pulmonary blood flow in the horse is likely a normal circulatory function, modulated either by regional or structural differences in vascular reactivity, changes in pulmonary vascular pressures, or through a yet unknown mechanism.

In vitro studies of equine pulmonary vessels demonstrated regional differences in endothelial reactivity to vasoactive substances. Equine pulmonary arteries (7mm diameter) located ventrally were found to contract in response to endothelium dependent vasodilators, such as methacholine, whereas dorsally located pulmonary arteries relax [21, 22]. Regional heterogeneity of smaller (100-400μm) pulmonary vessels to vasoactive substances is also present, but the response differs to larger vessels [23]. Small veins were found to relax in response to methacholine whereas pulmonary arteries demonstrated a response based on region, contracting in the caudodorsal regions and relaxing in the cranioventral region. Differences between the mechanical properties of regional pulmonary veins and arteries have been identified [24]. Caudodorsal arteries were found to be stiffer than cranioventral arteries. Conversely, cranioventral veins are stiffer walled than caudodorsal veins.

Regional differences in the density of pulmonary capillaries has been also been demonstrated in two horses [25]. Fewer capillaries were identified in the cranial lobes (54%) compared to the caudal lobes, but there was no difference in capillary density when comparing dorsal and ventral regions.

More than seventy percent of pulmonary blood flow is spatially fixed between rest and exercise [26]. This fixed pattern of blood flow is predetermined such that the greatest determinant of regional blood flow, is the blood flow at a previous time point; i.e. low flow regions at rest remain low flow during exercise, and vice versa. Only 30% of blood flow variation during exercise is redistributed, and this is predominantly sent dorsally. The variation in blood flow states can be incited with light trotting exercise, and further increase in exercise intensity does not alter blood flow patterns. The authors proposed that as pulmonary arterial pressure increases the recruitment of vasculature is greater in non-dependent regions of the lung; hence blood flow is redistributed dorsally [26].
1.3.4. Cardiopulmonary response to exercise

Maximal performance requires the coordination of multiple body systems operating either at, or close to, maximum capacity. During intense exercise such as racing, the metabolic demands increase more than 30-fold compared to the resting state [27]. Unlike the musculoskeletal system, the respiratory system does not undergo an adaptive response to training [28]. Ventilatory parameters do not change with training hence some physiologists conclude that the respiratory system is the major limitation to performance in the racehorse [27].

The major function of the respiratory system is gas exchange and to assist with heat dissipation. Breathing frequency can increase from 12-15 breaths/minute to more than 120 breaths/minute and tidal volume increases from 4.8 litres at rest to more than 13 litres during galloping [29]. This produces minute ventilation values greater than 1850 L/min in trained Thoroughbreds at maximum exercise [30]. The increased respiratory rate during exercise is modulated by: 1) stimulation from central and peripheral chemoreceptors detecting changes in carbon dioxide, oxygen and pH; 2) mechanoreceptors within the airway that detect change in lung compliance; 3) stimulation of phrenic and diaphragmatic afferent nerves; 4) locomotory stimuli linking breathing frequency with stride (locomotory respiratory coupling); and 5) central nervous system inputs [27].

The volume of blood expelled from the left or right ventricle is termed the cardiac output (CO) [31]. Cardiac output is the primary means of increasing muscle oxygen delivery during exercise and is calculated by multiplying the heart rate by the volume of blood ejected from the left or right ventricle per beat (stroke volume) and is expressed as litres per minute. Heart rate increases from 32 beats/minute at rest to 201-241 beats/minute (HR-max), and stroke volume has been reported to increase from 800-900 ml (2-2.5 mL/kg) at rest up to 1700ml (3.8±0.4mL/kg) during treadmill exercise in fit Thoroughbred [32].

The right side of the heart delivers its entire cardiac output to the pulmonary circulation, and as a result extremely high pulmonary vascular pressures are generated during exercise. Cardiopulmonary vascular pressures have been measured during treadmill exercise either at maximal heart rate (HR max), a set treadmill speed and incline, or under saddle in Thoroughbred and Standardbred racehorses [33–39]. Six Standardbreds were exercised at speeds equivalent to 75, 90 and 100% HR max [38]. Mean pulmonary arterial pressure (PAP) increased with exercise
peaking at 70.5±5.2 mmHg. Despite a large increase in cardiac output in response to exercise, the increase in PAP is dampened by a fall in pulmonary vascular resistance associated with recruitment and dilatation of vessels. The increase in PAP in Thoroughbreds is reportedly higher than in Standardbreds [37]. Exercising on a level treadmill at 15 metres/second for 90 seconds produced a mean PAP of 101.8±3.8mmHg and a mean transmural pulmonary arterial pressure (PAP minus oesophageal pressure) of 110.6±3.4mmHg [37]. Thoroughbreds have a significantly higher pulmonary capillary and pulmonary wedge pressure compared to Standardbreds when exercised at the same HR max [39]. Variability between studies examining vascular pressures at treadmill speeds rather than HR max may fail to account for this breed variability in speed and individual horse fitness [38] and/or ability.

Transmural pressure is the pressure applied to a membrane, essentially the intravascular subtract the extravascular forces [58]. The transmural pressure is considered of greater importance than just the vascular pressure alone, because extremely negative extravascular pressure, for example alveolar pressure, can summate with the positive vascular pressure, for example capillary pressure, and place enormous distending pressure on the barrier [58]. Pulmonary artery transmural pressure is estimated, often calculated by subtracting oesophageal pressure from PAP measurement.

A linear relationship between exercise intensity (treadmill speed) and pulmonary pressures has been identified [34, 35, 40]. However, when exercised at a specific intensity (percentage of HR max), rather than treadmill speeds, a linear relationship between pulmonary vascular pressure and intensity was not established after reaching 75% HR max [38]. Adding to this, pulmonary vascular pressure varies based on the rate of treadmill acceleration [41]. Slow, incremental increases in treadmill speed produced lower pulmonary artery pressure, right atrial pressure, pulmonary arterial wedge pressure and pulmonary capillary pressure compared to rapid acceleration to the same speed.

What mechanism is responsible for the high pulmonary vascular pressures during high intensity exercise? Manohar and Goetz (1999) identified that pulmonary vascular resistance (PVR) decreases significantly with exercise but reaches its nadir at moderate exercise (8m/sec), and further increases in speed did not produce further pulmonary vasodilation [42]. The initial reduction in PVR
is due to vasodilation and recruitment of vascular beds, but once the upper limit on this has been reached, PVR became fixed. Based on Ohm’s Law (pressure \( \alpha \) flow x resistance), pulmonary blood flow determines pulmonary artery blood pressure; hence the increase in cardiac output associated with higher intensity exercise, is likely responsible for further significant increases in pulmonary artery blood pressure [35, 43]. In humans despite both ventricles having similar cardiac outputs, PVR can only be reduced by 30-50% during exercise compared with systemic vascular resistance reducing in excess of 75% [44]. Consequently, the harder the exercise the greater the demands on the right ventricle compared to the left.

1.3.5. Pathophysiology of EIPH

Despite a high prevalence of disease there remains a poor understanding of EIPH pathophysiology. Our current understanding has largely stemmed from histopathology and the ultra-structural changes in lungs of affected animals post mortem. Multiple theories on pathophysiology have been proposed; the most widely accepted is stress failure of the pulmonary capillaries.

**a) Stress failure of the pulmonary capillaries**

Stress failure of the pulmonary capillaries has been described in several pathological and physiological states. One such physiological state known to induce stress failure of the pulmonary capillaries is intense exercise, and this has been documented in multiple species, including horses [45–47]. Evidence that intense exercise could induce disruptions to the Thoroughbred’s BGB was garnered when West *et al* (1993) galloped three horses on a treadmill for 3 minutes at 13.4-14 m/sec [45]. The horses were quickly euthanised and the lung tissue fixed for electron microscopy. Ultra-structural images of the lung tissue revealed breaks in the epithelial and/or the endothelial sides of the BGB allowing erythrocytes and oedema to accumulate within the alveolus and interstitium.

There are three stresses placed on the BGB [48] (Fig 1.3-5). Circumferential (Ttmp) or loop stress develop due to the capillary transmural pressure (Pcap). This stretches or causes the capillary endothelial cells to bulge into the alveolar space and can potentially stretch the alveolar
epithelial cells also. Surface tension (Tst) stress acts to support the capillary wall during expansion (bulging), creating an inward pressure on the endothelial cell. Lastly, during inflation of the lung, longitudinal tension (Tel) is applied to the alveolar wall. Failure of any structure is initiated when the material is loaded beyond its strength limit. High capillary wall stress and subsequent failure can be induced in two ways: by increasing the capillary transmural pressure or with high levels of lung inflation [16].

Figure 1.3-5. The forces acting on the blood gas barrier. Circumferential pressure (Ttmp) is associated with the capillary transmural pressure (Pcap). Surface tension (Tst) exerts an inward pressure supporting the capillary. Longitudinal tension (Tel) is associated with lung inflation. Taken from West et al (1991) [48]

Stress failure of the BGB was initially demonstrated in anaesthetized rabbits by raising the pulmonary capillary transmural pressure [48]. At capillary transmural pressures of ≥40mmHg endothelial and alveolar epithelial breaks were identified. In dogs and horses similar findings were identified when capillary pressure is increased beyond 70mmHg and 100mmHg, respectively [49, 50]. The difference in pressures required to disrupt the BGB between species appears proportional to its thickness. The barrier is the thickest in the horse, intermediate in the dog and thinnest in the rabbit and this is inversely proportional to the wall stress attained at a nominal pressure [51]. The micromechanics of failure of the BGB is not fully understood; disruptions are seen within the endothelial and/or epithelial cell, rather than at the intracellular junction [16, 52]. Often the basement membrane of the BGB remains intact [48, 53]. Elliott et al (1992) reported that most disruptions to the BGB are reversible once the capillary transmural pressure is reduced, particularly if the basement membrane remains intact [54]. The number of
endothelial and epithelial breaks identified decreased by almost 70% when the tissue was exposed to a reduced capillary transmural pressure for 3 to 6 minutes prior to fixation. The rapid reversibility of breaks in the BGB when the pulmonary capillary pressure returns to normal, suggests mechanical rather than biological repair, and that there may be an elastic component present within the BGB [54]. Type IV collagen has bending sites present, which permits distortion, and may contribute to the rapid reversibility of damage to the BGB [17].

In the horse damage to the BGB was predicted to occur if capillary transmural pressures exceeded 65mmHg [51]. Failure of the blood gas barrier has been observed with capillary transmural pressure (estimated using arterial-venous difference) between 72.5mmHg and 83±3.6mmHg [37, 55]. Observed pulmonary pressures recorded during galloping are higher than those reported to induce BGB failure [34–36]. Bronchoalveolar lavage (BAL), a procedure used to ‘wash’ the distal airways and examine retrieved cells, was used to correlate the luminal red blood cell concentration and transmural pulmonary artery pressure after treadmill galloping [37]. Free red blood cells reflect haemorrhage associated with pulmonary capillary rupture. At 15m/sec transmural pulmonary artery (PA) pressure (intravascular PA pressure minus oesophageal pressure) exceeded 95mmHg and erythrocyte counts were significantly increased above resting values (Fig1.3-6)

![Figure 1.3-6](image)

**Figure 1.3-6**: Change in concentration of red blood cells per mL of lavage fluid as mean transmural pulmonary arterial pressure increased. Solid horizontal line represents the regression through control and 9,11 and 13m/sec speeds. Dashed line represents the regression through 15m/sec values. Taken from Langestmo et al, 2000 [37].

Meyer et al (1998) reported similar results when the PAP exceeded 90mmHg resulting in significantly increased free erythrocytes in BAL fluid [56]. But pulmonary vascular pressures in
exercising horses were not significantly different between horses with and without EIPH, as diagnosed using tracheal endoscopy [34, 55]. This could reflect individual variation in BGB thickness or strength, or variability in pulmonary vascular pressures at set speeds [37]. Furthermore, in humans, pulmonary hypertensive states can induce cell-mediated signaling to increase the amount of type IV collagen present in the basement membrane [57]. A similar response in racehorses has not been documented, but if present would contribute to individual variation in susceptibility [45].

Failure of the BGB can also occur during sub-maximal speed exercise, not associated with high pulmonary vascular pressures [58, 59]. Epp et al (2006) reported that EIPH could be induced by low speed (trotting) exercise performed on an inclined treadmill to the point of fatigue [58]. Although the mean PAP was maintained at relatively low levels (<60mmHg) during exercise, maximal estimates of transmural PAP (calculated by subtracting minimum esophageal pressure from the highest PA pressure reached) exceeded 105mmHg as tidal volumes increased. Bronchoalveolar lavage revealed significant increase in erythrocytes confirming that damage to the BGB is not solely dependent upon PAP, but also extra-vascular pressures. The most negative extravascular pressure occurs in the caudodorsal lung region, the anatomic location of EIPH [60]. Consequently, transmural capillary pressure is expected to be greatest in this region [61]. Experimental manipulation of intra-pleural pressure can be achieved using inclined galloping on a treadmill. A reduced stride frequency associated with inclined running results in a greater negative intra-pleural pressure [62]. Inclined galloping also reduced breathing frequency, increased tidal volume, and reduced the mean peak PAP, but increased the severity of pulmonary haemorrhage compared to level galloping. It was theorized that inclined galloping created more negative alveolar pressures, thereby increasing transmural pressure, which increased EIPH severity highlighting the contribution of extra-vascular pressures to failure of the BGB.

Horses preferentially bleed into the alveolar space instead of developing pulmonary oedema, as occurs in humans during high altitude pulmonary capillary stress and subsequent failure. One explanation might be the rate at which the increase occurs [61]. In people with high altitude pulmonary oedema, the pulmonary capillary pressure increases over days, whereas racehorses attain maximal speed in seconds. Alternatively, pulmonary capillaries in Thoroughbreds may differ
from humans and adapt to repeated high-pressure events, possibly through collagen deposition in the basement membrane [61].

**b) Veno-occlusive remodeling**

Pulmonary veno-occlusive remodeling is characteristic of diseases associated with pulmonary venous hypertension in humans and other species [63, 64]. Experimental exposure to supra-maximal venous distension over 2 minutes in pigs is enough to initiate vessel remodeling [65]. Similarly, in sheep exposed to sustained pulmonary hypertension using a continuous air embolization model, vascular remodeling of pulmonary veins was initiated within 4 days [64]. Racehorses are known to experience high pulmonary vascular pressures during exercise and a similar remodeling response has been documented [66, 67]. Williams et al (2008) examined the lungs of 7 racing Thoroughbreds with a history of at least one episode of EAE [66]. Histopathological examination of the lung revealed region specific changes in the intralobular veins. In the caudodorsal lungs a prominent collar of mature collagen surrounded pulmonary veins, causing the vessel lumen to be reduced and, in some cases, occluded. The collagen was found within the adventitia and dispersed between the external elastic lamina and the vessel lumen. These changes were not identified in the ventral lung regions. Derksen et al (2009) reported similar changes, noting that the outer perimeter of remodeled veins did not increase in size, but rather the remodeling occurred in an inward direction only, reducing the lumen size by a third [67]. Regional veno-occlusive remodeling was always identified alongside regions of fibrosis and haemosiderin, and fibrosis and haemosiderin were not identified in regions without veno-occlusive remodeling [24, 66, 67]. In young, untraced control horses veins in the caudodorsal lung were markedly thinner walled without evidence of vascular remodeling [24, 67].

Veno-occlusive remodeling was absent in studies investigating post mortem lung changes associated with single and repeated autologous blood instillation in the bronchi suggesting that the physical presence of blood in the airway does not play a role in fibrosis and veno-occlusive remodeling [68, 69]. Veno-occlusive remodeling is a part of the characteristic lesions of EIPH. It is always present in areas of the lung affected by EIPH and is likely initiated by pulmonary hypertension associated with exercise. The consequence of increased vascular resistance due to venous
remodeling is to further increase the pulmonary capillary pressure upstream, likely exacerbating stress failure of the BGB during exercise and clinical EIPH, with on-going fibrosis and hemosiderin accumulation [67].

c) Locomotory impact–induced trauma

Variability in detecting tracheal blood after exercise despite no significant difference in pulmonary vascular pressures [55] along with evidence of EIPH during low intensity exercise [70] were highlighted as inconsistencies for stress failure of the BGB as the primary basis of EIPH [71].

The ears and the lung are the organs most susceptible to damage from stress waves secondary to bomb explosions [72]. When the chest is exposed to stress waves the impact is transmitted through the tissue in waves, intensifying in the apical regions of the lung causing tensile stress on the alveolar walls with resultant oedema and localized haemorrhage [73, 74]. It was proposed that EIPH resulted primarily from locomotory transmission waves originating from the forelimbs and transmitted to the scapulae, chest wall and then the lung [71]. The waves travel dorsally and caudally and are focused and amplified in the caudo-dorsal lung lobes. If impact waves are sufficiently strong tearing of tissue results. Schroter et al (1998) estimated that the impulse pressure exerted on the chest wall of a 500kg horse at galloping speed would exceed the magnitude required to cause oedema and haemorrhage [71]. As further evidence the authors suggested that electron microscopy findings of stress wave pulmonary trauma mimics that seen with BGB failure, with theoretical symmetric lesion distribution between left and right lung lobes [71]. That later argument has been challenged in that EIPH lesions are not necessarily symmetrical, and evidence of tracheobronchoscopic unilateral lung haemorrhage on occasions exist [75, 76]. Thorpe et al (2009) examined the change in shape of the equine thorax during exercise, and given that the gallop is an asymmetric gait these changes were examined both with the horse galloping on the preferred forelimb leg lead, and on the opposite forelimb lead [77]. Transverse and dorso-ventral thoracic dimensions are inversely related and negatively correlated with the trailing limb side. A finding described elsewhere [78]. Theoretically, stress wave propagation may be greater in lung lobes less inflated, and these findings may reflect asynchronous ventilation of the lung lobes, and hence an asymmetrical distribution for lung haemorrhage [77].
A two-dimensional computational model was developed to assess the propagation of stress waves originating on the anterior subscapular surface [79]. Stress waves were found to amplify and focus towards the caudodorsal lung tip. The lateral movement of waves in a three-dimensional plane is unknown, as is wave movement around the heart.

Clinical support of a locomotory induced traumatic aetiology of EIPH was suggested in an epidemiological survey of epistaxis [80]. Horses participating in steeplechase races were found to be at a significantly increased risk of epistaxis compared to horses racing in flat races, where no jumping occurred, a finding that has been identified elsewhere [81]. It was proposed that the act of jumping creates greater pressure waves increasing the risk of epistaxis. EIPH studies have also identified that jumping races pose an increased risk, but findings were reported in light of a small sample size and inadequate statistical analyses [9]. In an epidemiological survey of EIPH in competition draft horses, no horse had EIPH when examined 30-90 minutes after competition [82]. The authors argue this finding is relevant to this proposed aetiology of EIPH because draft horses impact the front of their harness with enormous force to overcome the inertia associated with moving a 1700-2000kg sedentary weight. It is not unreasonable to think that shock waves generated from this, could mimic the shock waves associated with foot strike during galloping, but this was not associated with EIPH. Swimming is a high intensity exercise, mimicking blood pressure and heart rates that occur during galloping exercise [83, 84]. Despite this, there are no published reports of EIPH associated with swimming. Certainly epistaxis has been reported to occur during swimming, but the haemorrhage is reported to originate in the pharynx, not the lung [85, 86]. Ventilation is markedly altered during swimming; horses have a sustained expiration against a closed glottis followed by an explosive and rapid inspiration [86]. It is proposed that smaller sub-atmospheric intrapleural pressures occur during swimming leading to reduced transmural pressures. Theoretically, this would translate into a reduced risk of stress failure of the pulmonary capillaries. In any case, the lack of EIPH associated with swimming does not necessarily favour locomotory impact trauma over capillary stress failure as mechanisms for EIPH.
\textit{d) Abdominal-visceral piston}

During asymmetric gaits such as the gallop and canter, respiration is coupled with stride frequency. Locomotion respiratory coupling (LRC) is coupled 1:1 during the gallop \cite{87}, although in horses with upper respiratory tract obstruction or lacking fitness the LRC could be erratic, switching to 2:1 or even 3:1 \cite{36, 88}. Inspiration at the gallop is confined to the off-loaded period, when the horse is either entirely off the ground or supported only by the rear limbs \cite{88, 89}. Exhalation commences as the first forelimb touches the ground and the thoracic limbs take the load. It is suggested that coupling the stride with respiration minimizes mechanical constraints to ventilation imposed by the limbs and contributes to exercise hyperpnoea. The mechanistic drive of LRC is contentious \cite{78, 87, 88, 90}, but the equine thorax is known to be rigid, and during galloping chest and abdominal wall expansion is minimal, and paradoxically, out of time with ventilation \cite{91}. Despite, tidal volume increasing 4-5-fold during galloping, chest circumference increases only minimally - 1-2cm, and abdominal circumference is unchanged. The longitudinal expansion of the abdomen associated with lumbar extension is the likely contributor to the augmented tidal volume. This is not surprising, given that if the chest wall was more compliant the increase in tidal volume and intrapleural pressure during intense exercise would collapse the lung inwards during inspiration. Contraction of the diaphragm during inspiration and the caudal movement of abdominal viscera are facilitated by back extension and abdominal muscle relaxation \cite{89}. Conversely, contraction of the abdominal muscles and back flexion facilitates expiration. Changes in back flexion and extension are likely the primary mechanism coupling respiration and locomotion \cite{90, 91}.

It was suggested that the abdominal viscera impacting on the diaphragm could cause trauma of the adjacent lung lobes thereby causing EIPH \cite{88, 92}. This concept lauded additional support with the belief that during galloping the abdominal viscera movement enhanced the LRC by facilitating expiration \cite{93}. However, it is now known that cranial displacement of the viscera against the diaphragm does not coincide exactly with expiration; in fact, the movement lags expiration by between a quarter and half per cycle \cite{90}. The visceral piston is not driving ventilation during exercise. It is unknown what effect the visceral piston has on lung trauma.
e) Upper airway obstruction

Any process that could increase transmural pressure could theoretically enhance stress failure of the BGB. Any extra-thoracic, inspiratory airway restriction or obstruction could cause an increase in the negative intra-thoracic pressure during inspiration, with resultant higher capillary transmural pressures and an increased likelihood of pulmonary capillary stress failure [94]. Ponies galloping on a treadmill with left laryngeal hemiplegia (LLH) were found to have markedly increased pleural pressure (up to 300%) compared to normal ponies [36]. In racehorses LLH was thought to contribute to EIPH by increasing transmural PAP, but initial research reported no increase in transmural PAP in affected horses [95]. Further work found that although LLH did not significantly increase transmural PAP, it did increase the pulmonary capillary pressure and the left atrial (wedge) pressure, supporting the theory that capillary pressure is more dependent on left arterial pressure than pulmonary artery hypertension [94]. Although theoretically likely to increase the likelihood and/or severity of EIPH, the results were not quantified with BAL cytology or tracheobronchoscopy. A more recent study in 93 horses identified no significant association identified between EIPH and either dynamic or static upper respiratory tract obstruction [96]. Dorsal displacement of the soft palate (DDSP), a purely expiratory obstruction, was associated with increased PAP during galloping but not transmural PAP. And it was concluded that DDSP was unlikely to contribute to EIPH [97].

A bit-induced asphyxia has been proposed to induce an airway obstruction via instability and dynamic collapse of the nasopharynx and larynx causing an increased negative pressure in the alveolus leading to EIPH [98, 99]. Currently there is no clinical evidence to support or refute this theory.

f) Haemorheologic aetiology

It was proposed that EIPH was caused by a defect in hemostasis [100, 101]. Examination of a small number of EIPH positive and EIPH negative horses (based on BAL fluid cytology) was unable to identify a difference in coagulation profiles at rest [100]. But in vitro platelet aggregation in response to agonists, such as adenosine di-phosphate, (ADP) was graded as poor in both rate and response in horses with EIPH. In contrast to this, a hyper-coagulable state has been reported in
horses with EIPH compared to controls when sampled after exercise [102]. The authors theorized that chronic haemorrhage could up-regulate coagulation pathways, as a response aimed at reducing future episodes of EIPH. Sampling during sub-maximal exercise identified increased coagulability and fibrinolytic activity that resolved after the cessation of exercise [103], a finding also reported in humans [104]. The authors hypothesized that rather than coagulation defect in horses with EIPH, that exercise caused increased fibrinolysis and this could contribute to EIPH. Others suggest EIPH could be associated with increased blood viscosity [105, 106] or a lack of erythrocyte deformability leading to capillary damage and subsequent EIPH [107]. Echinocytes are spiculated erythrocytes that are less deformable [108] and may contribute to higher pulmonary capillary wall stress [109]. Pretreatment with pentoxifylline, a phosphodiesterase inhibitor that improves RBC deformability was found to attenuate the exercise-associated increase in pulmonary vascular pressures in ex vivo pony lungs [107]. But blood samples from horses with an induced echinocytosis were more filterable compared to controls, had less cell to cell interaction, had a reduced sedimentation rate and did not increase blood viscosity [110]. Echinocytes were not rigid cells and were unlikely to inhibit or obstruct micro-vascular blood flow. Furthermore, no difference was identified in the proportion of deformed erythrocytes in horses with or without EIPH or EAE [106].

The role of polymorphic genes involved in hemostasis predisposing horses to EIPH has been investigated [111]. CD39 is a gene expressed on vascular endothelial cells and platelets and is involved in last step of converting AMP to adenosine. Twenty of 21 horses diagnosed with EIPH were either heterozygous or homozygous for the gene encoded CD39. Variability in severity between horses undertaking similar exercise regimes may be associated with the inheritance of distinct CD39 variants.

g) Inflammatory airway disease

Inflammatory airway disease (IAD) forms part of the spectrum of diseases termed “Equine asthma”; a range of respiratory conditions resembling human asthma [112]. Clinical signs of IAD include occasional coughing (often associated with exercise), excessive tracheobronchial mucus, poor performance without any signs of systemic illness or musculoskeletal disease, and BAL changes consistent with airway inflammation, including a mild increase in neutrophils, eosinophils
and/or metachromatic cells. There was a long-held perception that EIPH was unlikely to occur in “healthy lungs” and was caused by a pre-existing respiratory disease [6]. This was supported by post mortem studies demonstrating inflammatory changes, such as bronchiolitis, associated with EIPH lesions [113]. Small airway disease causing a subclinical airway obstruction and inhomogeneous ventilation was proposed as pathogenesis for EIPH [114], but the association between IAD and EIPH remains controversial [112].

A model of lung inflammation induced by instilling a diluted acetic acid solution into the bronchus was used to investigate the relationship between pulmonary inflammation and EIPH [115]. After instillation, horses were exercised to fatigue, and a BAL was performed on both the control and inoculated lung segment. The inoculated segment was 6 times more likely to have free erythrocytes in the BAL fluid than the control, indicating that localized lung inflammation was associated with EIPH. Similar results were reported in a small group of horses (n=11) exposed to aerosolized ovalbumin [116]. But in both these studies, haemorrhage was not limited to the caudodorsal lung lobes, but rather, limited to any segments exposed to the inflammatory agent. Inflammatory airway disease is regarded a diffuse disease, yet EIPHT occurs exclusively in the caudodorsal lung lobes [117, 118].

It is controversial if pulmonary inflammation is a cause or the result of, EIPH. Evidence of an association between IAD and EIPH was identified in a group of young Thoroughbreds in training [119]. A diagnosis of IAD was made using an aggregated scoring system comprising of tracheal mucus score and tracheal wash cytology. But this study was limited by the lack of uniformity of exercise intensity at the time of sampling. EIPH has been associated with pro-inflammatory markers [120] and increased nucleated cell counts in BAL fluid [121]. Yet others have found no association between haemosiderin-laden macrophages in tracheal secretions and IAD, mucus score, coughing or BAL neutrophil counts [70, 122–127].

Autologous blood instillation has been used to investigate the inflammatory response evoked by the physical presence of blood in the airway. Autologous blood instillation in various broncho-pulmonary segments produced a moderate inflammatory response including the recruitment of macrophages [128–130]. But this technique was not shown to alter bronchial reactivity or sensitivity during histamine provocation indicating there was no increased airway hypersensitivity associated
with the inflammatory response to airway blood [131]. Morphometric pulmonary changes secondary to blood instillation were reported to include increased alveolar septation and collagen deposition [132]. But this study recruited retired racehorses of various ages, which, although rested for a period of 3-months would be expected to have pre-existing lung changes from their time spent racing. Others report that single autologous blood instillation is rapidly and effectively cleared from the airway and does not induce collagen accumulation [68]. Furthermore, repeated instillations (up to 5 times over 10 weeks) did not increase perivascular or interstitial collagen, indicating that the pathogenic changes associated with EIPH are not driven by blood in the alveoli, but rather from within the vasculature [69].

**h) Cardiac aetiology**

Abnormal cardiac function is theorized to cause increases in pulmonary vascular pressure and increase the risk of EIPH. Left atrial pressures exert influence over the pulmonary vasculature and transmural pressures. Atrial fibrillation (AF) is a condition of random atrial depolarization, chaotic in nature, causing a loss of synchronized contraction [133]. It is associated with decreased cardiac output, increased pulmonary capillary wedge pressure and increased right atrial pressure in people [134]. Reported clinical signs of AF in racehorses include marked reduction in performance, epistaxis, and a brief period of ataxia, distress or even collapse [135]. Horses with AF will have higher heart rates at equivalent gaits than horses without AF and are more likely to develop ventricular arrhythmias during exercise [136]. The association between AF and EAE has been reported sporadically in case studies [137] followed by a retrospective evaluation of 106 horses with AF, of which 23.5% also exhibited EAE [138]. But large-scale epidemiological surveys have not confirmed this association. Associations between AF and EAE were examined from almost 40,000 race records in Japan [139]. Horses that finished more than 4 seconds behind the winner, did not finish the race or had epistaxis were auscultated within 2-3 minutes of racing to determine if they had AF. One hundred and fifteen horses were identified with AF, and epistaxis was identified in 78. Only two horses had both AF and EAE, no significant association was identified between AF and EAE. This study relied on the assumption that horses with AF always finished >4 seconds behind the winner.
and that veterinarians could accurately identify the dysrhythmia with auscultation. However adding to these findings no association was detected between BAL evidence of EIPH and cardiac disease (dysrhythmia or poor left ventricular function post exercise) in a retrospective evaluation of clinical records in Pennsylvania [140].

Collapse, with or without sudden death, has been associated with severe EAE [141]. It is unknown if a cardiac arrhythmia such as paroxysmal AF or AF-associated ventricular arrhythmias are the underlying cause of the collapse and EAE, or if this is a fulminant manifestation of EIPH [141, 142]. Deciphering the cause of fatal EIPH is complicated by acute pulmonary post mortem changes, such as haemorrhage, oedema and congestion. Pulmonary oedema and congestion are consistent with acute left sided pump failure and can occur with ventricular arrhythmia or catastrophic mitral valve failure. The former cannot be determined post-mortem.

It was thought that tricuspid and mitral valve regurgitation would increase the risk of EIPH [143]. The association between mitral valve regurgitation (MR), pulmonary venous hypertension and pulmonary oedema in congestive heart failure is well established, and the implication for EIPH appears justified. Although Warmbloods with moderate MR had increased pulmonary wedge pressure when compared with normal horses at similar speeds, there was no significant difference detected when MR was graded mild [43]. Similarly, no association was found between MR or tricuspid regurgitation (TR) and EIPH in National Hunt horses [144]. However, right ventricular lumen and internal dimensions in systole and diastole were positively associated with EIPH. Unfortunately, this study was severely limited by its classification of severe EIPH, relying on the trainer’s or primary care veterinarian’s impression of disease status, rather than objective measurement. In human athletes, although all four chambers undergo alterations in size, the changes in the right ventricle do not always mirror changes in the left ventricle despite the same cardiac output [145]. The wall stress disproportionally affects the right ventricle compared to the left in human endurance athletes [146]. In the racehorse, the right side of the heart undergoes changes in size with age and race fitness [147]. With exercise, there is a strong association between PAP and cardiac output; and the higher the pulmonary pressure the more work for the right ventricle compared with the left. Perhaps an association between right ventricular size and EIPH can be explained by an increased preload
(volume overload) and/or increased afterload (pressure overload) on the right ventricle, resulting in compensatory increase in wall thickness and lumen size [144].

**i) Thromboembolism and parasites**

Parasitism was proposed as a possible aetiology for EIPH based primarily on lesion location, with a predilection for parasites lodging in the caudal lung lobe [148]. This theory was based on pulmonary radiographic changes, and the authors postulated a subclinical parasitic infection might be responsible. This was supported at the time by lung histopathology from horses with suspected fatal EIPH describing “multiple oblong, oval or circular foci of degenerating eosinophils resembling parasite migration tracks observed in the pulmonary parenchyma” [142]. Aside from the above-mentioned observations, no association between EIPH and parasitism has been identified on BAL fluid, tracheal wash cytology or histopathology, and in recent years this aetiology has largely been ignored.

**j) Bronchial circulation as the cause of EIPH**

The lung has two blood supplies; the pulmonary circulation, which receives the entire cardiac output of the right ventricle and the bronchial circulation which receives about 1-2% of cardiac output from the left ventricle [133, 149]. The bronchial circulation arises from the broncho-oesophageal artery, a branch of the thoracic aorta [150]. The bronchial circulation supplies oxygenated blood to the airways, connective tissue and pulmonary vasculature. The architecture differs in the horse compared to other mammalian species in that it traverses not only axially along the airway but also provides an extensive sub-pleural vascular network [151]. Upon reaching the capillary bed, the bronchial arterial vessels can anastomose with the pulmonary circulation.

The source of blood in EIPH was first proposed to originate from the bronchial circulation based on gross lung pathology linking the dense network of sub-pleural bronchial arteries in the caudodorsal lung lobes with hemosiderin-stained tissue found in this region [76]. Marked bronchial artery proliferation in the caudodorsal lung lobe added to speculation that the bleeding was from the bronchial circulation [152]. Adding to this was that in humans, disruption to the bronchial circulation
is often associated with haemoptysis [153]. Research in exercising ponies identified that an increase in blood flow through the bronchial system is directly proportional to increasing PAP; consequently, the bronchial circulation could be susceptible to rupture as a consequence of bronchial hypertension [154].

Pulmonary hypertension in sheep experimentally induced with continuous air embolism resulted in remodeling not only of pulmonary veins and arteries, but also bronchial vessels [64]. The pulmonary venous remodeling is described above. The bronchial circulation increased not only in external vessel diameter compared to controls, but also in the number of vessels, increasing approximately three-fold after 4 days exposure to hypertension. It is therefore plausible that bronchial angiogenesis reported in racehorses with a history of EIPH is due to chronic exposure to intermittent bouts of pulmonary hypertension. Also, in other species, bronchial artery angiogenesis is a normal response to lung tissue repair, occurring in response to inflammation, asthma or cancer [155]. In humans, unlike the pulmonary circulation, the bronchial circulation has a large capacity for angiogenesis. This is similar to the systemic circulation, and perhaps the extensive proliferation of the bronchial circulation in racehorses is an effect, rather than cause of EIPH [133]. This is consistent with more recent evidence that the origin of lung haemorrhage in EIPH is from the pulmonary circulation [45].

Injection of labeled microspheres into the pulmonary and systemic circulations of exercising horses was used to determine the source of airway blood [156, 157]. BAL fluid collected post exercise identified spheres from the pulmonary circulation only, not the systemic circulation. However, given that the bronchial circulation receives only a small fraction of cardiac output it remains possible that any haemorrhage from the bronchial circulation could have been missed.

1.4. Epidemiology of exercise induced pulmonary haemorrhage

Exercise-induced pulmonary haemorrhage is most frequently described in Thoroughbred and Standardbred racehorses, but it is neither limited to these breeds, or these specific activities. The disease has been identified in racing Appaloosas and Quarter Horses, as well as horses involved in
other high intensity athletic pursuits, including showjumpers, 3-day eventers, barrel racers, steeplechasers and polo horses [125, 158–164].

The disease has been documented in other species undergoing strenuous physical exertion. Endoscopic examination of 20 poorly performing camels after racing 8-10 kilometres found blood in the airway of 6 camels [165]. Examination of racing greyhounds after a simulated 503-metre race revealed an increase in red blood cells (RBC) post-race in respiratory fluid indicative of EIPH [47]. There are sporadic case reports of EIPH in human athletes, such as marathon runners and cyclists [46, 166, 167].

The prevalence of EIPH within a population of horses varies depending on the method of detection, the intensity of the exercise being performed, and the frequency and timing of examination.

Blood from the nares after racing or intense physical exertion typically represents the most severe form of EIPH, and this form is most commonly encountered in the racehorse. Most worldwide racing jurisdictions fastidiously record episodes of epistaxis, which are readily available in the public domain. These conveniently accessible race records have permitted several epidemiological studies of Thoroughbred racehorses, assessing the prevalence of, and risk factors for, EAE.

A two-year review of epistaxis from race records in South Africa between 1948-1949 identified that “one in every 82 (1.2%) horses bleed during a race” [168]. The same author later published racing records for six yearling crops that were followed until they were 7 years old in which 2.41% (128) experienced epistaxis on at least one occasion [169]. A Korean study retrospectively examined 5 years of race records between 1993 and 1997 [170]. A total of 61,181-race starts from 2,963 racehorses were recorded during the study period. Four hundred horses were recorded as bleeding on one or more occasions. The incidence of bleeding in this population was 13.5%, the highest reported in the literature, equating to 0.84% of race starts.

In Hong Kong, data from the 2004-05 racing season, 49 Thoroughbred horses were reported with post-race epistaxis from a total horse population of 1358. This represents 3.68% of the population and equated to 0.55% of race starters. When epistaxis during training and racing were combined, 5.74% of the racing population experienced an episode of epistaxis [171].
Japan has a reported EAE prevalence of 0.15% per race start for Thoroughbred and Anglo-Arab racehorses [81], one of the lowest reported prevalence in the world. The low prevalence was attributed to the relatively young racing population surveyed (64% were less than 3 years old). The prevalence of epistaxis in Australian Thoroughbreds was assessed using racing records retrieved from New South Wales (NSW) and the Australian Capital Territory (ACT) for races between 1999 and 2008 [172]. One thousand two hundred and five episodes of epistaxis from 1085 horses were recorded from over 500,000 race starts, a prevalence of 0.24% per race start, affecting 1.9% of the racing population.

A UK study reported the prevalence of epistaxis in racing Thoroughbreds to be 0.08% per race start for flat, steeplechase and hurdling races combined [80]. The authors suggest this figure as a minimum estimate only as horses were not specifically checked or monitored after racing by on-course veterinarians, and a computerized recording system was not introduced in the UK until 2004, which may have led to some horses misclassified. The potential for misclassification of cases is supported by a more recent study in Great Britain observing a comparatively higher incidence of epistaxis [173]. Between 2001 and 2009 the incidence of epistaxis was 3.6 per 1000 in hurdle races (0.36%) and 5.3 per 1000 cases in steeplechase racing (0.53%).

As discussed earlier epistaxis denotes the most severe manifestation of EIPH, grossly under reporting disease prevalence. Detection of EIPH using tracheobronchoscopy within 120 minutes of racing equates to a disease prevalence ranging between 44 - 75% [8, 9, 124, 174–176]. The discrepancy in the range of reported prevalence of EIPH can be explained by study methods and design. Early studies reported a prevalence of EIPH in 43.8 - 46.8% in Thoroughbred racehorses [7, 8]. Endoscope length was 100cm, limiting complete examination of the distal trachea, increasing the proportion of false negative results. When a longer endoscope is used (140cm) and the entire length of trachea can be viewed, the prevalence of disease increases to 55.4 - 75.4% [124, 174, 177, 178].

The intensity of the exercise also contributes to discrepancies in reported prevalence. Tracheobronchoscopic examination of horses after ‘breezing’, essentially a training gallop, reports a lower prevalence of EIPH of 38.4% [9]. Whereas tracheobronchoscopic examination performed after racing, which is more strenuous and over longer distances, has a higher prevalence of EIPH, 74.5%.
Increasing prevalence of EIPH across different training speeds or gaits was also identified in a group of Thoroughbreds, confirming exercise intensity or speed is an important factor in determining EIPH prevalence [70]. Similarly, examination of endurance horses identified a minimum speed required for EIPH to occur; speeds greater than 240 metres/minute are necessary for a horse to have EIPH, and horses racing at speeds below this did not experience EIPH [159].

The prevalence of EIPH increases when horses are examined on multiple occasions after racing [8, 70, 119, 174, 178]. Examination of 168 Thoroughbreds after racing reported a disease prevalence of 73.8% [174], when 47 were examined a second time after racing the prevalence increased to 94.7%. When examined after racing on 3 occasions the prevalence increased further such that blood was identified on at least one occasion in every horse. Likewise, in sixty Standardbreds the prevalence of EIPH increased from 62% on their first examination to 87% when examined three times [178]. These results would suggest that almost all racehorses will have EIPH on at least one occasion during their racing career.

The time between racing and tracheobronchoscopy also affects EIPH prevalence. The rostral movement of blood to the trachea is a time and volume dependent process. Examinations conducted too soon after racing will increase the proportion of false negative results [124], thereby reducing true disease prevalence.

Bronchoalveolar lavage (BAL) is a technique used to wash the distal airways, and when performed blindly will sample the caudodorsal lung lobes, the region affected by EIPH [179]. Cytology consistent with EIPH includes the presence of free RBCs and/or macrophages containing the RBC pigment, haemosiderin. These macrophages are referred to as haemosiderin-laden macrophages or haemosiderophages. In a study of 6 horses, exercised intensively on a treadmill, there was no evidence of haemorrhage on tracheobronchoscopy post-exercise, but all horses had free RBCs in BAL fluid collected immediately post exercise, and haemosiderophages when sampled 1-3 weeks later [56]. Cytology of BAL fluid is considered the most sensitive indicator for detecting EIPH [37, 56, 180, 181]. A survey of BAL fluid cytology in Thoroughbred racehorses reported 90% of samples contained haemosiderophages demonstrating previous EIPH episodes [181]. Others have reported that all Thoroughbreds racehorses sampled have haemosiderophages, irrespective
of good or poor performance [180]. EIPH prevalence determined via BAL is dependent upon the level of exercise being performed [182].

1.5. Risk factors for EAE and EIPH

Risk factors have been identified for both EAE and EIPH. It is unknown if risk factors for EAE are pertinent and applicable to EIPH. Retrospective epidemiological surveys of EAE use conveniently accessible race records permitting analysis of large sample sizes. A Japanese study examined 251,609 race starts for Thoroughbred and Anglo-Arab horses racing between 1992 and 1997 [81]. The authors examined a range of horse and race characteristics including age, sex, race distance and the type of race (flat or steeplechase). All horses were examined within 30 minutes of racing for evidence of epistaxis, and if present underwent endoscopic examination of the trachea. If blood was identified in the trachea, EIPH related epistaxis was recorded. There were 369 episodes of EAE recorded from 325 horses. Multivariable logistic regression analyses were used. Epistaxis was more common following steeplechases races than flat races (P= <0.01; adjusted OR 5.58; 95% CI 3.18-10.47), a finding also identified by others [80]. This was suggested to be due to the larger weights being carried in these races compared with flat races and the act of jumping increasing pressure waves to the lung parenchyma from the forelimbs; supporting the locomotory-induced trauma pathogenesis for EIPH (see above) [80].

Older horses were found to be at increased risk of EAE compared to 2-year old horses (P<0.01; Adjusted OR 2.21; 95% CI1.27-4.25) [81]. Early epidemiological studies also reported a positive association between EAE and age with limited statistical analyses [6, 168–170]. A large retrospective study from Australia compiling 1,852,912 race-starts also supported this finding [183]. Age, in most instances reflects an accumulated time spent racing, examining more specific variables such as lifetime starts, and years spent racing, is arguably a more accurate reflection of the cumulative impact of racing [80]. Newton *et al* (2005) examined both age and years spent racing [80]. Both variables increased the risk of EAE, but age was considered a ‘proxy measure for time spent racing’, which was found to be a superior overall predictor of EAE. But the risk of EAE does not increase linearly with time spent racing, but rather plateaued and even declined with flat and hurdle races [80]. This was credited to a ‘healthy horse effect’, where horses that experience
epistaxis do so within the first few years of racing and are lost to racing thereafter. This ultimately results in a pool of older horses that are ‘healthy’, being less prone to EAE and can accrue longer racing careers.

The older a horse is at their first race start increases the risk of epistaxis (P<0.001; OR 1.13; 95% CI 1.07-1.2) [173]. This finding contradicts an effect of accumulated racing and the authors suggested a genetic predisposition for EAE or EIPH delaying the horses’ racing career, or similar to soft tissue and bone injuries, commencing racing at an earlier age has a conditioning or protective effect for EAE [184, 185].

Race distance is commonly reported as a risk factor for EAE, although with conflicting results. The frequency of epistaxis in hurdle races in Great Britain decreased as race distance increased (P<0.001, OR 0.75; 95% CI 0.64-0.87) [173]. Similar findings were confirmed in Japan, where after adjusting for race type, races that were less than 1600 meters were a risk factor for EAE compared with races that were between 1601 and 2000 metres long [81]. In contrast, others have identified middle to longer distance races to be a risk for epistaxis [168, 170], whereas some have been unable to identify any relationship [186]. Unfortunately, a lack of multivariable analyses in these later mentioned studies limits their interpretation.

A sex predilection for EAE also encounters mixed results in the literature. Takahashi and others [81] identified a higher risk of EAE in females compared to entire males (P= <0.01; OR 1.42; 95% CI 1.13-1.79), whereas other large epidemiological surveys identified females as having a reduced risk of EAE in hurdle races compared to males and geldings, but this effect is not present for flat, steeplechase or when all race types were combined [80]. This finding contrasts with Pfaff (1976) [169] who reported geldings were almost twice as likely to have EAE than females and entire males. This finding is consistent with larger Australian and South African studies that a higher proportion of Thoroughbred males had EAE compared to females [172, 186]. There is no biological explanation offered for a sex predilection for EAE. Financial driven management decisions capitalizing on the breeding potential of females, result in geldings having longer racing careers which, increases their racing exposure compared to females [172]. The largest retrospective study compiling 1,852,912 race starts found no effect of sex on epistaxis [183]. Females were found to
experience EAE earlier in their race careers compared to males, an effect that was unable to be explained.

Track ‘going’ or firmness has been investigated sparsely in the literature as a risk factor for EAE. Increasing track firmness was found to be a significant risk factor for EAE for steeplechase and hurdle races, but not flat races [80, 173]. But EAE in flat races was considered such a rare outcome that this model may have lacked statistical power to detect a significant effect of track firmness [80]. Studies examining only flat races have been unable to show any association between track firmness and EAE [183]. Faster winning speed was associated with an increased risk of EAE in flat races [80]. Faster winning speeds are recorded on firmer tracks and over shorter distances, and this finding was speculated to be associated with speed, a known factor to increase EIPH prevalence.

Certain environmental and geographical factors have been implicated with increased risk of EAE. In multiple South African studies, racing at sea-level, compared with racing at higher altitude (5,400 ft. above sea level), had a significantly higher incidence of epistaxis [168, 169, 186]. There are anecdotal reports that mid-Atlantic states in the USA (mostly at sea level) have a higher frequency of EAE compared with remainder of the United States [9]. Environmental factors such as season and temperature have been infrequently mentioned as risk factors for EAE. A significantly higher (P< 0.001) incidence of epistaxis was observed in winter and spring than in summer and autumn in South Africa [186]. But racing during the spring period (February, March and April) was associated with an increased risk of EAE compared to racing in winter [80]. This finding was overshadowed by a potential confounding factor that the period of increased risk coincided with the end of the National Hunt jumping races and could reflect an accumulated racing effect. A higher incidence of epistaxis was detected in South Korea during spring [170], and again in South Africa during winter [168, 169], but detailed analysis is lacking precluding meaningful interpretation of these results.

Increased risk of EAE has been associated with horses returning to racing after prosthetic laryngoplasty and ventriculocordectomy to treat recurrent laryngeal neuropathy, an upper respiratory tract obstruction [187]. This finding was reported in a small sample size of Thoroughbreds and the authors proposed that either the surgery failed to restore normal exercising transmural pressures or
that post-operative chronic airway contamination with saliva or food material predisposed the lung to haemorrhage.

The recurrence of EAE in horses returning to racing is higher than in the general population prevalence [81, 172, 173, 183, 188, 189]. In Great Britain, examination of 272,562 jump races starts revealed that horses with a history of EAE were 6 times more likely to have EAE than the general population (P< 0.001; OR 6.05; 95% CI 4.4-8.3) [173]. Furthermore, 71% of recurrent episodes occurred within a year of the initial event [188]. In Japan, EAE prevalence in horses previously diagnosed with EAE is 13%, compared to the general population prevalence of 0.15% [81]. Similarly, in Australia, Langford et al (2013) reported only 53.8% of horses return to racing after having EAE reported to racing authorities, but of those that do return, 20.8% had a second episode of EAE [172].

There is low quality evidence that a genetic predisposition to EAE exists [183, 189, 190]. The evidence was considered low quality due to challenges in identifying cases, misclassification of positive or negative cases, and because heritability may reflect factors that facilitate epistaxis rather than the severity of EIPH, such as passage of blood from the lungs to the nostrils [117]. Regardless of this, using the world’s second largest Thoroughbred population, data was collated and analysed for heritability of epistaxis in Australian Thoroughbreds racing between 2000 and 2011 [183]. Records for 1,852,912 race starts from 117,088 horses, identified 2,474 (2.1%) horses with one reported episode of epistaxis. Heritability of epistaxis was analysed in two ways: firstly, as a single, non-repeated event taken over the lifetime racing career of the horse; and secondly, at each individual race event (i.e. epistaxis reported or not reported for each race). Epistaxis was analysed as a binary threshold trait using a logistic generalized linear mixed model. Analysed as a single non-repeat event over a horses' career the heritability of epistaxis was 0.27 ± 0.02, and heritability of individual race epistaxis as a repeated measure was 0.5 ± 0.01. The strong genetic influence of epistaxis in this group of Thoroughbreds is on par with heritability of limb conformational traits [191] and is higher than heritability of performance traits, such as race time and speed [192, 193]. The genetic susceptibility may explain the variation in severity between horses exposed to the same environmental and non-genetic factors outlined above or the prevalence between racing populations. The authors theorized that a specific combination of haplotypes was required to increase the susceptibility of the blood gas barrier to failure during exercise, resulting in epistaxis. These findings
are supported by an earlier study from South Africa that reported a heritability estimate for sire models of 0.40, demonstrating a genetic link for EAE [190]. There is no evidence published about the heritability of EIPH.

Large epidemiological surveys investigating tracheobronchoscopic EIPH are limited in the literature. The high disease prevalence in the racing population requires large sample sizes to detect significant associations between EIPH and investigated risk factors and smaller surveys are vulnerable to type II statistical error. One such larger study prospectively examined 744 Thoroughbreds using post-race endoscopy and reported several risk factors for EIPH [124]. The authors investigated two models based on 1) the presence of EIPH (yes/no), and 2) the severity of tracheal haemorrhage when present. The variables retained in both models were ambient temperature, the presence of dirt in the trachea, and the time interval between finishing the race and examination.

The time interval between racing and tracheobronchoscopy is a risk factor for EIPH as it pertains to diagnosis [124]. The characteristic location of EIPH is in the caudodorsal lung lobes and the movement of haemorrhage from this location rostrally to the trachea is a time and volume dependent process. It is recommended that tracheobronchoscopic examination be conducted between 30-120 minutes after racing or breezing [194]. When examinations were conducted too soon after racing (mean time 31 minutes; 95%CI 19-46 min) it was associated with a decreased likelihood of detecting EIPH, and underestimating severity in 744 horses [124]. But, a similarly designed later study involving the same authors, reported opposite findings in 886 horses; the longer the time interval between race and examination the more likely to diagnose horses without EIPH [175]. In this study, 75% of horses were examined within 30 minutes (median 22 minutes, mean not provided). The authors suggested that this finding reflected the later examination of winners for regulatory reasons, compared to non-winners. The authors proposed that winners were less likely to be EIPH positive because they performed well and won, compared to non-winners that didn’t perform well and were therefore more likely to have EIPH. This may be an overly simplistic interpretation of the relationship between EIPH and performance. To date there has been no study that has determined an ideal time for tracheobronchoscopy after racing, but it is possible that the ‘ideal’ time may vary with disease severity. For the most severe EIPH grades timing after racing is
likely irrelevant, within reason, [175] as that grade will be maintained for a longer period of time. But lower grades are more susceptible to misclassification as blood is only transiently present in the trachea.

Dirt in the trachea was identified as a risk factor for EIPH [124]. Horses with dirt present in the trachea were less likely to have EIPH. Horses racing towards the tail of the field inadvertently inhale dirt stirred up by the leading horses. The authors assert that this finding is associated with dirt obscuring small quantities of blood.

A link between season and EAE has been described (see above). There are limited reports of an inverse relationship between endoscopic EIPH and temperature or season [119, 124, 178]. Ambient temperature was shown to be significantly associated with the presence and severity of EIPH over a relatively small temperature range (12.9-22.9°C) [124]. Horses racing at temperatures below 20°C were 1.8-2.0 times more likely to have EIPH present and be more severe than horses racing at temperatures greater than 20°C. A significant negative correlation between temperature and EIPH had previously been reported in Canadian Standardbreds, racing over a temperature range of -15 to +24°C [178]. The authors suggested that this finding could be a surrogate for the length of time in work, as colder temperatures coincided with the end of the racing season, although they could not discount a possible role of temperature. Spiers et al (1982) found no relationship between temperature and endoscopic EIPH in Standardbred racehorses in Australia, but the study was compromised by incomplete examination of the trachea and a small sample size [10]. An effect of season was also reported in racing Thoroughbreds in Rio de Janeiro, Brazil, [177] with horses racing during winter at increased risk of EIPH (OR= 1.78).

Age has been identified as a risk factor for EIPH in competition and racing horses [7–9, 158–160]. Pascoe et al (1981) reported a trend towards increased frequency of EIPH with age, with a larger proportion of older horses having EIPH, but a statistically significant association with age could not be established [7]. Raphel and Soma (1982) used a similar study design, except for a longer (140cm) fiber-optic endoscope, and identified a direct relationship between EIPH and age in racing and breezing (P< 0.05), but potential confounding variables were not accounted for [9]. In multivariable analyses cumulative measures of racing are significant, whereas age is not [124]. Hinchcliff et al (2010) identified that horses that had greater than 50 lifetime starts were 1.8 times...
more likely to have severe endoscopic EIPH, than horses that had less than 40 starts [124]. But in this study, and in Australian Racing generally [228], few horses would have had > 50 starts (mean number of starts 18.7; 95%CI 2-49). Morley et al (2015) reported no significant association between EIPH and age and did not examine the association between lifetime starts and EIPH [175]. The general persistence of these risk factors, albeit with varying but related definitions, across multiple epidemiological surveys pertaining to both EAE and EIPH suggests there is a cumulative effect of racing on EIPH, but additional longitudinal studies are required to confirm this.

Like EAE, there are mixed findings in the literature regarding the impact of race distance on EIPH. Some studies have identified an increased risk of EIPH over longer distance races (≥1600 metres) [9, 177], while others have identified increased risk in races less than 1400 metres [124]. Disagreement between studies could be attributed to study design, inadequate sample size, selection bias or statistical analyses. Costa et al (2006) performed endoscopic examinations 30-60 minutes post-race on 2,118 Thoroughbreds [177]. Multiple logistic regression analyses identified that horses that compete in longer races (1600-3500 metres) were 1.41 time more likely to have EIPH than horses racing over short or intermediate distance races (i.e. 1000-1500 metres). Conversely, in a diuretic-free racing population in Australia, race distance was not associated with the detection of EIPH (yes/no), but horses competing in races less than 1400 metres were at increased risk of moderate to severe EIPH than horses racing over distances greater than 1400 metres [124].

Race type was associated with the risk of developing EIPH in a small study with limited statistical analyses [9]. Tracheobronchoscopic examinations were performed post-race in 31 Thoroughbred steeplechasers competing over distances between 2.1-4.0 km, 14 Thoroughbreds racing on flat turf, and 3 Thoroughbred timber race competitors (4.8 km race jumping solid timber obstacles). The authors documented EIPH in 67.6% (21/31) steeplechasers, 14.3% (2/14) flat racers, and 66.6% (2/3) of the timber jumpers. Aside from reporting the prevalence with each race type no additional analyses were performed.

Rain affected tracks, referred to as ‘heavy’, were associated with increased risk of severe EIPH in Brazil [177]. The authors postulated that increased effort was required when the track was heavy, worsening EIPH. Others have reported no association between EIPH and track going, or
penetrometer reading, a measure of ground hardness or penetration [124, 175]. No studies have identified an association between EIPH and sex [124, 178], altitude or racetrack surface [175].

Moderate quantities of haemorrhage, detected with tracheobronchoscopy, were associated with increased likelihood of blood detected on subsequent occasions (P<0.001) [178]. This finding is supported by an earlier study of 76 Thoroughbreds examined on two occasions [9]. A good level of agreement was seen between observations (κ=0.523; P<0.001). Some studies have been unable to establish repeatability between examinations, but study design limitations, such as using a short (100 cm) endoscope, and inconsistencies in exercise intensity may account for this [8, 70]. Others reported that EIPH detected in the previous month increased the risk of identifying EIPH, using tracheal washes or tracheobronchoscopy [119].

Certain racing jurisdictions within the United States and worldwide permit the administration of furosemide (FUR; aka frusemide), a diuretic, pre-race to horses that have been identified in previous races as ‘bleeders’. Furosemide is the most commonly prescribed medication for the management and treatment of EIPH. Interpretation of results from epidemiological surveys conducted in a population treated race day with or without FUR is unknown [7, 174, 177].

1.6. Performance in the racehorse

Horse racing is a contest that is wagered upon; which horse will finish first under the set conditions of the race. If a horse wins or places, the performance is viewed favorably, if the horse finishes last or at the back of the field, the performance is considered poor. But race day performance is not that simplistic. Applied in this sense, a horses’ performance is ultimately determined by its own ability, not by the ability of its competitors. Some horses will be faster than others; some horses finishing towards the tail of the field could be performing at their maximum physiological limit, performing well, relative to their ability. Therein lies the challenge of separating ability from performance.

There is no consensus of a single or collective objective measure of performance in racehorses. Many authors researching surgical interventions, medical treatments, or the impact of disease have attempted to use performance in racehorses to quantify a positive or negative effect.
Reported measures of race day performance include finishing position, distance finished from the winner, race earnings and race time [124, 175, 195]. Reported career performance indices include maximum race class or handicap [196], lifetime earnings [175, 197], average earnings per start [198], lifetime wins and placings [175, 196, 199, 200], number of career starts [187, 196, 201], a performance index [197, 202, 203], speed rating or Beyer speed figure [204] and statistical standardization [199, 205, 206]. Subjective measures of performance such as trainer impression of performance have also been used [181, 197, 207, 208]. But numerous factors will affect these performance indices which are often overlooked, including age, sex, assigned weight, barrier, suitable race distance, trainer or jockey skill, track surface and rating, class of race, and other factors [209–211].

1.6.1. Performance measures used in clinical trials

(a) Race and sectional times: Race time is the time taken to complete a race, reflecting the animal’s speed over a set distance. Sectional times reflect the time taken to travel over a certain part of the race, such as the last 600 meters. Race and sectional time is right skewed due to the physical limits of racing at top speed, and no limit to how slow a horse can race [212]. Not surprisingly, longer races have slower average speeds than shorter races and vice versa [205]. Race time is often advocated as the most accurate measure of performance, in essence the horse that maintains the fastest speed wins and other measures such as finishing position and earnings are derivatives of race time [213].

Track type (dirt or turf) and track rating (muddy or firm) have a significant effect on race time [214]. It is estimated that 1.13 ms⁻¹ is gained on a ‘fast’ track compared to a heavy track [213]. Racecourse design differs in aspects such as the degree and length of an incline, the angulation of the bends and the length of the straight. Hence, race time over set distances varies between racecourses [214]. There is a significant inverse relationship between race time and prize money offered (P<0.0001) [205]. Larger purses attract better competitors with superior speed; purse is positively correlated with race class. Age affects race time; three-year-old horses have faster race times than two-year-old horses, and two-year-old’s attain faster race times as they accrue
race experience [215]. Mota et al (2005) found four-year-old horses to be significantly faster than all other ages for all distances excluding 1100 metres [213, 216]. Sex affects race time; male Thoroughbreds are faster than females, an advantage estimated at 1.5 lengths in all race distances [213, 217]. Sex superiority has also been identified in trotters [218, 219]. Some racing jurisdictions compensate for this with a 2 kg weight allowance for fillies and mares when racing against males [220]. The time a horse can run over a set distance alone is slower than what it can produce during racing [213]. The addition of a second horse doesn’t improve race time either, but thereafter each additional horse in the race, speed was increased by 0.02 ms\(^{-1}\). Others suggest increased number of competitors in a race has the potential to increase the race time for individuals due to an increased risk of interference [205].

Weight was found to positively correlate with finishing time, while barrier contributed a small but significant effect on race time; inside (closer to the rail) barriers have a faster race time than outside barriers [213, 216]. Summer months were associated with faster race times than other times of the year, but potential confounding factors such as 2-year-old racehorses accruing race experience in winter and spring and making fewer mistakes in summer could also explain this association [205].

(b) Finishing position: This is the ranked position in which horses pass the finishing post. The parameter can be expressed as a decile or can be used to calculate the “percentage of the field beaten”; variations expressed therein account for the differences in field sizes, which in Australia can range from 2 to 24 competitors [220].

Finishing position as a measure of performance is irrespective of race class. All races, notwithstanding the considerable difference in ability between a maiden race, and the top echelon Group 1 race, are treated equally. Some horses may not attain podium-finishing positions in major races, but their performance could still be considered more successful than winning or placing in a lower class of race, which is overlooked when comparing finishing position. Comparing finishing position of unplaced horses is also problematic as the finishing rank of these horses is not reliably reflective of competitiveness. If a horse is no longer in contention of a prize the race is not competitively finished. The jockey often eases the horse up prior to the finish, worsening their finishing position.
The odds of winning and placing are dependent upon the number of race starts. If a horse is
unplaced at one race start the next race start is not an independent event, but rather the horse
has a higher chance of earning because the trainer chooses a race more suited to the aptitude
of the horse [209]. In Thoroughbreds and French trotters the chance of being placed at a horse’s
first start is low, the chances of being placed at starts three or four increases to 50%, and
thereafter increase rapidly to almost 100% over ten race starts [209]. Winning or placing in
Thoroughbreds is affected by sex with male horses more likely to win and place compared with
aged-matched female horses [221, 222].

(c) Number of wins and places: This is expressed over the lifetime career of the animal and is the
numbers of wins, seconds or thirds that were accrued.

(d) Distance finished behind winner: This variable is usually expressed as ‘lengths’, which is the
average galloping length of a horse, approximately 2.4 metres. Like finishing rank, the distance
behind the winner can be exaggerated for horses that were not in contention of placing and
earning prize money because the jockey and horse relax over the concluding stages of the race.
In sprinting races, the distance finished behind the winner is condensed across the field, whereas
in longer distance races the distance finished behind the winner is exaggerated as horses’ tire
and there is no limit to how slow they can finish.

(e) Race and lifetime earnings: This measure reflects the earnings for a horse in a race or over a
lifetime. Variations of this have been expressed as annual amount or average earnings per start
[198, 223, 224]. Horses winning and placing up to position 10 can receive prize money. The race
class determines how many placed horses receive prize money [220]. Career and race earnings
have a highly skewed distribution, due to large numbers of non-earning horses and it is
problematic to statistically distinguish between non-earners and horses that didn’t race.

Using race and lifetime earnings to examine performance is often endorsed because it is
argued that each horse has equal opportunity to earn but not equal ability. Cheetham et al (2010)
reported lifetime earnings to be significantly impacted by the total number of starts [225], which
is not surprising given that horses that don’t race don’t earn prize money. Colon et al (2000)
suggested, without evidence, that average earnings decline with age due to young horses having
access to age restricted and purse supplemented racing [196]. Mean annual earnings were
highest in 3-year old Thoroughbreds and 4 and 5-year old French trotters [209]. These findings are on par with career profiling for racing Canadian Standardbreds, North American trotters and Thoroughbreds whereby earnings decline with age [218, 225]. Other factors that influence career earnings include sex, gait and track surface (dirt or turf) [225].

Race earnings are affected by training and time since their previous race start [210, 223, 226]. Horses that raced in the preceding 30 days won significantly more prize money in a race than horses that had not raced over that period [223].

(f) Career starts: Career starts reflects career longevity; it is the number of races participated in during a career. In 1998, the average number of career starts for Australian and New Zealand Thoroughbreds that competed in at least a single start was 15 (range 1 - 82), and comparison of male and female racehorses revealed significant differences in career duration [227]. The average career length for males and females were 1013 days and 461 days, respectively. This difference was attributed to the breeding potential of females, shortening their racing career. This finding has been identified in Australian Thoroughbreds [210, 228], Canadian Standardbreds [229] and racing American Quarter Horses [212]. The risk of retirement for entire males increases with increased earnings, whereas for geldings and females the risk decreases as earnings increased [230]. The number of starts and earning per start accrued during the first year of racing is a significant predictor of a horses’ career length, as unsuccessful horses are removed from racing at an earlier stage in their career [210, 230]. Horses that commence racing at 2 years of age have significantly longer careers than those that commence racing as 3 or 4 year old horses [210, 222, 229, 230]. The average distance raced was significantly associated with career duration in Hong Kong and Australia; longer distance racing extended career duration [228, 231].

(g) Race handicap: Race handicap is an official numeric value assigned to a horse based on the number of wins and places to date. The rating is assigned by the official handicapper and can be adjusted up or down after each race. The rating is used to determine eligibility for certain races. It is also used to assign the weight carried by allowing easy comparison between horses thereby facilitating the handicapping process for racing. The higher the rating the more superior the horse. Some authors record the highest class of race entered by the horse, but it is often not clear if they are competitive in this class, or just raced [196], whereas others have recorded the
highest rating achieved during a career [224].

(h) Performance index (PI): The PI is a point system based on finishing position and is often used to compare performances before and after a disease or an intervention, such as surgery [199, 202]. The PI is the mean of the points earned in ‘x’ number of races prior to the intervention and is compared to the mean points scored in the same number of races after an intervention [197]. Others variations of this measure includes a scalar value for race purse and multiplying this value by finishing position, then dividing the value when the horse had the intervention by the value when the horse did not have the intervention [203]. A PI >1, indicates improvement after the intervention, and PI<1, the reverse.

(i) Rating speed figure: Rating speed figures include the “Timeform” rating for Australia and the United Kingdom, and the “Beyer speed figure” (BSF) in North America. The BSF is a computer-generated number calculated based on race time, the racing surface and track. Timeform is a computer-based assessment calculated from race distance, race time, track surface, weight carried, age and other factors. Punters and handicappers and occasionally researchers have utilised this value [224].

(j) Regression model for standardizing performance: A multiple regression model was developed using data for 20,461 Thoroughbreds racing at 5 racetracks in Louisiana between 1981-85 [205]. The model incorporated the parameters track surface (muddy, good and fast), track, distance raced, age, prize money, race number (in the day), number of participants, weight carrier, barrier and season of the year and evaluated the impact of these on racing times. The coefficients could then be used to adjust finish times. The prediction error of this model is applied by subtracting the predicted finish time from the actual finishing time. A positive result indicated poor performance, and a negative result was interpreted as improved performance. This model has been applied only a limited number of times in the literature [199, 206].
1.6.2. Factors affecting performance measures

(a) Trainer factor: Trainers are engaged by owners to prepare a horse for racing. The trainer determines the exercise regime, management, and race programming for each horse. The trainer has been identified as a significant factor impacting performance measures on two levels; exercise training regime and an unmeasured trainer effect [210, 223, 226]. Using binary outcome multivariable analysis for winning (yes/no) and winning prize money (yes/no) the outcome of 860 horses racing in 5210 races was examined [223]. High-speed exercise in the preceding 30-days from the case race was a significant factor affecting winning (P=0.004) and winning prize money (P<0.001). The trainer was a significant variable also retained in the model for winning and earning prize money (P<0.001). After the inclusion of the pre-race exercise variables into the model, trainer remained a significant factor, suggesting that other factors associated with individual trainers are intrinsic to racing success. When horses that did not earn prize money (51% of observations) were excluded from the model, the trainer factor still remained significant for the amount of prize money won (P<0.001). These findings are similar to others where an unmeasured trainer factor influences performance [210], as well as the exercise regime preceding the case race [226]. These unmeasured factors may include appropriate race selection, fitness, veterinary care, and preference of a certain jockey, nutrition or other management factors. Analysis of 1804 Australian Thoroughbreds identified clustering at the level of trainer for total race earnings during the first year of racing, indicating that horses from within a stable have similar performance [210]. This could be attributed to training regime or the trainer’s reputation to attract horses of similar breeding, value and ability.

(b) Jockey Factor: The ability of horse and jockey are intrinsically related. As the reputation of the jockey increases so does the opportunities to be associated with greater ability horses. The jockey was found to be a significant factor for racing time (P<0.01) over all distances, on turf and dirt [232]. The effect of this factor increases as race distance increases. An investigation of performance in Polish Thoroughbreds examined race, horse and rider as random effects. All of these parameters were significant with a “rider” effect having the smallest component of variance (P= 0.0017; Wald-Z statistic 2.93) [213].

(c) Age: As discussed above, age influences race time, race and career earnings.
(d) Sex: As discussed above sex influences race time, race place and earnings.

(e) Weight carried: It is a law of physics that the magnitude of acceleration is inversely proportional to the mass of an object. The larger the mass, the slower the acceleration, which indirectly affects speed. Weight is used as a handicap to equalize the chances of all competitors. Better performing horses are asked to carry more weight in handicapped races thereby improving the chances of lower weighted rivals. The weight carried by the horse includes the jockey weight, saddle and if required, extra weight can be added to the saddle cloth to reach the assigned weight. As discussed earlier, weight positively correlates with finishing time. A five-pound (2.27 kg) weight difference was found to give a third of a length advantage to horses carrying less weight [205]. But Oki et al (1994) found linear regression coefficients for racing time on weight carried to have a negative value, indicating that horses with heavier weight produced faster racing times, but concluded that the weight allocated was perhaps not enough to compensate for the difference in ability [233]. It is likely that a set minimum weight to be carried in races negates some of the effect of handicapping. Weight allocation can impact performance depending on how the race is handicapped or if the weights are set, i.e., each horse carries the same weight (see below regarding race class).

(f) Race class: Not all races are handicapped races where horses are weighted according to their rating with the aim of equalizing each horses chance of winning. Some races, such as two or three-year-old races are restricted entry to these specific ages or sex, and each horse, irrespective of prize money or ability, carry the same weight, encouraging the best of that age group or sex to win. Other races, such as weight for age set weights, which are allocated on age, season and race distance rather than rating. In certain conditions, individual horses will be better suited to the conditions of the race, for example weight for age or set weights which encourage the superior to horse to win, rather than equalizing the chances of all competitors.

(g) Race distance: As implied this is the set distance of the race. Sprinting distances (≤1400metres) contain genuine sprinting horses, highly competitive over the shorter courses. However, they also often contain staying horses that start their racing in sprint events to gain fitness and race experience. Furthermore, young horses always start in sprinting races prior to being tried over
longer distances [213]. This affects the competitiveness of these later groups over shorter distance races.

(h) Barrier drawn: this is the starting position for the race. Outside barriers can translate to some horses being trapped “wide” (further from the inside rail or inner boundary) during the race thereby needing to cover additional ground than those horses positioned closer to the inside rail. Barrier contributed a small but significant effect on race time; inside (closer to the rail) barriers have a faster race time than outside barriers [205, 213, 216].

1.6.3. Defining poor performance

Poor athletic performance is considered common in racing. The term, poor athletic performance implies that the horse previously had satisfactory performance and now has not, or that race day performance does not reflect training performance. Clinical papers investigating groups of horses with poor performance are often without objective substantiation, relying on the owner or trainer’s subjective opinion that the horse is not performing to their expected level [234, 235]. This group of horses could have poor performance attributable to a variety of factors including fitness, management or race selection, or they could have limited ability, which is often overlooked by researchers. Poor performance evaluation requires careful consideration of various factors because, although a clinical anomaly may be identified, it may not be the sole contributor to the current performance.

Population based epidemiological surveys investigating poor performance often do so on a large scale, broadly identifying factors that may be related with good or poor race day and career performance. Many studies examining population-based diseases assume handicapping equalizes the ability of each competitor, allowing a straightforward comparison between competitors. But, not all races are handicapped on ratings and not all handicapping is fair for each horse, as discussed above.

Australian handicappers are bound by the Australian rule of racing 103(1) when allocating weight in races [220]. In 2008, the rule was altered to reduce the spread of the weight across the field. As it stands in Western Australia, the minimum top weight at nominations must not be less than
59 kilograms, and at time of acceptances to race, not be less than 58kg. The minimum weight that can be assigned is 54 kilograms. This rule excludes Group 1 (elite) and all 2-year old races, where the minimum top weight at nomination must be 58 kg, and the minimum weight can be 53kg. Weights compressed using this formula are a disincentive for lower rated horses to contest handicap races above their rating, because the weight advantage against the higher rated horse is not present, lowering their chances of winning. Consequently, the comparison of performance between horses, on the assumption that they are weighted equally, is unreliable. Weight for age and set weight races are also unreliable to compare performance, because these races generally favour the fastest, superior ability horses, and some horses are not suited to this type of handicapping. Hence a poor result under these circumstances does not necessarily reflect poor performance, but rather an unsuitable race for that individual. So how can we determine if a horse has (a) performed poorly; (b) has been placed in an unsuitably strong race, or; (c) isn’t suited to race conditions, when considering their performance? It is naive to think that each horse has an equal probability of winning, based on handicapping alone.

1.6.4. Using betting markets to predict performance

If we could use an equation to determine if a horse had poor performance it would be the actual finish position subtracted from the predicted finish, as based on specific criteria. A positive value would reflect a better than expected result, while a negative value may indicate poor performance. Ideally, the predicted finish would take into consideration the race class, the weight carried, age, sex, barrier, jockey ability, trainer ability, the race conditions, if the race distance was suitable and the aptitude of the competitors. The predicted finish would consider if the race was appropriate or winnable for an individual, incorporating the quality of the opposition and forecast the finishing order.

“The racetrack betting markets are surprisingly efficient. Markets odds are remarkably good estimates of winning probabilities” [236].

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In Australia there are three betting options for horseracing: a bookmaker either online or on-course, pari-mutuel totalizer (tote) and a betting exchange. The latter is a relative newcomer to betting and will be discussed later. The two main forms of betting markets are described as ‘market makers’ and make profit based on betting turnover. In pari-mutuel betting all bets are pooled. The racetrack retains a set percentage or ‘take’ of the pool, and winning bets are distributed from the amount remaining in the pool. The odds of a horse winning are determined by market demand minus the ‘take’, the more money that is wagered on a horse, the shorter the odds. The odds are not established until the close of betting when the race commences, although investors have a guide on movement of the market with odds updated every 30-45 seconds. But almost 40% of money wagered on an event is taken in the last minute of betting, which impacts on the final odds [237]. The opening quote by a bookmaker is their assessment of the winning chances of that horse. Subsequently, the volume of money wagered on each horse then dictates the odds. The odds are ‘fixed’ for the bettor at the time the bet is placed. It is unwise for bookmakers to ignore the bettor’s assessment of the market due to commercial liability if the horse wins; hence the odds are constantly adjusted based on the bettor’s behaviour.

Efficiency in a financial market is used to describe how well the market incorporates all publicly available information when setting an asset’s price in the market place [238]. For example, in the stock exchange, the security prices fully reflect all publicly available information. Basically, a market can be called efficient with respect to some information set, if prices of all assets within a market incorporate that information set, reflecting the true value of the asset [238]. Market efficiency implies that no one can outperform the market, except through luck and insider trading because all information is available to all investors, and prices update instantly reflecting this information. Informational efficiency is at the heart of all financial markets, betting markets included. Betting markets are an example of a simple financial market; they have a large number of investors (bettors) and rich informational resources, such as form guides, race replays, specialist opinions, insider information and information derived from price movements in the betting odds [238]. Betting markets also have the advantage of a well-defined end point where the value of the asset (or bet) is definite. These attributes make betting markets attractive for researchers testing market efficiency.
An investor or a bettor has a financial incentive to accurately judge the market. There is evidence that suggests that betting markets efficiently incorporate all public and monopolistically held (inside) information into the final market odds or starting price [238]. There is a strong positive relationship between the odds and the likelihood of winning [239]. The odds do not reflect the true probability of winning, as the sum of all implied odds is greater than one. This allows for the market maker (bookmaker or pari-mutual tote) to receive a ‘take’ or deduction and make a profit overall. The average bettor, gambling via these means trades at a loss over time [240]. The odds provided by a betting exchange (person-to-person betting) reflect a ‘truer’ probability of winning because of the absence of transaction costs [240].

There are exceptions where the market can be inefficient. The favourite-long shot bias (FLSB) has been well documented in the betting markets [238, 240–242]. Favourites win more often than the subjective probabilities imply, and long shots less often. There is a successive decline in financial returns for betting on longer odd horses. If we consider that the odds are the subjective probability of winning, over many races the objective probability of winning at those odds can be calculated. Horses that are the judged most likely to win (i.e. favourites) identified by having the shortest odds, win most often, approximately 33% of the time [236]. A British study identified that betting on horses with odds that are less than even money (i.e. less than 2/1; or having a greater than 50% chance of winning), produced a return of minus 0.4% [243]. This loss increased to minus 10.1% for horses wagered upon up to and including even money (2/1 on, or 50% chance of winning) and minus 16.2% for odds up to 14/1. The authors calculated that horses with odds on pari-mutuel betting of 20/1, the objective odds of success were actually 90/1. This finding is reiterated by Californian pari-mutuel betting data where horses at odds of 100/1 chances were actually 730/1 [236]. Similarly, odds of less than 0.92/1, the probability of success actually exceeds that implicit in the odds, whereas the reverse was true for all odds exceeding this amount [241]. This behavioral phenomenon leads to a FLSB, where risk loving characteristics of gamblers, or bookmakers attempting to limit financial exposure to long shots, see bettors over bet the long shot and under bet the favourite.

Informational costs also contribute to market inefficiency [240, 244]. In lower betting turnover races, higher numbers of casual bettors lacking information may whimsically back horses based on
number, jockey silks or the name. Casual bettors bet significantly less per person, and are poorly informed, they bet evenly across the market and accentuate the FLSB. High volume betting turnover races are high profile, top class races with media speculation on participants sometimes extending weeks prior to the event. Conversely, in these higher betting turnover events, there is improved information conveyance, form analysis and media coverage, and the bias is reduced [240]. In fact, the reverse can occur, with well-informed bettors overreacting to information and over betting the favourite [244].

The FLSB phenomenon is reduced in person-to-person betting exchanges [240]. Launched online in 2000, the betting exchange Betfair has over 1.7 million uses and processes over 1.2 billion bets per year with a trading value of £56 billion [245]. Betting exchanges match people wanting to bet on an outcome, with people that want to bet against that outcome. The person who bets at the price offered is referred to as the ‘backer’ the person who offers the price is the ‘layer’. People can bet and lay bets on the outcome of the same event over the course of the market. Betting exchanges have no interest in the outcome; it merely provides the forum to connect the backer and the layer. The betting exchange charges a small commission on net winnings, not turnover, to generate a profit. The FLSB is considerably lower in betting exchanges than in bookmaker or pari-mutuel betting. This is most likely due to reduced transaction costs and other factors [246]. Despite suggestion by the media that betting exchanges could promote corruption by fixing and laying horses to lose, this is largely unsupported in the literature [240, 247]. Others cite “market movers”, where a horse whose odds increase over the course of the market are extremely good lay bets and can generate profit, as these horses often fail to win or place [248]. This may indicate fraudulent activity; but overall betting exchanges significantly improve market efficiency [240, 249].

If we ignore the social stigma attached to horse race gambling, the market provides a subjective probability of a horse winning. Estimated by a financial market that has informational efficiency, potentially this market could highlight horses that perform below their anticipated performance, i.e. poor performance. The market odds could be useful in overcoming some of the limitations surrounding other performance measures.
1.6.5. EAE and racing performance

The appearance of blood at the nostrils after racing has safety and performance connotations in the racing industry worldwide. EAE may account for up to 18% of racing sudden deaths, [142, 250, 251] but only recently has there been evidence supporting a link between EAE and poor racing performance.

Cook (1974) cites anecdotal reports that there is often an obvious reduction in speed during racing, and that the loss of performance preceded the characteristic appearance of blood on the jockey’s breeches [6]. He acknowledges that some horses won despite bleeding from the nostrils, but successful performances were considered rare [6]. Newton et al (2005) examined the relationship between performance defined as ‘finishing position’ in the race and epistaxis for 27,191 horses racing over a 2-year period [80]. To account for variation in the number of runners per race the authors used the variable - proportion of the field beaten (POFB), calculated using the following formula:

\[
\frac{(\text{Number of runners - Finishing position})}{(\text{Number of runners - 1})} \times 100
\]

Results were then categorized as winning, having beaten >80%, 60-80%, 40-60% or <20% of the field, had finished last or had failed to finish. Using univariable analyses horses with EAE were significantly more likely to finish last or not finish the race than win or beat a greater proportion of the field (P<0.001). Poor finishers were more likely to be heavily scrutinized by stewards, which may add bias to this result. Multivariable analyses did not include finishing position because the authors argued that horses with poorer finishing values did so because of lung haemorrhage, rather than finishing position being a risk factor for epistaxis. Richard et al (2015) supported the findings of Newton and others when investigating epistaxis and POFB for horses participating in hurdle and steeplechase races in Great Britain between 2001 and 2009 [173]. They included POFB in multiple logistic regression analysis and it remained significantly associated with epistaxis in hurdle (P<0.001; OR 0.96; 95% CI 0.97) and steeplechase races (P<0.001; OR 0.98; 95% CI 0.98). They concluded that the relative odds of epistaxis decreased as the POFB increased. They also attributed poor performance to EAE, rather than being a risk factor for EAE.
An Australian study examined the race records over a 10-year period in Victoria [172]. They too defined performance by the POFB and examined the initial episode of EAE. A total of 1085 initial episodes of EAE were identified, of which 384 horses ran last or failed to finish, compared with 44 that were placed first. No further statistical analyses were reported.

Statistical analyses were reported of 400 episodes of EAE from 305 horses racing in Korea [252]. The authors identified a higher incidence of bleeding in horses that finished further back in the field. The three races preceding the epistaxis event, the epistaxis event, and the three races after the event were examined for average race finishing position. The average finishing position of horses with epistaxis was reduced in the race in which epistaxis occurred and in the next race start, although it was unclear if this value was adjusted for field size. Finishing time relative to the winner’s time was also examined. Over 74% of horses with EAE finished more than 1 second behind the winner, and almost half (43.6%) of this group finished more than 2 seconds behind the winner. Multivariable analyses were not performed.

1.6.6. EIPH and racing performance

Evidence of tracheobronchoscopic EIPH is anecdotally believed to negatively impact performance by trainers, jockeys and some veterinarians. If we consider the prevalence of endoscopically diagnosed EIPH in a population of racehorses is up to 75% [9] and then consider the large numbers of factors that can impact the outcome of a race, not least that the margin between winning and losing can be one hundredth of a second, it is not surprising that there have been challenges in identifying a clear link between EIPH and poor performance. Additionally, when trainers and veterinarians are presented with a performance below expectations, EIPH is reliably present in the majority of racehorses and is often held responsible in want of an alternative reason.

Early endoscopic surveys were either unable to detect an effect of EIPH on performance when defining performance by finishing position [7, 9, 10, 174], or identified superior performance in horses with EIPH [253]. Many of these studies were compromised by relatively small sample size, a lack of analysis of other performance variables, inadequate statistical analysis, and/ or incomplete examination of the trachea. More recently larger sample sizes, the use of adequate statistical
methods and expansion in how performance is measured has demonstrated a negative association between EIPH and performance [175, 177, 195].

Research from Brazil showed that despite the use of FUR, horses with EIPH had a greater chance of being unplaced than horses that did not have EIPH (OR 1.31) [177]. Horses were randomly selected from a list of certified ‘bleeders’ (horses with previous EIPH or EAE) and had received FUR prior to racing. Analyses were performed using the 2118 post-race tracheobronchoscopic examinations from 1,003 horses, without accounting for repeated sampling. Performance was defined by finishing position. Ordinal logistic regression of the data identified that finishing position was influenced by recurrence and severity of EIPH in FUR-treated horses (P ≤0.0028). Additionally, horses with EIPH, independent of FUR use, had a higher chance (OR 1.41) of being unplaced in races than horses that did not have EIPH.

A negative association between EIPH and performance was identified in a South African study of 886 Thoroughbreds [175]. Examinations were conducted between 7-110 minutes after racing, with the majority (74%) of horses examined within 30 minutes (IQR 16-30). Horses that won (P<0.001) or placed in a top 3 positions (P<0.001) were examined significantly later than others, with a median scope time of winners of almost 20 minutes later. It is not clear what impact the discrepancy in examination time between winners and non-winners had on results. The performance demographic in this population was also not reported. Horses without EIPH were more likely to win (OR 2.3; 95% CI 1.4-3.7; P=0.001) or finish one length closer to the winner than horses with EIPH of any severity (P=0.03).

In Australia, 744 Thoroughbreds were examined using tracheobronchoscopy after racing [195]. Horses were examined within 2 hours of racing and the volume of tracheal blood was graded using an established grading system. Individual career performance (up to the day of examination), and performance in the race on the day of examination were evaluated. Race day performance variables included race earnings, distance that the horse finished behind the winner, and finishing position. Finishing position was not normalised to field size. Horses with mild or no EIPH were 4 times more likely to win (P=0.006; 95% CI 1.5-14.3), 1.8 times more likely to place in the first three positions (P= 0.03; 95% CI1.05-3.07), and 3 times more likely to be in the 90th percentile for race earnings than horses with moderate or severe EIPH. Horses with EIPH finished significantly further
behind the winner (P=0.002) than horses without EIPH. No association was identified between EIPH and career performance indices, which included lifetime starts, wins, placing and earnings. The authors concluded that mild EIPH does not impact performance, but moderate to severe EIPH impairs race-day performance. The main limitations of this study are that enrollment were voluntary and many horses were examined within 30 minutes of racing, which in a separate publication using the same data, was identified as a risk factor for EIPH detection and severity [124].

This above-mentioned dataset was used 12 years later to examine if a one-off diagnosis of EIPH impacted future performance and overall career performance [200]. Indices of performance examined included career duration, number of race starts, total race earnings, number of wins and the number of places (second and third places). They were examined in two ways, career after the examination and overall lifetime racing career.

**Career performance after examination:**

a) Career duration: No association was identified between any grade of EIPH or grouped grades of EIPH based on increasing severity and career duration. However, when horses with the most severe EIPH grade (grade 4) were compared to horses without EIPH, they had significantly shorter careers.

b) Earnings: Horses with mild or no EIPH earned 3.3 times more prize money than horses with moderate or severe (≥2) EIPH (P=0.001; 95% CI 0.15-0.59).

c) Starts, wins and placing: Horses without EIPH had significantly more race starts than horses with EIPH, and this effect was also seen when comparing horses with mild or no EIPH with those that had moderate to severe EIPH. When the authors removed horses with the most severe grade of EIPH (grade 4), there was no significant difference detected between any grades of EIPH. There was no significant difference detected for any EIPH grade and number of wins or placings post endoscopy.

**Lifetime career performance:**

a) Career duration: There was no association between the presence of any EIPH, or moderate or severe EIPH, and total career duration. Horses with moderate to severe EIPH had fewer lifetime race starts than horses with mild or no EIPH. Again, this was highly influenced by horses with grade
4 EIPH. Upon removal of these horses, there was no significant difference between any grades of EIPH and career duration.

b) Lifetime earnings: There was no significant difference detected between lifetime earnings and any grade or severity of EIPH.

c) Wins and places: There were significantly more wins and places in horses with severe, grade 4 EIPH than other EIPH grades. This was attributed to one exceptionally performing grade 4 horse. This horse was deemed an outlier and removed, repeat analysis revealed no significant association between this indices and horses with or without EIPH.

In this study, there were 13 horses diagnosed with severe, grade 4 EIPH, of which, six horses never raced again. The reason for the management decision to retire this group of horses after examination is not made clear. Significant associations that were identified between grade 4 EIPH and markers of performance are weakened by the small number of horses that continued racing. Of the seven grade 4 horses that continued racing, one had exceptional lifetime and career performance. Overall, there was a lack of association between EIPH grades and the examined performance indices. The authors concluded that a one-off diagnosis of EIPH is an unreliable predictor of future or overall career performance.

The lack of long-term career implications for horses with EIPH is supported by a retrospective longitudinal study of veterinary records of Thoroughbreds in Hong Kong [254]. The study lacked careful attention to biases inherent to the study design and drew criticism for this [255, 256]. The study investigated 732 geldings imported from New Zealand between 2007-12 that had at least one tracheobronchoscopic examination. The majority (99%) of horses had more than one tracheobronchoscopic examination (med 15; IQR 3-15). Examinations were predominantly (97%) performed after fast exercise such as galloping, racing or trialing, but specific exercise intensity is not clarified in the analyses despite an expected prevalence variation of EIPH with exercise intensity. The timing of examination after exercise was also not discussed. In the statistical analyses potential confounding factors impacting retirement of racehorses in Hong Kong, such as career earnings and age at first race start [231], were also not accounted for. Findings included that horses with EIPH had longer careers (days from import to retirement) than those without EIPH. But in further
correspondence with the authors, approximately half of the horses (with or without EIPH) were still racing at the time of publication, which has an unknown effect on results [257].

There are limited reports investigating potential associations between athletic performance and BAL fluid cytology [121, 180, 181]. Cytological findings of BAL fluid that are indicative of EIPH includes the presence of free erythrocytes and an increased relative percentage of haemosiderophages. McKane et al (1993) found no significant relationship between BAL cytology and trainer judged performance when comparing groups of horses with average and above average performance [181]. Fogarty et al (1991) examined BAL fluid from successful (winning) Thoroughbred horses and from horses with severe exercise intolerance [180]. Successful horses had significantly less haemosiderophages in BAL fluid than horses with ‘severe exercise intolerance’ (P<0.05). But severe exercise intolerance is not a clinical sign associated with EIPH, and although this association is present it does not reflect causation. Couëtil et al (1999) compared BAL fluid cytology after an exercise test for horses that trainers thought raced poorly (failed to finish in the top four positions at their last 4 races) with a good performing group (placed in their last four race starts) [121]. Horse in the poor performing group were significantly more likely to have a higher percentage of haemosiderophages and greater haemosiderin scores than horses in the good performing group (P<0.01). The authors did not elaborate if the poor performance group previously had good performance, so are true “poor performance cases” or are limited in their ability.

### 1.7. Clinical signs of EAE and EIPH

Clinical signs associated with EIPH in the absence of epistaxis are vague. Epistaxis associated with high-speed exercise is almost always attributed to EIPH, occurring during or shortly after the completion of exercise. Other differential diagnosis for post exercise epistaxis include head or sinus trauma, nasal mucosa abrasions, ruptured varicosities, an ethmoid haematoma or guttural pouch mycosis. In the absence of epistaxis, the reported clinical signs attributable to EIPH are limited to poor performance; no other clinical sign has been reliably documented. A survey of 25 EIPH-positive Thoroughbred racehorses in Hong Kong identified only five with clinical signs attributable to the respiratory system [258]. Clinical examination at rest or after exercise is similar between horses
with and without EIPH [259, 260]. Auscultation of the lung fields using a re-breathing bag is unreliable at identifying EIPH-positive horses [259]. Excessive swallowing or coughing [7, 176] after racing has been reported in some horses with EIPH, but these are non-specific clinical signs and horses will cough or swallow after racing for other reasons, such as mucus accumulation or inadvertent inhalation of foreign material such as dirt, or water after racing. Furthermore, coughing was not associated with the relative percentage of haemosiderophages in BAL fluid [123]. In the absence of epistaxis, EIPH cannot be reliably diagnosed on the basis of clinical signs.

1.8. Diagnosis of EAE and EIPH

The diagnosis of EAE and EIPH can be made using a variety of methods. The accuracy of each method varies depending on the interval since exercise and the desired or required test sensitivity and specificity.

a) Observation of epistaxis

Observation of blood at both nostrils after exercise is strongly suggestive of EAE (figure 1.8-1). The diagnosis can be confirmed with tracheobronchoscopic examination of the trachea performed within a recommended 30-60 minutes of racing or exercise [194]. Racing jurisdictions often diagnose EAE solely on the presence of blood at the nares, and disqualification is applicable without tracheobronchoscopic confirmation of the origin [220]. Epistaxis as a marker for EIPH is highly insensitive, as only a very small percentage of race starters (<1%) will have epistaxis, grossly underestimating the disease prevalence [80, 169, 186, 210, 250, 254].
b) Tracheobronchoscopic examination

The procedure is typically performed in the standing, un-sedated horse. A handler restrains the horse. A nose twitch can be used to assist with restraint, as the procedure causes mild irritation when the endoscope is passed through the nasal passages. A 1.5-meter endoscope, usually <10mm in diameter is passed from the left or right nares, through the ventral meatus, to the larynx. The endoscope is then directed into the trachea to the level of the carina where the left and right mainstem bronchi are seen. A shorter endoscope can be used, but the distal trachea and carina cannot be seen, and the examination is incomplete. Evidence of blood in the trachea within 120 minutes of exercise is considered to be conclusive evidence of EIPH [260]. Two subjective scoring systems that semi-quantify the volume of haemorrhage are described [7, 261]. The most widely accepted with good inter-observer reliability, is a scoring system described by Hinchcliff et al (2005), and is as follows [261]:

Figure 1.8-1: Photograph depicting post-race epistaxis.
**Grade 0:** No blood present in the pharynx, larynx, trachea or main stem bronchi,

**Grade 1:** Presence of 1 or more flecks of blood, or ≤ 2 short (less than one quarter length of trachea), narrow (less than 10% of tracheal surface area) streams of blood in the trachea or main stem bronchi.

**Grade 2:** One long stream of blood greater than half the length of the trachea, or more than 2 short streams of blood occupying less than a third of the tracheal circumference.

**Grade 3:** Multiple, distinct streams of blood covering more than a third of the tracheal circumference, with no blood pooling at the thoracic inlet

**Grade 4:** multiple, coalescing streams of blood covering more than 90% of the tracheal surface with blood pooling at the thoracic inlet (figure 1.8-2).

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*Figure 1.8-2: Grades of severity of EIPH; Figure A-D corresponding to EIPH grade 1-4, respectively. Taken from Hinchcliff et al, 2005 [261].*

There are no reported limitations of this scoring system. However, from a user perspective there can be overlap between two scores and their descriptions. For example, horses with multiple coalescing streams of blood, covering more than 1/3 of the trachea but less than 90% of the tracheal surface can have blood pooling at the thoracic inlet, a characteristic limited to grade 4 horses only.

The true volume and distribution of pulmonary haemorrhage required to create these scores is unknown. Inter-observer agreement between three experienced observers using this scoring
system was 68.7% of recordings; when considering agreement between all or the majority of observers this increased to 99.4% of concordance on recordings [261].

Tracheobronchoscopy is highly specific but has questionable sensitivity [56]. Failure to detect blood in the trachea can be attributed to inadequate examination of the airways, examination too soon or too late after exercise, inadequate level of exercise, or minimal volumes of blood in the distal airways that does not reach the trachea [262]. Failure to observe EIPH with tracheobronchoscopy does not equate to a disease negative status. One of the major limitations for this diagnostic tool is timing. The optimum time to diagnose EIPH has not been established, but it is likely to vary slightly depending on the grade, with lower grades being most vulnerable to misclassification due to blood bring transiently present in the trachea. Examinations performed too soon after racing reduce the chances of blood being detected and can underestimate severity [124]. Grade 4 EIPH is consistently diagnosed across all time categories [175]. To improve the specificity of the test it is has been recommended to examine horses on 3 consecutive occasions after intense exercise or racing [263], but this does not increase test sensitivity to 100% [264]. The procedure does offer the advantage of being quick and minimally invasive. Most horses do not require sedation.

c) Bronchoalveolar lavage (BAL)

Adapted from human medicine BAL has been described in horses since the early 1980s [265]. The process involves lavage and aspiration of fluid from the distal airways in the caudodorsal lung lobes, followed by cytological examination of the retrieved fluid. The test has the advantage of being more sensitive than endoscopy at detecting lung haemorrhage [56] and can also detect previous EIPH episodes [181].

The BAL can be performed using a custom soft tube (BAL tube; 3.0m x 11mm OD) or an endoscope (>2.5m x 9-12mm OD) The BAL tube procedure is after Hoffman (2008) [266]. For the procedure to be performed safely the horse is typically sedated and can be additionally restrained with a nose twitch. The BAL tube is passed through the ventral meatus of the nasal passages and advanced blindly into the trachea. The tube is passed until it becomes wedged in the most distal bronchus, typically the left or right, fourth or fifth generation segment of the caudal lung lobe. The
tube passed blindly will always enter the dorsocaudal lung lobe [179, 267]. The cuff is then inflated to seal the airway, limiting sample collection to that segment of lung. A total of 250-500ml of isotonic saline is typically instilled, with half the volume injected as a bolus and retrieved and repeated. Fluid retrieval is usually between 50-70% of the instilled volume [112].

There is considerable variation in the published cytological profile in normal horses [181, 268, 269], attributed in part to variations in the volume of fluid instilled [270]. The use of smaller volumes of saline washes the bronchioles rather than the alveoli, and fluid cytology in these regions are different. Bronchiole washes have a higher proportion of neutrophils than alveolar washes.

The technique is considered safe, with minimal side effects [266, 271, 272]. The effect of the technique was examined by serially sampling 4 paddock rested horses and 10 Thoroughbred horses in training [271]. The authors reported that neither the procedure, nor exercise, evoked an inflammatory response in BAL fluid when sampled from the same (right) lung lobe on a weekly basis. When sampling is repeated after an interval of 48 hours, there were significantly increased neutrophils, indicative of a technique-induced inflammatory response [273]. The contralateral lung lobe demonstrates no inflammatory changes associated with the BAL procedure when sampled 24 or 48 hours later [121, 273]. In summary, this research suggests the BAL induces a short lived, localized neutrophilic inflammatory response in the sampled lung only; detected on repeated sampling after 48 hours but not after 7 days.

Detection of free red blood cells (RBC) or haemosiderophages is the hallmark of EIPH diagnosis with the BAL [266]. Haemosiderophages are present in BAL fluid from almost all racehorses [56, 181, 269], and RBCs have been reported in resting horses, speculated to be associated with the procedure itself [37]. Consequently, the presence of haemosiderophages or free RBCs overestimates the number and significance of EIPH positive horses. Reference ranges in clinically healthy horses for RBCs and haemosiderophages in respiratory fluids (BAL or tracheal wash) have not been published [274], although some publications include haemosiderophage percentages in ‘clinically normal, good performing racehorses’ that are utilized as controls [121, 180, 275, 276]. Reported haemosiderophage percentages in small groups of good performing “control” horses are 1.1 ± 2.5% [121, 275] and 16.6 ± 19.4% collected 1 hour after exercise, and 14.4 ± 10.7% collected 24 hours after treadmill exercise tests [121]. Variation in instilled fluid volumes (120ml vs. 60
250ml) could account for variation in the reported reference ranges. The proportion of macrophages that are haemosiderophages has been used to categorize clinically significant EIPH horses from non-clinically significant EIPH cases [119, 277]. More than 20% haemosiderophages, or alternatively, more than 50% of all macrophages contain haemosiderin, has been used as a cut-off to distinguish between clinical cases of EIPH and non-clinical cases of EIPH [119, 277].

A semi-quantitative human hemosiderin score was adapted and applied to horses to provide quantitative evaluation for haemosiderin found in BAL fluid [121, 264]. The haemosiderin score (THS) is based upon grading of the blue colouration (presence of iron deposits) in the cytoplasm of macrophages after Wright's staining. The scoring system varies between studies, but reflects increasing haemosiderin on a 0-4 scale [264], or a 0-3 scale [121]. Couetil et al (1999) reported that 13 racehorses' horse with EIPH (diagnosed on single endoscopic exam) had significantly higher THS than ten control horses that had been EIPH negative on one endoscopic examination [121]. Doucet et al (2002) divided 74 horses into two groups: no EIPH diagnosed on 3 consecutive endoscopic exams; and an EIPH-positive group that had either epistaxis, EIPH of any grade on endoscopic examination, or blood tinged BAL fluid [264]. All horses had haemosiderophages on BAL fluid analysis, but THS was significantly higher for the EIPH-positive group than the control group. The haemosiderin score was strongly associated with erythrophagocytosis in the EIPH-positive group but was only moderately correlated with erythrophagocytosis in the control group. Of interest in this study were three control horses that had high THS scores, suggesting that despite 3 consecutive negative endoscopic examinations these horses had evidence of pulmonary haemorrhage. Three EIPH-positive horses had low THS scores. The authors reasoned that false negative results in two of the three horses were attributed to the sampled lobe not coinciding with the lobe that the haemorrhage had occurred in, and in one case, sampling occurred too soon after the EIPH event. Using a cut off THS value of 75, the sensitivity of this test was calculated to be 94%, and the specificity 88%. The authors argue that this scoring system offers a more clinically applicable measure for EIPH diagnosis rather than noting the presence or absence of haemosiderophages. But this scoring system has had no further uptake in the literature to date.

The longevity of hemosiderophages in the lung is unknown. After autologous blood instillation in the lung there is a 3-day delay before initiation of erythrophagocytosis, the ingestion of
erythrocytes by macrophages, [128]. At day 10 after blood instillation macrophages develop golden brown granules indicating breakdown of hemosiderin. By days 14 and 21, stained macrophages contained darker blue staining representing more mature forms of hemosiderin. Step et al (1991) were also able to detect erythropagocytosis using tracheal wash cytology at days 1-3 after intrapulmonary blood inoculation in 8 horses, but not thereafter. Hemosiderophages were detected from 7 to 28 days, but not at 90 days [130]. Meyer et al (1998) reported that haemosiderophages were evident in BAL fluid 1 week after exercise and remained elevated for 21 days, before returning to pre-exercise levels by week four [56]. Others report anecdotally that haemosiderophages can persist in rested horses for up to a year, although it is unclear if the horses were paddock rested and could have undertaken galloping exercise at will [116]. The presence of haemosiderophages alone is a relative inaccurate indicator of EIPH severity due to persistence in the alveoli, and the likelihood that horses with continual mild EIPH could accumulate numerous haemosiderophages [182]. Furthermore, the number of hemosiderophages retrieved does not reliably correlate with the volume of blood inoculated into the lung [130], or the volume of blood imaged endoscopically [180, 277]. The percentage of haemosiderophages retrieved does correlate with pulmonary haemosiderin present on lung histopathology [278].

Free erythrocyte concentration has been used as a measure of EIPH severity [33, 37, 56]. The longer the interval between racing and sample collection, the less likely erythrocytes or erythropagocytosis will be present [264]. Red blood cells increased immediately after exercise, and are reported to return to normal within 1 week [56], although they have been detected at 14 and 21 days post exercise [128]. Although erythrocyte concentration in BAL fluid increases with exercise and pulmonary artery pressure there remains no clear quantified association with EIPH severity [37, 56]. Langsetmo et al (2000) reported that erythrocytes in BAL fluid significantly increased with speed when horses were galloped on a treadmill at 15m/s, compared with resting and slower speeds of 9, 11 and 13 m/s, and this corresponded to increased pulmonary artery pressure when galloping at 15m/sec. Demonstrating a direct relationship between erythrocyte concentration and pulmonary artery pressure, the author suggested this reflected severity: with the higher the pulmonary artery pressure, the more erythrocytes present in the BAL, the more significant the haemorrhage. Others refute this conclusion, reporting no significant relationship between erythrocyte concentration and
mean pulmonary artery pressure in a larger sample size of Thoroughbreds after controlling for the number of weeks in training, horse and FUR treatment [33]. The usefulness of a BAL to quantify the severity of lung haemorrhage remains questionable. Furthermore, examination of 8 Thoroughbreds galloping on a treadmill identified unilateral discrepancies between the left and right lung lobes; the left lung BAL sample contained significantly higher RBC counts than samples collected from the right lung [279]. These findings were supported by a study of 138 clinically normal racing French Trotters, where higher mean haemosiderophage percentages were present in BAL fluid samples collected from the right lung compared to the left lung [280]. The authors reasoned that a clinically significant EIPH diagnosis was based on a haemosiderophage/ macrophage ratio exceeding 20% in either the left or right lung BAL sample. Using these criteria there was only moderate agreement between the left and right lung lobe. EIPH was diagnosed in 44 horses; 19 horses had EIPH diagnosed in both left and right samples, while 25 horses had EIPH diagnosed from only one lung (8 and 17 horses diagnosed EIPH positive from the left and right lung BAL sample, respectively). Based on unilateral lung sampling 25 horses (56.8% of EIPH positive horses) could have been subjected to misclassification. This study was performed on a large sample size of racehorses in race training, whereas the aforementioned studies utilized very small groups of resting or respiratory diseased horses, which may account for differences in results. The BAL tube, when passed blindly as is common practice, enters the right bronchus in most horses [267, 281]. A tracheobronchoscopic survey of racehorses reported unilateral bronchial haemorrhage in 242 horses, 150 and 92 horses had haemorrhage from the right and left bronchus, respectively [75]. This suggests that a reasonable proportion of racehorses have unilateral haemorrhage and that EIPH can be a localized disease that is subject to misclassification using techniques that sample a single site.

**d) Tracheal aspirate**

Tracheal aspirates (TA) or tracheal washes can be performed via an endoscope or percutaneous perforation of the trachea. Endoscopically performed aspirates offer the advantage of inspection of the airways, and are quick and non-invasive, but in contrast to trans-tracheal aspirates the technique is not sterile. There is no significant difference in the cell counts retrieved via either
technique [268], but the technique performed with the endoscope is commonly utilized for EIPH diagnosis. Using an endoscope, the technique is the same as described for tracheobronchoscopy, except 20-30 mL of sterile saline is instilled into the tracheal lumen via a sterile catheter passed through the biopsy channel of the endoscope. The saline wash accumulates at the level of the thoracic inlet, is then aspirated and examined [277, 282]. Again, the detection of haemosiderophages or erythrocytes is the hallmark of EIPH diagnosis, but the presence of haemosiderophages is universal in horses in moderate intensity work [283], and erythrocyte presence can also be inadvertently caused by the procedure itself [129]. Similarly, to problems associated with the interpretation of BAL cytology, a haemosiderophage/macrophage ratio exceeding 50% in tracheal washes has been used to identify clinically significant EIPH cases [119].

Tracheobronchoscopy, TAs and blind BAL procedures were conducted on 50 French Standardbred trotters 60 minutes after a standardized high-speed treadmill exercise test [277]. The tracheobronchoscopy blood score correlated with haemosiderophages in TA (r=0.285, P=0.0483), but not in BAL fluid (r=0.257; P=0.081). Another study reported significant differences in the cell populations retrieved from BAL and TW samples collected simultaneously from 50 horses with respiratory disease [282]. Whilst this is not surprising, as they sample different areas of the lung, nine horses were found to have EIPH based on haemosiderophages in BAL fluid cytology. Haemosiderophages were observed in only 4 of the 9 paired TA samples, suggesting that BAL samples are more sensitive at detecting these cells and therefore diagnosing EIPH than TA’s.

e) Diagnostic imaging techniques

Thoracic radiographic changes for horses with EIPH have been described [148, 259, 284, 285] and include descriptions of an alveolar pattern and a bronchointerstitial pattern in the caudodorsal lung lobes at 7 days and 3 months respectively, after EAE [148]. The degree of radiopaque density present in the caudodorsal lung correlated well with haemosiderin and bronchial arterialization present on post mortem but was not able to predict the degree of lung involvement [284]. One horse deemed EIPH-negative clinically, had radiographic changes in the caudodorsal lung lobes consistent with EIPH, but gross assessment of the lung post mortem had no evidence of
haemosiderin or bronchial arterialization. Whilst other horses with extensive EIPH lesions at post mortem had only subtle radiographic changes documented.

A case controlled study of 10 EIPH negative horses, determined by 3 consecutive post exercise endoscopic examinations, and 10 EIPH-positive horses with tracheobronchoscopic evidence of EIPH on at least one occasion were examined with a variety of diagnostic tests, including thoracic radiography [259]. Radiographic lesions were assigned a score by a radiologist, which were then compared between groups. No significant difference was seen in radiographic scores given to both groups, and radiographic caudodorsal lung abnormalities were identified in both groups. Radiographic scores were not significantly associated with BAL cell counts in either group. The study concluded that radiographic scores were too variable between the EIPH and control groups, and although there are changes radiographically in some horses with EIPH, the modality is a poor diagnostic choice with a low sensitivity for a diagnosis of EIPH.

Thoracic ultrasonography has been used to diagnose EIPH in a mixed population of Thoroughbred and Standardbred racehorses [286]. Horses were classified EIPH-positive based on haemosiderophages detected in cytology of BAL fluid. Test specificity and sensitivity was then determined for post exercise endoscopy and ultrasound. Ultrasonographic evidence of EIPH included the presence of ring down artifacts (comet tails), hyperechoic reflections originating on the pleural surface of the lung, between the 10-17th intercostal spaces. Based on BAL fluid cytology, 127 horses (80.9%) were EIPH-positive. Ultrasound examination of the thorax was found to have high sensitivity (88.5%) but low specificity (25.7%) when compared to post exercise tracheobronchoscopy, which was both highly sensitive and specific (72.7 and 87.5%, respectively). Unfortunately, this study lacks detailed results or confidence intervals for significant associations.

f) Other

The use of scintigraphy to diagnose EIPH has been described [287, 288]. Votion et al (1999) undertook a pilot study to quantify the amount of haemorrhage associated with EIPH [288]. The theory behind using this modality is that small volumes of haemorrhage can be detected by administering radioactive labeled RBCs to exercising horses, which if not involved in haemorrhage
will be removed by the spleen post exercise, increasing the contrast between background activity and pulmonary haemorrhage. The feasibility of this modality was tested by direct placement of labeled RBCs in the caudodorsal lung with an endoscope. Twenty minutes later, the labeled RBCs could be detected with scintigraphy. The authors concluded that small volumes of haemorrhage could be detected but the level of background radioactivity in the lung maybe a limiting factor. There has been no further research using scintigraphy to diagnose EIPH primarily due to issues regarding radiation safety and cost.

More recently biomarkers for EIPH have been investigated as possible diagnostic tools. Angiotensin converting enzyme (ACE) was plausibly argued to increase with disease, as documented in human respiratory diseases [289, 290]. Activity of ACE has been associated with pulmonary macrophage activation, fibrosis and pulmonary diseases [291, 292]. The authors identified significantly increased ACE activity in horses with the highest grade of endoscopic EIPH compared with EPIH-negative horses (P=0.007; 95% CI -4.9 to -1.1), but no significant differences were detected between horses with lower grades of EIPH and negative animals [290]. An abstract published by the same authors reported ACE activity in a population of pre-race FUR treated horses [289]. Ignoring EIPH status, they found ACE activity in non-treated horses (n=46; ACE 91.7±15.9) to be significantly (P=0.002) higher than in treated horses (n=26; ACE 79.6±13.9). In EIPH-positive horses ACE activity was significantly (P=0.009) lower in treated (n=20; ACE 78.2 ±11.9) than non-treated animals (n=17; ACE 90±15.9).

Increases in serum 2,3-diphosphoglycerate (2,3-DPG) concentrations in horses with poor athletic performance were also examined [293]. Serum 2,3-DPG increases during periods of hypoxia, such as high-altitude training to facilitate oxygen release to the tissues [294]. Serum 2,3-DPG concentration were significantly (P<0.01) elevated in horses with poor performance; that included an EIPH-positive subgroup of horses, based on >10% haemosiderophages on BAL fluid cytology. When the EIPH subgroup was compared to the control group no significant differences in 2,3-DPG concentrations were identified. The study was based on a small sample size of horses with mild EIPH.

Treadmill exercise studies examining blood gas tensions have been examined for horses with and without EIPH [96, 121, 275, 295]. The studies differ in how they classify EIPH-positive
horses either with tracheal blood post exercise endoscopy, or haemosiderophages in BAL cytology, and controls were not always subjected to the same testing as EIPH groups. Some studies were unable to detect an association between EIPH and blood gas tensions [96], whereas others detected a more severe arterial hypoxemia in horses with EIPH compared with controls, but don’t provide confidence intervals for this effect [121, 295]. EIPH diagnosed on the basis of tracheal blood was associated with significantly higher blood lactate concentrations [121], but this finding is not reported universally [275, 295]. Examination of haematology, serum biochemistry and resting arterial blood gas analyses was not significantly different between EIPH-positive and control groups [259]. It is likely that EIPH induced changes on blood gas analysis are not sufficiently large enough to support a clinical application for diagnosis.

Post mortem examination provides gross and histological evidence of EIPH, often reported as an incidental finding. EIPH is an uncommon cause of death, and it is unknown if this fulminant form of pulmonary haemorrhage has the same aetiology as EIPH [251]. Horses euthanized within a short time of racing, for catastrophic orthopedic reasons, can have severe petechiation of the caudodorsal lung [260]. Macroscopic lesions for chronic EIPH include variable amount of blue/grey or blue/brown discolouration of the sub-pleural surface localized bilaterally and caudodorsally on the collapsed lung [66, 76]. The extent of discolouration varies and sharp demarcation exists between normal and abnormal tissue. The abnormal tissue is dense and is described as having a “spleen-like consistency” [76]. In severely affected lungs extreme hypertrophy of bronchial arteries is present, and in areas where the staining extends horizontally from the caudodorsal margin there are increased accumulation of bronchial vessels. Examination of the cut surface reveals that the discolouration is continuous in the dorsal portion of the slice, or that the discolouration is comprised of multifocal coalescing areas predominating in the dorsal aspect of the slice [152].

Histopathological evidence of vascular remodeling has been reported in both the arterial and venous vessels in affected regions of the lung [66, 296]. Williams et al (2008) describes numerous thick-walled vessels within the pleura and interlobular septa displaying marked medial smooth muscle hypertrophy and mild to moderate intimal hyperplasia and lacking well-developed internal and external elastic lamina [66]. In addition, a prominent collar of mature collagen surrounded interlobular veins, reducing the lumen size and in some instances creating veno-occlusion. The
bronchial vessels in affected regions are described as larger and tortuous, with thickened walls compared to bronchial vessels in non-affected regions of the lung [296]. There is increased fibrosis of the interlobular septa [296] and evidence of mineralization within the collagen bundles in this region [66].
1.9. **Treatment of EAE and EIPH**

“The diversity of these agents attests to the paucity of our knowledge about EIPH, the inventiveness of the prescribers, and the general frustration experienced by clinicians and horsemen in coping with this problem.” J. Pascoe, 1991 [297]

There are numerous different treatments for EIPH, which suggests that no single treatment is universally successful or accepted. Treatment is often empirical, and like many things in racing, propagated by successful racing performances rather than evidenced based controlled clinical trials or demonstrated efficacy under racing conditions. There are four main issues associated with clinical trials and research into possible EIPH therapies. The primary issue is that treatments are limited by our current lack of understanding of the pathophysiological basis of the disease. As a result, therapeutic interventions are often measured against an ability to address a specific pathophysiological theory, such as reducing pulmonary vascular pressure, or treating adverse sequelae of EIPH such as inflammation or fibrosis, rather than being measured against a reduction in tracheal blood post-race, for example. A second issue is that clinical trials historically have involved a small number of horses and have low statistical power. Poor statistical power is a critical flaw because failing to detect a difference does not equate to having no effect. Moreover, many clinical trials are performed on a treadmill, how apposite the treadmill is at generating race day conditions is unknown. Thirdly, the consistency of EIPH from one race start to the next is poorly understood. A lack of consistency could substantially reduce the impact of clinical trials or research where horses are categorized based on a single observation. And lastly, there is a lack of knowledge of the longitudinal progression of the disease. Although widely assumed to progressively worsen over time, this is not known. Fundamentally, this makes any treatment trial difficult to interpret unless the study involves a randomized, double blinded, cross over study design.

Proposed treatments for EIPH will be discussed according to the pathophysiology mechanism they are targeting.
1.9.1 Treatments preventing stress failure of the pulmonary capillaries

If stress failure of the pulmonary capillaries is the cause of EIPH, treatment theoretically could be targeted at strengthening the capillary wall; either by increasing the amount or strength of type IV collagen, or by reducing the capillary wall stress [45, 61].

(i) Strengthening the capillary wall

Currently, little is known about how to strengthen the blood gas barrier. Strengthening the BGB may be problematic for racing as any thickening of the structure will reduce gas exchange efficiency and by that, aerobic performance.

Bioflavinoids are antioxidants known for their positive effects on the cardiovascular system, principally through maintaining the integrity of the vessel wall [298]. The use of citrus bioflavonoids was reportedly useful for managing capillary haemorrhage in humans when taken preoperatively [299]. The rationale for use in racehorses is based on the premise that capillary weakness or fragility underlies EIPH. Anecdotal reports suggested bioflavonoids were helpful at reducing EIPH, so a clinical trial involving 45 Thoroughbred racehorses was conducted where horses were given 28 grams of hesperidin-citrus bioflavonoids orally in their feed daily for 90 days [300]. Using a chi-square test for association there was no difference in the incidence of EIPH between the treatment and control group. This study is limited by the unknown bioavailability of oral hesperidin-citrus bioflavonoids, the statistical analyses did not account for confounding factors, and the authors did not investigate potential improvement in EIPH severity.

“Kentucky Red”, or carbazochrome salicylate is classified as a ‘capillary stabilizer’ and is recommended for the treatment of haemorrhage secondary to capillary fragility [301]. The precise mechanism of action of carbazochrome salicylate is unknown but may be associated with blocking inflammatory chemical mediators to maintain an effective endothelial barrier [302]. Administered in combination with FUR to a small group of Standardbred horses (N=6) in a 3-way cross over placebo-controlled study, there were no significant differences in RBC counts obtained from a unilateral BAL sample between this treatment, the administration of FUR only, or the control group that received no treatment [303]. There was considerable variability between horses in this study and a low statistical power may have contributed to a lack of significant findings for both treatment groups.
Reducing pulmonary capillary wall stress

Reducing pulmonary vascular pressure decreases the stress placed on the BGB, and according to this pathophysiological principle, could reduce EIPH. Reducing the pulmonary capillary pressure could be achieved in two ways; either by reducing capillary pressure or by ensuring alveolar pressure does not fall transiently low [45].

(a) Lowering pulmonary capillary pressure

Lowering pulmonary artery pressure is achieved with the use of FUR. FUR has been used to manage EIPH in Thoroughbreds for decades and in some racing jurisdictions the drug is permitted on race days [304]. Dr. Alex Harthill, credited for first using FUR for bleeders, believed the condition was caused by high blood pressure and at the time FUR was used to treat this condition in humans [305]. FUR is a fast acting, potent high ceiling diuretic referred to as a ‘loop diuretic’ [306]. Loop diuretics reversibly bind to the luminal surface sodium-potassium-chloride (Na-K-Cl) co-transporter in the thick ascending limb of the loop of Henle [307]. Inhibition of this co-transporter facilitates the production of dilute urine by increasing sodium and chloride delivery to the distal tubule. The diuretic effect of FUR in the horse is quick, peaking at 15-30 minutes after intravenous injection, inducing a 40-fold increase in the rate of urine production [308], affecting the ability to concentrate urine for up to 6 hours after administration [309]. The rapid peak and decline of the diuretic effect is thought to be associated with rapid drug elimination. Intramuscular injection of FUR prolongs the diuretic response due to longer plasma levels [308]. The bioavailability of FUR is poor and variable when administered via the oral route or when nebulized [33, 309].

Increased water and sodium excretion causes packed cell volume and plasma protein to increase and reduces plasma volume and body weight if access to water is denied post injection. Plasma concentrations of potassium, chloride and calcium also decrease after FUR [308, 310–312]. The reduction in plasma volume nadirs at 15-30 minutes and resolves by 4hrs after intravenous injection [312]. The diuretic effect of FUR is further enhanced by renal vasodilation, a pathway mediated by prostaglandin [313]. Pre-treatment with non-steroidal
anti-inflammatory drugs, such as phenylbutazone, inhibits this effect, significantly reducing but not abolishing the diuretic effect of FUR in exercising horses [314].

FUR also has a cardiovascular effect, attenuating the increase in pulmonary vascular pressure that occurs with exercise [314–317]. The mechanism by which, FUR reduces pulmonary vascular pressure is unclear, with three schools of thought proposed: 1) a reduction in extracellular fluid volume associated with diuresis; 2) release of a vasoactive substance from the kidney; or 3) a direct vasodilator action on the pulmonary vasculature [318].

In humans the vascular effects of FUR were considered independent of the diuresis, as they occurred prior to renal losses [319]. This extra-renal effect has been reported in other species, including dogs [320]. In horses, the diuretic effect peaks at 15-30 minutes after FUR administration [312]. If diuresis were to be associated with pulmonary haemodynamic pressure changes, then this should coincide with diuresis. But when horses are exercised 1, 2, 3 or 4 hours after FUR administration the greatest reduction in pulmonary capillary pressure was at 2, 3 and 4 hours after injection [321]. Furthermore, pulmonary capillary pressure remains significantly lower at 3 and 4 hours after furosemide administration, despite restoration of plasma volume due to fluid redistribution [312]. But there is evidence that administrating intravenous fluids prior to exercise can ameliorate the cardiovascular effects of FUR [318]. This effect was also demonstrated in anesthetized splenectomized mares [322]. FUR administration to anaesthetized horses results in a reduction in right atrial and pulmonary artery pressure after FUR administration. When both ureters were ligated FUR had no effect on pulmonary artery or right atrial pressures [323]. This supports the notion that the hemodynamic effects are dependent on diuresis, or that replenishing lost fluid can overcome other extra-renal effects of FUR [318].

Release of a FUR-induced vasodilator, prostaglandin was proposed to cause the pulmonary haemodynamic effects with some studies demonstrating that hemodynamic vascular effects of FUR are negated with pretreatment of non-steroidal anti-inflammatories [314, 324]. But these findings are not universal. Others identify no change in pulmonary
capillary pressure with non-steroidal anti-inflammatory agents, concluding that the mechanism is unlikely related to prostaglandin release [325, 326].

The reduction in pulmonary vascular pressures with the administration of FUR in the exercising horse is between 7-10mmHg [315] and it is via this pressure drop that amelioration or elimination of EIPH may occur. The efficacy of FUR was initially contentious with early studies demonstrating that FUR was not curative for the disease. Pascoe et al (1981) reported on 56 horses receiving pre-race FUR, identifying that 30 (53.6%) remained EIPH-positive post-race [7]. This was supported by others that continued to observe haemorrhage after pre-race medication in a small sample size of horses [174, 300, 327, 328]. In contrast, studies that graded the severity of tracheal haemorrhage, FUR was associated with improvement [263, 329]. Under racing conditions, a randomized, placebo controlled crossover study was performed in South Africa where horses were raced in similar staged races (same weight, field, barrier) 7 days apart with or without pre-race medication with FUR [330]. Following the administration of FUR, horses had significantly lower odds of developing EIPH compared to saline administration, and significantly lower odds of developing EIPH grade ≥2. Furthermore, of horses that had EIPH after saline administration, 67.5% (81/120) had a reduction in severity score of at least one grade after racing with FUR. This study provided strong evidence of the efficacy of FUR for reducing or preventing EIPH. Pulmonary haemorrhage, classified by free red blood cells in BAL fluid, was significantly reduced compared with saline administered controls when horses were pre-medicatated with FUR prior to a standard treadmill exercise test conducted [331–333]. A recent meta-analysis reported a pooled (11 trials) relative risk of detecting EIPH in horses treated with FUR of 0.88 (95%CI 0.79-0.97), confirming a mild positive effect of FUR at preventing EIPH [334]. When this was further limited to high quality, low bias randomized clinical trials (2 trials) under race conditions, [263, 335] the relative risk of detecting EIPH in horses treated with FUR reduced further (RR 0.68, 95%CU 0.58-0.79).

Chronic use of loop diuretics in people and dogs leads to diuretic resistance, whereby the diuretic effect is diminished with continual use [336]. This phenomenon has recently also been described in resting horses receiving a standard dose of FUR weekly for seven weeks
This dosing regimen would be similar to what many racehorses receive while in training, extending use well beyond 7 weeks. If the hemodynamic effects of FUR are dependent on diuresis, which is diminished with chronic use, FUR may have a diminished capacity to reduce pulmonary artery pressure and therefore reduced efficacy in the treatment of EIPH.

A reduction in EIPH was also proposed to be mediated through bronchodilator effects of FUR. FUR increases dynamic compliance and reduces pulmonary resistance, when administered intravenously or aerosolized to a group of ponies with severe asthma [338], an effect also seen in human asthmatics [339], and clinically normal healthy Thoroughbreds [340]. If inflammatory airway disease is associated with the pathogenesis of EIPH, theoretically bronchodilators may be of benefit in reducing EIPH [304]. Alternatively, a decrease in airway resistance could reduce transmural pressures during exercise and reduce EIPH via this mechanism [304]. Other drugs with bronchodilator effects, such as ipratropium, was shown to have good results at preventing EIPH in a small number of horses; although it is unclear via what mechanism this improvement occurred [327].

Mediated via the diuretic effect, FUR causes a reduction in body weight of approximately 3-4% [312, 317, 318, 341]. Energy expenditure is directly related to the weight carried and speed travelled [342]. It was suggested that the administration of FUR to horses with EIPH improved performance, and this was due to a return to pre-EIPH performance levels [343]. This followed anecdotal reports at the time, that the administration of FUR markedly improved performance [344]. The effects of furosemide on racing performance were examined in a population of Thoroughbreds competing in wagering races in Pennsylvania [345]. The study design, which later drew criticism, [346] involved examining a group of non-medicated racehorses and separating them into EIPH-negative and EIPH-positive based on post-race tracheobronchoscopic results. EIPH-negative horses then, with permission from racing authorities, received FUR pre-race at their next race start. This group of horses, then if EIPH-negative again after race 2, went on to race a third time without FUR. Speed handicapping methods were used to standardize racing times. Geldings were found to have faster racing times with FUR administration than without, an effect unrelated to EIPH
status. These findings were supported by a retrospective analysis of race records documenting significantly faster racing times in Standardbred pacers pre-medicated with FUR compared to non-medicated controls, an effect that declined with age [347]. Average running times of pacers treated with FUR were 0.67 seconds faster than untreated pacers.

The mechanism for the altered performance was theorized to be due to the weight loss associated with diuresis improving efficiency [311]. The administration of FUR was found to improve the energetic response to exercise, an effect reversible by replacing the urinary and insensible weight loss associated with diuresis as weighted saddle pads prior to undertaking exercise [311]. Although aerobic respiration was not altered (VO₂ was unchanged), FUR administration reduced the contribution of anaerobic metabolism to generate energy to maintain a specific treadmill speed. CO₂ production, respiratory exchange ratio (VCO₂ divided by VO₂) and plasma lactate were all significantly reduced. This effect was partially reversible with the addition of the diuresis-associated weight loss. It makes sense that any reduction in body weight reduces the energy required to race a certain speed; horse race handicapping relies on this very principle. Others reported that FUR administration resulted in increased mass-specific VO₂ max during exercise tests compared with saline administration, and the authors suggested an ergogenic effect [348, 349]. A higher VO₂ max has been associated with faster racing times in Standardbred and Thoroughbred racehorses [350, 351]. Others identified that time to fatigue was significantly extended in horses pre-medicated with FUR during a standard exercise test also supporting an ergogenic effect [333]. An epidemiological survey analyzing the racing records of horses with and without FUR found that horses that received FUR had a faster race time, were more likely to earn prize money (OR 1.3; 95%CI 1.18-1.41), were more likely to win (OR 1.4; 95%CI 1.27-1.59) or finish in the top three positions (OR 1.2; 95%CI 1.09-1.37) than horses that did not receive pre-race FUR [352]. This study comprises of over 20,000 race records from almost 3,500 races presenting “clear and unequivocal evidence” of a performance enhancing effect of FUR that was unlikely to be related to any effect on improving EIPH.

Further controversy regarding the pre-race use of FUR is its ability to reduce to concentration of illicit drugs in the urine through its diuresis, or alter a drug’s excretion in urine.
Urine sampling for drug screening is not recommended during the diuretic period [353, 354]. Urine specific gravity should return to normal levels prior to sampling, recommended to be at least 4 hours after FUR administration [304, 356]. Drugs such as phenylbutazone and its metabolites have a reduced urinary excretion after FUR administration [353, 354]. Similarly, water-soluble metabolites such as apomorphine, morphine, narcotics and phenothiazine tranquilizers are reduced in urine after diuresis [355]. In contrast increased urinary excretion of fentanyl, procaine and methylphenidate occur with FUR administration [355, 356]. FUR does not alter drug plasma concentrations [357].

Other medications used to treat pulmonary hypertension in people have been trialed without success in horses with EIPH [358–361]. Fundamental differences between pathological pulmonary hypertension in people and exercise induced pulmonary hypertension in horses perhaps underlie the inability of these medications to replicate treatment results in horses with EIPH. Nitrous oxide is one of the major endogenous vasodilators in the pulmonary and systemic circulations. Released from the endothelium nitrous oxide regulates vascular tone [362]. Inhaled nitrous oxide has been shown to reduce PAP in the exercising horse [358, 359] but worsened EIPH, with red blood cells/ml of BAL fluid increased compared to exercise without nitrous oxide [359]. Intravenous nitroglycerin, an exogenous form of nitrous oxide, infused at a rate of 20µg/kg/min prior to, and during exercise in a small number of horses significantly reduced pulmonary vascular pressure at rest [363, 364] but these changes were not significantly different to control values during a standard exercise test and the intervention had no effect on EIPH [364]. A published abstract describes using a combined treatment of a type V phosphodiesterase (PDE-5) inhibitor (Sildenafil, E4021) followed by 30minutes of inhaled nitrous oxide then performed a near maximum intensity workout [361]. Sildenafil is marketed for erectile dysfunction in men but is also used for its vasodilation properties including in the PDE-5 rich lung tissue [360]. Sixteen horses participated in a randomized, controlled cross over study design, and treatment significantly decreased visual blood score on endoscopy post exercise and reduced the number of red blood cells in tracheal washes compared to controls [361]. But, when Sildenafil was administered to 12 Thoroughbreds prior to treadmill exercise, there were no appreciable
differences in maximum PAP during exercise, or free red blood cells in BAL fluid compared to controls [365]. Angiotensin converting enzyme (ACE) inhibitors, that produce generalized vasodilation and reduce pulmonary vascular pressure in people [366] had been advocated for use in horses with EIPH, although in six horses it was not found to alter pulmonary hemodynamics during exercise [367].

After maximal exercise elevated concentrations of endothelin-1 (ET-1) has been reported in Thoroughbreds [368]. ET-1 is a vasoactive peptide, affecting the neuroendocrine control of cardiovascular system, and administration of exogenous ET-1 caused vasoconstriction and increased PAP, effects of which are completely blocked by an ET-1 receptor antagonist [369]. ET-1 receptor antagonists were trialed during exercise to ascertain if a reduction in PAP could be induced during exercise [370]. The antagonist did not reduce PAP during intense exercise and did not reduce EIPH. Similarly, the α-adrenergic antagonist, phentolamine, was trialed without success in 7 horses prior to exercise to ascertain if inhibiting vasoconstriction will reduce pulmonary artery and aortic pressures [40].

Other practices include withholding water on race day and bloodletting [371]. The supposed rationale for withholding water was that dehydration might alter blood pressure and prevent or reduce EIPH. Despite the widespread withholding water on race day [372, 373] there is no scientific evidence of efficacy for the treatment of EIPH. Large volume phlebotomy (36ml/kg 22% reduction) has been trialed to reduce EIPH in RBC hypervolaemic Standardbreds [374]. Large volume phlebotomy was performed, reducing hematocrit, blood and plasma viscosity, PAP and pulmonary vascular resistance. EIPH tracheobronchoscopic scores improved in all in five horses. But after removal of 22% blood volume, horses had a shorter run time to fatigue making this treatment unviable for racing.

b) Lowering alveolar pressure

Horses are obligate nasal breathers, and unlike other species that are able to switch to mouth breathing to reduce airway resistance during exercise, the horse maintains nasal breathing during exercise [375]. Airway resistance describes the relationship between pressure and flow in the airways and is dependent upon the length and diameter of airways.
A reduction in diameter of an airway is the greatest contributor to increasing airway resistance. Upper airway resistance in the horse is reported between 10-19% of total airway resistance and the nares is the largest contributor to this [376]. During exercise the nares relies on dilator muscles to resist the suction induced by inspiration, the dynamic collapse of the nasal alae induces high negative inspiratory nasopharyngeal and tracheal pressures and theoretically, also reduces the extremely negative intrapulmonary and alveolar pressure which increases capillary transmural pressure, leading to EIPH.

A commercially available adhesive nasal dilator strip was released in 1999 and marketed as a tool to reduce EIPH by decreasing airway resistance during inspiration. The nasal strip tents the skin over the nasal valve, extends the dorsal conchal fold laterally, and examination with endoscopy reveals increased cross-sectional area of the dorsal meatus [376]. Horses exercising on a treadmill at 100-120% of maximal heart rate had significantly reduced inspiratory airway resistance and lowered negative peak tracheal inspiratory pressure during exercise with a nasal strip compared to exercise without [376]. The effect of the nasal strip at ameliorating EIPH is contentious. Twenty-three horses raced on two occasions, once with a nasal dilator strip and once without and were evaluated post-race with BAL cytology to quantitate EIPH [203]. On both occasions horses received FUR 4 hours prior to racing. No significant differences were detected in BAL fluid red blood cell counts between horses racing with and without nasal strips, but this study failed to account for potential confounding such as days between races, race distance, lifetime starts, and BAL sampling was performed blindly and unilaterally in this small sample size of horses. Tracheobronchoscopic examination after a treadmill exercise test reported EIPH was still present in 7 horses irrespective of wearing a nasal strip [377]. The severity of EIPH was not reported, only that nasal strips were not a cure for EIPH in this small group of horses. Others have demonstrated in sample sizes all less than 10, that application of a nasal strip during treadmill exercise mitigated EIPH based on RBC counts in BAL fluid [331–333, 378, 379].
1.9.2 Treatments for haemorrhheologic effects

A reduction in platelet responsiveness identified in horses with EIPH [100] as well as the reported exercise induced changes in platelet responsiveness [101] led to speculation that a coagulopathy contributed to EIPH.

Yunnan Paiyao (or Yunnan Baiyo) is a powdered homeostatic Chinese medicine reported to reduce bleeding and clotting times in rats, rabbits and humans [380]. But when administered to horses in a randomized, blinded cross over study, Yunnan Baiyo had no significant effect on any hemostatic variable [381]. Nevertheless, Yunnan Paiyao anecdotally reduced EIPH, which precipitated widespread use of the medication at racetracks [382]. But when administered for 3-days to a small sample size of horses (N=5), Yunnan Paiyao had no reduction in RBC counts in BAL fluid compared to controls [382].

Aminocaproic acid (ACA) is a lysine analog, an anti-fibrinolytic drug that prevents clot lysis by inhibiting the activation of the anticoagulant plasminogen without affecting plasmin activity [383]. The activity of ACA is limited to clot maintenance due to the inhibition of fibrinolysis occurring only after clot activation has been initiated. ACA has no effect on clot formation via intrinsic or extrinsic coagulation pathways [384]. The administration of ε-aminocaproic acid intravenously to a group of horses was performed to assess potential hemostatic and fibrinolytic effects [385]. The drug was well tolerated and caused a reduction in the mean fibrinogen concentration and an increase in antiplasmin activity, consistent with the drug’s known anti-fibrinolytic properties. Anecdotally ACA was touted to attenuate the volume and duration of blood loss associated with EIPH during exercise. A clinical study in a small sample size of horses (n≤8) reported that ACA administration prior to exercise did not significantly alter the BAL concentration of red blood cells compared to exercise conducted without ACA [386, 387]. Women taking oral contraceptives containing conjugated estrogens are reported to have a higher incidence of blood clots [388]. Subsequently, conjugated estrogen underwent widespread use on racetracks to control EIPH, but clinical trials were unable to substantiate any efficacy at reducing EIPH [387]. Vitamin K, oxalic and malonic acid have been administered to horses with EIPH as pro-coagulants to assist with EIPH [389]. Currently there are no clinical trials that have evaluated the effect of Vitamin K, oxalic acid or malonic acid on EIPH. Paradoxically, proposed increased platelet aggregation in horses with a history of EIPH [390], lead
to some treating EIPH with aspirin [391]. Clinical trials examining the effect of aspirin on EIPH have not been conducted.

Proposed shearing stress in the lung vasculature was considered as a contributing factor to EIPH [392, 393]. Research demonstrated that FUR altered the deformability of red blood cells and increased blood viscosity [394–396]. Pentoxifylline is a phosphodiesterase inhibitor, clinically used to improve micro-vascular blood flow. Proposed pharmacological effects include increased white and red blood cell deformability, decreased RBC and platelet aggregation, thrombolysis, vasodilation and inhibition of neutrophil function [397, 398]. In vitro equine RBCs exposed to pentoxifylline had improved RBC filterability without altering size, chloride or potassium concentration. This suggested an effect on the ability to deform the RBC membrane, a mechanism that may improve microvasculature blood flow [395]. Pentoxifylline significantly reduced RBC packed cell volume and improved RBC filterability but did not alter viscosity in resting horses [396]. Pentoxifylline administered every 12 hours for 7 days, induced changes in red blood cell deformability that persisted when examined during a standard exercise test [399]. However, pulmonary hemodynamics and EIPH status were unchanged compared to controls after a one-off dose of pentoxifylline (8mg/kg, IV) was administered 15 minutes prior to exercise [400]. RBC plasticity was not tested, and it is unknown if administration 15 minutes prior to exercise is efficacious. But when twenty horses that had EIPH on two occasions were given pentoxyifylline (8.5mg/kg, q12h, PO) for 7 days prior to a 1000 metre stimulated race, EIPH was significantly improved or resolved compared to twenty EIPH positive horses that did not receive pentoxyifylline [401].

1.9.3. Treatments to reduce inflammatory airway disease

The concomitant relationship between EIPH and pulmonary inflammation and the widely held assumption that airway inflammation perpetuates EIPH [113] leads to many racehorses being treated with medications to reduce pulmonary inflammation and/ or bronchoconstriction. Certainly, it appears prudent to alleviate any infectious or non-infectious lower respiratory tract disease to maintain lung health, but widespread treatment with inhaled or systemic anti-inflammatories has
become habitual in racing despite no studies examining the efficacy of these treatments on naturally occurring EIPH.

Clenbuterol is a $\beta_2$ adrenergic receptor agonist resulting in bronchodilation and transient pulmonary and systemic vasodilation [402]. Clenbuterol was speculated to be of use in managing inflammatory airway disease and EIPH. Clinical trials were unable to show any change in pulmonary vascular pressure after clenbuterol administration, and the drug did not attenuate post exercise tracheal blood [403]. Combining FUR and clenbuterol also did not attenuate EIPH [404] but EIPH severity was not assessed in either of these studies. A 2006 study investigated the effect of autologous blood instillation in the trachea and administration of clenbuterol (2ml/100kg, PO, BID) for 9 days [405]. Clenbuterol treatment reduced the number of RBC in BAL fluid compared to controls at day 3; an effect attributed to a possible anti-inflammatory action or enhanced mucociliary clearance [406, 407]. No further research investigating naturally occurring EIPH and the effects of clenbuterol has been conducted.

Dexamethasone, a corticosteroid, was administered to 7 horses (0.1mg/kg, iv, SID) for three days prior to an exercise test [408]. Horses had developed EIPH in the control exercise treadmill tests prior to the initiation of dexamethasone therapy. Despite treatments, all horses still developed EIPH after exercise. Severity was not reported. An intra-pulmonary blood inoculation model was used to study the effects of an inhaled (beclomethasone) and an oral corticosteroid (prednisolone) to reduce the damage to alveolar tissue after haemorrhage [405]. Both treatments significantly reduced RBC count in BAL fluid collected on day 3 and oral prednisolone significantly increased macrophage percentage (P<0.05), suggesting this treatment as an aid for blood clearance from the alveoli. But neutrophil counts were not significantly (P<0.05) different between the groups, suggesting corticosteroid or bronchodilator treatment did not have an intra-pulmonary anti-inflammatory effect or that BAL cytology is an inaccurate reflection of active inflammation. It is unclear if these therapies may provide long-term benefit to lung tissue affected by EIPH, or if the autologous blood instillation model appropriately replicates the microscopic cellular infiltration and immune responses associated with EIPH.

A transpirator, a device designed to deliver warm water vapor to the airways to aid mucus clearance associated with IAD, was purported by the manufacturer to prevent and decrease the
incidence of EIPH. Independent controlled clinical trials were unable to document any difference in EIPH presence or severity between treatment and control groups using either post exercise endoscopy or BAL [409]. Straw bedding was speculated to contribute to EIPH due to lung inflammation associated with dust generation. An investigation examining the incidence of EIPH on straw or paper bedding found no significant difference between bedding materials [410].

Concentrated equine serum (CES) was theorized to reduce EIPH by reducing pulmonary inflammation via immunomodulation [411]. In humans, immunomodulation is successfully used to treat asthmatics [412]. CES is collected from multiple draft horse donors containing high levels of immunoglobulins, complement and other serum proteins, and when administered to 10 horses pre-exercise test was found to significantly reduce EIPH and associated inflammation after maximal exercise compared to placebo treated controls [411]. A reduction in EIPH was concluded based on a reduction in RBC counts in BAL fluid.

Alternative therapies such as omega-3 fatty acids, purported to reduce airway inflammation via their action on the arachadonic acid cascade have been advocated to reduce inflammatory airway disease and therefore also EIPH [373, 413]. Unpublished data suggested that horses fed a diet rich in long-chain omega-3 fatty acids for 83 and 145 days showed a reduction in EIPH [413].

1.9.4. Rest

Rest is an obvious recommendation for horses with EIPH. Without further racing or galloping exercise, this treatment option will prevent further episodes of EIPH. But in all likelihood the disease will recur when galloping or racing recommences. The viability of this treatment is unproven; no study has examined the impact of rest of any duration, on disease presence or severity. The economic viability of this treatment is questionable if the severity of the disease is unaffected by rest and recommences when training resumes.
1.10. Prognosis for EAE and EIPH

“Forecasting the probable course of a disease, including the chances of recovery” - The Oxford Dictionary.

The prognosis for racing with EIPH is multifaceted. Prognosticating the progression and course of the disease is of indisputable importance, but EIPH has other important ethical, financial and regulatory implications that can override and dictate the racing future of the horse more so than the disease course.

Regulatory controls for EAE are present in many racing jurisdictions. Australian rule of racing 53A (AR53A) prevents any horse being nominated for a race for a period of 3 months after an episode of epistaxis and mandatory retirement after 2 EAE episodes [220]. Similar restrictions apply in Hong Kong, while in Japan horses are banned from racing for 1 month [414, 415]. Restrictions on horses with EAE are in place due to safety concerns for jockeys and horses, because although extremely rare, EIPH is associated with collapse and sudden death [142, 251]. In Australia, the majority of racehorses are removed from racing after their first reported episode of epistaxis and invoking of AR53A [183, 250]. Whilst in Hong Kong, 90% of horses with EAE are retired prematurely, prior to any compulsory action being taken [254]. Potential drivers of this decision to voluntarily retire after EAE include ethical, safety or financial concerns. The association between EAE and poor performance is well documented, as is, the increase risk of EAE in this group of horses upon returning to racing [81, 172, 173, 183, 188, 189, 250]. Perceived future poor performance in horses with EAE may cause some owners to consider these horses financially untenable to return to racing, and poor performance is a major driver of early retirement [210]. In fact, if a horse returning to racing after EAE maintains its performance (measured as a percentage of wins before and after the first episode), they are more likely to have a second EAE episode than horses that return to racing with a lower percentage of winning starts [250].

Severe EAE or EIPH causing sudden death or collapse is reported [142, 251]. But haemosiderophages, markers of chronic EIPH, were frequently unreported in horses that died from acute pulmonary haemorrhage suggesting perhaps these horses were not a fulminant progression of chronic EIPH [251]. Others describe haemosiderophages in nine horses that died from pulmonary
failure secondary to EIPH, but 5 horses had previous history of EIPH and haemosiderophages presence is not surprising in these horses [142]. Furthermore, only four horses had epistaxis despite the premise that if EIPH were severe enough to cause pulmonary failure and death one would reasonable expect epistaxis to be present. It is unknown if chronic EIPH ascribes to a progressive disease resulting in fatal EAE or collapse, or if the pulmonary haemorrhage in these cases is the result of another underlying cause, such as cardiac arrhythmia. There are inherent challenges in assigning a definitive cause of death, as well as post mortem change and probable previous EIPH episodes complicate assigning a definitive cause of death.

The prognosis for racing for horses with severe EIPH is described as guarded due to the widely held belief that the disease is progressive, but ultimately the prognosis is unknown [260]. A pattern of increasing severity on endoscopic evaluation has not been described [8, 254]. Post mortem examination would suggest the disease is progressive, with lesions appearing to spread in a horizontal fashion over the dorsal bronchopulmonary segments and horses that have not undergone racing or intense exercise do not have these lesions [24, 67, 76]. But meaningful longitudinal studies on EIPH are scarce, and it is unknown if the volume of tracheal blood is even an appropriate marker to gauge progression. The majority of endoscopic epidemiological surveys of racing horses focus on a single observation or are limited to anecdotal comments regarding repeatability within a small group of horses [8, 70, 174, 254]. These studies describe the disease as unpredictable, with “some horses exhibiting considerable haemorrhage whilst on subsequent occasions little or none” [8].

There is evidence that EIPH will reoccur after being diagnosed on one occasion, with three studies demonstrating that the presence of EIPH on one occasion significantly increases the chances of EIPH detection on future occasions, but these studies have been conducted on small sample sizes, and haven’t controlled for FUR administration or other confounding variables [9, 178, 263]. A Canadian study examined 60 Standardbred horses after racing on three occasions, grading tracheal blood on a 0-2 scale of increasing severity [178]. Using McNemar’s test for paired proportions, horses that were diagnosed with grade 2 EIPH, had EIPH detected more frequently than horses that had EIPH ≤1. In 76 Thoroughbred racehorses examined after racing or breezing on 2 occasions, there was moderate agreement for the presence of EIPH between the two examinations
(κ=0.52) [9]. Others report good agreement between two post-race examinations (κ=0.59), although the distal trachea was not visualized [263].

Most horses (72%) with EIPH experience a variety of grades throughout their career, or part thereof [254]. In a study from Hong Kong, that collated veterinary records, found the majority of horses have consistently low grades of EIPH during their career [254]. Horses that experience higher grades of EIPH (grade 3, 4 or epistaxis), most (70%) also experience a lower grade of EIPH at some point during their careers. Anecdotal comments were made attesting to the capricious nature of the disease after diagnosis of severe EIPH, although statistical analyses or supporting data is not provided. The study also reported that horses with EIPH grades ≤3 were more likely retired for reasons other than EIPH, although not all horses reached retirement during the study period. This study is the first attempt to longitudinally examine EIPH over a racehorses’ career and although criticized for bias and methodological limitations, [416] the authors suggest that EIPH does not affect career longevity. Further studies prospectively examining progression of EIPH, under racing conditions are required to decipher the importance of EIPH on future performance.

**Experimental Aims and hypothesis**

1. Using a population of racing Thoroughbreds performing under normal conditions, identify what risk factors, if any, determine disease presence and severity. Based on previous research our primary hypothesis is that ambient temperature will impact both disease occurrence and severity.

2. Using a larger population of racing Thoroughbreds, we aim to determine if disease occurrence or disease severity will have an impact on athletic performance. Our hypothesis is that EIPH will negatively impact some markers of performance.

3. The consistency and progression of EIPH over time will be investigated using a subpopulation of racing Thoroughbreds examined on 2 or more occasions. Our hypothesis is that EIPH is a progressive disease over time and the progression can be slowed by longer periods of rest between racing campaigns.
Chapter Two.  Material and Methods

2.1. Study design

A prospective, longitudinal, observational study design was used to examine a population of racing Thoroughbreds competing at selected racetracks in metropolitan and regional Western Australia between May 2012 and April 2013, and between July 2014 and December 2015.

Western Australia’s two metropolitan racetracks conduct race meetings exclusively for six months of the year. Belmont racecourse conducts race meetings over the winter months (May to October) and Ascot racecourse, the summer months (October - April). Belmont racecourse has superior track drainage and is better equipped to handle racing during winter. The provincial racetrack where examinations also occurred is located 170km southwest of Perth. This racetrack is also utilised seasonally from October through to April.

2.2. Recruitment

Information regarding the study was initially promoted to racing participants through radio interviews, advertising in the racing calendar, audio-visual presentations at racetracks and postal communication. During the study period, trainers and owners were regularly approached at the races to remind them about the study, encourage enrolment and discuss the importance of diagnosing respiratory tract disease in racehorses. Endoscopic examinations of the upper respiratory tract were offered free of charge. Any obvious abnormalities of the larynx, evidence of EIPH or mucus were reported to the trainer and, or owner to help make enrolment worthwhile for all parties. Potential biases procured through owners or trainers preferentially permitting access to certain horses, and not others were managed by encouraging the enrolment all horses in their care. Providing trainers with additional information such as upper airway abnormalities helped build relationships with trainers and reiterate the importance of utilising this free service. This helped to boost enrolment and ensure horses were available for re-examination.
2.3. Horses

All races were conducted on a flat turf surface. Pre-administration of any medication on race day is prohibited in accordance with the Australian Rules of Racing [220]. Any horse racing on the day when examinations were being conducted was eligible for enrolment. Enrolled horses must have finished the race to be examined on that occasion.

Horses were presented to a designated location on-course no earlier than 30 minutes and no later than 220 minutes after racing. A number, assigned chronologically, was given to each examination. This number was written down and filmed as part of, and prior to, each examination. This served to identify each examination by number, blinding the horses’ identity. The same number was then also recorded in the race book next to the horses’ name, facilitating the coupling of the examination and data entry.

The horse was restrained by the trainer or strapper provided by the trainer. On occasion, sedation was required to facilitate tracheobronchoscopy, and 200mg xylazine (Xylazil-100, 100mg/ml, Troy, NSW) combined with 10mg acepromazine (ACP10, 10mg/ml, Troy, NSW) was administered intravenously. If the horse continued to be fractious in a manner deemed dangerous the examination was aborted. A nose twitch was usually applied and a 1.5 metre endoscope (AOHUA model LG-200 or Pentax, Model EPM-3000) was passed down the left or right nares into the trachea to the carina (figure 2.1). The larynx was assessed for symmetry and any abnormalities noted after complete inspection of the trachea had been performed. All examinations were recorded. The time at examination and the presence of epistaxis were documented. Videos were labelled with the examination number for reviewing purposes.
2.3. Detection and quantification of EIPH

Two experienced veterinarians blinded to the identity and performance of the horse reviewed the tracheobronchoscopic examinations independently. A previously described 0-4 graded scoring system was used [261]. Briefly, grade 0 is assigned to cases where no blood is observed in the trachea or nasopharynx; grade 1 has ≥ 1 blood specks or ≤ 2 short (≤ ¼ trachea length) and narrow (<10% of the tracheal surface area) streams of blood; grade 2 is a single long stream of blood (>½ tracheal length) or > 2 short streams of blood occupying ≤⅓ of the tracheal circumference; grade 3 is multiple, distinct streams of blood covering more >⅓ of the tracheal circumference, but without blood pooling at the thoracic inlet; and grade 4 is multiple, coalescing streams of blood covering >90% of the tracheal surface, with blood pooling at the thoracic inlet.

The primary reviewers entered the allocated EIPH grade next to the examination number in a pre-formatted excel workbook. The reviewers' workbooks were combined and if there were discrepancy between the primary reviewers' score, a third experienced veterinarian would independently and blindly review and grade that examination. The median score was then adopted for statistical analyses.
2.4. Race, meteorological and betting data

Racing records were retrieved from a public database maintained by the racing regulator in Western Australia (Racing and Wagering Western Australia, RWWA) [417]. Variables captured included trainer, racetrack, time and date of the race, weight carried, jockey, position on the turn (generated at the 400metre mark), finishing position, distance finished behind the winner, overall race time, last 600m sectional time, number of starters in the race, race earnings, race distance, track rating, penetrometer reading, age, sex, date of the first start of the current racing preparation, date of the last start of the previous racing preparation, date of the previous race start, the number of starts in the current racing preparation, number of career starts and career earnings recorded from the horses’ first career start to the day of examination.

Categorical outcomes of these variables included winning (yes/no), finishing in the top 3 positions (yes/no) and collecting race earnings (yes/no). Average race speed (m/sec), average speed over last 600m (m/sec), and the average early/mid race speed (m/sec) were calculated using the horses’ race time and the last 600m time. The early/mid race speed was then compared to the last 600m speed to ascertain if the horse accelerated or decelerated their average race speed over the last 600m of the race (yes/no). The position on the turn minus the finish position was used to assess field movements relative to other competitors over the final 400m of the race; a positive number infers that the horse overtook competitors, a negative number indicated the reverse. The number of days in the current racing preparation was calculated using an excel formula subtracting the date of examination from the date of the first race in the current preparation. Similarly, spell length was calculated by subtracting the date of the first race start in the current preparation from the date of the last race start in the previous racing preparation. Days since the last race start was also calculated in a similar fashion. The time between racing and examination was calculated using the race time and the recorded time of examination. Finishing position was recorded as a decile of the number of competitors in the race to account for variation in field size.

The starting price (SP) for each horse was retrieved from the betting exchange website, Betfair Australia using the win pool market [418]. Horses were also ranked based upon their SP (shortest to longest odds) and this rank was used as a prediction of finishing order. If a horse was
ranked ≤4 (shortest odds), the horse was examined for the predicted finish minus actual finishing position; a negative value indicated a performance below market expectation; and a positive value, the reverse.

Climatic variables were obtained from the Australian Bureau of Meteorology using the closest weather station recording temperature, apparent temperature, relative humidity, dew point and wind speed at the nearest time point to the race start time [419]. Due to the proximity of metropolitan racetracks, the same weather station was used. This weather station was located 3.1 and 6.3km from Ascot and Belmont Park, respectively. Season was categorised into periods of three months beginning the first days of December (summer), March (autumn), June (winter) and September (spring).

Any additional gear worn during the race was retrieved from the RWWA online gear list for each horse [417]. This includes the use of a tongue-tie (yes/no), the type of bit used (standard, Norton, lugging or tongue bit), non-standard shoes (NSS) (yes/no), the type of NSS (bare, bar shoes, concussion plates, glue-on shoes, pads), the foot the NSS was applied to, nasal flair strips (yes/no), or a bubble cheeker (yes/no). The type of bar shoe (egg, straight or heart bar) was not specified in the database.

i. **Risk Factor Variables**

Risk factors for EIPH were investigated to determine if any horse or race day variable could be manipulated to reduce or eliminate EIPH. Specific risk factors included in this analysis were; age, sex, number of career race starts, and the number of race starts during the current racing preparation. Lifetime starts was examined categorically; <20 or ≥20 race starts as this appeared the natural breaking point in the data. Race and track selection factors examined included weight carried, number of race starters, weight carried, track, track rating, penetrometer reading and race distance. All climatic and gear variables mentioned above were examined. The time between racing and examination was also included as a risk factor as it pertains to diagnosis. Performance variables such as finishing position were not included in risk factor analyses, as they were considered attributed to, rather than a risk factor for EIPH.
ii. **Performance variables**

A comprehensive approach to race day performance was taken in an attempt to overcome problems that are encountered defining poor and good performances. Indices documenting race day performance included finishing position (as a decile of the field), if the horse won or placed in the first three positions, if the horse collected race earnings, the amount of race earnings, distance finished from the winner, position on the turn minus finishing position, last 600metre speed, average race speed, average early/mid race speed, if the horses’ average speed accelerated or not over the last 600 metres, Betfair starting price and the predicted finish (SP rank) subtract the actual finishing position. Career performance measures included the number of career starts and career earnings to date.

iii. **Longitudinal progression of disease variables**

Variables captured for longitudinal analysis included the number of career race starts, the number of days and races in the current racing preparation, the number of days since the horses’ last race start, the number of days between racing preparations (spell length), weight carried, race distance and non-standard shoes (yes/no). A racing preparation was defined as one or more races without a break of more than 60 days between consecutive races. More than 60 days between two races denoted the end of that racing preparation and the commencement of the next racing preparation.
2.7. Data analysis

i. Risk factor data analysis

The analysis involved a subset of examinations conducted only at the two metropolitan racetracks between May 2012 and April 2013. Two models, categorising EIPH severity were used in the analyses. These models were selected to remain consistent with other published literature investigating risk factors [124]. Model A was based on the presence or absence of blood (i.e. EIPH grade 0 vs. EIPH grades ≥1), and model B contrasted EIPH grades ≤1 with EIPH grades ≥2. Initially each variable was examined using univariable analyses (Chi-squared test of independence and Kruskal-Wallis ANOVA) and those with a P ≤0.25 were included in multivariable logistic regression analyses. To account for the potential association between track (Ascot/Belmont Park) and environmental factors, track was initially included as a random effect in a logistic normal multiple regression model. Significance of track dictated subsequent inclusion of this variable in the model as a random effect. Backward elimination was used to determine the variables dropped from the multivariable models. The model fit was evaluated by calculating a Hosmer-Lemshow statistic. Two-way interaction terms among the explanatory variables were examined after identification of the reduced set main effects. Each interaction was added sequentially to the model and the significance assessed. Statistical analyses were conducted in SPSS (SPSS statistics v21), Statistix (Statistix v9.0) and Egret (Egret v2.0.3). Variables with a P<0.05 in the final model were considered significant and therefore retained.

ii. Performance data analysis

Continuous response variables were assessed for normality. The variable ‘distance finished behind the winner’ was highly right skewed associated with the physiological limitations on maximal speed and no limit to how slow a horse could race. Horses that won were removed from analysis of response variable and the remaining observations were log transformed. Race and lifetime earnings were also right skewed due to the large number of non-earners. One dollar was added to each observation and the value was log transformed.

The association between the performance response variable and EIPH were assessed in two models. These models varied from the models used in the risk factor analysis. Firstly, EIPH
grade 0 was compared to EIPH grades 1-4; and secondly EIPH grade ≤2 was compared to EIPH grades ≥3. EIPH group comparison was categorised in this manner based on the significant associations between performance findings comparing grade 0 to the other grades, and the lack of significant findings when the analyses were performed comparing EIPH ≤1 to EIPH ≥2. Factors that could potentially predict or affect the outcome of the race or the performance response variable were considered as potential confounders and were held as fixed effect, specifically weight carried, sex, race distance, career race start, start this racing preparation, ambient temperature, track rating, nonstandard shoes and racetrack. Interaction effects between these factors were assessed. Due to the six-monthly use of one metropolitan racetrack over winter, the interaction between racetrack and ambient temperature, and ambient temperature and track rating were incorporated into the model. Collinearity between the fixed effects was considered and if highly correlated the variable that was subjectively considered to be the superior predictor was used. For example, age and career race starts were highly correlated; age was considered a proxy measure for career starts and was removed from the model.

Associations between continuous response variables and EIPH were assessed using the lme() function in R for linear mixed effect models. The models were adjusted for potential confounding variables and a random intercept was incorporated to account for repeated sampling of individuals. Model checking when including or removing predictor/confounding variables from the model was performed using ANOVA () function in R. Underlying distributional assumptions of normality and homogeneity of variance for the models were assessed using QQ plots and residual plots. Maximum likelihood mean estimates and 95% confidence intervals (CIs) were obtained for the effect sizes of EIPH and other confounding variables included in the model.

Associations between categorical response variables (winning, finishing in the top 3, accelerate/ decelerate, collect race earnings) and EIPH were examined using multiple logistic regression models using generalised estimating equations. The R function geeglm() was used based on an independent correlation structure to account for the repeated sampling of horses and the models were adjusted for the potential confounding variables. Odds ratio and Wald statistics based on 95%CIs were calculated for the regression coefficients of EIPH grades, and other confounding
variables included in the model. A $P<0.05$ was considered significant for both continuous and categorical response variables models. Data are reported as mean and 95% CIs.

iii. **Longitudinal progression of disease data analysis**

Horses with two or more observations were included in the analysis. Observations were included into two datasets; all observations collected over the study period; and observations conducted after sequential races that were separated by a break of more than 60 days (i.e. last race of one preparation and the first race start of the next racing preparation). Collinearity between the fixed effects was considered and if highly correlated the variable that was subjectively considered to be the superior predictor was used. The number of days and the number of races in the current racing preparation were highly correlated, as was age and the number of career race starts. The number of days in the current racing preparation and the number of career race starts was used for analysis.

Using the second or greater observation for each horse, the association between the current and the preceding EIPH score was investigated using a linear mixed effects model. The `lmer` function of the `lmer4` package in R was used to assess the strength of the relationship between the two EIPH scores (0-4), adjusting for possible confounding variables. Potential confounding factors that could affect the current EIPH score were included as fixed factors in the model. These included ambient temperature, number of days since the last race start, sex, weight carried, number of days in the current racing preparation, the number of career starts and race distance. A random intercept was included to account for the repeated sampling of horses. Model selection when including or removing predictor/confounding variables was performed using the `Anova` function in R.

Factors associated with a change in EIPH score were modeled using multiple ordinal regressions with a random effect term for each horse. For this analysis, EIPH grades 0-4 were reclassified into three states: no or mild EIPH (grade ≤1); moderate (grade 2); and severe EIPH (grade ≥3). These multiple ordinal regressions essentially model the observed sequence of EIPH states for each horse as a time inhomogeneous Markov chain. To achieve this, all ordinal regressions used the same set of regressor variables, but the regression parameters were allowed to differ based on the EIPH state the horse was transitioning out of. This was to ascertain whether a predictor had a notable change in behaviour across EIPH states.
Longitudinal analysis was performed using a semi-parametric approach to fit a smooth curve, namely a spline, using the number of career starts as a marker for time. At each observation, fixed effects including ambient temperature, race distance, weight carried, the number of days in the current racing preparation, the number of races between examinations and the number of days since last racing were incorporated into the model. A horse specific intercept and slope was included to permit the spline to vary slightly between horses. The shape of the mean population spline over time (career starts) was examined to assess disease progression. The population mean response represents the average horse racing, racing in average conditions with average other covariate values.

Underlying distributional assumptions for normality and homogeneity of residuals were checked using QQ and residual plots. Maximum likelihood mean estimates and 95% confidence intervals were obtained for the effect sizes of the lagged EIPH score and other confounding variables included in the models. Significance was based on a p-value <0.05 and data analysed using the R environment for statistical computing.
Chapter Three. Bar shoes and ambient temperature are risk factors for exercise induced pulmonary haemorrhage in Thoroughbred racehorses

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Key words: Thoroughbred; horse; EIPH; risk factor; haemorrhage; lung;

Ethical animal research
Approval for this study was obtained from Murdoch University's Animal Ethics Committee (R2474/12). Owner informed consent was given for each horse included in this study.

Author’s declaration of interests
The authors have no conflicts of interest

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Authorship
E.J. Crispe contributed to study design and execution, data collection, statistical analysis and manuscript preparation. G.D. Lester contributed to study design and execution, statistical analysis and manuscript preparation. C.J. Secombe contributed to study design and execution and manuscript preparation. ID Robertson contributed to the statistical analysis and manuscript preparation.
Summary

*Reason for performing the study:* Ambient temperature has been identified as a risk factor for exercise-induced pulmonary haemorrhage (EIPH) in racing Thoroughbreds. This warranted a more expansive investigation of climatic conditions on the incidence and severity of EIPH. The impact of other variables, such as the type of bit used, tongue ties, and non-standard shoes, has not been reported and also warrant investigation.

*Objectives:* To examine the effect of various climatic variables as contributing risk factors for EIPH. Other previously uninvestigated variables, as well as standard track and population factors will also be examined.

*Methods:* Thoroughbred racehorses competing at metropolitan racetracks in Perth, Western Australia were examined, 30-200 minutes post-race with tracheobronchoscopy. Examination took place at 48 race meetings over a 12-month period. Examinations were graded (0 to 4), independently by 2 experienced veterinarians. Univariable analyses were performed, and variables with a P <0.25 were entered into a multivariable logistic regression analysis. The analysis was performed twice using the presence of blood (EIPH grade 0 vs. grades ≥1) and EIPH grades ≤1 vs. EIPH grades ≥2 as dependent variables.

*Results:* EIPH was diagnosed in 56.6% of observations. Lower ambient temperature was significantly associated with EIPH grades ≥1 (OR 0.95; 95% CI 0.93-0.98) and EIPH grades ≥2 (OR 0.97; 95% CI 0.94-1.0). Bar shoes were significantly associated with EIPH grades ≥1 (OR 6.35; 95% CI 2.17-18.54) and EIPH grades ≥2 (OR 2.72 95% CI 1.3-5.68). Increasing race distance was significantly associated with EIPH grade ≥1, and increasing lifetime starts was significantly associated with EIPH grade ≥2.

*Conclusions:* Ambient temperature is a risk factor for EIPH in Thoroughbred racehorses, with lower temperatures associated with increased risk. Bar shoes are a novel risk factor for EIPH in this population.
Introduction

Exercise-induced pulmonary haemorrhage (EIPH) is a common condition of Thoroughbred and Standardbred racehorses worldwide. Tracheobronchoscopy after racing has been reported to be a reliable technique in identifying pulmonary haemorrhage and there are several scoring systems that have been used to semi-quantitate EIPH severity [7, 261]. The point prevalence of EIPH using tracheobronchoscopy within 120 minutes of racing varies between 44 and 75% [8, 9, 124, 174, 176]. The percentage of racehorses that demonstrate EIPH increases when individuals are examined after multiple races [8, 174, 178].

The pathophysiological basis of EIPH remains controversial. A plausible and well-accepted theory is that failure of pulmonary capillaries occurs secondary to substantial and sustained increases in transmural pressures associated with high intensity exercise [45, 420]. Locomotory induced trauma, inflammatory airway disease, upper airway obstruction and haematopoeitic abnormalities have also been proposed as initiators or contributing factors for EIPH [80, 96, 119, 421, 422]. The identification of risk factors may provide further understanding of disease causality.

Epistaxis after racing typically represents the most severe form of EIPH. Exercise-associated epistaxis has been examined through conveniently accessible race records [6, 80, 81, 170, 172, 186]. Some of these reports are lacking suitable statistical analyses precluding meaningful interpretation, but several larger retrospective studies identified race type, accumulated time spent racing, age, track hardness, and race distance as significant risk for exercise-associated epistaxis [80, 81]. Epistaxis under-represents EIPH prevalence, consequently risk factors for this entity may differ from EIPH diagnosed via endoscopy. Several groups have reported risk factors for EIPH [7, 8, 10, 124, 174, 178, 300].

A prospective study of Australian Thoroughbreds using post-race endoscopy reported several factors that were significantly associated with EIPH [124]. The study reported two models based on EIPH grade and, depending on the model used, reported increased risk with increased lifetime starts, lower ambient temperatures (<20°C), longer time intervals between racing and examination, and shorter race distances. Others have alluded to an association between environmental conditions and the incidence of EIPH, but have not identified a clear association [9,
Although ambient temperature was significant in both models used in the Australian study the temperature range was relatively small (mean 17.9°C; 95% CI 12.9-22.9°C) [124]. The primary aim of this study was to examine the effect of environmental conditions on the occurrence and severity of EIPH over a continuous 12-month period. The secondary aim was to identify if other horse or track factors were significantly associated with EIPH in this population of racing Thoroughbreds. Our hypothesis was that lower ambient temperatures are associated with increased risk for EIPH.

Materials and methods

Study design

A prospective cross-sectional design was used, structured to capture a wide spectrum of local climatic conditions. During the period from May 2012 to April 2013, inclusive, 48 race meetings were attended at two metropolitan racetracks in Perth, Western Australia. This included 22 meetings at Belmont Park between May and October and 26 meetings at Ascot between October and April. Belmont Park racecourse has superior drainage and is utilized exclusively over the winter months, during the period of highest rainfall. The racetracks are situated 3.2 kilometers apart. The university’s Animal Ethics Committee (R2474/12) approved the study.

Horses

All horses that completed the race were eligible for enrollment and horses were voluntarily enrolled by trainers or owners. Horses were only examined once. All races were conducted on a flat turf surface. Premedication with furosemide, or any other medication, is not permitted on race day under the Australian Rules of Racing [220]. After completion of the race, horses were recovered as per normal. Horses were brought to a designated location on-course for endoscopic examination. Horses were presented no earlier than 30 min after racing. A 1.6 m videoendoscope (Pentax, Model EPM-3000i) was passed through the nares into the trachea to the level of the carina. Sedation, if required, was 200 mg xylazine (Ilium Xylazil-100) with 10mg acepromazine (Ilium Acepril-10) administered intravenously. All examinations were recorded, and tracheal blood was later graded by two experienced veterinarians, familiar with the grading scale, and blinded to race date and horse.
identity. The time interval between racing and the examination was recorded. A previously described scoring system was used with a range of 0-4, inclusive: Grade 0 is assigned to cases where no blood is observed in the trachea or nasopharynx; grade 1 has ≥ 1 blood specks or ≤ 2 short (<¼ trachea length) and narrow (<10% of the tracheal surface area) streams of blood; grade 2 is a single long stream of blood (>½ tracheal length) or > 2 short streams of blood occupying <½ of the tracheal circumference; grade 3 is multiple, distinct streams of blood covering more >½ of the tracheal circumference, but without blood pooling at the thoracic inlet; and grade 4 is multiple, coalescing streams of blood covering >90% of the tracheal surface, with blood pooling at the thoracic inlet [261]. Where there was discrepancy in the score between the primary reviewers, a third independent reviewer was used, and the resultant median score was used in the statistical analysis. The third reviewer was an experienced equine veterinarian and received the same training as reviewers 1 and 2. This reviewer was also blinded to horse identity and date.

Race records

Race records were retrieved from a public database maintained by the racing regulatory body in Western Australia [6]. Data obtained from this database included: horse name, age, sex, trainer, race date, race day (Wednesday or Saturday), race distance, finish place, number of race starters, weight carried, track, track rating, penetrometer reading, starts for this racing preparation (a hiatus of more than 60 days between racing was considered the end of a racing preparation), lifetime starts, and epistaxis. Any additional gear worn during racing was recorded, including use of a tongue-tie, the type of bit (standard, Norton, lugging, tongue), non-standard shoes (bare, bar shoes, concussion plates, glue-on shoes, pads), nasal flair strips, or use of a bubble cheeiker (also known as a ‘bit burr’). Additional information for non-standard shoes included the type of non-standard shoe and the foot the non-standard shoe was applied to. The type of bar shoe (egg, straight or heart) was not specified in the database. Final race position was categorised into quartiles. Lifetime starts were classified into <20 or ≥20 starts, as this appeared to be a natural break point in the data set.

Track rating was performed by the racing regulatory body in a standardised manner that is consistent across Australian racing jurisdictions. Tracks were assigned an overall rating from fast (firm) to heavy (wet) based on use of a penetrometer. A grading system from 1-10 was also
used to more precisely reflect the overall firmness of the track. A value of 1, being described as fast, or firm, through to 10 which is described as very soft and wet.

Climatic variables were obtained from the Australian Bureau of Meteorology using the same weather observation station located 3.1 km and 6.3 km from Ascot and Belmont Park, respectively. Weather observations are reported at 30-minute intervals. Data were collected at the closest time point to the race start time. Variables included season, ambient temperature, apparent temperature, relative humidity, dew point, and wind speed. Season was categorized in 3-monthly periods, beginning the first day of December (summer), March (autumn), June (winter), and September (spring).

Data analysis

Two models were developed based on the median EIPH score for each observation. Model A was based on the presence or absence of blood (EIPH grade 0 vs. EIPH grades ≥1), whereas model B contrasted EIPH grades ≤1 with EIPH grades ≥2. Initially each variable was examined using univariable analyses (chi-squared test of independence and Kruskal-Wallis ANOVA) and those with a P ≤0.25 were included in multivariable logistic regression analyses. To investigate the potential association between track (Ascot/ Belmont Park) and environmental factors, track was initially included as a random effect in a logistic normal multiple regression model. Significance of track dictated subsequent inclusion of this variable in the model as a random effect. Backward elimination was used to determine the variables dropped from the multivariable models. The model fit was evaluated by calculating a Hosmer-Lemeshow statistic. Two-way interaction terms among the explanatory variables were examined after identification of the reduced set main effects. Each interaction was added sequentially to the model and the significance assessed. Statistical analyses were conducted in SPSS, Statistix and Egret. In the final model variables with a P < 0.05 were considered significant and therefore retained.

Results

Data were collected from 48 race meetings over a 12-month period. There were 583 horses that underwent tracheobronchoscopic examination over the study period. Of these, 373 examinations were performed at Belmont Park racecourse and 210 at Ascot racecourse. Horses
ranged in age from 2-9 years, and consisted of 205 females, 357 geldings and 21 entire males. 121 trainers participated, enrolling between 1 and 36 horses.

The primary reviewers concurred on 86% of examinations. The third reviewer score agreed with one of the primary reviewers on each occasion. Blood was detected in the trachea in 330 horses (56.6%). There were 253 (43.4%) horses with grade 0; 159 (27.3%) grade 1; 111 (19%) grade 2, 45 (7.7%) grade 3 and 15 (2.6%) were grade 4. Therefore, there were 171 horses with an EIPH score ≥2. Five horses had unilateral or bilateral epistaxis, and of these four were graded as 4, and one horse was grade 3. Thirty-one horses wore one or more bar shoes. Two horses had bar shoes on both hind feet, five horses wore both front and hind bar shoes, and twenty-four horses had bar shoes on one or both front feet.

Twenty-seven variables were examined using univariable analysis for both models: EIPH grades ≥1 (model A) and EIPH grades ≥2 (model B) (Table 3.1). Fifteen variables had a p-value <0.25 on univariable analysis, and were included in the multivariable regression model A, 13 variables were presented in the multivariable regression model B.

The mean time interval between racing and examination was 51.7 minutes (Table 3.1). Univariable analysis of the time after racing to examination and the detection of blood (Model A) had a P-value >0.25. Univariable analysis of the time after racing to examination and increasing EIPH severity (Model B) was had a P-value <0.25 and was included in the multivariable analysis but was not retained in the final model. When track was initially included as a random effect in the logistic regression analyses it was not significant in either model (P=0.529 and P=0.488 for Models A and B, respectively), and therefore standard logistic regression analyses were performed.

The mean ambient temperature was 22.9 °C with a range of 13.5-37.7 °C. Ambient temperature, the addition of bar shoe(s) and increasing race distance were significantly associated with the presence of blood (EIPH grade 0 vs. EIPH grades ≥1) (Table 3.2). An inverse relationship was identified between EIPH and ambient temperature. The Hosmer-Lemeshow statistic for this model was 13.04 with a P-value of 0.11.

Ambient temperature, the addition of bar shoe(s) and increased lifetime starts were all associated with increased EIPH severity (EIPH grade ≥2) (Table 3.3). An inverse relationship was
identified between ambient temperature and EIPH grade ≥2. The Hosmer-Lemeshow statistic for this model was 11.11 with a P-value of 0.20.

Discussion

The primary aim of this study was to examine the relationship between climatic conditions and EIPH in a population of Thoroughbreds racing in a temperate climate. Ambient temperature was significantly associated with EIPH in both multivariable models. For comparative reasons the endoscopic grading system and model selection was based on a similar study investigating EIPH risk factors [124]. In most studies the presence of blood in the large airway (EIPH grade 0 vs. grades ≥ 1), irrespective of volume, is the dependent variable of interest. A second model (EIPH grades ≤ 1 vs. grades ≥ 2) is based on evidence that grade 1 EIPH is not associated with impaired race performance [195].

There are limited reports of an inverse association between EIPH and temperature [124, 178]. Hinchcliff and others identified ambient temperature as a risk factor for EIPH despite monitoring horses over a relatively small temperature range [124]. They concluded that horses racing at ambient temperatures below 20°C were 1.8-2.0 times more likely to develop EIPH than horses racing at temperatures above 20°C. A Canadian study reported a similar significant negative correlation between temperature and EIPH in Standardbreds monitored over a temperature range of -15 to 24°C [178].

Human athletes competing in cold conditions are known to have a higher prevalence of respiratory disease and airway hyper-responsiveness than athletes competing in temperate climates [423–426]. Inspiration of dry cold air in horses results in airway cooling, mucosal injury, and local induction of cytokines with neutrophil influx [427–429]. Exercise in cold air could therefore lead to airway inflammation, which could increase the risk of EIPH. The temperature range in this study was clement, but it is important to note that endoscopic findings and temperature values were recorded at the time of racing; training would have occurred at cooler temperatures, although almost exclusively at temperatures above 0°C.

We are unaware of any other reports of a relationship between EIPH and bar shoes in
racehorses. Although not specifically defined in this study, the addition of a bar shoe is normally due to underlying foot problems, such as a resolving sub solar abscess, foot soreness, third phalanx support, heel pain, or for penetrating injuries to the sole. The type of bar shoe worn was not specified, but straight bar shoes are the most commonly used in this population. There is no obvious explanation for the relationship between EIPH and bar shoes. It is possible that horses with bar shoes continue to experience foot pain and this has been associated with a prolonged elevation in heart rate during exercise [430–432]. Aside from changes to heart rate, foot pain could lead to other cardiovascular alterations including increased pulmonary artery pressure. Prolonged elevation in pulmonary artery pressure increases the risk of EIPH [58]. Foot strike and impact shock transmission to the thorax, a proposed pathogenesis for EIPH [80], could not be excluded as bar shoes may accentuate this effect. A limitation of this study is that horses with bar shoes were not examined for lameness, and information was not sought regarding the transition from a standard racing plate to a bar shoe.

Race distance was significant in model A (EIPH grade 0 versus EIPH grades ≥1) in the present study. Others have identified an increased risk of EIPH with increasing race distance [9, 177], while Hinchcliff et al. reported the risk of EIPH ≥2 was lower for horses racing over 1400 – 2400 metres compared to horses racing over shorter distances (≤1400m) [124]. Lifetime starts was significant in Model B (EIPH grades ≤1 versus EIPH ≥2). Multiple studies have identified an association between increased racing, whether expressed as age, time spent racing, or the number of lifetime starts and endoscopic EIPH or epistaxis [8, 9, 80, 81, 119, 124, 170, 186]. These findings suggest accumulated racing activity has an association with EIPH. It is important to note that most of these studies are based on single point observations and longitudinal studies are needed to verify these observations.

The time between racing and tracheobronchoscopy has been identified as a risk factor for the detection of EIPH when horses are examined within 2 hours of racing [124]. It is generally accepted that the rostral movement of blood to the trachea is both a time- and volume-dependent process. Examinations conducted too soon after racing may be associated with an increased likelihood of false negative results or an underestimation of EIPH severity. In the present study the time between
racing and examination was not significantly associated with EIPH in either model, noting that horses were not examined within 30 minutes of racing.

In conclusion environmental temperature has an inverse association with EIPH. We have also identified bar shoes as a risk factor for EIPH in this population of racing Thoroughbreds.

Endnotes:

i Pentax EPM-3000 Tokyo, Japan


iv IBM SPSS statistics version 21, New York, United States

v Statistix ver 9.0, Analytical Software, Tallahassee Florida, United States

vi Egret ver 2.0.3, Cytel, Inc, Cambridge MA, USA
<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Median</th>
<th>Lower 5%</th>
<th>Upper 5%</th>
<th>P – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time lapse race to examination (min)</td>
<td>51.69</td>
<td>48</td>
<td>51.1</td>
<td>53.27</td>
<td>0.408</td>
</tr>
<tr>
<td>Race distance (per 100 metres)</td>
<td>1370</td>
<td>1300</td>
<td>1344.76</td>
<td>1395.18</td>
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<td>Race day (Wednesday 1; Saturday 2)</td>
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<td></td>
<td></td>
<td>0.083</td>
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<td>Starts this racing preparation</td>
<td>3.36</td>
<td>3</td>
<td>3.161</td>
<td>3.559</td>
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<td>Lifetime starts continuous data</td>
<td>15.36</td>
<td>11</td>
<td>14.25</td>
<td>16.467</td>
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<td>Lifetime starts categorized</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20 race starts</td>
<td>11.59</td>
<td>12</td>
<td>11.38</td>
<td>11.8</td>
<td>0.06</td>
</tr>
<tr>
<td>≥20 race starts</td>
<td>11.59</td>
<td>12</td>
<td>11.38</td>
<td>11.8</td>
<td>0.06</td>
</tr>
<tr>
<td>Finishing position expressed as quartiles</td>
<td>55.95</td>
<td>56</td>
<td>55.81</td>
<td>56.09</td>
<td>0.725</td>
</tr>
<tr>
<td>Weight carried (kg)</td>
<td>55.95</td>
<td>56</td>
<td></td>
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<td></td>
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<tr>
<td>Season</td>
<td>4.23</td>
<td>4</td>
<td>4.1</td>
<td>4.345</td>
<td>0.422</td>
</tr>
<tr>
<td>Track (Ascot/ Belmont Park)</td>
<td>6.308</td>
<td>6.3</td>
<td>6.298</td>
<td>6.317</td>
<td>0.098</td>
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<td>Track rating (scale 0-10)</td>
<td>3.235</td>
<td>3</td>
<td>3.192</td>
<td>3.278</td>
<td>0.305</td>
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<tr>
<td>Age (years)</td>
<td>3.235</td>
<td>3</td>
<td>3.192</td>
<td>3.278</td>
<td>0.305</td>
</tr>
<tr>
<td>Sex (gelding/ female/ entire male)</td>
<td>4.23</td>
<td>4</td>
<td>4.1</td>
<td>4.345</td>
<td>0.422</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>22.86</td>
<td>21.9</td>
<td>22.37</td>
<td>23.34</td>
<td>&lt;0.001</td>
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<tr>
<td>Apparent temperature (°C)</td>
<td>18.72</td>
<td>17.6</td>
<td>18.24</td>
<td>19.22</td>
<td>&lt;0.001</td>
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<td>Dew point (°C)</td>
<td>7.28</td>
<td>7.25</td>
<td>6.86</td>
<td>7.69</td>
<td>0.238</td>
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<td>Relative humidity (%)</td>
<td>30.9</td>
<td>30.9</td>
<td>30.9</td>
<td>30.9</td>
<td>0.042</td>
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<tr>
<td>Wind speed (km/hr)</td>
<td>18.75</td>
<td>18</td>
<td>18.21</td>
<td>19.54</td>
<td>0.997</td>
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<td>Tongue tie (n=112)</td>
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<tr>
<td>Norton (pulling) bit (n=20)</td>
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<tr>
<td>Lugging bit (n=67)</td>
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<td>Non-standard shoes (n= 45)</td>
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<td>Bar Shoe(s) (n= 31)</td>
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<tr>
<td>Nasal flair strips (n= 4)</td>
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<tr>
<td>Bubble cheeker (n= 29)</td>
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Table 3.1: Univariable analysis of variables and summary statistics; n = 583 cases
<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>S.E</th>
<th>Wald</th>
<th>Odds ratio (95% C.I.)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Race distance (per 100 metres)</td>
<td>0.105</td>
<td>0.029</td>
<td>12.745</td>
<td>1.11 (1.05-1.18)</td>
<td>&lt;0.001</td>
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<tr>
<td>Temperature (°C)</td>
<td>-0.049</td>
<td>0.015</td>
<td>11.230</td>
<td>0.95 (0.93-0.98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bar Shoe(s)</td>
<td>1.849</td>
<td>0.547</td>
<td>11.424</td>
<td>6.35 (2.17-18.54)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Constant</td>
<td>-0.122</td>
<td>0.534</td>
<td>0.053</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.2: Variables retained in the final multivariable logistic regression model for EIPH ≥1; n = 583 cases

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>S.E</th>
<th>Wald</th>
<th>Odds ratio (95% C.I.)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>-0.033</td>
<td>0.016</td>
<td>4.203</td>
<td>0.97 (0.94-1.00)</td>
<td>0.040</td>
</tr>
<tr>
<td>Bar shoe(s)</td>
<td>1.002</td>
<td>0.375</td>
<td>7.129</td>
<td>2.72 (1.30-5.68)</td>
<td>0.008</td>
</tr>
<tr>
<td>Lifetime starts (&lt;20 starts vs. ≥20 starts)</td>
<td>0.404</td>
<td>0.195</td>
<td>4.285</td>
<td>1.50 (1.02-2.20)</td>
<td>0.039</td>
</tr>
<tr>
<td>Constant</td>
<td>-0.733</td>
<td>0.456</td>
<td>2.592</td>
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<td></td>
</tr>
</tbody>
</table>

Table 3.3: Variables retained in the final multivariable logistic regression model for EIPH ≥2; n = 583 cases
Chapter Four. The association between exercise-induced pulmonary haemorrhage and race-day performance in Thoroughbred racehorses

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Keywords:
Thoroughbred; EIPH; pulmonary haemorrhage; performance; horse; poor performance

Ethical animal research
Approval for this study was obtained from Murdoch University's Animal Ethics Committee (R2651/14, R2474/12). Owner informed consent was given for each horse included in this study.

Author’s declaration of interests
The authors have no conflicts of interest

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Racing and Wagering Western Australia

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Authorship
E.J. Crispe contributed to study design and execution, data collection, statistical analysis and manuscript preparation. G.D. Lester contributed to study design and execution, statistical analysis and manuscript preparation. C.J. Secombe contributed to study design and execution and manuscript preparation. D.I Perera contributed to the statistical analysis and manuscript preparation.
Summary

Background: Exercise induced pulmonary haemorrhage (EIPH) is commonly implicated as a cause of poor athletic performance but there is limited and conflicting evidence for this association.

Objectives: The aim of this study was to determine if EIPH, based on endoscopic examination after racing, is associated with a variety of novel and established performance parameters.

Study design: Prospective, observational cross-sectional study.

Methods: Thoroughbred racehorses competing between 2012-2015 were examined on-course no earlier than 30 minutes after racing. Examinations were recorded and graded blindly by experienced veterinarians using a 0-4 scale. Linear mixed effect models were used for analysis of continuous response variables with horse name incorporated as a random effect to account for repeated sampling and horse variability. Generalized estimating equations (GEE) were used for analysis of binary responses. Performance variables were examined in two models, comparing EIPH grade 0 to grades 1-4, and EIPH grade ≤2 compared with EIPH grades ≥ 3, controlling race factors that could influence performance.

Results: There were 3,794 observations collected from 1,567 horses. EIPH was detected in 55.1% of observations. Horses with grade 4 EIPH were significantly more likely to have a lower finishing position and finish further behind the winner, less likely to place in the first 3 positions and collect race earnings, collected less earnings per race start and were slower over the last 600m of the race than horses without EIPH (grade 0). Similar associations were seen in model 2, with horses with EIPH grade ≥ 3 having inferior performance when compared to horses with EIPH ≤2.

Limitations: Enrolment was voluntary.

Conclusion: Mild to moderate haemorrhage was not associated with inferior race day performance in this population of Thoroughbred racehorses.
Introduction

Exercise induced pulmonary haemorrhage (EIPH) is anecdotally regarded within racing communities as a major cause of poor athletic performance, but until recently there had been minimal scientific evidence to support this. Early studies investigating EIPH and finishing position could not identify an association, but these studies had methodological limitations or large type II error [7, 9, 10, 174]. Whilst others that did identify an association had selection bias or failed to control for potential confounding [8, 177].

There are inherent challenges in defining variables to objectively evaluate race day performance. The characterization of performance based on finishing position alone is overly simplistic. Individual performance is ultimately determined by the ability of the competitors as well as other unrelated factors, including race class and conditions, jockey skill and race tactics. Consideration of other parameters, including distance finished behind the winner, sectional times, ratings, earnings per start and finishing in the first three positions mitigate some of the challenges faced in quantifying performance.

Recent epidemiological surveys [175, 195] investigating EIPH and performance have applied a more comprehensive appraisal of race day performance. This, in combination with larger sample sizes and improved statistical methods has identified significant associations between EIPH and athletic performance. Evidence of this association was identified in an Australian study examining 744 Thoroughbreds post-race with endoscopy [195]. In that study associations were examined using two models based on EIPH presence and severity: horses with EIPH grades ≤1 were significantly more likely to win, place in the first three positions and be in the 90th percentile of race earnings compared to horses with EIPH grades ≥2. EIPH-positive horses also finished further from the winner than horses without tracheobronchoscopic evidence of EIPH. A similarly designed study of 886 Thoroughbreds concluded that horses with endoscopic EIPH were less likely to win, finished further behind
the winner and were less likely to be in the 90th percentile for race earnings than horses without endoscopic EIPH.

The aim of this study was to further define an association between tracheobronchoscopic EIPH on a range of routine and uninvestigated performance parameters in a population of racing Thoroughbreds not medicated with furosemide. Our hypothesis was that EIPH would be associated with inferior performance.

Materials and Methods

Study design

A prospective, observational study design was used to examine a population of Thoroughbreds racing at two metropolitan and one provincial racecourse in Western Australia between May 2012 and April 2013, and July 2014 and December 2015.

Horses

Information regarding the study was promoted to racing participants through radio interviews, advertising in the racing calendar, presentations, and postal and direct communication on race day. Enrollment was voluntary, and trainers and owners could enroll horses at their discretion. Trainers were encouraged to enroll all horses in their care to limit bias associated with volunteering only certain horses to be examined. Any horse racing on the day of examination was eligible to be enrolled. Enrolled horses must have finished the race to then be included in the study.

All races were conducted on a flat, turf surface. Pre-race administration of any medication is prohibited in accordance with the Australian Rules of Racing [220]. Horses were presented to a designated location on course for endoscopic examination. Examination occurred no earlier than 30 minutes and no later than 220 minutes after racing. A 1.5 metre endoscope (AOHUA model LG-200) was passed through the nares into the trachea to the
carina. Videos of all examinations were digitally recorded and the time at examination and presence of epistaxis were recorded.

**Detections and quantification of EIPH**

Two experienced veterinarians, blinded to the identity and performance of the horse, reviewed the tracheobronchoscopic examinations independently. A previously described 0-4 graded scoring system was used [261]. Briefly, grade 0 is assigned to cases where no blood is observed in the trachea or nasopharynx; grade 1 has ≥ 1 blood specks or ≤ 2 short (< ¼ trachea length) and narrow (<10% of the tracheal surface area) streams of blood; grade 2 is a single long stream of blood (>½ tracheal length) or > 2 short streams of blood occupying <⅓ of the tracheal circumference; grade 3 is multiple, distinct streams of blood covering more >½ of the tracheal circumference, but without blood pooling at the thoracic inlet; and grade 4 is multiple, coalescing streams of blood covering >90% of the tracheal surface, with blood pooling at the thoracic inlet. If there were discrepancy between the primary reviewers’ score, a third experienced veterinarian would independently and blindly review and grade the examination. The median score was then adopted for statistical analyses.

**Performance variables**

Racing records were retrieved from a public database maintained by the racing regulator in Western Australia. Variables captured included trainer, racetrack, time of the race, weight carried, position on the turn (recorded 400m from the finishing post), finishing place, distance finished behind winner, overall race time, last 600m sectional time, number of race starters, race distance, track rating, penetrometer reading, age, sex, non-standard shoes (yes/no), number of starts this preparation (more than 60 days between racing or trialing was considered the end of a racing preparation), race earnings, number of lifetime starts and lifetime earnings recorded from the horse’s first career race start to the endoscopic
examination. Categorical outcomes of these variables included winning (yes/no), finishing in the top 3 positions (yes/no) and collecting race earnings (yes/no). Average race speed (m/sec), average speed over last 600m (m/sec), and the average early/mid race speed (m/sec) were calculated using the horses’ race time and the last 600m time. The early/mid race speed was then compared to the last 600m speed to ascertain if the horse accelerated or decelerated over the last 600m of the race. The position on the turn minus the finish position was used to assess field movements relative to other competitors over the final 400m of the race. The length of the home straight varied between racetracks from 300-348m. To account for variations in the number of competitors in races, finishing position was converted to a decile. The starting price (SP) for each horse was retrieved from the betting exchange website, Betfair Australiaii using the win pool market. Horses were also ranked based upon their SP (shortest to longest odds) and this rank was used as a prediction of finishing order. If a horse was ranked ≤4 (shortest odds), the horse was examined for the predicted finish minus actual finishing position; a negative value indicated a performance below market expectation; and a positive value, the reverse. Climatic variables were obtained from the Australian Bureau of Meteorologyiii using the closest weather station recording temperature, apparent temperature and humidity at the nearest time point to the race start time.

Data analysis

Continuous response variables were assessed for normality. Distance finished behind the winner was highly right skewed. Rather than assigning a zero value to winners, winners were removed from this outcome and remaining observation values were log transformed. Race and lifetime earnings were also highly right skewed due to the large number of non-earners. One dollar was added to each observation and the value log transformed.

The association between the response performance variable and EIPH were assessed in two models using statistical program Riv. Firstly, EIPH grade 0 was compared to EIPH
grades 1-4; and secondly EIPH grade ≤2 was compared to EIPH grades ≥3. Factors that could potentially predict or affect the outcome of the race or the response variable were considered as potential confounders and were held as fixed effects, specifically weight carried, sex, race distance, lifetime starts, starts this racing preparation, ambient temperature, track rating, non-standard shoes, and racetrack. Interaction effects between these factors were assessed. Western Australia’s metropolitan racetracks are exclusively used for 6 months of the year based on season due to one racetrack having superior drainage. The interaction between racetrack and ambient temperature, and ambient temperature and track rating were incorporated into the model. Collinearity between the fixed effects was considered and if highly correlated the variable that was considered to be the superior predictor was used. For example, age and lifetime starts were highly correlated; age can be considered a proxy measure for lifetime starts and was removed from the model.

Associations between continuous response variables and EIPH were assessed using the lme() function in R for linear mixed effects models. The models were adjusted for potential confounding variables and a random intercept was incorporated to account for repeated sampling of individuals. Model checking when including or removing predictor/confounding variables from the model was performed using the anova() function in R. Underlying distributional assumptions of normality and homogeneity of variance for the models were assessed using QQ plots and residual plots. Maximum likelihood mean estimates and 95% confidence intervals were obtained for the effect sizes of EIPH grades, and other confounding variables included in the model.

Associations between categorical response variables (winning, finishing in the top 3, accelerate/ decelerate, collect race earnings) and EIPH were examined using multiple logistic regression models using generalized estimating equations. The R function geeglm() was used based on an independent correlation structure to account for the repeated sampling of horses and the models were adjusted for the potential confounding variables. Odds ratios and Wald
statistics based 95% confidence intervals were calculated for the regression coefficients of EIPH grades, and other confounding variables included in the model. A P-value <0.05 was considered significant for both continuous and categorical response variable models.

Results

Tracheobronchoscopic examinations were performed at 155 metropolitan and 17 provincial race meetings. A total of 3,794 tracheobronchoscopic examinations were performed on 1,567 horses (Table 4.1). There were 226 trainers that enrolled between 1 and 126 horses. There were 587 females, 937 geldings and 43 entire males examined. Age ranged between 2 and 10 years. The mean interval between racing and examination was 48 minutes (95%CI 47.1,48.4; range 30-220min; Fig 4.1). Of the 3,794 examinations, 442 (11.6%) examinations were from horses that won, and 1,220 (32.2%) were from horses finishing in the first three positions.

Agreement between primary reviewers was very good (κ= 0.75; 95%CI 0.7,0.8). Disparity between reviewers was limited to one grade on each occasion. The third reviewer always agreed with one of the primary reviewers. Blood was detected in over half of examinations (2,092; 55.1%). Of the total population 44.9%, 31.6%, 17.2%, 5.1% and 1.2% were recorded for grades 0 to 4 respectively (Fig. 4.2). Epistaxis was recorded in 22 horses, of which 13 horses were grade 4, 6 horses were grade 3, 2 horses were grade 2, and 1 horse grade 0 (epistaxis caused by head trauma in the starting barrier prior to racing).

Model 1: EIPH grade 0 versus. EIPH grades 1-4

Horses with the highest grade of EIPH had significant negative associations with many performance variables (Table 4.2). Horses with EIPH grade 4, were significantly less likely to finish in the first three positions (P=0.04; OR 0.3; 95%CI 0.1-0.9; Fig 4.3) compared to horses graded 0. Horses with EIPH grade 4 had a significantly (P<0.001) higher finishing decile or lower finishing position (mean; 95%CI, 7.2; 6.1-8.4) than horses with EIPH grade 0 (5.6; 5.3-
Horses with EIPH grade 1, 3 and 4 finished significantly (P<0.001, P= 0.02 and P<0.001, respectively) further behind the winner (3.0; 2.5-3.6, 3.3; 2.5-4.3, and 4.8; 3.2-7.3 lengths, respectively) than horses with EIPH grade 0 (2.7; 2.4-3 lengths). Horses with EIPH grade 4 were significantly (P=0.003) less likely to collect race earnings (OR 0.3; 95%CI 0.2-0.7) and collected significantly (P<0.001) less race earnings per start (9.1; 1.9- 43.9) than horses with grade 0 (72.4; 46.8- 112.2).

Horses with EIPH grade 4 had a significantly (P<0.001) slower average speed over the last 600m (16.84; 16.6-17m/sec) compared to horses with grade 0 (17.07; 16.99-17.1 m/sec). Horses with grade 4 EIPH were significantly (P=0.002) more likely to be passed by other competitors over the last 400 m of the race (turn position minus finish position) (-2.0; -3.6 to -0.4) than horses graded 0 (-0.2; -0.7 to 0.2). Horses with EIPH grades 1 and 2 were significantly (P=0.01 and P<0.001, respectively) more likely to overtake competitors (turn minus finish position) over the last 400m of the race (0.1; -0.6 to 0.9 and 0.4; -0.4 to 1.2, respectively) compared to horses graded 0.

Horses with EIPH grades 3 and 4 were significantly (P=0.02 and P=0.03, respectively) faster over the early/mid sections of the race (16.43; 16.32- 16.55 m/sec and 16.48; 16.32-16.65 m/sec, respectively) compared to a horse with grade 0 (16.37; 16.3-16.43 m/sec). When comparing the average early/mid race speed with the average speed over the last 600metres, horses with EIPH grade 3 or 4 were significantly more likely to decelerate (P=0.03 and P=0.01, respectively) compared to horses with grade 0. Horses with EIPH grade 0 were between 1.6 (95%CI 1.1-2.5) and 2.4 (95%CI 1.2-4.8) times more likely to accelerate their average speed over the last 600 m than a horse with EIPH grade 3 and 4, respectively.

Model 2: EIPH grades ≤2 versus EIPH grades ≥3

Similar findings were seen in model 2, whereby horses with EIPH≥3 were significantly (P=0.002) faster over the early/mid sections of the race (mean; 95%CI. 16.45; 16.34-16.55 m/sec) compared to horses with EIPH≤2 (16.37; 16.32- 16.43 m/sec). Horses with EIPH
grades ≥3 were significantly more likely to decelerate their average speed over the last 600 meters (P=0.006) compared to horses with EIPH grade ≤2. Horses with EIPH≤2 were 1.7 (95%CI 1.2- 2.4) times more likely to accelerate their average speed over the last 600 metres than horses with EIPH ≥3.

Horses with EIPH≥3 were significantly (P<0.001; Table 4.3) slower over the last 600m (16.96; 16.82- 17.1 m/sec) than horse with EIPH≤2 (17.07; 16.99- 17.15 m/sec). Horses with EIPH≥3 were more likely to have a higher finishing decile (lower finishing position) (P=0.005), to finish further behind the winner (P=0.002), to be passed by other competitors over the last 400m of the race (P=0.001), were less likely to collect race earnings (P=0.03) and collected less race earnings per start (P=0.03) than horse with EIPH≤2 (Table 4.3). Horses with EIPH≥3 were not more or less likely to place in the top three positions than horse with EIPH ≤2 (P=0.06). Lifetime earnings, winning, Betfair starting price, Betfair rank minus finishing position, and average race speed were not significantly associated with EIPH in either model.

There was a direct, positive relationship between weight carried and collecting race earnings (P<0.001), the amount of race earnings collected (P<0.001), lifetime earnings (P<0.001), winning (P<0.001) and finishing in the top 3 positions (P<0.001). An inverse relationship existed between weight carried and distance finished from the winner (P<0.001) and decile finish position (P<0.001). An inverse relationship existed between the number of lifetime starts and collecting race earnings (P<0.001), the amount of race earnings (P<0.001), winning (P<0.001) and finishing in the top three positions (<0.001). In contrast, the number of starts in the current racing preparation had a direct positive association with collecting race earnings (P<0.001), the amount of race earnings (P<0.001), winning (P=0.02) and finishing in the top 3 positions (P=0.001). As expected there was a significant inverse relationship between all speed measurements and race distance (P<0.001), and track rating (P<0.001). Horses wearing non-standard shoes were less likely to collect race earnings (P=0.01),
collected less race earnings (P=0.01), finished further behind the winner (P=0.04) and had a lower finishing position (P=0.03) (supplementary information items 1 and 2).

Discussion

This study supports the finding of other investigators that severe EIPH is associated with inferior athletic performance, but differs in respect to being unable to detect a significant difference between lower grades of EIPH and grade 0 [175, 195]. The difference in findings could be attributed to a multitude of factors, including differences in population, the time interval between race and examination, and sample size.

Successful racing performances are often determined by relatively small margins and the prevalence of EIPH post-race using tracheobronchoscopy is reported between 44-75% [8, 9, 124, 174, 176]. Consequently, large sample sizes are required to detect significant associations between disease and performance. This is the largest EIPH study, conducted prospectively and observationally under race day conditions and controlled for confounding variables that could affect performance. This study also recruited a relatively larger proportion of winners and top-3 placed horses compared to others [195], which was likely because of blanket enrollment of horses from several large racing stables. Differences in the performance demographic as well as horse, race and environmental variation could account for differences between studies.

A minimum interval of 30 minutes between racing and examination was used in this study. The ideal time to detect EIPH has not been conclusively established, although it is recommended that examinations be performed 30-120 minutes after racing [260]. A risk factor study where horses were examined between 13 and 175 minutes (median 30min) after racing concluded that examinations conducted too soon after racing were significantly less likely to detect blood and underestimated disease severity [124]. In a similarly designed study, imposing a minimum time lapse of 30 minutes (median 48min), the time between racing and examination was not significantly associated with EIPH detection or severity [433].
In the current study, horses with grade 4 EIPH had impaired athletic performance over many of the examined performance parameters. This is not proof of a causal relationship between severe EIPH and poor performance but strengthens arguments in support of this. Career implications of tracheobronchoscopic EIPH based on a single observation has been reported [200]. In that study, it was reported that horses with grade 4 EIPH had fewer race starts after endoscopy diagnosis and fewer lifetime starts than horses with grade 0. Although these findings are suggestive that EIPH negatively impacts long-term performance, almost half of the horses diagnosed with grade 4 EIPH never raced again after examination, obscuring whether this is due to incapacity to race or management decisions limiting the opportunity.

In the current study, horses with EIPH grade 1 and 2 were significantly more likely to improve their position in the field over the final 400m compared to horses with grade 0. In barrel racing horses, horses with EIPH grade 4 were significantly faster than EIPH grade 0 animals when all race distances were considered together [164]. While it is highly improbable that EIPH confers an athletic advantage to these horses, the results warrant the use of secondary models comparing categorized groups of EIPH based upon severity.

We were unable to detect an association between average overall race speed and EIPH, which is consistent with others [178]. The capacity to now report sectional times creates opportunities to examine speed over different sections of the race. Horses with EIPH grades ≥3 were significantly faster over the early/mid sections of the race and were more likely to decelerate over the last 600 metres in both models. The causality of these findings is unclear: Is the deceleration due to EIPH, or to fatigue associated with a faster early/mid average race speed, and could this racing pattern contribute to severe EIPH? It is known that rapid acceleration triggers higher pulmonary vascular pressures than a gradual incremental increase to the same speed [41]. Although all racing would be considered rapid acceleration, perhaps horses that race at faster speeds initially, reach the breaking threshold of the
pulmonary capillaries at an earlier stage in the race compounding the severity of the disease compared to horses that start the race at a slower speed. It could also be possible that horses in which a positive pacing strategy is adopted (i.e. fast start with declining speed) fatigue and decelerate over the last 600 m of the race irrespective of EIPH.

Not all horses have an equal chance of winning irrespective of the handicapper’s best intentions. Results of this study would indicate that horses that carried more weight were more likely to be associated with positive performance outcomes, implying weight allocation was not enough to compensate for differences in ability, a finding that has been identified elsewhere [233]. Australian racing imposes a minimum weight that must be carried in handicap races [220]. Lower rated horses contesting races above their class are at a distinct disadvantage, as they cannot be allocated a weight below the minimum. Weight allocation is therefore not proportional to ability and reduces their chances of winning. Studies comparing performance, relying on the assumption that horses are weighted proportional to ability, could be unreliable and misinformed when racing under these conditions if they have not considered this confounding factor. Similarly, ‘lifetime starts’ and ‘starts this racing preparation’ were significantly associated with many performance variables, although in opposing directions. Horses that are unplaced at one start have a higher chance of earning at their next start because the race selected is usually more suited to the aptitude of the horse [209]. Lifetime starts are inversely associated with performance parameters due in part to restricted sex and age races available to younger horses, often with purse supplementation [196]. Average earnings decline with age [218], a proxy measurement for lifetime starts.

A horse’s likelihood of winning is not solely dependent upon their ability, but also the innate ability of its competitors and the set conditions of the race. The starting price can be regarded as the public summation of the individual’s ability, the opposition and the race conditions reflecting the subjective probability of that horse winning. There is a large body of evidence that suggests betting markets efficiently incorporate all publically and
monopolistically held information into the starting price [237, 238]. A strong positive relationship exists between the market odds and the likelihood of winning [239]. Betting exchange markets are regarded as the most efficient financial betting market [240, 246, 249]. Ignoring the social stigma attached to horse race gambling, the market provides a subjective probability of winning, and could highlight horses performing below their anticipated performance. Despite a lack of significant associations between this marker and EIPH, this novel approach has potential applicability in performance analyses.

Voluntary recruitment requires careful management due to biases associated with owners or trainers preferentially permitting access to certain horses, and not others. We attempted to curb this by encouraging trainers to enroll all horses in their care. The large proportion of winners and top three-placed horses is testament to the attempt to manage this bias.

This population does not receive pre-race furosemide. Trainers are permitted to use furosemide during training, abiding by withdrawal times when racing. Despite the Australian rules of racing permitting the use of nasal flair strips in Thoroughbred racing, their use has been almost negligible in Western Australia. In conclusion, our results add weight to the findings that severe EIPH is associated with impaired performance, as defined by placing in the first 3 positions, decile finishing position, distance finished from the winner, collecting race earnings, the amount of race earnings, being passed by competitors in the last 400m of the race and slower average last 600 m sectional times. Mild to moderate haemorrhage was not associated with inferior race day performance in this population of Thoroughbred racehorses.

Manufacturers:

Supplementary information:

S4.1: Significance of associations between variables in model 1.

Supplementary item S4.2: Significance of associations between variables in model 2.
<table>
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<th>Variable</th>
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<th>Median</th>
<th>Q3</th>
<th>Maximum</th>
<th>Mean</th>
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<td>41</td>
<td>53</td>
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<td>1200</td>
<td>1400</td>
<td>1600</td>
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<td>6.5</td>
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<td>3</td>
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<td>1</td>
<td>2</td>
<td>6</td>
<td>126</td>
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</tr>
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<td>Number of examinations enrolled by trainer</td>
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<td>1</td>
<td>3</td>
<td>9</td>
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<td>16.8</td>
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<td>2</td>
<td>4</td>
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<td>22.6</td>
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</tr>
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<td>Nonstandard shoes (n=169)</td>
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<td>N/A</td>
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<td>N/A</td>
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</tr>
<tr>
<td>Race prize money earned ($)</td>
<td>0</td>
<td>400</td>
<td>3,500</td>
<td>619,500</td>
<td>4,492</td>
<td></td>
</tr>
<tr>
<td>Lifetime prize money earned ($)</td>
<td>0</td>
<td>14,821</td>
<td>42,947</td>
<td>107,201</td>
<td>1,616,600</td>
<td>82,128</td>
</tr>
<tr>
<td>Finishing position (of field)</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>16</td>
<td>5.8</td>
</tr>
<tr>
<td>Finishing position as decile (of field)</td>
<td>0.1</td>
<td>0.4</td>
<td>0.6</td>
<td>0.9</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Distance finished behind the winner in lengths (n=3352)</td>
<td>0.05</td>
<td>2</td>
<td>3.75</td>
<td>6.25</td>
<td>86.75</td>
<td>4.8</td>
</tr>
<tr>
<td>Turn position minus finish position</td>
<td>-15</td>
<td>-2</td>
<td>0</td>
<td>3</td>
<td>13</td>
<td>0.1</td>
</tr>
<tr>
<td>Last 600 metre speed (m/sec) (n=3580)</td>
<td>12.5</td>
<td>16.5</td>
<td>16.8</td>
<td>17.1</td>
<td>18.6</td>
<td>16.8</td>
</tr>
<tr>
<td>Average race speed (m/sec) (n=3791)</td>
<td>13.9</td>
<td>17.6</td>
<td>16.1</td>
<td>16.4</td>
<td>16.6</td>
<td>16.3</td>
</tr>
<tr>
<td>Average early/mid race speed (m/sec) (n= 3580)</td>
<td>12.3</td>
<td>15.7</td>
<td>16</td>
<td>16.3</td>
<td>17.45</td>
<td>16.0</td>
</tr>
<tr>
<td>Betfair Starting price ($)</td>
<td>1.23</td>
<td>5.9</td>
<td>11.9</td>
<td>26</td>
<td>874</td>
<td>29.6</td>
</tr>
</tbody>
</table>

Table 4.1: Race and horse characteristics in 1,567 Thoroughbreds racing in Western Australia (unless specified observations = 3794).
<table>
<thead>
<tr>
<th>Variable</th>
<th>EIPH Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Average speed last 600m (m/sec) (n=3580)</td>
<td>17.07; 16.99-17.1</td>
</tr>
<tr>
<td>Finishing position (decile)</td>
<td>5.6; 5.3-5.6</td>
</tr>
<tr>
<td>Distance finished behind winner in lengths (n=3552)</td>
<td>2.7; 2.4-3</td>
</tr>
<tr>
<td>Position on the turn (400m mark) minus finish position</td>
<td>-0.2; -0.7 to 0.2</td>
</tr>
<tr>
<td>Amount of race earnings collected ($)</td>
<td>72.4; 46.8-112.2</td>
</tr>
<tr>
<td>Betfair starting price ($)</td>
<td>4.67; 4.33-5.01</td>
</tr>
<tr>
<td>Lifetime earnings ($)</td>
<td>2944; 2335-3713</td>
</tr>
</tbody>
</table>

Table 4.2. Performance variables (mean; 95%CI) in Thoroughbreds racing in Western Australia with EIPH grade 0 and EIPH grades 1-4 (model 1; 3,794 observations collected from 1,567 horses). *Significantly (P<0.05) different from the control group, EIPH grade 0.
<table>
<thead>
<tr>
<th>Variable</th>
<th>EIPH Grades ≤ 2</th>
<th>EIPH Grades ≥ 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average speed last 600m (m/sec) (n=3580)</td>
<td>17.07; 16.99-17.15</td>
<td>16.96; 16.82-17.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Average early/mid race speed (m/sec) (n=3580)</td>
<td>16.37; 16.32-16.43</td>
<td>16.45; 16.34-16.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Finishing position (decile)</td>
<td>5.7; 5.3-6</td>
<td>6.2; 5.5- 6.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Distance finished behind winner (lengths) (n=3352)</td>
<td>2.8; 2.5- 3.15</td>
<td>3.5; 2.75- 4.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Position on the turn (400m mark) minus finish position</td>
<td>-0.2; -0.4 to 0.4</td>
<td>-0.9; -1.82 to 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Amount of race earnings collected ($)</td>
<td>67.6; 44.5-102.8</td>
<td>38.0; 14.7-97.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lifetime earnings ($)</td>
<td>2965; 2360-3724</td>
<td>2926; 1920-4459</td>
</tr>
<tr>
<td>Average race speed (m/sec) (n=3791)</td>
<td>16.32; 16.30-16.34</td>
<td>16.33; 16.27-16.38</td>
</tr>
<tr>
<td>Starting price ($)</td>
<td>4.77; 4.44-5.1</td>
<td>4.87; 4.15-5.59</td>
</tr>
</tbody>
</table>

Table 4.3. Performance variables (mean; 95%CI) in Thoroughbreds racing in Western Australia with EIPH grades ≤2 and grades ≥3 (model 2; 3,794 observations collected from 1,567 horses). *Significantly (P<0.05) different from the control group EIPH grades ≤2.
Figure 4.1: Time elapsed from race to tracheobronchoscopic examination compared with the severity of haemorrhage detected in Thoroughbreds racing in Western Australia (3,794 observations collected from 1,567 horses)
Figure 4.2: Severity of EIPH detected post-race among Thoroughbreds racing in Western Australia (3,794 observations collected from 1,567 horses).
Figure 4.3: Forest plot depicting odds ratio and 95% confidence intervals for the likelihood of finishing in the top three positions compared with the severity of haemorrhage detected, graded using a 0-4 scale (n=3794). *Significantly (P<0.05) different from the control group grade 0.
<table>
<thead>
<tr>
<th>Performance variable</th>
<th>Estimate</th>
<th>Weight carried (kg)</th>
<th>Sex</th>
<th>Race distance (m)</th>
<th>Lifetime starts</th>
<th>Starts this racing preparation</th>
<th>Non-standard shoes (Y/N)</th>
<th>Track Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average speed last 600m (m/sec) (n=3580)</td>
<td>17.07; 16.99-17.14</td>
<td>P&lt;0.001 17.07; 16.97-17.14</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.002; 17.08; 16.99-17.15</td>
<td>NS</td>
<td>P&lt;0.001 16.95; 18.85-17.04</td>
</tr>
<tr>
<td>Average early/mid race speed (m/sec) (n=3580)</td>
<td>16.37; 16.31-16.43</td>
<td>P&lt;0.001 16.42; 16.33-16.51</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.001 16.27; 16.2-16.35</td>
</tr>
<tr>
<td>Finishing position (decile)</td>
<td>5.6; 5.3-5.9</td>
<td>P&lt;0.001 5.4; 5.1-5.8</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.001 5.7; 5.3-5.6</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Finish in top 3 positions</td>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.001 5.5; 5.2-5.8</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Distance finished behind winner in lengths (n=3552)</td>
<td>2.7; 2.4-3.1</td>
<td>P&lt;0.001 2.6; 2.3-3</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.001 2.7; 2.4-3.1</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>Position on the turn (400m mark) minus finish position</td>
<td>-0.23; -0.68 to -0.2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.001 -0.26; -0.71 to 0.19</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>Amount of race earnings collected ($)</td>
<td>72.44; 46.76-112.2</td>
<td>P&lt;0.001 83.18; 49.43-137.93</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.001 70.79; 44.72-109.88</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>Collect race earnings (Y/N)</td>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.001 81.28; 48.91-132.51</td>
<td>P&lt; 0.003 27.54; 9.3-30.80</td>
<td>N/A</td>
</tr>
<tr>
<td>Lifetime earnings ($)</td>
<td>2944; 2335-3712</td>
<td>P&lt;0.001 1333; 648-2745</td>
<td>NS</td>
<td>P=0.001 2945; 2334-3714.7</td>
<td>P&lt;0.001 3212; 2533-4074</td>
<td>P=0.001 3068; 2391-3936</td>
<td>P&lt;0.004 4188; 2621-6691</td>
<td>N/A</td>
</tr>
<tr>
<td>Accelerate/ Decelerate speed over final 600m</td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>Betfair starting price ($)</td>
<td>4.67; 3.45</td>
<td>P&lt;0.001 3.36; 3.97-4.75</td>
<td>5; 4.39-5.63</td>
<td>NS</td>
<td>NS</td>
<td>4.73; 4.39-5.09</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Average race speed (m/sec)</td>
<td>16.32; 16.29-16.35</td>
<td>P&lt;0.001 16.32; 16.29-16.35</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.001 16.32; 16.29-16.35</td>
<td>NS</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Supplementary item 4.1: Significance of associations between variables in model 1 (mean; 95%CI). Sex comparison group is female; M= entire male; G= gelding. Weight carried mean centered at 56.05kg. Race distance mean centered at 1406m. Track rating: 1-10 scale (1-dry, hard track, 10- very soft and wet, heaviest category). Non-standard shoes: standard racing plates used as the reference group to compare horses with one or more non-standard shoes. NS: not significant; N/A not considered in this model.

The estimate for each performance variable is for a weight of 56.05kg, race distance of 1406m and a track rating of 1.
<table>
<thead>
<tr>
<th>Performance variable</th>
<th>Estimate</th>
<th>Weight carried (kg)</th>
<th>Sex</th>
<th>Race distance (m)</th>
<th>Lifetime starts</th>
<th>Starts this racing preparation</th>
<th>Non-standard shoes (Y/N)</th>
<th>Track Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average speed last 600m (m/sec)</td>
<td>17.07; 16.99-17.15</td>
<td>NS</td>
<td>G</td>
<td>NS</td>
<td>P&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>(n=3580)</td>
<td>17.06; 17.0-17.14</td>
<td>NS</td>
<td>M</td>
<td>16.99-17.15</td>
<td></td>
<td>17.00; 17.0-17.16</td>
<td>NS</td>
<td>16.86-17.04</td>
</tr>
<tr>
<td>Average early/mid race speed (m/sec)</td>
<td>16.37; 16.31-16.43</td>
<td>NS</td>
<td>G</td>
<td>NS</td>
<td>P&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>(n=3580)</td>
<td>16.41; 16.32-16.51</td>
<td>NS</td>
<td>M</td>
<td>16.31-16.43</td>
<td></td>
<td>16.37; 16.31-16.43</td>
<td>NS</td>
<td>16.28-16.35</td>
</tr>
<tr>
<td>Finishing position (decile)</td>
<td>5.7; 5.4-6</td>
<td>NS</td>
<td>G</td>
<td>NS</td>
<td>P&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.001</td>
</tr>
<tr>
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<td>5.5; 5.1-5.8</td>
<td>NS</td>
<td>M</td>
<td>5.4-6</td>
<td></td>
<td>5.7; 5.4-6</td>
<td>NS</td>
<td>6.2; 5.4-6.9</td>
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<tr>
<td>Finish in top 3 positions</td>
<td>NS</td>
<td>NS</td>
<td>G</td>
<td>NS</td>
<td>P&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>2.7; 2.4-3.1</td>
<td>NS</td>
<td>M</td>
<td>2.5-3.2</td>
<td></td>
<td>2.9; 2.5-3.2</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>Distance finished behind winner in lengths (n=3552)</td>
<td>2.8; 2.5-3.2</td>
<td>NS</td>
<td>G</td>
<td>NS</td>
<td>P&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>2.7; 2.4-3.1</td>
<td>NS</td>
<td>M</td>
<td>2.5-3.2</td>
<td></td>
<td>2.7; 2.4-3.1</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>Position on the turn (400m mark) minus finish position</td>
<td>-0.02; -0.4 to 0.4</td>
<td>NS</td>
<td>G</td>
<td>NS</td>
<td>P&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>-0.2; -0.5 to 0.4</td>
<td>NS</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>Amount of race earnings collected ($)</td>
<td>47.61; 44.47-102.78</td>
<td>P&lt;0.001</td>
<td>G</td>
<td>NS</td>
<td>P&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>77.62; 47.18-126.77</td>
<td>NS</td>
<td>M</td>
<td></td>
<td></td>
<td>66.06; 42.51-100.57</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>Collect race earnings (Y/N)</td>
<td>NS</td>
<td>NS</td>
<td>G</td>
<td>NS</td>
<td>P&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>1.08; 0.95-1.13</td>
<td>NS</td>
<td>M</td>
<td></td>
<td></td>
<td>1.06; 0.93-1.19</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>Lifetime earnings (+)</td>
<td>2964; 2361-3724</td>
<td>P&lt;0.001</td>
<td>G</td>
<td>NS</td>
<td>P&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>3159; 2452-4070</td>
<td>NS</td>
<td>M</td>
<td></td>
<td></td>
<td>1346; 657-2759</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>Betfair starting price ($)</td>
<td>4.77; 4.44-5.1</td>
<td>P&lt;0.02</td>
<td>G</td>
<td>NS</td>
<td>P&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>4.46; 4.08-4.84</td>
<td>5.11; 4.5-5.7</td>
<td>M</td>
<td></td>
<td></td>
<td>4.84; 4.5-5.17</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>Average race speed (m/sec)</td>
<td>16.32; 16.30-16.35</td>
<td>NS</td>
<td>G</td>
<td>NS</td>
<td>P&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>16.32; 16.29-16.34</td>
<td>P&lt;0.02</td>
<td>M</td>
<td></td>
<td></td>
<td>16.33; 16.30-16.35</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>16.33; 16.3;16.35</td>
<td>P&lt;0.001</td>
<td>M</td>
<td></td>
<td></td>
<td>16.33; 16.30-16.35</td>
<td>NS</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Supplementary item 4.2: Significance of associations between variables in model 2 (mean; 95%CI). Sex comparison group is female; M= entire male; G= gelding. Weight carried mean centered at 56.05kg. Race distance mean centered 1406m. Track rating: 1-10 scale (1-dry, hard track, 10- very soft and wet, heaviest category). Non-standard shoes: standard racing plates used as the reference group to compare horses with one or more non-standard shoes. NS: not significant; N/A not considered in this model.

The estimate for each performance variable is for a weight of 56.05kg, race distance of 1406m and a track rating of 1.
Chapter Five. Exercise-induced pulmonary haemorrhage in Thoroughbred racehorses: a longitudinal study

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Keywords: Thoroughbred; horse; EIPH; pulmonary haemorrhage; progression; longitudinal

Ethical animal research

Approval for this study was obtained from Murdoch University’s Animal Ethics Committee (R2651/14, R2474/12). Informed consent was given for every horse included in this study.

Author’s declaration of interests

The authors have no conflicts of interest

Source of funding

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Authorship

E.J. Crispe contributed to study design and execution, data collection, statistical analysis and manuscript preparation. G.D. Lester contributed to study design and execution, statistical analysis and manuscript preparation. C.J. Secombe contributed to study design and execution and manuscript preparation. D.I. Perera, A.A. Manderson and B.A. Turlach contributed to statistical analysis and manuscript preparation.
Summary

Background: Exercise induced pulmonary haemorrhage (EIPH) is considered a progressive disease based on histopathology, but it is unknown if tracheobronchoscopic EIPH severity worsens over time.

Objectives: The aim of this study was to examine tracheobronchoscopic EIPH changes over time in a population of Thoroughbred racehorses. A secondary aim was to identify factors that affect changes in tracheobronchoscopic EIPH severity between observations.

Study design: Prospective, longitudinal, observational cross-sectional study.

Methods: Thoroughbred racehorses were examined with tracheobronchoscopy no earlier than 30 minutes after racing. Examinations were recorded and graded blindly by experienced veterinarians using a 0-4 scale. Horses with 2 or more observations were included in the analysis. The association between the previous and current EIPH score was investigated using a linear mixed effect model. Factors associated with transitioning from a lower to a high EIPH grade and vice versa were examined using multiple ordinal regression. A semi-parametric regression model was used to examine progression using the number of career starts as a marker for time. Models were adjusted for potential confounding variables.

Results: There were 2,974 tracheobronchoscopic examinations performed on 747 horses. Blood was detected in over half of all examinations (55.6%). The population prevalence of EIPH increased as the number of examinations for each horse increased. The preceding EIPH score was significantly associated with the current EIPH score. Significant variables associated with moving between EIPH grades were the number of days since last racing, ambient temperature and weight carried. Tracheobronchoscopic EIPH is mildly progressive over the first thirty career starts.

Limitations: Enrolment was voluntary. Horses were not followed for their entire career.

Conclusion: Limiting the number of days in the current racing preparation and spacing races for horses with moderate to severe EIPH may be beneficial for reducing tracheobronchoscopic EIPH severity. The association between ambient temperature and EIPH warrants further investigation.
Introduction

Exercise-induced pulmonary haemorrhage (EIPH) is a highly prevalent disease of racehorses; significant due to associations with inferior race day performance [195, 434], potential regulatory implications [220], as well as horse welfare and safety concerns [251]. The prognosis for clinically significant EIPH is considered guarded due to the likelihood of disease progression, but the prognosis for worsening of EIPH, based on tracheobronchoscopy, is unknown [260]. Histopathological pulmonary lesions in horses with EIPH suggest the disease is progressive and irreversible [66, 76]. Accumulated racing activity (increasing age and lifetime starts) has been associated with a higher incidence of epistaxis and EIPH, also supporting a progressive disease process [80, 124, 433]. It would be reasonable to conclude that if tracheobronchoscopic EIPH worsens with increased racing, a one-off diagnosis of EIPH should impact career performance; but recent research questions this [200]. And others suggest that career longevity is not adversely affected by EIPH [254].

Tracheobronchoscopic studies examining disease changes over time are not available. Two studies have demonstrated moderate agreement between detecting tracheobronchoscopic EIPH on two occasions, but did not elaborate on severity [9, 263]. Other studies report increased disease prevalence with repeated observations, but aside from anecdotal comments that EIPH severity is unpredictable, further statistical analysis was not performed [70, 174, 254].

The aim of this study was to examine tracheobronchoscopic EIPH changes over time in a population of Thoroughbred racehorses. A secondary aim was to identify variables that affect changes in tracheobronchoscopic EIPH severity between races. Our hypothesis was that EIPH presence and severity would increase with repeated observations over the study period. We further hypothesized that a break of more than 60 days between races would reduce EIPH severity.
Materials and Methods

Study design

A prospective, observational, longitudinal study design was used to examine a population of Thoroughbreds racing at two metropolitan and one provincial racecourse in Western Australia between May 2012 and April 2013, and between July 2014 and December 2015.

Horses

Information regarding the study was promoted to racing participants through radio interviews, advertising in the racing calendar, presentations, and postal and direct communication on race day. Enrollment was voluntary, and trainers could enroll horses at their discretion. Trainers were encouraged to enroll all horses in their care to limit bias. Any horse racing on the day of examination was eligible to be enrolled. Horses were enrolled prior to racing and must have finished the race to be examined.

All races were conducted on a flat turf surface. Pre-race administration of any medication is prohibited [220]. Horses were presented to a designated location on course for tracheobronchoscopy. Examination occurred no earlier than 30 minutes and no later than 220 minutes after racing. A 1.5 metre endoscope (AOHUA model LG-200) was passed through the nares into the trachea to the carina. A video recording of each examination was collected. The time at examination and if epistaxis was present were recorded. Horses with two or more observations were included in the study. Horses were not examined after every race start or for the entire study period due to retirement, periods of extended convalescence, ownership or trainer changes or did not race at racecourses where examinations were occurring.

Detections and quantification of EIPH

Two experienced veterinarians, blinded to the identity and performance of the horse, independently reviewed the tracheobronchoscopic video recordings. A previously described 0-4 graded scoring system was used [261]. Briefly, grade 0 is assigned to cases where no blood is
observed in the trachea or nasopharynx; grade 1 has ≥ 1 blood specks or ≤ 2 short (< ¼ trachea length) and narrow (<10% of the tracheal surface area) streams of blood; grade 2 is a single long stream of blood (>½ tracheal length) or > 2 short streams of blood occupying <½ of the tracheal circumference; grade 3 is multiple, distinct streams of blood covering more >½ of the tracheal circumference, but without blood pooling at the thoracic inlet; and grade 4 is multiple, coalescing streams of blood covering >90% of the tracheal surface, with blood pooling at the thoracic inlet. If there were discrepancy between the primary reviewers' score, a third experienced veterinarian independently and blindly reviewed and graded the examination. The median score was adopted for statistical analyses.

Racing variables

Racing records were retrieved from a public database maintained by the racing regulator1. Variables captured at each observation included the number of career race starts, age, sex, the number of days and races in the current racing preparation, the number of days since the horses' last race start, weight carried, race distance and non-standard shoes (yes/no). A horse's race career was divided into racing preparations; training without reaching the races for the purpose of this study was not considered a racing preparation. More than 60 days between two consecutive races was considered indicative of a break in training, denoting the end of one racing preparation, and the commencement of the next racing preparation Race-day performance variables were not included in analyses. Climatic variables were obtained from the Australian Bureau of Meteorology2 using the closest weather station recording apparent temperature at the nearest time point to race start time.

Data analysis

The number of days and the number of races in the current racing preparation were highly correlated, as was age and the number of career race starts. The number of days in the current racing preparation and the number of career race starts were used in the analysis.
The association between the current and the preceding EIPH score was investigated using a linear mixed effects model. The imer function of the imer4 [435] package in R³ was used to assess the strength of the relationship between the two EIPH scores (0-4). Potential regressor variables that could affect the current EIPH score were included as fixed factors in the model. These included ambient temperature, number of days since the last race start, sex, weight carried, number of days in the current racing preparation, the number of career starts and race distance. A random intercept was included to account for the repeated sampling of horses. Model selection when including or removing regressor variables was performed using the ANOVA function in R. The regressor variable with the highest p-value was removed from the model in a stepwise manner. Significance was based on a p-value <0.05.

In a second model, designed to establish which regressor variables were related to changes in EIPH, variables were modeled using multiple ordinal regressions with a random effect term for each horse. EIPH was categorised according to severity: no or mild EIPH (grade ≤1); moderate (grade 2); and severe EIPH (grade ≥3). These multiple ordinal regressions essentially model the observed sequence of EIPH scores for each horse as a time inhomogeneous Markov chain, where the EIPH score to which a horse transitions to at their next observation depends only on the current score; however, the probability of transitioning between scores differs as they depend on regressor variables. To achieve this, all ordinal regressions used the same set of regressor variables, but the regression parameters were allowed to differ based on the EIPH score the horse was transitioning out of. This was to ascertain whether a regressor variable had a notable change in behaviour between EIPH scores. For example, the model allows the effect of temperature on EIPH at the next time point to vary based upon the current EIPH score. The effect of temperature on a horse that had previously EIPH≤1 can be different to the effect on a horse that had previously EIPH≥3. Significance was based on a p-value <0.05. An additional longitudinal analysis was also performed using a semi-parametric approach to fit a smooth curve, namely a spline, using the number of career starts as a marker for time. At each observation, fixed effects including ambient temperature, race distance, weight carried, the number of days in
the current racing preparation, the number of races between examinations and the number of days since last racing were incorporated into the model. A horse specific intercept and slope was included to permit the spline to vary slightly between horses. This semi-parametric model was fitted using the Stan probabilistic programming language and its interface to R, Rstan\textsuperscript{4}[436]. The shape of the mean population spline over time was examined to assess disease progression. The population mean response represents the average horse racing in average conditions with average other covariate values.

Underlying distributional assumptions for normality and homogeneity of residuals were checked using QQ and residual plots. Maximum likelihood mean estimates and 95\% confidence intervals were obtained for the effect sizes of the lagged EIPH score and other confounding variables included in the models. Significance was based on a p-value <0.05 and data analysed using the R\textsuperscript{5} environment for statistical computing.

\textbf{Results}

There were 2,974 tracheobronchoscopic examinations performed on 747 horses (number of examinations range 2-19; median 3; IQR 2,5) (table 5.1). There were 129 trainers that enrolled between 1 and 89 horses (median 5). There were 272 females, 457 geldings and 18 entire males. At the first observation, age ranged between 2 and 10 years (median 4), and the number of career race starts ranged from 1 to 93 (median 8). Sixty-eight horses were examined at their first race start (age 2-4 years) and 177 horses had an examination performed during their first racing preparation. The number of races between examinations ranged from 0 to 36 (median 0; IQR 0, 2). When examinations were performed after sequential race starts, the number of days between examinations ranged from 3 to 511 (median 14; IQR 13,21, number of observations 1,747; n=471).

There was no significant difference between the number of tracheobronchoscopic examinations performed on horses diagnosed as EIPH positive or EIPH negative at their first observation. Blood was detected in over half of all observations (55.6\%; 1655 observations). Of the total observations 44.4\%, 32.4\%, 17.7\%, 4.5\% and 1.0\% were graded 0 to 4, respectively. All
horses that had 7 or more observations (n=107) had EIPH diagnosed at least once. Horses that had three, four, five or six observations, 72.5%, 85.4%, 92.1% and 96.2%, respectively, had EIPH detected at least once. Of the horses that had grade 4 EIPH recorded (n=25), 17 were re-examined on at least one occasion. There was variability in EIPH severity from one observation to the next (figure 5.1). Horses previously diagnosed as grade 4 EIPH, 11.1%, 33.3%, 27.8%, 22.2% and 5.6% were diagnosed with EIPH grade 0 to 4, respectively, at their next observation. Whereas, horses previously diagnosed EIPH grade 0, the majority (59%) remained EIPH grade 0 at their next observation.

The preceding EIPH score was significantly (P<0.0001) associated with the current score (table 5.2). A direct and positive association (P<0.0001) was present between the number days in the current racing preparation and the EIPH score. Ambient temperature was inversely and significantly (P<0.0001) associated with the EIPH score.

Significant variables associated with moving between EIPH categories (EIPH≤1 EIPH 2; EIPH≥3) included the number of days since last racing, ambient temperature and weight carried (table 5.3). For horses with previous severe EIPH (grade ≥3), or moderate EIPH (grade 2), the number of days since their last race had the largest estimated effect (coefficient -0.39; 95% CI -0.81, -0.01 and coefficient -0.28 95%CI -0.58, -0.0004, respectively), implying that increasing the number of days between races was more likely to result in transition to a lower EIPH grade at the next observation. For horses with previously no or mild EIPH (grade ≤1), ambient temperature had the next largest effect size (coefficient -0.25, 95%CI -0.41, -0.09), implying that horses with EIPH ≤1 were more likely to move to higher grades of EIPH when racing at a lower ambient temperature. For horses previously with EIPH≤1 reducing the weight carried in the race was associated with a transition to a higher EIPH grade at the next observation (coefficient -0.16; 95%CI -0.31, -0.005).

The mean population spline indicates tracheobronchoscopic EIPH mildly progressed in severity (figure 5.2). The study population mean increases over the first 30 career starts after which the spline plateaued. Beyond approximately 35 career race starts, sparse data leads to
considerable uncertainty in estimating progression. Significant fixed effects in this model included ambient temperature and the number of days in this racing preparation (Table 5.4).

There were 147 horses with examinations performed after sequential race starts that were separated by more than 60 days. The number of days between observations varied from 61 to 511 days (median 140). The duration of time between racing preparations was not significantly associated with the current EIPH score (P=0.07). The preceding EIPH score was significantly associated with the current EIPH score (P<0.0001). There was a significant inverse relationship between the current EIPH score and ambient temperature (P=0.001).

Discussion

This is the first study to document tracheobronchoscopic EIPH changes over time in a large number of horses, conducted prospectively under race conditions. The current EIPH score is associated with the previous observation, but this does not mean that EIPH scores remain the same from one race start to the next. This study is in agreement with others that tracheobronchoscopic EIPH severity can vary between races [70, 174, 254].

Increasing the number of days between races for horses previously with moderate (grade 2) or severe (grade ≥3) EIPH was more likely to be associated with a transition to a lower grade. Autologous blood instillation in the respiratory tract evokes a mild inflammatory response, [128] and EIPH has been associated with pro-inflammatory markers and increased nucleated cell counts in bronchoalveolar lavage (BAL) fluid [120, 121]. If the inflammatory response to EIPH perpetuates the disease, increasing the time between races may allow this response to dissipate or reduce, decreasing EIPH severity at the next race start. The role of airway inflammation in perpetuating EIPH is controversial [112]. Some investigators have reported no association between haemosiderophages and neutrophil counts in BAL fluid or tracheal aspirates, and no association between EIPH and clinical signs of inflammatory airway disease, such as coughing or tracheal mucus [122–126, 181]. The pulmonary pathology associated with EIPH is not driven by the repeated physical presence of blood in the airway [69]. Rather they are likely determined
by changes in the interstitium and the vasculature. Recently, regions of pulmonary interstitial oedema have been described in horses with EIPH [437]. Associated with areas of extensive venous remodeling and fibrosis, the interstitial oedema was suggested to be a sequel to the initial lesion of vascular remodeling and haemorrhage. Perhaps lengthening the time between races is beneficial for allowing the pulmonary interstitium to resolve oedema and undergo fibrosis, a process that may assist to maintain the integrity of the lung to withstand hypertensive episodes in the future.

An inverse relationship between ambient temperature and EIPH has been identified in multiple studies [124, 178, 433]. Ambient temperature was a persistently significant variable throughout these analyses; associated with the current EIPH score, the transition from a lower to a higher EIPH score and progression modeling. Strenuous exercise in cold conditions is associated with chronic airway inflammation and bronchial hyperactivity in human athletes [438] and a similar response has been documented in equine athletes [429, 439]. Additionally, cold-induced pulmonary hypertension has been reported in calves exposed to cold temperatures (3-5°C), and in sheep and rats [440–442]. We are not aware of a comparable study in horses exposed to cold temperatures, but a similar mechanism could plausibly affect EIPH severity.

Decreasing the amount of weight carried in a race for horses previously diagnosed with EIPH≤1 was more likely to be associated with a transition to higher EIPH grades. The basis for this finding is unknown. The allocation of weight is to ensure racing is competitive. Horses that move up to a higher class of race will be allotted a lower weight to equalize their chance of winning against a better field of competitors. Weight is negatively correlated with speed [213]. Average race speed or race time has not been associated with EIPH, but average race speed in sections of the race has [178, 434]. A faster early/mid average race speed has been identified in horses with severe (≥3) EIPH, and horses with EIPH grades 1 and 2 are more likely to overtake competitors in the final 400 metres of the race compared to horses without EIPH [434]. Rapid acceleration causes higher pulmonary vascular pressures compared to an incremental increase
to the same speed [41]. A reduction in weight, coupled with a higher standard of competitors, may result in increased speed or faster acceleration and that could increase the likelihood of EIPH.

This study is in agreement with other surveys of Standardbred and Thoroughbred racehorses where increased post-race tracheobronchoscopic scrutiny increases the population prevalence of disease [174, 178].

Pulmonary changes associated with EIPH have been described as permanent and progressive, characterized by fibrosis, bronchial neovascularization and haemosiderophage accumulation [296]. More recently, remodeling of the small pulmonary veins has been reported as a characteristic lesion accompanying haemosiderophages and interstitial fibrosis [66]. High vascular pressures and blood flow during exercise likely underpin this remodeling process [437], a process that has been documented in young, unraced Thoroughbreds in training [59]. These pathological changes are likely progressive with continued racing. Single point observations have reported an association between EIPH and a cumulative racing measure, lifetime starts [124, 433]. In this study, inclusion of specific shorter-term cumulative measures such as, the number of days in the current racing preparation were significantly associated with the EIPH score and progression modeling. This suggests that racing intensity even within a racing preparation can affect EIPH severity.

The large number of confounding variables inherent in racing, and irregularly spaced observations complicates longitudinal analysis compared to single point observations. A semi-parametric approach to disease progression was used to provide a more flexible population mean structure than conventional regression. Using this approach, the population mean tracheobronchoscopic EIPH score mildly increased over the first thirty race starts, after which the disease severity plateaued. We expected to identify a stronger positive association between EIPH severity and progression over time. The association between tracheobronchoscopic EIPH score and pulmonary histological changes has not been substantiated. It is possible that tracheobronchoscopic EIPH severity is not an accurate marker of pulmonary disease or progression.
There are logistical challenges in following every horse for every race start, notwithstanding change of trainer or ownership over the study period, and racing at multiple venues across a large geographical area. As a result, a limitation of this study is that not all horses were examined after every race start, or for the entire study period. Furthermore, the study period does not necessarily encapsulate a horses' entire career. Another limitation of this study is enrollment. Voluntary enrollment is inherently biased as owners and trainers can permit access to certain horses and not others. Participating in this study did not disadvantage trainers or owners. There are no regulatory implications for tracheobronchoscopic EIPH. Horses with bilateral epistaxis are subjected to regulatory bans [220].

Trainers were aware of endoscopy results at the time of the examination. Initiated treatments, such as the administration of furosemide during training, or changes to the training or racing regime could have altered the disease course. This population does not receive any pre-race medication. Currently, there is no evidence to support or refute the efficacy of furosemide in training for reducing or preventing race-day EIPH.

We report that the tracheobronchoscopic EIPH score can vary from one race start to the next. Factors associated with moving from a lower to a higher EIPH score include lower ambient temperature and reduced weight carried. The number of days between race starts is associated with movement from a higher to a lower EIPH score. Limiting the number of days in the current racing preparation and spacing races for horses with moderate to severe EIPH may be beneficial for reducing tracheobronchoscopic severity. The role ambient temperature plays in the pathogenesis of EIPH warrants further investigation.

Footnotes:
RStan Stan Development Team (2017) *RStan: The R interface to Stan*. R Package version 2.16.2


Supplementary information item:

S5.1 Jittered plot of longitudinal tracheobronchoscopic EIPH over time (career starts) for 747 Thoroughbred racehorses
Figure 5.1: Bar graphs demonstrating EIPH score variability in Thoroughbred racehorses. The previous EIPH score (0-4) is demonstrated on the right y-axis, corresponding to the current EIPH score (0-4) on the x-axis. N=747; number of observations= 2,227
Figure 5.2: Tracheobronchoscopic EIPH progression over time (career starts) for 747 Thoroughbred racehorses (observations 2,974). The population mean spline is plotted with a solid line and its 95% credible intervals is plotted with a dashed line.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Minimum</th>
<th>Q1</th>
<th>Median</th>
<th>Q3</th>
<th>Maximum</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time between racing and examination (min)</td>
<td>30</td>
<td>34</td>
<td>41</td>
<td>53</td>
<td>220</td>
<td>48</td>
</tr>
<tr>
<td>Race distance (m)</td>
<td>1000</td>
<td>1200</td>
<td>1400</td>
<td>1600</td>
<td>2400</td>
<td>1417</td>
</tr>
<tr>
<td>Weight carried (kg)</td>
<td>50</td>
<td>55</td>
<td>56</td>
<td>57.5</td>
<td>62</td>
<td>56</td>
</tr>
<tr>
<td>Ambient temperature (°C)</td>
<td>5.6</td>
<td>15.5</td>
<td>18.7</td>
<td>22.9</td>
<td>36.8</td>
<td>19.4</td>
</tr>
<tr>
<td>Days in the current racing preparation</td>
<td>1</td>
<td>14</td>
<td>35</td>
<td>70</td>
<td>329</td>
<td>48.7</td>
</tr>
<tr>
<td>Races in the current racing preparation</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>Days since last race, within a racing preparation (observations= 2395; n=737)</td>
<td>3</td>
<td>11</td>
<td>14</td>
<td>19</td>
<td>60</td>
<td>14.9</td>
</tr>
<tr>
<td>Days since last race, over study period (n=679)</td>
<td>3</td>
<td>13</td>
<td>14</td>
<td>24</td>
<td>1071</td>
<td>41</td>
</tr>
<tr>
<td>Career race starts at first observation</td>
<td>1</td>
<td>3</td>
<td>8</td>
<td>17</td>
<td>93</td>
<td>12</td>
</tr>
<tr>
<td>Career race starts over the study period</td>
<td>1</td>
<td>7</td>
<td>14</td>
<td>24</td>
<td>95</td>
<td>17.5</td>
</tr>
<tr>
<td>Age at first observation</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Age over the study period</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>11</td>
<td>4.5</td>
</tr>
<tr>
<td>Days between racing preparations (n=147)</td>
<td>61</td>
<td>109</td>
<td>140</td>
<td>179</td>
<td>511</td>
<td>152</td>
</tr>
<tr>
<td>Observations performed per horse</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>Number of horse enrolled per trainer</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>89</td>
<td>7</td>
</tr>
<tr>
<td>Number of observations contributed per trainer</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>14</td>
<td>404</td>
<td>23</td>
</tr>
<tr>
<td>Days between observations</td>
<td>3</td>
<td>14</td>
<td>28</td>
<td>112</td>
<td>1103</td>
<td>93</td>
</tr>
<tr>
<td>Races between observations</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>36</td>
<td>1.7</td>
</tr>
<tr>
<td>Days between observations conducted after sequential races (n=471)</td>
<td>3</td>
<td>11</td>
<td>134</td>
<td>20</td>
<td>60</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 5.1: Horse and race characteristics from 747 Thoroughbreds racing in Western Australia that were examined with tracheobronchoscopy post-race (number of observations 2,974).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Std. Error</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>0.52</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous EIPH score</td>
<td>0.30</td>
<td>0.02</td>
<td>0.26, 0.34</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ambient temperature</td>
<td>-0.016</td>
<td>0.003</td>
<td>-0.022, -0.009</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Days in the current racing preparation</td>
<td>0.0017</td>
<td>0.0004</td>
<td>0.0009, 0.0023</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 5.2: Mean and 95% confidence intervals for variables significantly associated with the current EIPH score. N=747, number of observations 2,227
<table>
<thead>
<tr>
<th>Variable</th>
<th>Means</th>
<th>95% credible interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature, formerly no or mild EIPH*</td>
<td>-0.2500</td>
<td>-0.4128</td>
<td>-0.0939</td>
</tr>
<tr>
<td>Temperature, formerly moderate EIPH</td>
<td>-0.1891</td>
<td>-0.4123</td>
<td>0.0391</td>
</tr>
<tr>
<td>Temperature, formerly severe EIPH</td>
<td>0.0544</td>
<td>-0.3479</td>
<td>0.4416</td>
</tr>
<tr>
<td>Weight carried, formerly no or mild EIPH*</td>
<td>-0.1563</td>
<td>-0.3077</td>
<td>-0.0052</td>
</tr>
<tr>
<td>Weight carried, formerly moderate EIPH</td>
<td>0.1919</td>
<td>-0.0317</td>
<td>0.4135</td>
</tr>
<tr>
<td>Weight carried, formerly severe EIPH</td>
<td>-0.3466</td>
<td>-0.8153</td>
<td>0.1232</td>
</tr>
<tr>
<td>Race distance, formerly no or mild EIPH</td>
<td>0.0887</td>
<td>-0.0843</td>
<td>0.2564</td>
</tr>
<tr>
<td>Race distance, formerly moderate EIPH</td>
<td>0.0382</td>
<td>-0.2142</td>
<td>0.2937</td>
</tr>
<tr>
<td>Race distance, formerly severe EIPH</td>
<td>0.1011</td>
<td>-0.3870</td>
<td>0.5881</td>
</tr>
<tr>
<td>Career starts, formerly no or mild EIPH</td>
<td>0.0866</td>
<td>-0.8683</td>
<td>0.2674</td>
</tr>
<tr>
<td>Career starts, formerly moderate EIPH</td>
<td>0.1198</td>
<td>-0.1630</td>
<td>0.4011</td>
</tr>
<tr>
<td>Career starts, formerly severe EIPH</td>
<td>0.0532</td>
<td>-0.6467</td>
<td>0.7654</td>
</tr>
<tr>
<td>Races between exams, formerly no or mild EIPH</td>
<td>0.0685</td>
<td>-0.0766</td>
<td>0.2062</td>
</tr>
<tr>
<td>Races between exams, formerly moderate EIPH</td>
<td>-0.0811</td>
<td>-0.3711</td>
<td>0.1969</td>
</tr>
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<td>Races between exams, formerly severe EIPH</td>
<td>-0.3934</td>
<td>-0.9936</td>
<td>0.0899</td>
</tr>
<tr>
<td>Days in current preparation, formerly no or mild EIPH</td>
<td>0.0707</td>
<td>-0.0919</td>
<td>0.2285</td>
</tr>
<tr>
<td>Days in current preparation, formerly moderate EIPH</td>
<td>0.1525</td>
<td>-0.1354</td>
<td>0.4388</td>
</tr>
<tr>
<td>Days in current preparation, formerly severe EIPH</td>
<td>0.0976</td>
<td>-0.3728</td>
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</tr>
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<td>Days since last race, formerly no or mild EIPH</td>
<td>0.0106</td>
<td>-0.1513</td>
<td>0.1645</td>
</tr>
<tr>
<td>Days since last race, formerly moderate EIPH*</td>
<td>-0.2825</td>
<td>-0.5818</td>
<td>-0.0004</td>
</tr>
<tr>
<td>Days since last race, formerly severe EIPH*</td>
<td>-0.3913</td>
<td>-0.8075</td>
<td>-0.0070</td>
</tr>
</tbody>
</table>

Table 5.3: Factors associated with a change in EIPH states from one race start to the next: model summary with coefficients and 95% credible intervals for 747 Thoroughbred racehorses (2,227 observations) *p-value < 0.05.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Sd</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient temperature*</td>
<td>-0.0834</td>
<td>0.0152</td>
<td>-0.0533</td>
<td>-0.1125</td>
</tr>
<tr>
<td>Weight carried</td>
<td>-0.0183</td>
<td>0.0152</td>
<td>0.0155</td>
<td>-0.0485</td>
</tr>
<tr>
<td>Race distance</td>
<td>0.0262</td>
<td>0.0224</td>
<td>0.0695</td>
<td>-0.0172</td>
</tr>
<tr>
<td>Races between exams</td>
<td>0.0120</td>
<td>0.0158</td>
<td>0.0422</td>
<td>-0.0187</td>
</tr>
<tr>
<td>Days in the current racing preparation*</td>
<td>0.0824</td>
<td>0.0191</td>
<td>0.1196</td>
<td>0.0452</td>
</tr>
<tr>
<td>Days since last racing</td>
<td>-0.0116</td>
<td>0.0161</td>
<td>0.0197</td>
<td>-0.0436</td>
</tr>
</tbody>
</table>

Table 5.4: Fixed effects in the semiparametric model of tracheobronchoscopic EIPH progression in 747 Thoroughbred racehorses (observations 2,974). Mean, standard deviation and the 95% credible interval around the mean. *Significant factor (i.e. 95% credible interval does not cross zero).
Suppl 5.1: Jittered plot of tracheobronchoscopic EIPH over time (career starts) for 747 Thoroughbred racehorses (observations 2,974). The population mean spline is plotted with a solid line, and its 95% credible interval is plotted with a dashed line.
Chapter Six. Evidence of acute severe pulmonary haemorrhage and collapse in a Thoroughbred racehorse without evidence of chronic EIPH

Introduction

EIPH is perhaps the most widely recognized disease of racehorses having significant negative ethical and welfare implications for the racing industry. The consensus within the industry is that EIPH is progressive and can culminate in profuse bilateral EAE, collapse or sudden death during racing. Although collapse or sudden death during racing is extremely rare the potential implications of such an event are substantial for horse and rider. Many racing jurisdictions address these fundamental safety and welfare concerns by imposing bans or retirement on horses that experience EAE [220, 414]. But there are no longitudinal studies or case reports that document chronic tracheobronchoscopic EIPH or EAE culminating in collapse or sudden death during racing or training.

Case details

A four-year old Thoroughbred gelding was examined with post-race tracheobronchoscopy on 7 occasions between August 2014 and March 2015. Following the first observation on August 8, 2014, the horse had one more race start at a racetrack where examinations were not occurring and was then paddock spelled for a routine period of 6 weeks. The gelding returned to training in October 2014 and had one trial in late December before resuming racing on January 7, 2015. Tracheobronchoscopy was performed on-course, after racing on 6 occasions in 2015 (table 6.1). All examinations were recorded and graded by two experienced veterinarians, blinded to the identity of the horse, using a previously described 0-4 scale [261]. Of the 7 observations, the first observation was graded EIPH 1, followed by 6 consecutive observations graded EIPH 0. The last post-race observation was recorded on March 14, 2015.

On March 28, 2015 the gelding was performing a routine track gallop. Coinciding with the end of the gallop the horse developed profuse, bilateral epistaxis and collapsed. The horse remained
recumbent on the training track for approximately 1 minute with haemorrhage emanating from both nostrils. The gelding did not lose consciousness. The gelding then regained his feet and was taken back to the hose down stalls before being walked slowly back to the stabling complex. The horse underwent clinical examination and tracheobronchoscopy approximately 60 minutes later. The examination was recorded and again, graded blindly and independently by two veterinarians. The examination was graded EIPH 4, with haemorrhage from the left and right bronchi. Clinical examination revealed a bilateral epistaxis but was otherwise unremarkable. Auscultation of the heart revealed normal cardiac rhythm, but an electrocardiogram was not performed.

Conversation with the track rider described the presence of blood on his clothing and hands towards the end of the track gallop and prior to the gelding collapsing. The track gallop was of standard quality and routine speed and distance. No medication had been administered prior to track work. The horse was subsequently retired from racing.

**Discussion**

While there are critical omissions in the clinical work up of this case to determine the cause of the pulmonary haemorrhage and collapse, this case highlights the possibility that horses that experience severe EAE and collapse during racing may not necessarily be experiencing a worsening of chronic EIPH, but rather an acute manifestation of this or another disease resulting in EAE and collapse. There are no longitudinal studies, or case reports describing previous post-race tracheobronchoscopic results in horses that collapse or die from fulminant EIPH during racing or training.

Markers of chronic pulmonary haemorrhage, including haemosiderophages, chronic inflammation and fibrosis were inconsistently recorded in horses that were deemed to have died from acute pulmonary haemorrhage during or within an hour of racing [251]. Pulmonary haemorrhage was identified in the majority (188/269) of these lesions, but the cause was only attributed to this lesion in a minority of cases (50/268). The pathologists varied in their interpretation of pulmonary oedema, congestion and haemorrhage contributing to death and some described these changes as a result of cardiac failure rather than pulmonary failure. In an Australia study investigating racetrack sudden
death in Thoroughbreds, 17 of 77 horses reportedly died from acute pulmonary oedema, congestion and haemorrhage. But not all horses (6/17) had evidence of previous pulmonary haemorrhage. Lyle et al (2011) suggest that the cases of acute pulmonary haemorrhage causing death were not necessarily fulminant cases of chronic EIPH [251], and this thought process may align with the longitudinal post-race endoscopy results in this case.
<table>
<thead>
<tr>
<th>Exam date</th>
<th>Time to exam (min)</th>
<th>Weight carried (kg)</th>
<th>Finishing position</th>
<th>Ambient temperature (°C)</th>
<th>Distance finished from winner (L)</th>
<th>Race distance (m)</th>
<th>Race earnings ($)</th>
<th>Starts this preparation</th>
<th>Career race starts</th>
<th>Days since last race</th>
<th>Post race EIPH score</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/08/14</td>
<td>33</td>
<td>56</td>
<td>2</td>
<td>23.7</td>
<td>0.30</td>
<td>1000</td>
<td>3000</td>
<td>6</td>
<td>11</td>
<td>13</td>
<td>1</td>
</tr>
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<td>55</td>
<td>6</td>
<td>34.1</td>
<td>2.25</td>
<td>1000</td>
<td>225</td>
<td>1</td>
<td>13</td>
<td>147</td>
<td>0</td>
</tr>
<tr>
<td>17/01/15</td>
<td>33</td>
<td>55</td>
<td>9</td>
<td>38.1</td>
<td>7.00</td>
<td>1000</td>
<td>0</td>
<td>2</td>
<td>14</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>31/01/15</td>
<td>30</td>
<td>54.5</td>
<td>6</td>
<td>29</td>
<td>3.25</td>
<td>1100</td>
<td>400</td>
<td>3</td>
<td>15</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>25/02/15</td>
<td>33</td>
<td>57</td>
<td>3</td>
<td>33</td>
<td>2.50</td>
<td>1000</td>
<td>1350</td>
<td>5</td>
<td>17</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>4/03/15</td>
<td>32</td>
<td>53</td>
<td>1</td>
<td>32.4</td>
<td>0.00</td>
<td>1000</td>
<td>14400</td>
<td>6</td>
<td>18</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>14/03/15</td>
<td>32</td>
<td>51.5</td>
<td>4</td>
<td>25</td>
<td>3.75</td>
<td>1000</td>
<td>2000</td>
<td>7</td>
<td>19</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 6.1: Race characteristics, results and post-race EIPH score for a 4-year old Thoroughbred racehorse that collapsed during track work with profuse bilateral epistaxis
Chapter Seven. General discussion

To the best of my knowledge this is the largest study investigating tracheobronchoscopic EIPH in Thoroughbred racehorses. Conducted prospectively and under race day conditions, this is also the first body of work investigating the longitudinal changes in race day tracheobronchoscopic EIPH severity.

This research project developed from a desire to examine the association between EIPH and climatic variables. After ambient temperature was pinpointed as a risk factor for EIPH by others [124, 178], our aim was to capture a wider range of climatic conditions on race day and examine the association with EIPH. It was somewhat unexpected how persistent the variable, ambient temperature, would be throughout each analysis given that the environmental conditions in Western Australia are considered relatively mild. Ambient temperature was significantly and inversely associated with the detection and severity of EIPH, the transition from a lower to a higher grade of EIPH and was a significant variable in disease progression modeling.

We proposed that the association between EIPH and cold weather could be due to cold-induced hypertension, inflammatory airway disease secondary to hyperpnoea of cold air or another yet unknown mechanism. The association between ambient temperature and cardiovascular hypertension has been demonstrated in several animal models at a temperature of 5°C [442, 443]. This animal model is the only ‘naturally-occurring’ form of experimental hypertension; induced without surgical, medical, hormonal or genetic intervention [444]. Although ambient temperature on race day is typically above 5°C in Western Australia, training would have occurred at, or below this temperature during the cooler months. There is evidence in rats of chronic hypertension persisting despite ambient temperature returning to normothermic conditions, demonstrating that cold-induced hypertension is not immediately reversible [445]. Intermittent exposure of rats to cold, in a sigmoidal fashion akin to the time of the day, also induces hypertension [446]. The association between adverse cardiovascular effects of cold weather in people is well documented, but not fully understood [447]. It is possible that a similar
cardiovascular hypertensive response also occurs in the horse, which could theoretically increase EIPH prevalence and severity via the most well accepted pathophysiological mechanism; stress failure of the pulmonary capillaries.

Given the strong and persistent association between ambient temperature on race day, it would be prudent to avoid racing or training horses with moderate or severe EIPH during the colder months.

Race distance was initially identified as a risk factor for increasing the risk of diagnosing any grade of EIPH (EIPH grade 0 vs. EIPH ≥1). This finding was based on analysis of 583 horses. When the analysis was expanded to include the entire data set (n=3794) or even the subset used in longitudinal analysis (n=2974), race distance was not significantly associated with EIPH in any analysis. It is possible a Type II statistical error occurred during the risk factors analysis aspect of this study when using a smaller, albeit 583 horse, sample size. Other studies investigating risk factors for EAE or EIPH report variable findings on the risk of race distance on EIPH. Some studies identified increased risk in races 1600m or longer, while others report increased risk of EIPH in races less than 1400m. The high prevalence of EIPH coupled with a small variation of typical race distance (1000-2400m) reiterates the importance of utilizing very large sample sizes when investigating this disease.

The application of one or more bar shoes was significantly associated with EIPH detection and severity. The reasoning behind this is unknown and we speculated that horses with bar shoes had subclinical hoof pain during racing and this could somehow increase cardiovascular pressures during racing. In addition to this association with EIPH, horses with bar shoes were less likely to collect race earnings, collected less race earnings and finished further behind the winner than horses without non-standard shoes. Irrespective of the association with EIPH, horses with bar shoes race inferiorly compared to horses racing in standard plates. Trainers should reconsider or delay racing a horse that requires a bar shoe for a short-term foot issue.

This study supports tracheobronchoscopic examinations being performed between 30 and 220 minutes after racing. The optimum time to diagnose EIPH has not been established, but it is
likely to vary slightly depending on the grade. Horses with grade 4 EIPH however, are consistently diagnosed during all time categories, a finding also reported by others [175].

The association between grade 4 EIPH and almost every examined index of race day performance is compelling and certainly strengthens arguments that there is a causal relationship between severe EIPH and inferior race day performance. But the performance implications of EIPH were largely limited to horses with the severe form of the disease. The lack of association between EIPH grades 1 and 2, and race day performance differs from the work of others [175, 195] and possible explanations for this has been discussed previously.

Horses with EIPH ≥3 race significantly faster average speeds during the early/ mid aspect of the race and were more likely to decelerate their speed over the final 600m. We proposed that horses that raced in this manner reached the breaking threshold of the pulmonary capillaries at an earlier stage in the race, and this compounded the severity of the disease. This theory was supported by evidence that horses that rapidly accelerate on a treadmill reached a higher pulmonary artery pressure than a gradual incremental increase to the same speed [41]. Although the causal relationship between severe EIPH and deceleration over the final 600m appears straightforward, if we also examine the alternative hypothesis; adopting a positive pacing strategy can result in fatigue and deceleration irrespective of EIPH occurring - a phenomenon seen in many athletes adopting this pacing strategy [448]. It would be judicious for trainers to instruct jockeys riding horses with a history of severe EIPH to refrain from racing in this manner. This is not always possible, but if the horse can settle in a mid-field or back-marker position, off the pace, it potentially could reduce the severity of race day EIPH and this may improve race day performance.

We identified that horses with EIPH grades 1 and 2 were more likely to improve their position in the home straight compared to horses graded EIPH 0. A plausible explanation is simply that horse that are ridden competitively to the finish are functioning at their maximal physiological limit, compared to horses that are eased up over the finishing stages of the race because they are not in prize contention or are affected by interference in the home straight. We reasoned that
a proportion of horses that don’t finish the race competitively do not reach breaking threshold of the pulmonary capillaries, or the breaks in the blood gas barrier are rapidly reversed by the reduction in vascular pressure upon reducing exertion at an earlier time point compared to other competitors.

The analysis of performance in racing is complicated and a solid understanding of racing and handicapping is required. There is no consensus for a single or collective objective measure of race day performance. Many studies rely on handicapping to equalize the chances of all competitors permitting easy comparison, but as shown in our work, weight allocation in Australian racing [220] is not enough to compensate for a difference in ability. Reliance on handicapping to equalize racing can be misinformed. Other factors such as the number of races within a racing preparation, and lifetime starts also affect if a horse wins or places in race. Although the ranked starting price was not a significant variable in this analysis, this marker of performance has merit and potential applicability in other performance analyses. For example, if a horse that has odds of 100-1 and finishes last, we argue that this horse has not performed poorly, but rather performed to expectations. Meanwhile, a horse that has odds of 2-1 or less and finishes last, has performed below expectations. Based on the comparison of routine performance parameters, these two horses are equal; but in our opinion, only one horse has poor performance. The inclusion of the ranked starting price allows horses performing below their anticipated performance to be pinpointed. This novel parameter potentially could counteract some of the challenges encountered when assessing poor performance.

Risk factors reported for EIPH initially included age [9]. The risk factor, age, was superseded by a stronger predictor of racing intensity, lifetime starts [124]. We too, described lifetime starts as a risk factor for detecting moderate or severe EIPH, however inclusion of more specific, short-term racing intensity variables such as ‘the number of days, or races within a racing preparation’ became a stronger predictor. The number of days in the current racing preparation was significantly and directly associated with the current EIPH score and progression modeling. This reflects a straightforward short-term cumulative association between racing and training, and tracheobronchoscopic EIPH severity. Trainers frequently recognize that horses with EIPH
‘perform well fresh’, meaning that they perform well early in a racing preparation and then their performance can taper off. This finding could support these anecdotal comments based on the association between EIPH and race day performance. From a management point of view, limiting the number of days in a racing preparation for horses with EIPH is a viable and practical solution for owners or trainers to implement, which could reduce EIPH severity.

Tracheobronchoscopic EIPH severity can be erratic, which agrees with the comments of others [70, 174, 254]. We identified three factors that can contribute to the tracheobronchoscopic EIPH score changing between observations. Increasing the number of days between races was associated with a transition from a higher grade of EIPH to a lower grade at the next observation; racing in cooler weather and a decrease in weight carried was associated with a transition from a lower to a higher EIPH grade at the next observation. There are also likely to be unmeasured intra-horse and race factors that could also account for the variation in EIPH scores from one race start to another. Because there are race and environmental factors that can affect tracheobronchoscopic EIPH severity, and the score can change between race starts based on extrinsic factors such as weight carried and the number of days between races, it is unlikely that this marker of disease is suitable to gauge the extent of pathological change that is present in the lung. Tracheobronchoscopy remains an excellent diagnostic tool for assessing EIPH as a cause of poor race day performance.

Using our entire data set we reported no association between any grade of EIPH and career earnings. If endoscopic EIPH worsened over time, a one-off diagnosis should impact career performance variables, but this isn’t the case. Sullivan et al (2015) reported no association between career performance variables and a one-off diagnosis of most grades of EIPH [200], despite identifying race day performance implications in the same population a decade earlier [195]. We reported that tracheobronchoscopic EIPH severity is only mildly progressive over a career. Despite the findings reported by Sullivan et al (2015) [200] we still expected to detect a stronger association between EIPH progression and time. Racing participants and veterinarians emphatically believe that endoscopic EIPH worsens over time, but this assertion is without any longitudinal analysis of severity ever being performed. It is surprising that this is the first published
work examining the longitudinal changes of tracheobronchoscopic EIPH severity given that the
disease is so prevalent and has significant economic costs associated with treatment and
management. The findings of Sullivan et al (2015) and the work reported here serve as a timely
reminder that research into EIPH is not finished and the long-term implications of this disease are
not necessarily aligned with conventional opinions.
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