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**Bar shoes and ambient temperature are risk factors for exercise induced pulmonary haemorrhage in Thoroughbred racehorses.**

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**Keywords:** horse; thoroughbred; EIPH; risk factor; haemorrhage; lung

**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.

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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.

*Study Design:* Cross-sectional study.

*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  $\geq 1$ ) and EIPH grades  $\leq 1$  vs. EIPH grades  $\geq 2$  as dependent variables.

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

*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.

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## 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 [1, 2]. The point prevalence of EIPH using tracheobronchoscopy within 120 min of racing varies between 44 and 75% [3-7]. The percentage of racehorses that demonstrate EIPH increases when individuals are examined after multiple races [3, 5, 8].

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 [9, 10]. Locomotory induced trauma, inflammatory airway disease, upper airway obstruction and haematopoietic abnormalities have also been proposed as initiators or contributing factors for EIPH [11-15]. 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 [14, 16-20]. 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 [14, 19]. 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 [2-5, 8, 21, 22].

A prospective study of Australian Thoroughbreds using post-race endoscopy reported several factors that were significantly associated with EIPH [4]. The study reported 2 models based on EIPH grade and, depending on the model used, reported increased risk with increased lifetime starts, lower ambient temperatures ( $<20^{\circ}\text{C}$ ), 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 [7, 15, 17, 20, 23]. Although ambient temperature was significant in both models used in the Australian study, the temperature range was relatively small (mean  $17.9^{\circ}$ ; 95% CI  $12.9\text{-}22.9^{\circ}\text{C}$ ) [4]. 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 2 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 utilised exclusively over the winter months, during the period of highest rainfall. The racetracks are situated 3.2 km apart. The university's Animal Ethics Committee (R2474/12) approved the study.

### ***Horses and Tracheobronchoscopic Examination***

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 [24]. 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-3000<sup>a</sup>) 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 10 mg acepromazine (Ilium Acepril-10) administered intravenously. All examinations were recorded and tracheal blood was later graded by 2 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  $\geq 1$  blood specks or  $\leq 2$  short ( $< \frac{1}{4}$  trachea length) and narrow ( $< 10\%$  of the tracheal surface area) streams of blood; grade 2 is a single long stream of blood ( $> \frac{1}{2}$  tracheal length) or  $> 2$  short streams of blood occupying  $< \frac{1}{3}$  of the tracheal circumference; grade 3 is multiple, distinct streams of blood covering more  $> \frac{1}{3}$  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 [1]. 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<sup>b</sup>. 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 cheeker (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  $\geq 20$  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<sup>c</sup> 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 min 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 categorised 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  $\geq 1$ ), whereas model B contrasted EIPH grades  $\leq 1$  with EIPH grades  $\geq 2$ . Initially each variable was examined using univariable analyses (chi-squared test of independence and Kruskal-Wallis ANOVA) and those with a  $P \leq 0.25$  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<sup>d</sup>, Statistix<sup>e</sup> and Egret<sup>f</sup>. 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. One hundred and twenty-one trainers participated, enrolling between one 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  $\geq 2$ . Five horses had unilateral or bilateral epistaxis, and of these 4 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, 5 horses wore both front and hind bar shoes, and 24 horses had bar shoes on one or both front feet.

Twenty-seven variables were examined using univariable analysis for both models: EIPH grades  $\geq 1$  (model A) and EIPH grades  $\geq 2$  (model B) (Supplementary Item 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 min (Supplementary Item 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

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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  $22.9^{\circ}\text{C}$  with a range of  $13.5\text{-}37.7^{\circ}\text{C}$ . Ambient temperature, the addition of bar shoes(s) and increasing race distance were significantly associated with the presence of blood (EIPH grade 0 vs. EIPH grades  $\geq 1$ ) (Table 1). 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  $\geq 2$ ) (Table 2). An inverse relationship was identified between ambient temperature and EIPH grade  $\geq 2$ . 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 [4]. In most studies the presence of blood in the large airway (EIPH grade 0 vs. grades  $\geq 1$ ), irrespective of volume, is the dependent variable of interest. A second model (EIPH grades  $\leq 1$  vs. grades  $\geq 2$ ) is based on evidence that grade 1 EIPH is not associated with impaired race performance [25].

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There are limited reports of an inverse association between EIPH and temperature [4, 8]. Hinchcliff and others identified ambient temperature as a risk factor for EIPH despite monitoring horses over a relatively small temperature range [4]. 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 [8].

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 [26-29]. Inspiration of dry cold air in horses results in airway cooling, mucosal injury, and local induction of cytokines with neutrophil influx [30-32]. 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

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heart rate during exercise [33-35]. 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 [36,37]. Foot strike and impact shock transmission to the thorax, a proposed pathogenesis for EIPH [14], 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  $\geq 1$ ) in the present study. Others have identified an increased risk of EIPH with increasing race distance [7, 23], while Hinchcliff *et al.* reported the risk of EIPH  $\geq 2$  was lower for horses racing over 1400 – 2400 m compared to horses racing over shorter distances ( $\leq 1400$  m) [4]. Lifetime starts was significant in Model B (EIPH grades  $\leq 1$  versus EIPH  $\geq 2$ ). 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 [4, 5, 7, 14, 15, 17, 19, 20]. 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 h of racing [4]. 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 min 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.

#### **Authors' declaration of interests**

No competing interests have been declared.

#### **Ethical Animal Research**

Murdoch University's Animal Ethics committee approval has been sought for this study: Permit number R247/12. Horses were voluntarily enrolled in the study with owner informed consent.

#### **Source of funding**

This study was funded by the College of Veterinary Medicine, Murdoch University.

#### **Authorship**

E.J. Crispe contributed to study design, data collection, statistical analysis and preparation of the manuscript. G.D. Lester contributed to study design and execution, data analysis, manuscript preparation and review. I.D. Robertson contributed to study design, statistical analysis and interpretation of the data, and manuscript review. C.J. Secombe contributed to study design, data analysis, and manuscript review.

## Manufacturers' addresses

<sup>a</sup>Pentax EPM-3000 Tokyo, Japan.

<sup>b</sup>Racing and Wagering Western Australia website: <http://www.rwwa.com.au/cris/>

<sup>c</sup>Australian Government Bureau of Meteorology <http://www.bom.gov.au/>

<sup>d</sup>IBM SPSS statistics version 21, New York, USA.

<sup>e</sup>Statistix ver 9.0, Analytical Software, Tallahassee, Florida, USA.

<sup>f</sup>Egret ver 2.0.3, Cytel, Inc, Cambridge, Massachusetts, USA.

## Table Legends:

**Table 1:** Variables retained in the final model for EIPH 0 vs. EIPH  $\geq 1$ .

**Table 2:** Variables retained in the final model for EIPH  $\leq 1$  vs. EIPH  $\geq 2$ .

## Supplementary Information

**Supplementary Item 1:** Univariable analysis of variables and summary statistics.

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**Table 1:** Variables retained in the final multivariable logistic regression model for EIPH  $\geq 1$ ; n = 583 cases.

Variable	$\beta$	s.e.	Wald	Odds ratio (95% C.I.)	P value
Race distance (per 100 m)	0.105	0.029	12.745	1.11 (1.05-1.18)	<0.001
Temperature ( $^{\circ}$ C)	-0.049	0.015	11.230	0.95 (0.93- 0.98)	<0.001
Bar Shoe(s)	1.849	0.547	11.424	6.35 (2.17- 18.54)	<0.001
Constant	-0.122	0.534	0.053		

**Table 2:** Variables retained in the final multivariable logistic regression model for EIPH  $\geq 2$ ; n = 583 cases

Variable	$\beta$	s.e.	Wald	Odds ratio (95% C.I.)	P
Temperature ( $^{\circ}$ C)	-0.033	0.016	4.203	0.97 (0.94-1.00)	0.04
Bar shoe(s)	1.002	0.375	7.129	2.72 (1.30- 5.68)	0.008
Lifetime starts (<20 starts vs. $\geq 20$ starts)	0.404	0.195	4.285	1.50 (1.02-2.20)	0.04
Constant	-0.733	0.456	2.592		