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**EXERCISE-INDUCED PULMONARY
HAEMORRHAGE: STATE OF
CURRENT KNOWLEDGE**

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Editors: D. J. Marlin, K. W. Hinchcliff and J. F. Wade

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EDITORS' FOREWORD

Exercise-induced pulmonary haemorrhage (EIPH) is a pervasive and important problem of athletic horses, in particular racehorses. The condition, when diagnosed by a single endoscopic examination of the trachea soon after exercise, occurs in 55–80% of racehorses whilst repeated scoping suggests it is ubiquitous. The cost of EIPH to the Thoroughbred and Standardbred racing industries in the United States alone is estimated to be between \$115,000,000 and \$225,000,000 annually, not including the cost of racing and training days missed and the shortened racing career of affected horses. Moderate to severe haemorrhage, which affects approximately 20% of horses, impairs race performance and reduces earnings.

Recognising the need for further research into this important problem, the Havemeyer Foundation sponsored a workshop on EIPH. The purpose of the workshop was to review current knowledge of EIPH and, perhaps more importantly, to identify areas of future investigation, including definition of specific research problems and approaches to addressing these issues. Input from researchers, regulators, attending veterinarians, and funding bodies was an important feature of this workshop. This workshop was an opportunity to gather an international group of experts involved in EIPH research and to hear from those involved with EIPH as a regulatory or health and welfare issue.

The aims of the workshop were to review:

- the epidemiological information currently available on EIPH;
 - the pathological features of EIPH;
 - the theories of causation and aetiology of EIPH;
 - evidence for effects of EIPH on pulmonary function and performance;
 - the efficacy of treatments or management systems to reduce EIPH;
 - the scale of EIPH and management and regulatory procedures in major world racing jurisdictions.
- The goal of these reviews was to identify specific deficits in current knowledge and understanding of EIPH. As a group, the importance of these deficits was assessed and ranked and a prioritised list of research needs developed to assist funding bodies in making decisions about funding priorities.
- Priorities for research into EIPH in horses were determined. High priority research topics identified by this group were:
- determination and quantification of risk factors for EIPH;
 - further studies into the association of EIPH with race and career performance of horses;
 - a comprehensive assessment of the economic impact of EIPH;
 - the efficacy of furosemide in treatment of EIPH under racing conditions;
 - management of EIPH including the need for rest;
 - the health and welfare aspects of pre-race dehydration of horses as a management for EIPH.

These high priority topics address areas in which new information would be likely to have a high impact in the management of EIPH in horses. The common theme was the study of horses under actual racing and training conditions, or in experimental situations that mimic these conditions.

We thank the Havemeyer Foundation for funding this meeting and for providing logistical support to the organisers and the participants. This meeting again highlighted the importance and usefulness of bringing together a group of experts for a focused and goal-oriented discussion of a problem.

Dave Marlin and Ken Hinchcliff
Workshop Organisers

HAVEMEYER SCIENTIFIC WORKSHOPS

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Organiser: Dr D. F. Antczak
- 1982 **Second International Workshop on Lymphocyte Alloantigens of the Horse**
October - Cornell University, Ithaca, New York, USA
Organiser: Dr D. F. Antczak
- 1983 **Third International Workshop on Lymphocyte Alloantigens of the Horse**
April - New Bolton Center, University of Pennsylvania, USA
Organiser: Dr D. F. Antczak
- 1984 **First International Symposium on Equine Embryo Transfer**
October - Cornell University, Ithaca, New York, USA
Organisers: Drs D. F. Antczak and W. R. Allen
- 1985 **Fourth International Workshop on Lymphocyte Alloantigens of the Horse**
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- 1986 **Workshop on *Corynebacterium equi* Pneumonia of Foals**
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October - Lexington, Kentucky, USA

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Equine Placentitis Workshop

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1999

Equine Genome Project

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European Equine Gamete Workshop

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November - Barbados, West Indies

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2000

Equine Genome Project

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DIAGNOSIS, DETECTION AND QUANTIFICATION OF EIPH

D. J. Marlin

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Diagnosis is the act of identifying a disease from its signs and symptoms and accurate diagnosis is essential for effective treatment, accurate prognosis and in understanding the aetiology of disease. In exercise-induced pulmonary haemorrhage (EIPH), it is not clear if we are dealing with a disease or a dysfunction. Historically, 'EIPH' was diagnosed only on the presence of blood at the nostrils following exercise, however prior to the introduction and widespread use of the rigid and subsequently flexible endoscope, it was not known with any certainty that the lung was the origin of the haemorrhage in the majority of cases. Thus, EIPH *per se* is a disease or condition that has only been studied for the last 30 years.

EIPH can be diagnosed on the basis of current or recent occurrence, a history of previous EIPH or signs suggestive of EIPH. Current or recent EIPH can be diagnosed on the basis of the appearance of blood at the nostrils following exercise and confirmed as pulmonary in origin by endoscopy, the presence of blood visualised in the trachea post exercise by endoscopy or the presence of significant numbers of red blood cells in tracheal wash (TW) or bronchoalveolar lavage (BAL) taken post exercise. A history of previous episodes of EIPH can be inferred from the presence of red blood cells (RBC) in BAL or TW several days following intense exercise (Meyer *et al.* 1998), haemosiderophages in TW or BAL in horses in training, Whitwell and Greet (1984) Ocharacteristic lesions or haemosiderin staining in lung biopsies or lung tissue obtained at post mortem (O'Callaghan *et al.* 1987; Oikawa 1999). Finally, less specific signs suggestive of EIPH may include epistaxis post race (without confirmatory endoscopy), radiographic changes or scintigraphic abnormalities in perfusion,

especially in the dorso-caudal region, poor or loss of performance and coughing post exercise.

Is there such a thing as typical EIPH? There are certain manifestations of EIPH that may be considered as atypical, for example, EIPH associated with atrial fibrillation, development of sudden, severe epistaxis in a horse with a history of mild tracheal EIPH, severity of EIPH greater in a ventral lung region than in the dorso-caudal region, a marked difference in severity between left and right lung haemorrhage, severe focal versus mild diffuse haemorrhage and haemorrhage from the bronchial as opposed to pulmonary circulation.

A further possibility for consideration is whether there are different EIPH phenotypes that can be identified and which may help in understanding of the basic mechanisms of this disease or dysfunction.

It is also clear that there are some limitations in the use of endoscopy to detect EIPH and that increased frequency of examination leads to an increased prevalence. Thus, for a single examination, around 40–60% of horses examined may have blood in the trachea following racing, but if these animals are examined on 3 separate occasions the prevalence will be near 100% (Birks *et al.* 2002). Thus, there appears to be race or horse specific factors that produce variations in the severity of haemorrhage, at least as detected or quantified by endoscopy of the trachea, which at present are poorly understood.

Currently there appear to be inconsistencies in terminology of EIPH between researchers, clinicians, regulators and owners/trainers, which may lead to confusion. For example, the term 'bleeder' is widely used. However, does this refer to a horse in which blood has been detected in the trachea post exercise, or a horse with a Grade 4 or

5 score out of 5 in the trachea post exercise, or a horse with epistaxis?

Some suggestions for consideration relative to the diagnosis, detection and quantification of EIPH include:

- Precise definition of what constitutes a 'bleeder'
- Adoption of a universal grading system for EIPH
- Recommendation for time post exercise for examination
- Recommendation for number of examinations
- Recommendation for BAL timing, volume instilled and method (endoscopic versus blind)
- What is typical versus atypical EIPH?
- Is epistaxis severe EIPH or a condition with a different aetiology?
- How do the severity of clinical signs of EIPH (epistaxis and endoscopic examination) relate to the degree of pulmonary damage?

- Can we begin to describe different phenotypes of EIPH?

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EPIDEMIOLOGY OF EIPH

J. R. Newton

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INTRODUCTION

Despite exercise-induced pulmonary haemorrhage (EIPH) being recognised by equine veterinary science for several decades, the epidemiology of the naturally-occurring condition has only very recently been studied in detail. In epidemiological studies sufficiently large to detect statistically meaningful results, risk factors for EIPH are identified and the strengths of their associations with the condition are determined. Application of appropriate statistical methods allow the simultaneous effects of multiple factors to be accounted for and the potentially important influence of repeated observations in individual animals to be dealt with. In order to minimise the damaging effects of misclassification bias, epidemiological studies require robust and easily applied case and control definitions. In relation to EIPH this has resulted in most studies to date looking at epistaxis ie blood at the nostrils (Kim *et al.* 1998; Takahashi *et al.* 2001; Wiedeman *et al.* 2003; Newton *et al.* 2005) rather than the more prevalent definition of endoscopically visible blood. However, studies of respiratory disease in racehorses in training in the UK have allowed the epidemiology of EIPH defined by visible tracheal bleeding or large proportions of haemosiderophages in tracheal lavages to be evaluated (Newton and Wood 2002).

STUDIES OF EIPH-ASSOCIATED EPISTAXIS

Epistaxis in racehorses invariably originates from the lung and is therefore specific to EIPH. In addition, because its occurrence is frequently systematically recorded as part of routine data collection on racecourses this makes it a

convenient outcome measure for epidemiological studies. Several recent studies have made use of these kinds of data in identifying and quantifying risk factors for epistaxis (Kim *et al.* 1998; Takahashi *et al.* 2001; Wiedeman *et al.* 2003; Newton *et al.* 2005).

Risk factors for epistaxis were investigated among Thoroughbred and Anglo-Arab horses racing in Japan between 1992 and 1997 (Takahashi *et al.* 2001). Results showed that epistaxis was more prevalent following steeplechase than flat races, in older horses than 2-year-olds, among races $\leq 1,600$ m long than in races between 1,601–2,000 metres in length and in females than sexually intact males (Table 1).

A survey of 400 epistaxis episodes among 2,963 Thoroughbred racehorses and 61,181 race starts in Korea found increased prevalence of the condition in older horses, females, horses originating from England or Ireland, higher grade performers, heavier horses and those carrying heavier weight, those in middle or longer distances, handicap racers and during the spring season (Kim *et al.* 1998). In a study performed in South Africa, 1,287 episodes of epistaxis were recorded among 778,532 race starts between 1986 and 2001 (Wiedeman *et al.* 2003). Univariable analyses identified racing at sea-level, between the months of May and October, after 1995 and on Fridays and Sundays as potential risk factors. There was also evidence that the risk increased with age. Unfortunately however, lack of appropriately conducted multivariable analyses precludes meaningful interpretation of these data.

Several risk factors for epistaxis among all race starts between 1996–1998 in Great Britain have recently been investigated and this has provided evidence for the previously unsupported theory that locomotory impact-induced trauma

TABLE 1: Risk factors for epistaxis associated with EIPH among horses racing in Japan between 1992–1997 (from Takahashi *et al.* 2001)

Variable	Category	Crude OR	Adjusted OR	95% CI	P-value
Race type	Flat	1.0	1.0	Referent	
	Steeplechase	6.88	5.58	3.18 – 10.47	<0.01
Age	2-year-old	1.0	1.0	Referent	
	3-year-old	1.98	2.21	1.27–4.25	<0.01
	4-year-old	4.91	4.90	2.79–9.44	<0.01
	≥5-year-old	6.66	6.43	3.63–12.48	<0.01
Distance raced	≤1,600 m	1.43	1.59	1.23–2.08	<0.01
	1,601–2,000 m	1.0	1.0	Referent	
	>2,000 m	3.90	1.08	0.59–1.85	0.79
Sex	Filly/Mare	1.12	1.42	1.13–1.79	<0.01
	Colt/Stallion	1.0	1.0	Referent	
	Gelding	1.09	0.67	0.26–1.39	0.34

OR = Odds ratio

CI = Confidence interval

TABLE 2: Risk factors for epistaxis (n=176) among horses racing in all race types on British racecourses between 1996–1998 (from Newton *et al.* 2005)

Variable	Category	Adjusted OR	95% CI		LRS P-value
			Lower	Upper	
Race type	Flat	1.0		Referent	<0.0001
	NH flat	1.5	0.3	6.9	
	Hurdle	2.4	2.1	5.5	
	Chase	5.0	3.0	8.3	
Going	Firm	2.7	1.5	4.9	0.001
	Good to firm	1.2	0.8	1.7	
	Good	1.0		Referent	
	Good to soft	0.5	0.3	0.9	
	Soft	0.5	0.3	1.0	
	Heavy	0.5	0.2	1.7	
	Standard (AW)	1.0	0.5	2.2	
	Slow (AW)	2.5	0.3	19.5	
Years spent racing any race type	0 years	1.0		Referent	0.006
	1 year	1.6	0.8	3.2	
	2 years	2.8	1.4	5.6	
	3 years	2.6	1.2	5.3	
	≥4 years	2.7	1.3	5.3	
Season	Winter	1.0		Referent	0.017
	Spring	1.6	1.1	2.4	
	Summer	1.1	0.7	1.8	
	Autumn	0.8	0.5	1.4	

Random effects Horse-level

0.001

LRS = Likelihood ratio statistic OR = Odds ratio CI = Confidence interval

TABLE 3: Risk factors for epistaxis among horses racing in a) Flat (n=44), b) Hurdle (n=65) and c) Steeplechase (n=65) races on British racecourses between 1996–1998 (from Newton *et al.* 2005)

Variable	Category	Adjusted OR	95% CI		LRS P-value
			Lower	Upper	
<u>Flat races</u>					
Years spent racing	Year ¹	2.5	1.4	4.6	0.0004
[Years spent racing] ²	Year ¹	0.9	0.8	1.0	0.006
Winning speed	<35 mph	1.0		Referent	0.004
	35–37.5 mph	3.3	1.2	9.0	
	>37.5 mph	5.1	1.8	14.5	
<i>Random effects</i>	<i>Horse-level</i>				<i>0.018</i>
<u>Hurdle races</u>					
Going	Firm	2.6	1.0	6.8	0.001
	Good to firm	1.0	0.6	1.8	
	Good	1.0		Referent	
	Good to soft	0.3	0.1	0.9	
	Soft	0.2	0.1	0.7	
	Heavy	0.7	0.2	3.1	
Sex	Male	1.0		Referent	0.011
	Female	0.4	0.2	0.9	
Years spent racing this race type	0 years	1.0		Referent	0.037
	1 year	1.1	0.5	2.3	
	2 years	2.6	1.3	5.2	
	≥3 years	1.3	0.6	2.7	
<i>Random effects</i>	<i>Horse-level</i>				<i>0.031</i>
<u>Steeplechase races</u>					
Going	Firm	3.0	1.1	8.1	0.02
	Good to firm	1.6	0.9	2.8	
	Good	1.0		Referent	
	Good to soft	0.4	0.2	1.2	
	Soft	0.8	0.3	2.0	
	Heavy	0.5	0.1	3.6	
Weight carried	≤150 lbs	1.0		Referent	0.019
	>150 lbs	2.0	1.1	3.7	
<i>Random effects</i>	<i>Horse-level</i>				<i>0.20</i>

LRS = Likelihood ratio statistic

OR = Odds ratio

CI = Confidence interval

(Schroter *et al.* 1998) might be contributing to the pathogenesis of EIPH (Newton *et al.* 2005). The association of epistaxis with a wide range of race-, horse- and start-level variables was examined in multivariable mixed effect logistic regression analyses. Four multivariable analyses were conducted, one for all race types considered collectively (Table 2) and one each for flat, hurdle and chase race types considered separately (Table 3).

Risk of epistaxis was significantly increased for hurdle and chase race types compared to both flat and National Hunt flat races. In 3 of the 4 final models there was a significant biological trend for increasing risk of epistaxis with increasing ground hardness ('going') and accumulated years spent racing. However, in flat races epistaxis was such a rare outcome (0.33 cases per 1,000 starts) that this subset analysis had insufficient power to measure the detectable effect of 'going' as statistically

TABLE 4: Final ordinary logistic regression models including horse-level random effect terms for visible tracheal bleeding and haemosiderophage positive episodes (Newton and Wood 2002)

Variable	Category	Visible tracheal bleeding				Increased haemosiderophages			
		Crude OR	Adjusted OR	95% CI	P-value	Crude OR	Adjusted OR	95% CI	P-value
EIPH the previous month	Absent	1.0	1.0	Referent		1.0	1.0	Referent	
	Present	9.4	2.7	1.0–7.3	0.055	4.2	2.0	1.5–2.8	<0.001
Inflammation score	0	1.0	1.0	Referent		1.0	1.0	Referent	
	1	2.3	2.8	1.3–5.9	0.010	2.0	2.3	1.7–3.2	<0.001
	2	5.7	12.0	4.2–34.3	<0.001	2.2	4.4	2.5–7.8	<0.001
	3	4.0	10.6	2.5–44.5	0.001	1.0	1.5	0.7–3.3	0.257
Age	≤2 years	1.0	1.0	Referent		1.0	1.0	Referent	
	3 years	1.4	2.5	1.0–6.0	0.048	1.7	1.5	1.1–2.2	0.023
	≥4 years	4.4	3.4	1.1–10.0	0.028	1.9	1.4	0.8–2.5	0.228
Season	Nov–Jan	1.0	1.0	Referent		1.0	1.0	Referent	
	Feb–Apr	1.1	0.7	0.2–2.1	0.507	1.4	1.3	0.9–2.0	0.207
	May–Jul	1.5	1.2	0.4–3.5	0.713	2.7	3.3	2.1–5.2	<0.001
	Aug–Oct	2.5	2.5	0.9–7.0	0.077	2.8	3.5	2.2–5.6	<0.001
Trainer	1	1.0	1.0	Referent		1.0	1.0	Referent	
	2	1.3	0.9	0.2–4.4	0.876	1.3	0.8	0.3–1.8	0.516
	3	0.4	0.2	0.02–1.3	0.087	1.3	0.6	0.3–1.4	0.225
	4	4.2	2.2	0.5–9.8	0.285	2.1	0.9	0.4–2.2	0.851
	5	0.9	0.7	0.1–3.8	0.709	0.8	0.6	0.3–1.4	0.216
	6	-	-	-	-	0.4	0.2	0.1–0.5	<0.001
	7	1.4	0.8	0.1–5.8	0.844	3.1	1.1	0.4–3.0	0.841
Fungal material in TW	Absent	1.0	1.0	Referent		1.0	1.0	Referent	
	Slight	4.3	3.9	1.1–14.0	0.037	2.3	2.8	1.9–3.9	<0.001
	Moderate	4.5	3.1	0.7–14.5	0.143	4.4	5.4	3.1–9.3	<0.001
<i>Random effect</i>	<i>Horse-level</i>				0.003				<0.001

OR = Odds ratio

CI = Confidence interval

significant. Despite this, findings were considered consistent with the theory that locomotory impact-induced trauma contributes to exercise-induced epistaxis (Schroter *et al.* 1998). However, further validation of this hypothesis through application of similar methods to the condition of endoscopically visible EIPH and through biomechanical studies is warranted.

STUDIES USING VISIBLE TRACHEAL HAEMORRHAGE OR HAEMOSIDEROPHAGE QUANTITIES

In an epidemiological study of risk factors for EIPH in young Thoroughbreds in the UK in which 148 horses contributed 1,614 horse-months of data, there were 64 (4%) episodes of

endoscopically visible tracheal bleeding and 824 (51%) episodes of increased quantities of haemosiderophages in tracheal washes (Newton and Wood 2002). There were increases in prevalence and risk of EIPH by both definitions with age from ≤2 years to ≥4 years, season of sampling from winter (Nov–Jan) to autumn (Aug–Oct) and several different measures of airway inflammation, including tracheal mucus, neutrophil proportion, inflammation score and fungal material in tracheal washes. There was considerable variability in the prevalence of EIPH between trainers. EIPH in the preceding month significantly increased the risk of the condition the following month. There was no evidence that EIPH was associated with infection of the airways with even large numbers of *Streptococcus*

zooepidemicus or *Pasteurella*-like spp., which are significantly associated with airway inflammation in younger racehorses. Multiple logistic regression modelling that took account of random variability between horses and the effects of each trainer and an episode the preceding month, confirmed that after controlling for the other risk factors, EIPH was still significantly associated with increasing age, different seasons, airway inflammation and evidence of airway fungal material (Table 4).

The findings of an association between EIPH and apparently non-septic airway inflammation in this study were consistent with a number of previous observational (Cook 1974), theoretical (Robinson 1979, Robinson and Derksen 1980), experimental (Derksen *et al.* 1992) and pathological (O'Callaghan *et al.* 1987) studies of EIPH. However, although this study provides epidemiological evidence for an association between inflammatory airway disease and EIPH in horses in training, it does not and cannot resolve the issue that this is necessarily a causal relationship.

FUTURE DIRECTIONS FOR EPIDEMIOLOGY, EPISTAXIS AND EIPH

Contention remains as to whether epistaxis and more prevalent forms of EIPH are in fact different entities and as such have different risk factors. It has been proposed, but as yet remains unfunded, to use an epidemiological approach to determine the effect of different risk factors, including ground firmness, on severity of EIPH as defined by endoscopically visible tracheal blood, thereby directly addressing whether the same risk factors are important for EIPH as epistaxis. The study would involve sampling horses racing on different classes of ground firmness in different types of races and might lead for the first time, through appropriate modifications to locomotory related factors, to practical measures (eg through track watering and changes to obstacle design and landing side conditions), to reduce the burden of EIPH among racehorses.

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EIPH AND PERFORMANCE

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Exercise-induced pulmonary haemorrhage (EIPH) occurs commonly in Thoroughbred and Standardbred racehorses throughout the world. Blood can be detected by means of tracheobronchoscopic examination of the airways in >50% of Thoroughbred horses after a race. The high incidence of EIPH has prompted speculation that EIPH is an important cause of impaired performance in Thoroughbred racehorses. But although this belief is strongly held by many horsemen and veterinarians involved in the care of racehorses, others have suggested that EIPH may be associated with superior performance, being reflective of greater racing effort, and there currently is little scientific evidence to support either eventuality.

Retrospective studies of horses with epistaxis have found a strong association between the presence of epistaxis and poor performance. It is assumed that most horses with epistaxis during or after racing have EIPH, but this was not confirmed by endoscopic examination of all horses in most studies. Epistaxis is assumed to be indicative of the most severe form of EIPH. Thus, studies that rely on the use of epistaxis as an indicator of EIPH do not provide information about the effect of haemorrhage of lesser severity, which is much more common than is epistaxis, on performance.

Previous studies of Thoroughbred horses in which tracheobronchoscopic examination was performed after racing to detect EIPH have not found an association with performance. Studies of Standardbred racehorses have reported either no association between EIPH and performance, an association between EIPH and poor performance, or a tendency for EIPH to be associated with superior performance. However, the ability of previous studies to detect an association between EIPH and performance may have been impaired

by inadequate statistical power, non-random selection of subjects, and administration of furosemide. Because races are won or lost by small margins, relative to the overall length of the race, examination of low numbers of horses would result in low statistical power and could prevent detection of an important effect of EIPH on performance. Furthermore, a large number of factors can affect the athletic performance of horses, and analysis of epidemiological information of race performance requires appropriate sampling and use of sophisticated statistical analyses to account for colinearity among independent variables. Finally, although furosemide administration has been found to be associated with superior performance in racehorses, its effect on the occurrence of EIPH has not been objectively demonstrated in racehorses.

On the basis of this evidence, it is unclear whether EIPH is associated with altered performance in racehorses. Therefore, we performed an observational cross-sectional study to determine whether EIPH was associated with racing performance in Thoroughbred horses not medicated with furosemide or using nasal dilator strips. Two- to 10-year-old Thoroughbred horses (n=744) racing in Melbourne, Australia were enrolled prior to racing, and a tracheobronchoscopic examination was performed after one race. This study is described in detail in Hinchcliff *et al.* (2005).

Severity of EIPH was graded on a scale from 0–4. Statistical analysis included examination of distance finished behind the winner, race earnings, and finishing position as indicators of performance. The modal value of the EIPH severity grades assigned by the 3 observers was used in all analyses. Presence of EIPH was defined

as a dichotomous (no vs yes) variable in 2 ways: severity grade of 0 (no) versus severity grade ≥ 1 (yes), and severity grade ≤ 1 (no) versus severity grade ≥ 2 (yes). To control potential confounding, all variables that may have affected or predicted a horse's performance were included as covariates in the analyses. Principal component analysis was used to create orthogonal (uncorrelated) scores for these independent covariates. With distance finished behind the winner and race earnings as dependent variables, potential associations with the occurrence of EIPH (EIPH severity grade, severity grade = 0 vs severity grade ≥ 1 , and severity grade ≤ 1 vs severity grade ≥ 2) were examined by means of multivariable ANOVA. Multivariate logistic regression was used to determine whether occurrence of EIPH was associated with various categorical assessments of finish position and race earnings (ie, winning [yes vs no], finishing in the first 3 positions [yes vs no], earning any money in the race [yes vs no], and being in the 90th percentile or higher for earnings in the race [yes vs no]). The Bonferroni method for multiple comparisons was used to adjust comparisons of least-square means derived from ANOVA models. Odds ratios (ORs) and 95% confidence intervals (CIs) derived from likelihood ratio statistics were calculated from the logistic regression models. Data are given as mean \pm SE. For all analyses, values of $P < 0.05$ were considered significant.

Overall, 52.1% of horses eligible for participation in the study were examined, and horses that were examined did not differ from horses that were not examined in regard to age or sex distribution or proportion of horses that won or finished in the first 3 positions. Horses with EIPH grades ≤ 1 were 4.0 times as likely to win, 1.8 times as likely to finish in the first 3 positions, and 3.03 times as likely to be in the 90th percentile or higher for race earnings as were horses with

grades ≥ 2 . Horses with EIPH grades ≥ 1 finished significantly further behind the winner than did horses without EIPH. However, odds that horses with grade 1 EIPH would win or finish in the first 3 positions were not significantly different from odds for horses without EIPH.

The results of this study provide justification for consideration of aggressive management of horses with EIPH of Grade 2 or greater, and illustrate the need for further studies of this disorder. Moreover, the present study revealed a consistent association, for Thoroughbred horses racing in Melbourne, Australia, between the presence of EIPH of severity grade ≥ 2 and a lower odds of winning or finishing in the first 3 positions, a longer distance finishing behind the winner, and a lower likelihood of being in the 90th percentile or higher for race earnings. It is concluded, therefore, that EIPH is associated with impaired racing performance among Thoroughbred horses racing without treatment with furosemide or application of nasal dilator strips. Detection of an association between EIPH and performance in the present study does not prove causation. However, the high prevalence of EIPH severity grade ≥ 2 (18.6%) in the present study and its association with measures of performance, combined with the well-documented effects of spontaneous or experimentally induced EIPH on lung function and arterial oxygen tension during exercise, suggest that EIPH is an important cause of impaired performance in Thoroughbred horses.

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PATHOLOGY OF EIPH

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INTRODUCTION

The clinical manifestations of exercise-induced pulmonary haemorrhage (EIPH) are those of epistaxis associated with recent intense exercise and the findings of diagnostic procedures that identify blood within airways coming from the lower respiratory tract. The documentation of lesions found in cases of EIPH has essentially only considered changes that are related to pulmonary structures. No systematic study of any extrapulmonary lesions has been undertaken, but lesions associated with other causes of epistaxis and pulmonary haemorrhage (and not associated with exercise) are well documented. An understanding of the pulmonary lesions associated with EIPH comes from evaluation of both assessment of naturally-occurring cases and experimental models.

NATURALLY OCCURRING DISEASE

Acute and 'typical'

Based on clinical methods of detection, most cases of EIPH are not life-threatening to the animal, and so the 'typical' case rarely goes to post mortem. For this reason there is no comprehensive published study that has examined an association between the clinical severity of the disorder and the extent and character of pulmonary lesions. Sporadic cases submitted where the animal dies from other causes after intense exercise (usually catastrophic musculoskeletal injury) have been the usual source for diagnostic material, and under many circumstances interpretation of acute lesions is complicated by the use of barbiturates for euthanasia. Rapid intravenous administration of

barbiturates often induces immediate and intense pulmonary congestion, and variable degrees of oedema and haemorrhage thus making identification of the presence of acute lesions prior to euthanasia problematic. However, tissues from these cases are suitable for investigation of more chronic changes associated with EIPH and such changes are well described.

The acute lesions are characterised by multifocal areas of haemorrhage usually restricted to the most dorsal and caudal aspects of both lungs that may not be symmetrical. In lungs where no evidence of chronic haemorrhage exists, foci of acute haemorrhage appear macroscopically as raised red to black firm areas that elevate the pleura and which are distributed mainly in subpleural areas of lung. Foci commonly vary from tiny petechia to coalescing spherical foci 10–25 mm in diameter, suggestive that haemorrhage occurs from multiple sites, at least some of these at the capillary level. In more severely affected cases, suffuse irregular haemorrhages may be present and large amounts of blood or bloody foam may be present within airways and insufflated blood may form extensive spatter patterns throughout the lungs. In these cases where bleeding has been extensive, and in those rare cases where sudden death is attributable to EIPH, usually the blood is so extensively distributed through the lungs that seeking a particular single site for haemorrhage is fruitless. Despite such obvious gross changes, histological lesions are surprisingly bland, with blood (usually unclotted) present in lung tissue without any light microscopic evidence for pulmonary capillary endothelial injury. Two studies have described the acute ultrastructural and light microscopic changes in lungs subjected to high pulmonary vascular pressures (West *et al.* 1993; Erickson *et*

al. 1997) but one study was conducted in ponies and the other on very few animals, so the relationship of the capillary changes noted in both these studies to those which occur spontaneously when horses exercise intensely needs further investigation.

Acute and catastrophic - sudden death associated with exercise, 'atypical cases'

Lesions attributed to EIPH from investigation of cases of sudden death during or immediately after intense exercise and unrelated to catastrophic musculoskeletal injury have been reported only for a limited number of cases (Gunson *et al.* 1988). Recently we reported on results of a similar prospective study (Slocombe *et al.* 2005). Pulmonary lesions from both these studies are essentially the same and cases are characterised by extensive coalescing acute pulmonary haemorrhages (Fig 1). In addition to the pulmonary haemorrhages noted in the study conducted at the University of Melbourne, all cases had concurrent intense pulmonary congestion, 85% had pulmonary oedema and in 20% of cases oedema was thought to be the predominant lesion, and 55% of cases had lesions consistent with concurrent chronic EIPH. Post mortem evaluations also noted a range of extrapulmonary lesions including acute haemorrhages under the parietal pleura, on both serosal surfaces of the dorsal diaphragm and with

dorsal diaphragmatic musculature, haemorrhage within thoracic lymph nodes and less commonly petechia within the dorsal splenic capsule and pancreas. Laryngeal haemiplegia was found in only 1 of 20 such cases of sudden death attributed to EIPH, but the inability to identify dynamic upper airway abnormalities at post mortem is acknowledged. Histological evidence for cardiac disease that could account for the acute pulmonary lesions was absent, but it is also acknowledged that conduction disorders could occur without the presence of lesions. In addition, the interpretation of acute pulmonary lesions derived from these studies as being representative of the 'typical' case of acute EIPH needs careful consideration because haemorrhage, congestion and oedema are likely pulmonary consequences of acute cardiac failure and the parameters for distinction between these 2 entities as causes of sudden mortality during exercise remain to be defined.

Subacute/chronic and 'typical'

Because of the ready distinction of more chronic lesions from acute haemorrhagic processes, there is greater experience with recognising the persisting lesions associated with past episodes of pulmonary haemorrhage, whether cases originate from catastrophic deaths while exercising, or where animals are submitted for post mortem for investigations unrelated to a suspicion of EIPH. As for the 'typical' acute

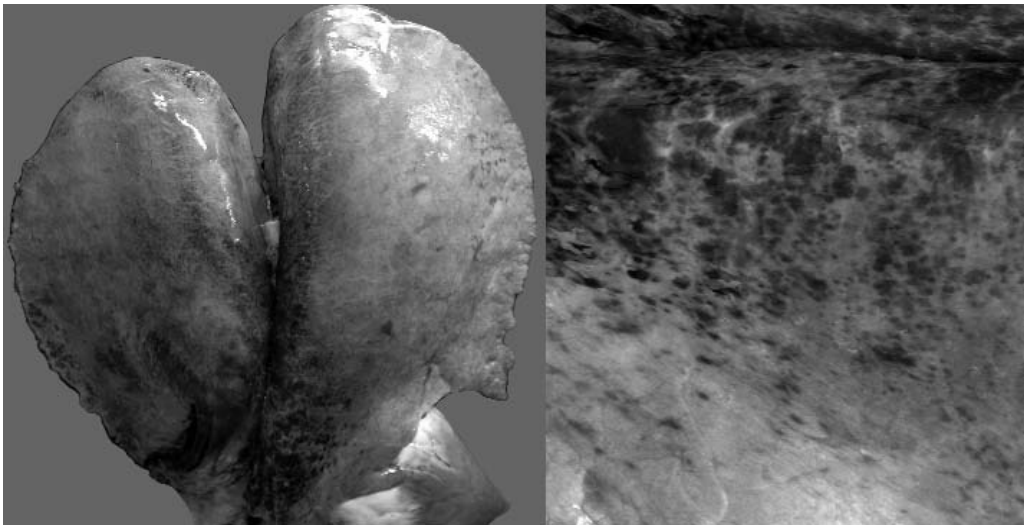


Fig 1: Left panel illustrating lungs with acute multifocal haemorrhagic areas predominantly affecting the dorsal areas of lung, and severe pulmonary oedema. Right panel illustrates detail of subpleural haemorrhages of varying sizes.

case, chronic cases of EIPH are clinically silent and the association between lesion character and severity with reduced performance is suspected rather than proven. The lesions of chronic EIPH have been reported in a number of studies (O'Callaghan *et al.* 1987a,b,c; Oikawa 1999), would be widely recognised by diagnostic pathologists involved in race track investigations and can be summarised as consistently having the following features: subpleural deposits of iron, which appear grossly as brown to blue-black areas. This is attributable to both the accumulation of macrophages containing haemosiderin and also to the precipitation of iron complexes within the connective tissue that develops in areas of scarring; fibrosis, which ranges in thickness, the distribution appears to begin in the caudal dorsal lung extremities and then extend cranially with progression of lesions. Macroscopically, the lesions appear as pale, firm thickened plaques on the pleural surface that sometimes appear approximately in register with intercostal spaces, but generally in older horses, broad regions of the dorsal lungs are uniformly and symmetrically affected. Histologically, fibrosis can be demonstrated to involve the pleura and extend into the interstitium, often involving and distorting subpleural bronchioles and alveoli. Adventitial fibrosis of small airways and blood vessels is also commonly reported; concurrent chronic small airway diseases reported, with bronchiolar distortion, mucosal mucous cell hyperplasia, sometimes mucostasis or obstruction with inflammatory exudates, intraluminal and peribronchiolar accumulations of haemosiderophages, and fibrosis. Less commonly, accumulation of eosinophil-rich infiltrates have been described. Without case-matched controls, there is difficulty in determining whether these changes are integral to the pathogenesis of EIPH or merely a reflection of concurrent sources of lung injury.

Alveolar septal remodelling is described, and grossly affected areas of lung often fail to collapse fully or are poorly inflated, presumably related to the scarring of tissues. A less consistent finding is alveolar lining cell proliferation and hypertrophy, and probably this simply reflects altered alveolar function and susceptibility to other sources of injury once an area becomes fibrosed.

Vascular remodelling with bronchial-pleural anastomoses and hypertensive arterial changes

have been described. These changes were originally proposed as the probable source of haemorrhage in cases of chronic EIPH, but in more recent times, capillary stress failure and haemorrhage from alveoli have been considered more likely, at least acutely (West *et al.* 1993; Erickson *et al.* 1997). However, the evidence for either in cases of chronic EIPH is not compelling. It is curious that in chronic lesions, haemorrhage appears to typically adjacent to, rather than within, scarred areas (Fig 2) consistent with a view that shear forces may be established between normal and fibrosed lung regions during exercise sufficient to cause haemorrhage. This view is also consistent with the concept of a repetitive injury and perhaps explains why lesions appear to progressively migrate rostrally with increasing chronicity. To date, studies to clarify if the mechanisms that lead to initial episodes of EIPH are the same as those that operate in lungs damaged by past episodes of EIPH have not been done.

EXPERIMENTAL MODELS

Controlled exercise-intensity studies

Although there are numerous published studies that use graded exercise and treadmill tests to investigate aspects of EIPH, these typically do not involve destruction of animals and detailed examination of the respiratory tract. Lung biopsy has been shown to have little merit as an investigative tool because the areas involved are not accessible with this technique (Doucet and Viel 2002). Only one study has described

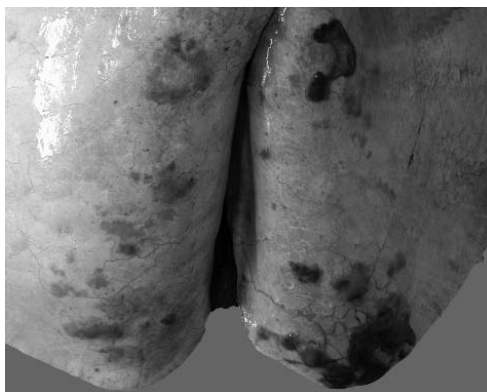


Fig 2: Irregular areas of chronic fibrosis and haemosiderosis with superimposed areas of more recent and resolving haemorrhages.

pulmonary lesions in groups of horses where the intensity of exercise was controlled (Oikawa 1999), lesions are essentially the same as those described for naturally occurring cases but studies of this type are unattractive for both ethical and financial reasons.

Models of pulmonary inflammation

Derksen *et al.* (1992), were able to demonstrate that segments of lung challenged with antigen after systemic sensitisation, developed haemorrhagic lesions after treadmill exercise. Given the intensity of the inflammatory reaction to allergin in this study, it remains unclear whether less intense inflammation of peripheral lung segments would also predispose to EIPH.

Autologous blood inoculation

McKane and Slocombe (2002), described the effects of autologous blood installation into segments of lung and were able to characterise cytological and histopathological changes over time after a single installation of blood. They described the development of an inflammatory response, noted the persistence of both erythrocytes and increased macrophages in lesions for the duration of studies and reported increased connective tissue deposition associated with inoculation of blood. Attempts to reproduce these findings by Derksen and Robinson (personal communication) have to date been unsuccessful, and therefore validation of segmental blood inoculation as a model for structural studies of chronic EIPH remains to be determined.

Naturally-occurring cases

Information regarding the structural changes seen with EIPH has been largely derived from spontaneously occurring cases. To date these studies have lacked adequate case-matched controls. However, with improved consistency of clinical methods of assessment and given the prevalence of EIPH within the equine racing populus, it should be possible to develop protocols for prospective studies using the animals that come available through other causes of wastage.

METHODS FOR STANDARDISATION OF PATHOLOGICAL STUDIES

One major limitation in the capacity to compare studies of the pulmonary pathology associated with EIPH is the lack of quantitative or semi-quantitative methodology in the assessment of lesions. For the assessment of acute lesions, particularly those associated with sudden death at racetracks, the scoring system needs to account for the major changes of oedema and haemorrhage, but with the lesions associated with chronic EIPH, estimates of fibrosis and haemosiderosis are currently considered the most important. While biochemical/analytical methods are possible for analysis of tissue specimens obtained at post mortem, this has not been done in a systematic way. Outlined below are some scoring systems but none have universal acceptance.

Acute EIPH

Quantitative estimates of lung water, and volume of haemorrhage are possible, but are difficult given the size of the lung and the usual requirement for diagnostic samples for concurrent provision of diagnostic descriptions at the histological level. A common problem in case material obtained from routine diagnostic material is the bias attributable to selection of the most apparent lesions rather than a selected sampling approach. For cases in the prospective racetrack fatality study at the University of Melbourne, the protocols have recently been modified to address this issue and currently samples are collected from dorsal, middle and ventral aspects of both lungs in a matrix that includes cranial, central and caudal lung. A scoring system for both macroscopic and histological acute changes in the lungs has been introduced. (Tables 1 and 2).

Chronic EIPH

In addition to the system outlined in Table 1, in the studies reported by O'Callaghan *et al.* (1987a), a scoring system was developed based on the extent of lesions in the dorsal-caudal area of the lungs (O'Callaghan *et al.* 1987a), lesions identified by the distinctive colour changes associated with fibrosis and deposition of iron pigments. More recently Marlin (personal communication)

TABLE 1: Classification of macroscopic lesions of EIPH

Grade	Description
<i>Acute pulmonary oedema</i>	
+	Patchy dependent areas of wet lung that fail to collapse and exude fluid from minor airways
++	Diffusely wet lungs that do not collapse, stream fluid from cut surfaces and have stable foam in minor airways
+++	Diffusely wet and heavy lungs that fail to collapse, stream fluid from cut surfaces and have stable foam in both minor and major airways including the primary bronchi and trachea
<i>Acute pulmonary congestion</i>	
+	Lungs discoloured uniformly or patchily dark pink and heavier than normal
++	Lungs diffusely deep red and heavy
+++	Lungs diffusely plum-coloured, heavy and wet
<i>Acute pulmonary haemorrhage</i>	
+	Discrete red petechial or ecchymotic parenchymal haemorrhage visible beneath the pleura but no obvious blood staining of any oedema fluid
++	Small confluent areas of red to blue black parenchymal haemorrhage and pink to red fluid in airways
+++	Large confluent areas of deep red to black parenchyma with obvious haemorrhage into airways and blood staining of any oedema fluid
<i>Chronic EIPH lesions</i>	
+	Small areas of fibrosis and yellow brown discolouration of the subpleural parenchyma at the dorsocaudal extremity of diaphragmatic lobes
++	Broad zones of fibrosis, yellow brown discolouration and/or mineralisation involving up to 50% of the subpleural parenchyma of the dorsal region of the diaphragmatic lobes
+++	Fibrosis, yellow brown discolouration and/or mineralisation involving more than 50% of the subpleural parenchyma of the dorsal region of the diaphragmatic lobes

developed a system using analysis of digital images of lungs to assess the distribution of iron pigments based on light spectra specific for haemosiderin. Blood is washed from the lungs prior to photography and the surface area of the dorsal lung affected is compared to the total surface area from the dorsal image.

Obviously adaptability of methods is essential to meet individual circumstances, and for example estimates of the amount of haemosiderin or fibrosis in lungs may not have much direct bearing on the volume of blood found in cases where both acute and chronic lesions are present.

FUTURE DIRECTIONS

The basic tissue response to haemorrhage in equine lungs seems no different to that from other tissues and other animals. However, the associations between these lesions and many other aspects of EIPH are largely unknown. There are

many questions that remain unanswered with regard to the lesions and lesion progression with EIPH. Where does blood come from and is it the same in acute and chronic cases, are sudden death cases truly just an extreme form of EIPH, are extra-pulmonary lesions a reflection of yet unrecognised mechanisms that affect the expression of EIPH, what is the history of progression of lesions, what factors affect the rates of blood clearance, the development of scarring and the resolution of lesions, and how do the severity of structural lesions relate to the clinical assessments of lung injury? Is scarring protective or detrimental? Are there horses that develop subclinical lesions of EIPH early in their racing careers and then completely resolve, or is any EIPH lesion permanent and progressive? Do continuing bouts of exercise influence the progression of lesions once they become established, and if so, how does the frequency of exercise bouts influence lesion size and

TABLE 2: Classification of microscopic lesions of acute EIPH

Grade	Description
<i>Acute pulmonary oedema</i>	
+	interstitial/alveolar fluid with low protein
++	interstitial and < 25% alveolar involvement, fluid with slight /moderate eosinophilia
+++	interstitial and adventitial fluid accumulation with >25% alveolar involvement. Fluid generally has eosinophilic staining properties
<i>Acute pulmonary congestion</i>	
+	focal capillary distension, 1-2 rbc profiles
++	multifocal to diffuse, 2-3 rbc profiles
+++	multifocal to diffuse, 3+ rbc profiles
<i>Acute pulmonary haemorrhage</i>	
+	+predominantly interstitial, and sparse multifocal lesions
++	< 25% alveolar involvement, numerous multifocal lesions, some coalescing
+++	>25% alveolar involvement, typically with blood also in airways, subpleura and with some septal effacement

progression. Is there a correlation between accumulation of iron pigments in tissues, tissue fibrosis and the total volume of blood lost into lung tissue and is evidence of upper or lower airway disease, or haemorrhage in other tissues simply epi-phenomena?

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EIPH: IMAGING HAEMORRHAGE AND LUNG REMODELLING

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INTRODUCTION

A method to accurately detect and repeatedly quantify lung haemorrhage in exercise-induced pulmonary haemorrhage (EIPH) would be of great interest to define the relationship between bleeding and performance as well as to assess response to therapy or to prophylactic measures. In a few studies, investigators have attempted to determine whether lung imaging could be of value in EIPH detection and quantification. Apart from endoscopy, the potential imaging techniques for confirmation of EIPH are radiography and scintigraphy. Ultrasound is likely to be of limited use because ultrasound findings in EIPH are not specific and are very similar to other conditions such as pulmonary oedema, recurrent airway obstruction or to scarring from previous pleuropneumonia (Reef 1998).

RADIOGRAPHY

With radiography, a specific pattern of lung density may be found in horses suffering from EIPH. This pattern consists of an increase in bronchointerstitial opacities limited to the dorsocaudal regions of the lungs. Variation in the pattern of this opacity may be observed according to the time since the bleeding episode (O'Callaghan and Goulden 1982) and a gradual clearance of these opacities has been documented (Pascoe *et al.* 1983). Serial radiographic examinations of confirmed bleeders showed that the resolution of pulmonary opacities may last for several days to several months (Pascoe *et al.* 1983). Disappearance of radiological changes is most likely dependent on degree of initial changes, persistence of initiating factors and/or problems secondary to the initial changes. Complications secondary to EIPH such as

cavitation have also been reported (Pascoe *et al.* 1983). When EIPH lesions are detectable with radiography, changes correlate significantly to actual lesion severity as determined by post mortem examination of the lungs (O'Callaghan *et al.* 1987a). However, while radiographic opacities are readily identified in some cases of EIPH, the great majority of horses with clinical signs of the condition have few or only equivocal radiographic signs (O'Callaghan *et al.* 1987a). In these horses, the vaguely discernible increase in interstitial density in the dorsocaudal lungfields may not be differentiated from other causes of bronchointerstitial patterns (Fig 1) and most probably, the inflammatory process associated with blood flooding the airways partially contributes to radiographic opacities (Art *et al.* 2002). Furthermore, a case-control study found that the considerable inter-subjects variability in radiographic findings for both groups (ie controls and horses with confirmed EIPH) impedes obvious discrimination of groups (Doucet and Viel 2002).

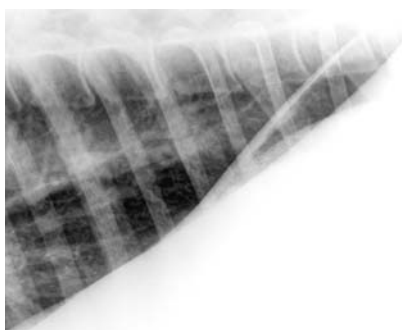


Fig 1: Radiograph of the dorsocaudal lungfields of a horse confirmed for exercise-induced pulmonary haemorrhage (EIPH). No focal or localised lesions are seen. The bronchointerstitial pattern found is not pathognomonic of EIPH and may be found in several other pulmonary conditions.

Therefore, an accurate prediction of lung pathology in individual cases based on radiographic criteria alone is most frequently hard to achieve.

SCINTIGRAPHY

Regional radioactive counts detected over the lungfields with a gamma-camera reflect the density of radioactive tracer's distribution within the target organ. Theoretically, scintigraphy has the capability to quantify the function and/or parameter visualised. In addition, scintigraphy may be performed repeatedly in horses with diagnosed abnormalities to follow the course of a disease process and/or to monitor response to therapy. However, only of few publications report the use of scintigraphy in the investigation of EIPH. The time-consuming nuclear procedures and necessary images analysis have most probably contributed to limit its role as a diagnostic and investigation tool. Benefit of lung scintigraphy in detection and quantification of EIPH has still to be proven but preliminary studies about the ventilation-perfusion relationship, resting and exercising perfusion and about the use of labelled red blood cells (RBC) suggest that this nuclear imaging technique might be a valuable technique.

Ventilation-perfusion studies

Ventilation/perfusion ratios (V/Q) images computed from ventilation-perfusion scans obtained at rest from horses with recent histories of EIPH showed moderately under ventilated areas and marked perfusion deficits in the dorsocaudal lungfields (O'Callaghan *et al.* 1987b). Determination of V/Q mismatches might be of interest to quantify functional disturbance induced by EIPH (Votion *et al.* 1997) but the relationship between V/Q mismatches and the quantification of bleeding seems more hazardous.

Perfusion studies

Regional pulmonary vascular perfusion can be evaluated by imaging lungfields after intravenous injection of radioactive particles which are entrapped in the pulmonary capillary bed. Lung perfusion scanning assumes that the regional distribution of radioactive particles in the lung is a function of blood flow. The radioactive tracer used to visualise the perfusion may be injected to horses in a resting state as well as to horses performing an exercise on a treadmill. Resting and perfusion



Fig 2: Perfusion redistribution induced by exercise (image of the left lung in a healthy horse). Computed image showing the areas more perfused at exercise than at rest. Exercise redistributes blood flow to the dorsocaudal region of the lung, ie the bleeding site in EIPH.

studies may be sequentially performed after a delay corresponding to radioactive decay of the tracer. From exercising and resting perfusion images, computed exercising to resting perfusion ratio images may be obtained. Such images demonstrated that exercise redistributes blood flow to the dorsocaudal regions of the lungs (Harmegnies *et al.* 2002; Fig 2). This particular redistribution may contribute to the location of bleeding sites in EIPH (O'Callaghan *et al.* 1987c). Perfusion studies might contribute to improve the knowledge of the pathophysiological processes involved in EIPH and to assess therapeutic or preventive measures that aim at decreasing blood flow at the bleeding sites.

Red blood cell labeling

In human medicine, the use of labelled autologous RBC is an established means of diagnosing occult gastrointestinal bleeding. The labelling of equine RBC is feasible (Votion *et al.* 1999). To detect pulmonary haemorrhage following intravenous administration of labelled RBC to the exercising horse, the contrast between background activity (ie resulting from circulating radioactive RBC not involved in the haemorrhage) and radioactivity at the bleeding sites must be increased. Altering human RBC with heat during the labelling process stimulates uptake of these cells by the spleen and enables quicker removal of the tracer from the blood pool thus favouring detection of bleeding sites. Applied to horses, this technique did not contribute to remove the background either more quickly or more efficiently, most probably because the active macrophage-monocyte system of the equine lung trapped the heated denatured RBC (Votion *et al.* 1999). Another attempt was made to remove the unwanted background activity using a double isotope scintigraphy. The method was the following: 2 scans were successively acquired. The first scan was obtained following intravenous administration of labelled RBC, withdrawal of a small amount of radioactive blood (ie mixed with normal blood) and instillation of this blood into the

airways of horses to simulate EIPH. The second scan was acquired following administration of RBC labelled by another radioactive element to the resting horse. Knowing that activities of different radioelements may be recorded on 2 different channels by the gamma-camera, it was possible to remove the background vascular radioactivity by computer processing. This method enabled visualisation of the surrogate of bleeding (Votion and Lekeux 2003).

Among the unknowns about EIPH, the quantity of blood released into the airways during an episode is indefinite. Evaluation of bronchoalveolar lavage contributes to assess the severity of bleeding but it does not give the volume of blood that has been released into the lungs. Using phantom studies (studies of the relationship between gamma-camera counting and blood volume by means of instillation of radioactive blood to the dorsocaudal lung fields as surrogate of bleeding), an estimate of the volume of pulmonary haemorrhage might potentially be performed in the future using labelled RBC.

Miscellaneous

Development in the field of radiopharmacy has enlarged the spectrum of nuclear medicine to molecular imaging. Several target-specific agents start to be available such as antibodies and peptides that have affinity for specific receptor. Radiolabelled peptides that target fibrin and that are used in the detection of deep venous thrombosis might constitute an interesting tool for EIPH detection and perhaps quantification.

CONCLUSION

There have been only a small number of field investigations of radiographic findings in horses with EIPH. All of the reports found in the literature conclude that, due to the large variability in findings, interpretation of chest radiographs is of poor value for diagnosing EIPH. *A fortiori* the sensitivity of radiography is too poor to quantify EIPH and lung remodelling. Scintigraphical techniques to detect and quantify EIPH have not yet been validated and most probably will have primarily research applications. However, preliminary studies indicate that scintigraphy might potentially diagnose EIPH, assess the severity of bleeding, monitor the site and time course of pulmonary haemorrhage and follow response to therapy and/or prophylactic measures.

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ROLE OF AIRWAYS IN EIPH

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While several mechanisms may be involved in pulmonary bleeding during exercise in horses, evidence is accumulating that pulmonary capillary wall stress failure is important in the pathogenesis of the condition (West *et al.* 1993). Capillary wall stress failure occurs when the capillary transmural pressure, the difference between intraluminal and extraluminal capillary pressures, exceeds capillary wall strength. Much attention has been paid to the high intravascular pressures in the equine pulmonary circulation during intense exercise as the cause of EIPH. Indeed, during intense exercise pulmonary capillary pressures have been estimated to exceed 100 mm Hg (Manohar *et al.* 1994). However, transmural pressure is the difference between intraluminal and extraluminal pressures, and changes in extraluminal capillary pressures could also be important in the pathogenesis of this disease. The pressure outside the pulmonary capillary, in the alveolar interstitium, is determined primarily by the alveolar pressure. During inhalation, alveolar pressures become negative relative to atmosphere, whereas during exhalation, positive alveolar pressures force air out of the respiratory system. Thus, during exhalation positive alveolar pressure minimises pulmonary capillary transmural pressure, whereas during inhalation the negative alveolar pressure enhances transmural pressure. Interestingly, it appears that pulmonary capillary pressure swings are in phases with airway pressure changes (Ducharme *et al.* 1999). That is, pulmonary capillary pressures are most positive when airway pressures are most positive, whereas pulmonary capillary pressures are more negative when airway pressures are negative. This phasic relationship minimises transmural capillary pressure.

Upper airway obstructions, including recurrent laryngeal neuropathy (RLN), are

common in horses. When affected animals exercise, inspiratory airway pressures are more negative to allow adequate alveolar ventilation in the face of the airway obstruction (Derksen *et al.* 1986). Under these circumstances, it is expected that pulmonary capillary transmural pressures are greater in RLN-affected horses. In spite of this, there is no direct evidence that upper airway obstructions such as RLN contribute to EIPH, and horses with RLN do not have an increased risk of lung bleeding during exercise (Raphel 1982). However there are no large studies in the literature examining this question, and the methodology used to determine severity of EIPH, such as endoscopic score or bronchoalveolar lavage (BAL) fluid analysis, may not be sufficiently sensitive.

Circumstantial evidence suggests that in exercising horses during inhalation, negative airway pressures contribute to EIPH. Most important is a series of studies demonstrating that use of a nasal strip decreases the number of red cells in BAL fluid after exercise (Geor *et al.* 2001; Kindig *et al.* 2001; Valdez *et al.* 2004). In horses, the majority of inspiratory resistance to airflow is located in the upper airway (Art *et al.* 1988). The nasal valve region, located just cranial to the nasoincisive notch is a high resistance region, not supported by bone or cartilage. These characteristics make this region particularly susceptible to collapse during inhalation. Application of the nasal strip to this region prevents nasal collapse, and decreases upper airway resistance during exercise (Holcombe *et al.* 2002). This in turn is expected to reduce negative alveolar pressure during inhalation, and decrease transmural capillary pressures. In horses exercising on the treadmill, application of the nasal strip decreases EIPH (Geor *et al.* 2001;

Kindig *et al.* 2001), and when EIPH is severe, the nasal strip also decreases lung bleeding in Thoroughbreds in training (Valdez *et al.* 2004).

Furosemide, like the nasal strip, decreases the number of red cells in BAL fluid collected after exercise (Geor *et al.* 2001). Furosemide's ability to decrease calculated pulmonary capillary pressure in exercising horses is most likely in part responsible for this beneficial effect (Manohar *et al.* 1994). However, furosemide is also a potent bronchodilator in horses with recurrent airway obstruction (Broadstone *et al.* 1991). This effect is mediated via bronchodilator prostenoids (Rubie *et al.* 1993). In normal resting horses, bronchodilators have no measurable effects on pulmonary function, suggesting that airways are fully dilated. Little is known about airway caliber in exercising horses, but in people, exercise-induced bronchospasm is common, particularly in dry and cold conditions. If this is true in horses, the beneficial effect of furosemide on EIPH may be explained in part by its ability to dilate airways, and to decrease alveolar pressure swings and pulmonary capillary transmural pressures during exercise. Together these data demonstrate that airway caliber has an important influence on EIPH.

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INFLAMMATORY AIRWAY DISEASE AND EIPH: IS THERE A LINK?

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DEFINITION OF IAD

Inflammatory airway disease (IAD) is a mild form of lower airway disease commonly encountered in racehorses that has been recognised recently as a separate entity from recurrent airway obstruction (RAO) and other pulmonary diseases (Robinson 2001). Subsequently, the Dorothy Russell Havemeyer Foundation sponsored a workshop focused on better defining IAD. A working definition of IAD was proposed at the conclusion of the workshop which included:

- Cough, excess airway mucus, and decreased performance.
- Horses are not febrile, not systemically ill, and have normal haematology.
- Mild airway inflammation.
- Some horses exhibit mild airway obstruction and hyper-responsiveness but they do not exert increased respiratory efforts at rest.

EPIDEMIOLOGY OF IAD

In the majority of cases, RAO and IAD may be differentiated based on clinical grounds. However, some have argued that, over time, horses with IAD may progress into RAO (Viel 1997). The prevalence of IAD in racehorses may vary depending on the diagnostic criteria used (endoscopy, cytology) and the conditions of examination (ie pre- versus post exercise). In Standardbreds, the prevalence was reported to be 22% based on post race endoscopy (MacNamara *et al.* 1990). In Thoroughbreds, the prevalence of IAD ranges between 10–40% per month based on a clinical score including visualisation of mucus by endoscopy and cytological analysis of tracheal

wash fluid (Burrell *et al.* 1996; Wood *et al.* 2005). Nasal discharge is noted in only 4% of affected horses whereas 37–48% display neutrophilia of tracheal secretions (>20% cells are neutrophils) (Malikides *et al.* 2003; Wood *et al.* 2005).

Age is a significant risk factor for IAD. Two-year-old Thoroughbred racehorses have a 7 times higher risk of IAD than 3-year-olds (Burrell *et al.* 1996). Also, the prevalence of excess airway mucus decreases significantly between yearlings entering training and racehorses 4 years of age and older (Wood *et al.* 2005). Contrarily, the severity of airway neutrophilia is similar regardless of age whereas, the number of alveolar macrophages containing hemosiderin increases as racehorses get older (McKane *et al.* 1993; Wood *et al.* 2005).

Exercise is another factor influencing the prevalence of IAD. Endoscopic examination of racehorses within 1 h of strenuous exercise reveals presence of excess tracheal mucus in approximately 60% of the time but in only 10–20% of examinations conducted after rest or jogging (Burrell 1985). Similarly, neutrophil count increases in respiratory secretions up to 24 h after exercise thereby increasing the rate of false positive diagnosis of IAD (Couëtîl and Denicola 1999; Martin *et al.* 1999).

Isolation of bacteria from tracheal wash samples collected from racehorses in training is more common in young animals entering training (1–2 years old) and is associated with the degree of neutrophilic inflammation (Wood *et al.* 2005; Chapman *et al.* 2000). No infectious aetiology is evident in 27–90% of horses with IAD. Exposure to inhaled dust, endotoxin and other airway irritants appears to play a role in IAD pathophysiology as well (Hodgson *et al.* 2005). Viral infections are rarely associated with IAD.

EFFECT OF IAD ON LUNG FUNCTION

Horses with IAD exhibit various degrees of bronchiolitis resulting in mild peripheral airway obstruction. Airway obstruction may be detected using sensitive tests of lung function or by measuring blood gases exchange during exercise which provides a physiological explanation to the adverse effect of IAD on performance (Hoffman *et al.* 1998; Couëttil *et al.* 2001; Sanchez *et al.* 2005).

EVIDENCE IN FAVOUR OF A LINK BETWEEN IAD AND EIPH

The possible role of small airway obstruction in the pathophysiology of exercise-induced pulmonary haemorrhage (EIPH) was first proposed in 1978 (Robinson and Sorenson 1978). A comprehensive study of racehorses with EIPH revealed that the bleeding occurs almost exclusively in the caudo-dorsal lung areas and is associated with macrophagic bronchiolitis and fibrosis (O'Callaghan *et al.* 1987). Subsequent experimental studies in ponies sensitised to ovalbumin demonstrated EIPH in lung areas with severe ovalbumin-induced neutrophilic bronchiolitis (Derksen *et al.* 1992). However, pulmonary hemorrhage occurred in all lung areas instilled with ovalbumin regardless of location (cranial versus caudo-dorsal lung) and was also observed in ponies that were not exercised.

More recently, an epidemiological study of Thoroughbreds in training demonstrated evidence of an association between IAD and EIPH (Newton and Wood 2002). The study found a 3–12 fold increase in the odds of observing blood endoscopically in horses with mild to severe IAD. Horses with mild to moderate IAD had also higher odds (2.3–4.4) of having >50% haemosiderophages in tracheal secretions. The main issue with these findings is that horses were examined every month and the timing of endoscopy and tracheal wash compared to exercise was not taken into account. As discussed above, the intensity of exercise and the timing of examination have major effect on both airway mucus and cytology.

EVIDENCE AGAINST A LINK BETWEEN IAD AND EIPH

Several epidemiological studies have found no association between the number of

haemosiderophages in tracheal secretions and IAD, cough, or the number of neutrophils (Burrell 1985; Chapman *et al.* 2000; Christley *et al.* 2001). Similarly, no correlation has been reported between haemosiderophage and neutrophil counts collected by bronchoalveolar lavage or tracheal wash (Clark *et al.* 1995; Martin *et al.* 1999; Sanchez *et al.* 2005).

The discrepancy between these studies is likely to originate from differences between methodologies, case definitions and heterogeneity of the IAD phenotype. In particular, bronchoalveolar lavage and tracheal wash fluid cytologies do not correlate (Derksen *et al.* 1989; Malikides *et al.* 2003). Furthermore, bronchoalveolar lavage fluid cytology correlates well with lung histopathology but tracheal wash does not (Viel 1983; Larson and Busch 1985; Winder *et al.* 1989). Exceptions to this rule are focal or localised lung diseases such as bronchopneumonia or pulmonary abscess that are likely to be missed by bronchoalveolar lavage (Rossier *et al.* 1991).

FUTURE DIRECTIONS

Better definitions and characterisation of IAD phenotypes are critical to the success of future studies of equine pulmonary diseases. In addition, the timing of diagnostic tests such as endoscopy and sampling of respiratory secretions will have to be included in the study design. Finally, a better understanding of IAD and EIPH pathophysiology is needed in order to study the potential interaction between the 2 diseases.

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EIPH AS A CAUSE OF AIRWAY INFLAMMATION AND REMODELLING

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Cook (1974) theorised that the 2% of racehorses which displayed epistaxis originating from pulmonary haemorrhage probably had a predisposing pulmonary inflammatory disease. Both acute viral respiratory disease and chronic bronchiolitis were implicated by Cook in this idea, although the involvement of acute viral disease was, according to Cook himself, largely based on circumstantial evidence and no serological evidence of infection was ever gathered from the horses. In fact even with the knowledge of exercise-induced pulmonary haemorrhage (EIPH) available today, it still seems likely that horses not involved in intense exercise work which suffer severe episodes of EIPH may have underlying inflammatory disease which predisposes them to haemorrhage during moderate exercise.

After post mortem examinations of 117 retired racehorses and histological evaluation of the lungs from 55 of these, Mason *et al.* (1983) observed gross haemorrhagic lesions located bilaterally in the caudal tips of the caudal lung lobes in 82% and histological evidence of old haemorrhage in the same regions in 96% of the horses evaluated. There was histological evidence of bronchiolitis in the haemorrhagic regions, as well as a mild mononuclear cell infiltrate, excessive peribronchiolar fibrous tissue and alveolar scarring. Mason *et al.* (1983) concluded that the histological changes present were not consistent with chronic obstructive pulmonary disease, but did not comment on their relevance to viral disease or whether or not the changes were a cause or effect of EIPH.

In 1987 a series of 8 papers was published in which the authors' conclusions supported the theory that predisposing pulmonary inflammatory disease was the cause of EIPH (O'Callaghan *et al.* 1987). O'Callaghan *et al.* reported that areas of

darker staining appeared less compliant and contained substantial fibrotic change when examined grossly. Microscopic examination of haemosiderin stained lesions within the lung demonstrated extensive bronchiolar artery proliferation and widespread bronchiolitis with peribronchiolar fibrosis, which was concluded to result from viral inflammatory pulmonary disease. More recently an epidemiological study (Newton and Wood 2002) confirmed an association but not a causal link between EIPH and pulmonary inflammation in racehorses. This study found that changes in bronchoalveolar lavage cytology consistent with inflammation were found in horses that had suffered EIPH. This inflammation did not appear to be related to bacterial bronchopneumonia, but such a study could not identify whether prior viral injury could be the cause of peribronchial fibrosis that might predispose to EIPH. In the study by O'Callaghan *et al.* (1987) it was observed that the dark blue lesions grossly visible on the surface of the lung appeared to originate at the tips of the caudal lobe and extend cranially, with areas of lighter brown staining, consistent with fresh episodes of EIPH found adjacent to the older areas. The investigators confirmed this by noting haemosiderophages and fibrous tissue reaction and haemorrhaged erythrocytes respectively (O'Callaghan *et al.* 1987), indicating that EIPH lesions may extend outward from the caudal lung tip with the development of new episodes of haemorrhage, which may explain the increase in severity as horses age.

The theory of pulmonary inflammatory disease predisposing to all cases of EIPH is difficult to accept based on the prevalence, since almost every Thoroughbred racehorse is expected to display EIPH at least once throughout its racing

career. This theory would infer a massive prevalence of undiagnosed equine respiratory disease. An alternative hypothesis is that blood induces airway inflammation and that this leads to the observed peribronchial fibrosis and mononuclear cell infiltrates. Two studies by McKane and Slocombe (1998 and 2002) demonstrated that autologous blood inoculated into the airways was removed quite slowly and that by 3 days modest airway inflammation developed, that persisted for as long as 21 days. Initially this response was neutrophil dominated, but then a more chronic and persistent phase occurred that was characterised by increased macrophage numbers and morphological signs of marked macrophage activation and erythrophagocytosis.

The most noticeable change in cell morphology occurred in the macrophages which were observed to increase in size and vacuole content within the first 3 days of blood inoculation, prior to substantial erythrophagocytosis occurring. At about Day 10 a number of macrophages were noted to develop golden brown granules of various sizes, indicating the early stages of haemosiderin production. Small numbers of macrophages were also observed to undergo transformation to epithelioid and giant cell forms, consistent with high levels of macrophage activation. Neutrophil percentages were observed to more than double within the first 24 h from 3.5% to 10.5% of all leucocytes present. After Day 3 the neutrophil percentage decreased toward pre-inoculation levels and remained there for the rest of the sampling period. There was a surprisingly slow removal of erythrocytes from the lung, with erythrocytes evident in the lavages of all horses at both 14 and 21 days following inoculation. This has implications for the management of racehorses which are known to suffer adversely from episodes of EIPH, particularly under racing conditions where horses are raced as frequently as once a week for 3 or more months during a racing campaign or season. The results of this study suggest that this may be too frequent to allow sufficient removal of erythrocytes and resolution of respiratory inflammatory damage between races and training gallops, causing an accumulation of erythrocytes within the airways of the lung and cumulative adverse effects on the health of the lung and performance of the horse.

Morphometric analysis of histological sections was used to quantify the effects of this macrophage activity during erythrophagocytosis,

on alveolar cell populations and the physical structure of the alveolar walls. Signs of chronic inflammation and peribronchial fibrotic remodelling, in response to the presence of blood in the airways, included increased macrophage activity and erythrophagocytosis, increased alveolar macrophage numbers ($10,688 \pm 1,708$ cells/ μm^3 to $30,957 \pm 6,831$ cells/ μm^3), increased septal thickness (4.1 ± 0.4 μm to 6.1 ± 0.5 μm) and an increased alveolar septal collagen content ($6.6 \pm 0.5\%$ to $27.5 \pm 3.3\%$). These results confirmed that intrapulmonary blood was capable of inducing a prolonged macrophage dominated inflammatory response, which resulted in increased alveolar septal thickening and the development of alveolar fibrosis. Signs of chronic inflammation including increased macrophage activity and erythrophagocytosis coincided with increased alveolar macrophage numbers ($10,688 \pm 1,708$ cells/ μm^3 to $30,957 \pm 6,831$ cells/ μm^3), septal thickness (4.1 ± 0.4 μm to 6.1 ± 0.5 μm) and alveolar septal collagen content ($6.6 \pm 0.5\%$ to $27.5 \pm 3.3\%$). Sections obtained 8 and 15 days after blood inoculation were observed to contain accumulations of amorphous material within the alveolar septa that stained positive for collagen in Masson's trichrome preparations.

The data suggest that intrapulmonary blood has a number of dramatic effects on the lung that centre on the macrophage response and removal of the erythrocytes. In fact blood appeared to produce an overall difference in many morphometric values including the thickness of alveolar septa, the amount of septal collagen present, and the number of alveolar macrophages. These results confirm the role of intrapulmonary blood in being able to induce a macrophage dominated inflammatory response and provoke alveolar septal fibrosis. The results suggest that intrapulmonary blood induces a macrophage dominated inflammatory response, septal thickening, and the development of alveolar fibrosis. Since serum had no effect on alveolar cell populations, it appears that the erythrocytes directly provoke the alveolar inflammatory response. Presumably there are changes that occur in the erythrocytes once they have haemorrhaged into the alveolar space which trigger erythrophagocytosis, however the signals that direct phagocytosis of autologous erythrocytes are poorly understood at this time. The precise signalling pathway for recognition of the erythrocytes as foreign, the activation of the

macrophage population and subsequent erythrophagocytosis are unknown, but it is likely that this activation of the macrophage population results in the production of transforming growth factor- β and subsequent fibrosis.

Following the development of alveolar fibrosis full restoration of normal pulmonary mechanical properties is unlikely. Normally the alveolar septa contain little collagen and are therefore very compliant, however as the content of collagen within the septa increases the compliance will decrease. Robinson and Derksen (1980) proposed that local inhomogeneities in compliance resulting from pulmonary scar tissue formation could lead to local alterations in pulmonary capillary transmural pressures and capillary rupture. The characteristics of EIPH to originate in a focal region of each lung and then expand cranially may be related to a concurrently expanding region of alveolar fibrosis that develops with subsequent episodes of EIPH at the interface between regions of different tissue compliance. If alveolar fibrosis can predispose to EIPH, then the horse is presented with a condition that will incite

haemorrhage, inflammation, further fibrosis and then new haemorrhage throughout its life, with the probability that the episodes of haemorrhage will increase in severity as the amount of parenchymal tissue involved increases. Circumstantial evidence of this may be the finding that EIPH severity increases in older horses (Mason *et al.* 1983; McKane *et al.* 1993).

Alveolar fibrosis and bronchiolitis was once suspected to be the originating cause of EIPH. Instead it is likely that EIPH is more often the cause of alveolar fibrosis and bronchiolitis. This does not however preclude a role for inflammation in the aetiology of some cases of EIPH. Even though the theory that all cases of EIPH result from previous pulmonary inflammation is unlikely, it does not preclude the fact that some cases of EIPH may well be predisposed to or made more severe by strenuously exercising horses that are suffering from pulmonary inflammatory disease in any form. Finally it is speculated that alveolar fibrosis may play a role in determining the distribution, recurrent nature, and increasing severity of EIPH in older horses.

CARDIAC DISEASE AND EIPH

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INTRODUCTION

Exercise-induced pulmonary haemorrhage (EIPH) and tricuspid (TR) and mitral valve (MR) regurgitation occur frequently in conditioned performance horses (Pascos *et al.* 1981; Raphael and Soma 1982; Patteson and Cripps 1993; Kriz *et al.* 2000; Young and Wood 2000) and the prevalence of all 3 conditions increases with time in intensive training (Raphael and Soma 1982; Oikawa 1999; Young and Wood 2000). Although the precise pathogenesis of EIPH remains elusive and the condition is generally considered to be multifactorial (Marlin 2003); all available evidence nevertheless points to stress failure of pulmonary capillaries, caused by high transmural pressures, being pivotal in its aetiology (West *et al.* 1993). The left atrium can also exert independent influences on pulmonary capillary and thence transmural pressure: the positive association of EIPH and atrial fibrillation, a condition that independently increases left atrial pressure is well-recognised in equine athletes (Fregin and Deem 1980). However, atrial fibrillation is not the only commonly occurring cardiac condition able to elevate left atrial, thence pulmonary venous pressures. Once left atrial compliance has been exceeded, MR also causes pulmonary venous hypertension; indeed this is the underlying pathogenesis of the pulmonary oedema that occurs in congestive heart failure.

The horse's ability to generate a cardiac output in excess of 250 l/min at maximum exercise (Poole 2004) and the resultant increases in pulmonary vascular and left atrial pressures (Erickson *et al.* 1990; Manohar 1993; Manahar *et al.* 1997) also create a unique set of loading conditions and stresses for the equine right

ventricle compared to other mammals (Young 2003). In all species, the right ventricle (RV) is a structurally complex chamber with an elongated irregular crescent-like configuration that endows a very large surface area relative to its intracavitary size; though difficult to measure accurately using ultrasound, it is nevertheless, ideally adapted to efficiently discharge a large volume of blood into the normally low resistance pulmonary circulation. Its anatomical position, wrapped around the cranial surface of the left ventricle facilitates this 'bellows-like' action; only minimal myocardial fibre shortening being needed to cause sufficient axial excursion of the RV free wall towards the interventricular septum and displace the stroke volume (Jiang *et al.* 1994). In comparison to the left ventricle, the structural and functional responses of the equine RV to high intensity exercise and training has not been well explored. Yet the uniqueness of the Thoroughbred's pulmonary circulation during high-speed exercise make the adaptations of the equine RV particularly intriguing. Recent data has shown that the equine right ventricle undergoes significant change in chamber size in response to exercise and training (Lightfoot *et al.* 2006) and that its internal dimensions in systole and diastole are positively associated with the presence of TR assessed by colour flow doppler (CFD) (Helwegan *et al.* 2006). As TR is a sequel to athletic training in racehorses and as RV adaptations to exercise will be influenced by the loading conditions imposed by the pulmonary circulation, which might in turn be modified by EIPH, we performed a study to examine the possible associations between the 2 conditions and to explore whether EIPH had independent effects upon RV chamber size.

MATERIALS AND METHODS

An echocardiographic and auscultation study was conducted in 121 race-fit National Hunt Thoroughbreds in 3 commercial training yards. Horses were aged 6.2 (± 2.0) years and weighed 497 kg (± 24) kg. Cardiac auscultation and echocardiography were performed. A guided M Mode image of the RV just below the tricuspid valve was obtained from a right parasternal location and CFD was used to interrogate the tricuspid valve and right atrium. The mitral valve was similarly examined from the left haemithorax. An experienced echo-cardiographer retrospectively graded the severity of TR and MR by CFD using a 1–9 scale. Binary data on EIPH, based on whether the horse was perceived to have a clinically significant problem, were determined retrospectively for each horse by the horses' primary care veterinary surgeon from medical and other records. Data were analysed using a standard logistic regression analysis approach.

RESULTS

The prevalence of EIPH in our population was 20.7% (25/121) and that of TR and MR measured by CFD was 83.5% (101/121) and 40.5% (49/121), whilst that of murmurs TR and MR was 40.5% (49/121) and 16.5% (20/121). The mean size of RV lumen in diastole (RVIDd) was 5.72 cm (s.d. 0.90) and that in systole (RVIDs) was 4.45 cm (s.d. 0.97). EIPH was significantly and positively associated with the systolic and diastolic dimensions of the RV ($P=0.017$ and 0.011 respectively) and this association was not sensitive to the effects of age or weight. There were no significant associations between EIPH and TR or MR by auscultation or CFD (TR: auscultation $P=0.1$; CFD $P=0.2$ and MR: auscultation $P=0.07$; CFD $P=0.37$).

DISCUSSION

The data failed to demonstrate significant associations between EIPH and AV regurgitation in conditioned Thoroughbred racehorses, although the P value for the association of EIPH with murmurs of mitral valve regurgitation (Grade $> 3/6$) was 0.07 and thus approached statistical significance.

Of interest, however, was the positive association demonstrated between EIPH and RVIDd and RVIDs, the dimensions of the right

ventricular lumen, measured at a standard position relative to the LV. These associations suggest that the factors that result in EIPH might directly, or indirectly affect RV remodelling in horses. Unfortunately the present dataset do not allow the mechanisms of this association to be explored further as this would require invasive haemodynamic studies. However, RV chamber dilation is most likely to be a response to increased volume loading of the RV, not to increased pressure. Provided that RV lumen width is directly proportional to RV volume - an assumption that regrettably cannot be tested in the irregularly shaped RV - our data might support the assertion that horses affected by EIPH are subject to increased volume loads during exercise; indirectly supporting the hypothesis that pulmonary vascular pressures at high intensities are influenced more by cardiac output than by pulmonary vascular resistance (Manohar and Goetz 1999). Alternatively, increased pressure load on the RV will result in compensatory increases in wall thickness to reduce wall stress according to La Place's law. Nevertheless, evidence of increased vascular pressures during exercise in horses affected by EIPH is not supported by current literature, as thus far workers have failed to demonstrate differences in pulmonary arterial pressures in affected compared with non-affected horses (Erickson *et al.* 1990; Manohar and Goetz 1996).

We have recently shown that RVIDs and RVIDd are also positively associated with the severity of TR assessed by colour flow Doppler echocardiography, however as there was no association in the present dataset between TR and EIPH, it seems likely that the association of EIPH with the dimensions of the right ventricular lumen are independent of any tricuspid valve incompetence.

This study was limited by the method used to classify EIPH, but there was no association between EIPH and horse age, MR, TR or weight in this population of Thoroughbreds. Nevertheless, RV size was greater in horses severely affected by EIPH, suggesting that factors resulting in EIPH may directly or indirectly affect RV remodelling in athletic horses.

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EFFECTS OF LIMB LOADING AND RETRACTION ON THORACIC SHAPE DURING GAIT

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INTRODUCTION

The equine thorax has been thought to be relatively rigid, and as such, only capable of minor changes in shape during ventilation (Bramble 1989). However, classic descriptions of the horse's breathing strategy as a biphasic process imply that the intercostal muscles and associated movements of the ribs are involved at least in the expiratory phase to force residual air from the lungs (Koterba *et al.* 1988). Compounding this process are the interactions of the forelimbs with the chest wall, as the horse couples its locomotor pattern to its breathing pattern. Bramble and Carrier (1983) showed that cyclic locomotor movements and ventilation both involved movements of the ribs, sternum and associated musculature, and Ainsworth *et al.* (1996) suggested the horse couples its ventilatory pattern to its stride pattern to minimise the mechanical constraints to ventilation imposed by these limb movements. The supposed 'visceral piston' is also thought to contribute to ventilation, although Young *et al.* (1992) have shown that inertial movements of the abdominal viscera do not coincide completely with ventilatory air flow. Marlin *et al.* (2002) used respiratory impedance plethysmography to track changes in total chest circumference alongside air flows, and showed that there were only very small changes in

circumference despite very large changes in tidal volume at the faster gaits. Against this confusing and somewhat contradictory background, we have evaluated the changes in transverse dimension of the right and left hemithorax during gait, and also changes in the dorso-ventral (D-V) dimension.

EXPERIMENT ONE

Two horses performed an incremental exercise test on the treadmill. Reflective markers were glued to the dorsal midline of the horse over the tips of the spinous processes, to the lateral chest wall over the positions of ribs 10 and 16, and to the forehooves. Four infrared cameras were positioned bilaterally at the four corners of the treadmill to capture the motion of these markers in 3-D. Ventilatory air flows were recorded simultaneously using ultrasonic flow transducers in a breath-by-breath system. Data were collected from the horses standing at rest on the treadmill immediately before and after exercise, and at walk, trot, canter and gallop.

Table 1 shows that the amplitudes of change in thoracic hemidiameter were approximately symmetric at walk and trot, but were asymmetric at canter and gallop. The changes in thoracic hemidiameter were larger on the side of the trailing forelimb at canter and gallop, and this

TABLE 1: Amplitudes of excursion in right and left transverse hemidiameter (mm) measured at ribs 10 and 16 in 2 horses. Both horses cantered and galloped on the right lead

Rib	Standing (Pre-Ex)	Walk (1.8 m/s)	Trot (3.5 m/s)	Canter (6.0 m/s)	Canter (8.0 m/s)	Gallop (10.0 m/s)	Standing (Post-Ex)
R Rib 10	15	32	34	25	30	35	22
L Rib 10	10	33	30	50	60	68	27
R Rib 16	13	48	34	31	37	46	21
L Rib 16	10	46	32	36	45	49	21

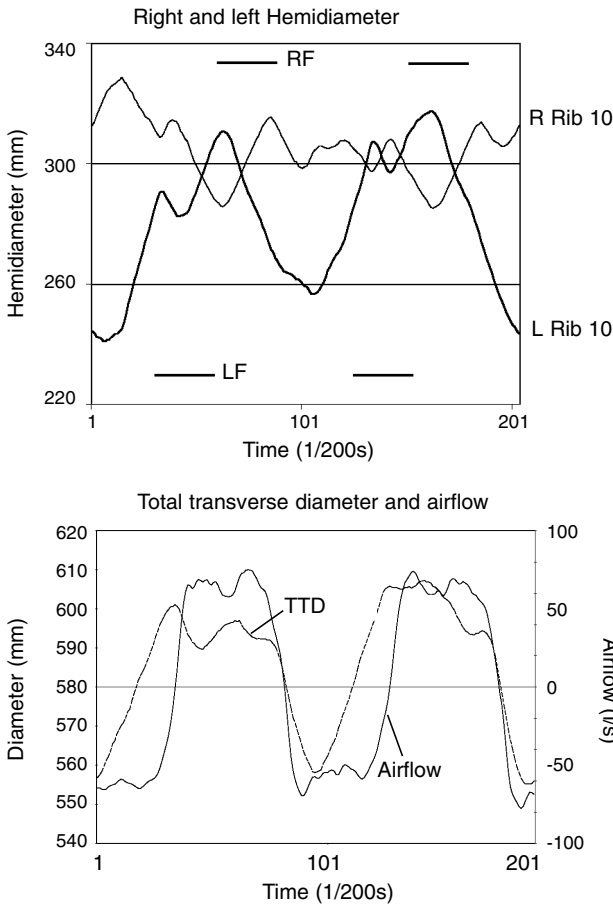


Fig 1: Two canter stride cycles (8.0 m/s) by a horse on the right lead. Changes (mm) in leading side (solid line) and trailing side (dashed line) thoracic hemidiameter at rib 10 are shown in the left graphs. Right (RF) and left (LF) forelimb stance phases are shown as horizontal lines. Airflow (solid line) and total transverse thoracic diameter (dashed line) are shown in the right graphs. Positive airflow values are expiratory, and negative values are inspiratory.

discrepancy was greater at rib 10 than at rib 16. A representative canter trial (8.0 m/s) is shown in Figure 1. On the side of the trailing forelimb, change in thoracic hemidiameter is approximately double the amplitude of change on the side of the leading forelimb. Increases in hemidiameter occurred during ipsilateral stance on both sides. Total transverse diameter changed by about 5cm per breathing cycle, and was not completely in phase with airflows.

EXPERIMENT TWO

To evaluate D-V changes in thoracic dimension alongside the transverse changes, reflective markers were glued to the tip of the T10 spinous process, and circumferentially around the lateral and ventral thoracic wall. Data were recorded from both sides independently, but only one side was evaluated per test, with all 4 cameras located on that side for maximal marker tracking and accuracy. Four horses

underwent tests at trot (3.5 m/s), slow canter (6.0 m/s) and fast canter (8.0 m/s) on the level treadmill, and on the treadmill inclined to 10% slope. They were also each tested on separate days cantering on right and left leads to evaluate lead:slope interaction. Pearson product-moment correlations were calculated for transverse and D-V movements.

At trot (Table 2) there were no left-right differences in transverse hemidiameter, either on the level or inclined treadmill, although the excursion measured was slightly larger when on the incline. Likewise, the D-V marker excursions were symmetric. There were negative correlations between transverse and D-V movements on the level ($r = -0.288, P < .05$) and inclined ($r = -0.431, P < .05$) treadmill. At canter, the side of the trailing forelimb underwent a larger transverse displacement than did the leading side, as observed in Experiment 1. For example, at 8.0 m/s (Table 3) the trailing side went through transverse excursions of 49 mm and 50 mm when on the right

TABLE 2: Mean amplitudes of change in left and right transverse hemidiameter (mm) and dorso-ventral diameter (mm) in 4 horses trotting on the level and inclined treadmill at 3.5 m/s

Trot	Transv. Hemid.		D-V Diam.	
	Left	Right	Left	Right
Level	35	38	34	34
Incline	39	40	32	33

and left leads respectively, while the D-V displacements were 49 mm and 54 mm. At 6.0 m/s on the level treadmill there were negative correlations between the transverse and D-V displacements for the leading ($r = -0.263$, $P < .05$) and trailing ($r = -0.702$, $P < .01$) sides. On the inclined treadmill these were ($r = -0.038$, ns) for the leading side, and ($r = -0.653$, $P < .01$) for the trailing side. At 8.0m/s on the level treadmill there was a positive correlation between transverse and D-V motions on the leading side ($r = 0.259$, $P < .05$) and negative on the trailing side ($r = -0.783$, $P < .01$). On the inclined treadmill, there was likewise a positive correlation for the leading side ($r = 0.335$, $P < .05$) and a negative correlation for the trailing side ($r = -0.687$, $P < .01$). At both canter speeds, there was an apparent lead:slope interaction. On the inclined treadmill, the trailing side increased its excursion, but only when on the right lead.

DISCUSSION

Schroter *et al.* (1999) proposed a model of wave transmission through the thoracic wall and lung after forelimb impact with the ground and showed that the effects of an impact-induced wave would be focussed in the dorsocaudal lung, where exercise-induced pulmonary haemorrhage is most often found. Our findings suggest this model would need to take into account the apparent asymmetry in thoracic geometry, and the different effects of the leading and trailing forelimbs. Ground reaction forces at canter have been shown to vary between leading and trailing forelimbs (Merkens *et al.* 1993). There is only a very small braking phase after contact of the trailing fore, and this limb is mostly propulsive. In contrast, there is a larger braking action by the leading fore, and a smaller propulsive phase. The muscles responsible for forelimb retraction originate from the ribs and dorsal fascia caudal to the scapula (*latissimus*

TABLE 3: Mean amplitudes of change in left and right transverse hemidiameter (mm) and dorso-ventral diameter (mm) in 4 horses cantering on left and right leads on level and inclined treadmill at 8.0 m/s

Canter		Transv. Hemid.		D-V Diam.	
		Left	Right	Left	Right
Level	R lead	49	37	49	33
	L lead	34	50	27	54
Incline	R lead	64	42	56	39
	L lead	38	48	46	57

dorsi, serratus ventralis, cutaneous trunci). It is likely that the trailing forelimb is forcefully retracted after hoof contact, and that the ribs are pulled cranially as the muscles shorten to pull the trunk forward over the planted hoof. However, the hemidiameter traces show that both sides increase their hemidimension during ipsilateral forelimb support at canter.

The markers on the dorsal midline may have been affected to some degree by lateral bending or axial rotation of the spine. Faber *et al.* (2001) have shown that the spine has a single period of lateral bending during canter, in that it bends away from the supporting forelimb. How this might affect the measurements in these studies is unknown, although it would be reasonable to expect the ribs to move laterally with the spine as they are attached to it.

It is difficult to assess the apparent lead effect at canter on the incline. Horses are known to have preferred leads, and the data shown here suggest that they 'pull' themselves uphill with their trailing forelimb at canter, and that this may cause further cranial displacement of the ribs on that side.

The thoracic compartment appears to change its shape during locomotion by expanding transversely at the expense of the D-V dimension, possibly yielding only a small change in thoracic circumference. Whether the individual lungs are ventilated differently according to leading limb remains to be determined.

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METABOLIC, CARDIOVASCULAR AND RESPIRATORY RESPONSES TO SWIMMING IN HORSES

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Exercise-induced pulmonary hemorrhage (EIPH) occurs, as the name specifies, in association with intense exercise in horses, eg, running at high speeds or pulling against heavy loads. Because swimming is heavy exercise for horses requiring significant increases in aerobic power and O₂ delivery, it seems a likely activity also to be associated with the occurrence of EIPH. However, review of the literature finds no documented reports of EIPH occurring with swimming.

Swimming is a popular mode of exercising horses either for training or as a means of maintaining cardiorespiratory fitness during layups due to musculoskeletal injuries. Nicholl *et al.* (1978) reported that swimming horses occasionally experienced epistaxis, but did not indicate if the blood was pulmonary in origin. Veterinarians of the Japan Racing Association have extensive experience with swimming horses in pools at their Miho and Ritto Training Centers. They observe that a number of horses experience epistaxis after swimming. When examined with endoscopy, however, they invariably have found that the blood originates from the pharynx, not the lungs. Trainers at the Macao Jockey Club occasionally observe horses with EIPH after swimming, confirmed by endoscopy. However, those horses had run on a training track prior to swimming, so there is no way of determining whether they actually experienced EIPH while running and the blood simply arrived at the nares after swimming (Birks, personal communication).

Given that it is possible that horses exercise heavily while swimming without experiencing EIPH, it is of interest to compare how metabolic and cardiopulmonary variables change while swimming compared to intense running exercise. Differences in the physiological responses to these 2 forms of exercise could suggest what factors are

of greater significance in terms of causing EIPH to occur. To facilitate making such comparisons, we have compiled in Table 1 data from the major peer-reviewed papers that have reported on the metabolic and cardiopulmonary responses of horses to swimming, and have included a set of similar data collected during treadmill galloping for comparison. Those findings are summarised below.

It is somewhat difficult to compare the results of different swimming studies because the ages of horses used, protocols employed, variables measured, etc. varied greatly between studies. Even external work rates of different studies are hard to compare because some studies were conducted with the horses tethered and pulling against a strain gage, whereas, others employed horses swimming around a circular pool or in a straight line at different speeds. Heart rate (HR) is one variable that was measured in all studies and offers a means of relating relative metabolic intensities between studies because the fraction of a horse's maximum HR is closely correlated with the fraction of its maximum rate of O₂ consumption (VO_{2max}). Comparing raw HR values must be done with some caution because the same HR may represent a significantly different percentage of maximum HR for horses between studies due to age differences or individual variation between animals.

Oxygen consumption (VO₂) during swimming has only been measured in one study (Thomas *et al.* 1980). The peak mass-specific value they reported (2.0 ml O₂ (STPD) s⁻¹ kg⁻¹) during tethered swimming is only approximately 70–80% the expected maximum rate of VO_{2max} of a trained horse. These workloads elicited plasma lactate accumulation rates (<3 mM min⁻¹) and heart rates (200 min⁻¹) that both indicate metabolic power was submaximally aerobic. Misumi *et al.* (1994) recorded higher HR (205–220 min⁻¹) approaching

TABLE 1: Summary of metabolic and cardiopulmonary data reported for swimming horses. Abbreviations and coding as follows: Authors – source of data, ‘Treadmill data’ are for horses running at VO_{2max} on an equine treadmill at UC Davis; Pool type – “circ pool” is circular pool; Time – duration of experiment, ‘tet’ is tethered swimming; N – number of horses studied, ‘SB’ is Standardbred, ‘TB’ is Thoroughbred, ‘WB’ is Warmblood; Train – training history; HR – heart rate; RR – respiratory rate; RR+1 – respiratory rate 1 min after swimming; PCV – packed cell volume; [Hb] – blood haemoglobin concentration; [LA] – end-swim blood or plasma lactate concentration; VO_2/kg – mass-specific O_2 consumption; Q/kg – mass-specific cardiac output; V_E/kg – mass-specific minute ventilation; P_a – mean arterial blood pressure; P_{pa} – mean pulmonary arterial blood pressure; P_{ra} – mean right atrial pressure; PaO_2 – arterial O_2 tension; $PaCO_2$ – arterial CO_2 tension; pH_a – arterial pH; $T_I:T_E$ – ventilatory duty cycle; P_{trach}^I – intratracheal peak inspiratory and peak expiratory pressures; P_{oesoph} – oesophageal (\pm intrapleural) peak inspiratory and expiratory pressures; values in parentheses are maximum values reported, others are means or ranges

Authors	Pool	Speed type $m\ s^{-1}$	Time min	N #/breed	Age yr	Train	HR min ⁻¹	RR min ⁻¹	RR +1 min ⁻¹	PCV %	[Hb] g dl ⁻¹	[LA] mM
Asheim et al. 1970	'pool'	1.3	4	6 SB	2-9	yes (?)	190 (212)	48 (60)		57 (62)	21 (23)	6 (9)
Fregin and Nicholl 1977	circ	NA	2/tet 1	6 mix	9	3 wk swim	174	24	45	54	19	
Fregin et al. 1977	circ	NA	2/tet 4	6 TB-X	4-15	6 wk swim	163	26	57	57	19	
Milne et al. 1977	circ	NA	2/tet 4	5 TB/ TB-X	4-15	6 wk swim	159	28				
Thomas et al. 1980	circ	NA	tet 2/3	3 TB/ SB	6-10	?	185-200					4-10
Misumi et al. 1994	100 m pool	1.3	1.3	7 TB	2	4 mo run/swim	220-205					11-3
Hobo et al. 1998	circ	1.1	5	5 TB	3-4	?	195	27	59	57		2
Jones et al. 2002 (Treadmill data)	circ	1	2	2 TB/ 2 WB	4	5 mo tread	184	28				
		15	2.5	5 TB	5	6 mo tread	216	132		64	23.4	28

Authors	VO_2/kg $ml\ s^{-1}\ kg^{-1}$	Q/kg $ml\ s^{-1}\ kg^{-1}$	V_E/kg $ml\ kg^{-1}$	P_a torr	P_{pa} torr	P_{ra} torr	PaO_2 torr	$PaCO_2$ torr	pH_a	$T_I:T_E$	P_{trach} torr	P_{oesoph} torr
Milne et al. 1977	5.8			196			77	45		7.3		
Thomas et al. 1980	1.2-2	6.7-9	31	234-254	90-110	45-55						
Hobo et al. 1998							90	51	7.28	1:2	-34/ +38	
Jones et al. 2002 (Treadmill data)	2.61	12.3	60	241	114	58	64	56	7.1	1:1		-29/ +26

those expected at VO_{2max} during running when swimming horses in a straight pool. Their horses had more prior exercise training than in any other swimming study except that of Jones *et al.* (2002), and they recorded blood lactate accumulation rates as high as $9\ mM\ min^{-1}$, suggesting they were exercising near VO_{2max} . In human athletes other than elite swimmers, there is a tendency for

VO_{2max} during swimming to be lower than that measured during running or cycling. Heart rates at maximum exercise during swimming tend to be lower in humans than during maximum exercise on land.

Thomas *et al.* (1980) measured mean blood pressures in the carotid artery (250 torr), pulmonary artery (105 torr) and right atrium (50 torr) during

tethered swimming in horses with HR of 185–200 min^{-1} . All blood pressure values are similar to those of horses exercising at $\text{VO}_{2\text{max}}$ on a treadmill, when EIPH can occur. Mass-specific cardiac output (Q/M_b , where M_b is body mass in kg) while swimming at this intensity was approx 70% that of horses at $\text{VO}_{2\text{max}}$ on a treadmill, although the extent of aerobic conditioning that the swimming horses had received is unknown. Milne *et al.* (1977) reported lower Q/M_b in swimming horses with lower HR.

Both Milne *et al.* (1977) and Hobo *et al.* (1998) measured arterial blood gases of horses swimming with HR of 159 and 195 min^{-1} , respectively. Milne *et al.* (1977) reported mild hypoxemia (77 torr), mild hypoventilation (45 torr) and mild acidosis (7.3), whereas Hobo *et al.* (1998) measured no hypoxemia (90 torr), modest hypoventilation (51 torr) and mild acidosis (7.28). None of these values was as extreme as during treadmill exercise at $\text{VO}_{2\text{max}}$.

Ventilation during swimming is slower than during galloping (0.5 Hz vs. 2.2 Hz) and deeper. During swimming horses maintain a ventilatory duty cycle (inspiratory time:expiratory time) of 1:2, with nearly 2/3 of the ventilatory period being apneic at full inspiratory volume with a closed glottis. Inspiratory and expiratory flow times are approx equal. Jones *et al.* (2002) measured oesophageal pressures (P_{oesoph}) during swimming and galloping and found that when swimming, horses had higher expiratory pressures (100 cm H_2O vs. 35 cm H_2O) but reduced magnitude in subatmospheric inspiratory pressures (-19 cm H_2O vs. -39 cm H_2O).

In general, it appears that in studies in which cardiopulmonary variables have been measured in swimming horses, the horses have not reached $\text{VO}_{2\text{max}}$. Maximum HR and Q reached in swimming studies tend to be lower than at $\text{VO}_{2\text{max}}$ on a treadmill, however, mean blood pressures are similar. Arterial blood gas values reach less extreme values during swimming than at $\text{VO}_{2\text{max}}$ on a treadmill. Ventilatory pattern is markedly altered during swimming, with sustained expiration against a closed glottis followed by

explosive expiratory flow and rapid subsequent inspiration.

There are no documented reports of horses experiencing EIPH during swimming. Although mean pulmonary arterial and right atrial pressures during swimming are similar to those of horses that experience EIPH when running near $\text{VO}_{2\text{max}}$ on a treadmill, it is possible that the smaller subatmospheric intrapleural pressure excursions during swimming result in smaller transmural pressures associated with capillary stress failure in the lungs.

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ALTERNATIVE THERAPIES FOR EIPH

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Exercise-induced pulmonary haemorrhage (EIPH) is a major health concern and cause of poor performance in the equine athlete (Erickson and Poole 2002; Erickson and Hildreth 2004; Hinchcliff *et al.* 2005). Significant progress has been made in recent years to diagnose EIPH and to understand the pathogenesis, prevention, and treatment of EIPH. The development of effective therapies for EIPH has been difficult due to controversy regarding the mechanisms causing EIPH and the ability to quantify EIPH. Some alternatives to furosemide that are used to prevent and treat EIPH include nasal dilators, concentrated equine serum, nitric oxide, herbal formulations, conjugated oestrogens, a diet rich in omega-3 fatty acids, and rest.

NASAL DILATORS

During quiet breathing and during exercise, 40–50% of the total pulmonary resistance is located within the nasal passages (Art *et al.* 1988). During inspiration, the extrathoracic airways account for more than 90% of the total resistance. Horses are obligate nasal breathers, so nasal resistance is of much greater importance than in humans. During exercise, partial collapse of the unsupported nasal passages may occur during inspiration. The FLAIR nasal strip has been developed for horses to prevent or reduce the collapse of the nasal passages, to decrease upper airway resistance (particularly nasal resistance), and to prevent intrapleural and alveolar pressure swings that may contribute to high pulmonary capillary transmural pressures and EIPH. Holcombe *et al.* (2002) conducted studies which concluded that the nasal strip tents the skin over the nasal valve and dilates that section of the nasal passage, resulting in decreased airway resistance

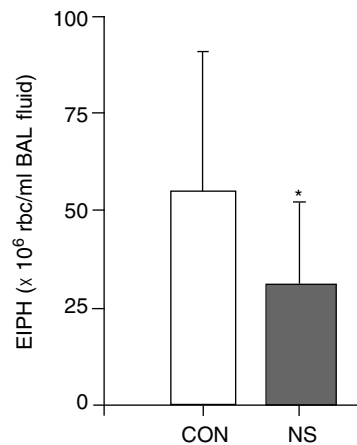


Fig 1: EIPH incurred during near-maximal exercise was reduced significantly by the nasal strip (NS). * $P < 0.05$. From Kindig *et al.* 2001a.

during inspiration. Horses evaluated on the treadmill under control conditions and wearing the nasal strip demonstrated a significant reduction in oxygen uptake and carbon dioxide production with the nasal dilator (Erickson 2000; Poole *et al.* 2000). Bronchoalveolar lavage (BAL) demonstrated a 33% reduction in EIPH with the nasal strip, with the largest reduction in EIPH in those horses that bled the most. These results have subsequently been confirmed by other studies (Fig 1, Geor *et al.* 2001; Kindig *et al.* 2001a; McDonough *et al.* 2004; Valdez *et al.* 2004) with larger reductions in EIPH.

CONCENTRATED EQUINE SERUM

Inflammatory airway disease may be an important component of EIPH (McKane *et al.* 1993; Newton and Wood 2002). Seramune is a concentrated equine serum (CES) product composed of serum collected

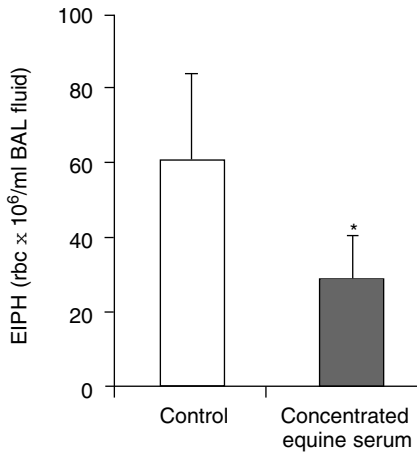


Fig 2: Severity of EIPH is reduced by treatment with concentrated equine serum *P<0.05.

from multiple draft horse donors and contains high levels of immunoglobins and other serum proteins. The product is given in a series of 5 injections 24 h apart (20 cc intratracheal and 10 cc iv) with subsequent weekly boosters thereafter during training and performance. This treatment regimen is based on field studies completed at various racetracks over a 5-year period where a reduction in EIPH and mucus was observed when Seramune was used to treat chronic bleeders on the race track. In a study on the treadmill, CES resulted in a 53% decrease in the number of red blood cells and a 32% decrease in the white blood cells in the BAL fluid (Fig 2, Erickson and Hildreth 2004). CES may have immuno-modulatory and anti-inflammatory effects that are beneficial in reducing small airway disease which may be one of the mechanisms responsible for EIPH. CES may reduce EIPH through an immune-mediated mechanism that may improve the healing of the lung tissue, reduce scar formation, and improve lung function.

NITRIC OXIDE

Nitric oxide is a vascular smooth muscle relaxing factor that is produced by the action of NO synthase on L-arginine within vascular endothelial cells. Inhaled nitric oxide reduces the pulmonary arterial pressure during exercise (Mills *et al.* 1996a; Mills *et al.* 1996b); however, the severity of EIPH increases with NO inhalation (Fig 3, Kindig *et al.* 2001b). These findings support the notion that extremely high pulmonary artery

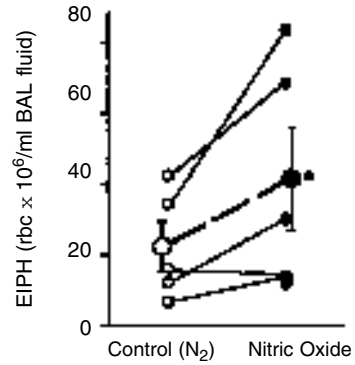


Fig 3: Severity of EIPH is exacerbated by inhalation of nitric oxide. Large symbols denote mean data. *P<0.05. From Kindig *et al.* (2001b).

pressures may reflect, in part, an arteriolar vasoconstriction that serves to protect the capillary bed from the extraordinarily high pulmonary arterial pressures during maximal exercise in the horse. These data also suggest that exogenous NO treatment during exercise in horses may not only be poor prophylaxis, but may actually exacerbate the severity of EIPH.

HERBAL FORMULATIONS

Herbal formulations are used to treat horses with EIPH; however, very few scientific studies have been done to determine the effectiveness of herbal remedies. Herbal formulations are used to decrease inflammation and oedema in the lung and move stagnated blood out of the airways. Herbal formulations have also been designed to address coagulation defects, such as platelet function, that may contribute to EIPH. Two commonly used herbal formulations (Yunnan Paiyao and Single Immortal) were recently evaluated, but were not found to be effective, at this particular dose and duration of treatment, in reducing EIPH (Epp *et al.* 2005).

CONJUGATED OESTROGENS AND ANTIFIBRINOLYTICS

Haemostatic agents are often used to treat uncontrolled bleeding in patients with systemic fibrinolysis. Conjugated oestrogens and

antifibrinolytics are used on the race track to prevent EIPH. However, there is no scientific evidence that horses with EIPH have increased fibrinolysis or defective coagulation (Bayly *et al.* 1983; Johnstone *et al.* 1991).

OMEGA-3 FATTY ACIDS

Many equine supplements are high in omega-3 fatty acids which are hypothesised to reduce EIPH via their action on the arachadonic acid cascade and reduction in airway inflammation. Since inflammatory airway disease may be an important component of EIPH, a diet rich in omega-3 fatty acids may prevent and reduce EIPH.

CONCLUSIONS

In summary, there are alternatives to furosemide to reduce and treat EIPH. However, only the nasal strip and concentrated equine serum have demonstrated scientific efficacy at this time. Rest and a reduced training schedule should also be a part of the treatment regimen to allow healing of traumatized lung tissue and clearance of blood from the airways.

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NOVEL THERAPIES FOR EIPH

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A number of therapeutic modalities are used in attempts to prevent/limit exercise-induced pulmonary haemorrhage (EIPH). Treatments fall into several discrete categories with specific proposed aetiologies and varying degrees of efficacy. These treatments include, but are in no way limited to: 1) reducing pulmonary disease; 2) correcting airway obstructions or other excessive resistance to ventilatory airflow; 3) reducing pulmonary vascular pressures; and 4) modulation of coagulation pathways. Recently, a hypothesis has been proposed that involves locomotory-impact as another possible aetiology of EIPH. Selected aspects of therapies in these categories will be discussed briefly. Epidemiological studies have shown that EIPH can exist even in the absence of specific pulmonary disease or airway obstructions. While there may be multifactorial influences acting in individual horses, it appears that reducing pulmonary vascular pressures may provide the best option for limiting EIPH in normal, healthy athletic horses.

The most commonly utilised 'treatment' for EIPH in racehorses has been the loop diuretic furosemide (eg Lasix/Salix). Although most studies have demonstrated a reduction in pulmonary vascular pressures following furosemide administration, reports on its efficacy in preventing/reducing EIPH are somewhat equivocal.

A novel approach to EIPH has been adapted from research into treatments for cardiac angina in

human patients. Nitric oxide donor therapies (eg nitroglycerine, nitroprusside) have been shown to treat angina effectively. Research into the mechanisms related to these actions led to the discovery of organ-specific compounds with vasodilatory properties, most notably Type V phosphodiesterase inhibitors such as sildenafil, vardenafil, tadalafil, and zaprinast. Some of these also have pulmonary actions, with sildenafil recently being approved by the US-FDA to treat human pulmonary hypertension.

Reduced pulmonary vascular pressures have been reported in horses breathing nitric oxide mixtures. Because of an extremely short biological half-life, nitric oxide must either be continuously inhaled or its effects prolonged. Continuous inhalation is impractical during competitions, but prolonging vasodilatory actions is possible with certain Type V phosphodiesterase inhibitors. Significant reductions in pulmonary arterial pressures have been observed during exercise in horses given the lung-directed inhibitor, E4021, followed by brief inhalation of nitric oxide, on treadmills and at racetracks. Significant reductions in EIPH compared to controls (visual endoscopic as well BALF RBC numbers) were also seen following the treatment. This effect has been observed during exercise up to 2 h following treatment. These studies suggest that selective pulmonary vasodilation during intense exercise may prove to be an effective treatment for EIPH in equine athletes.

FUROSEMIDE AND EIPH

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Administration of furosemide to Thoroughbred and Standardbred horses on the day of racing costs the racing industry in the United States between \$17,000,000 and \$35,000,000 annually. This cost is incurred because furosemide is administered to over 90% of Thoroughbred horses and 50–70% of Standardbred horses starting a race. The large costs for use of furosemide to treat exercise-induced pulmonary haemorrhage (EIPH) in racehorses are incurred for a drug for which there is no scientific evidence of efficacy under the conditions in which it is used (Hinchcliff 2005).

Day-of-racing use of furosemide also raises important issues concerning the integrity of racing. The common administration of furosemide, a drug that is unequivocally associated with superior performance in both Thoroughbred and Standardbred racehorses, raised concerns about the impression by the public of fairness and moral integrity of racing. This concern is only exacerbated when the lack of known efficacy of furosemide in treatment of EIPH is considered.

The issues of EIPH, furosemide use and athletic performance by racehorses in the United States are closely linked and can be summarised by 3 questions:

1. Does EIPH affect performance?
2. Does furosemide affect performance?
3. Does furosemide reduce the incidence or severity of EIPH?

We recognise that there are other areas of uncertainty regarding understanding of the causes and effects of EIPH but argue that these other issues are secondary to the above questions.

Questions 1 and 2 have been addressed, but there is little information about the 3rd question.

As discussed elsewhere in this monograph, the prevalence of EIPH in racehorses exceeds 50%. Because of this high prevalence among racehorses and the perception that EIPH impairs performance, most Thoroughbred and Standardbred horses racing in the United States are administered furosemide. Although the cost of administering furosemide to Standardbred and Thoroughbred racehorses exceeds \$17,000,000 annually there is no conclusive evidence obtained from racehorses that furosemide reduces either the incidence or severity of EIPH. Current guidelines for evidence based medicine classify evidence of efficacy of treatment into several levels with level one evidence, the highest level, being demonstration of drug efficacy in the target population using randomised, placebo-controlled trials. No level one evidence exists that furosemide reduces severity or incidence of EIPH in racehorses.

Exercise-induced pulmonary haemorrhage is an important economic issue because it is associated with impaired performance and shortened racing career (see preliminary data). Until very recently, the relationship between the presence of EIPH and impaired performance and shortened racing career was conjectural and based on anecdotal evidence, although this was a strongly held belief among veterinarians attending to racehorses. Our recent work clearly indicates that among horses not treated with furosemide EIPH is associated with an approximately 4 fold decrease in the likelihood of winning, a 1.8 fold decrease in the likelihood of placing, and a 3 fold decrease in likelihood of earning in the top 10% of prize monies. Furthermore, the distance a horse finishes behind the winner is associated with

severity of EIPH, indicating a severity-dependent effect of EIPH on performance (Hinchcliff *et al.* 2005).

The high prevalence of EIPH in populations of racing horses, its effect on performance, the apparently progressive nature of the disorder, and its putative effect in adversely affecting career longevity of severely affected horses have spawned a variety of treatments, none of which has been scientifically proved to be effective in racehorses. Of the potential treatments for EIPH, furosemide is among the most widely used and the only one pharmacological treatment for EIPH permitted on race day in the United States. Because of its frequency of use and importance to the racing industry in the United States, there are detailed studies of its pharmacokinetics, pharmacodynamics, drug interactions, effect on performance, and efficacy in reducing experimental EIPH (rather than listing all these studies, several recent reviews that included extensive lists of references are cited). However, the efficacy of furosemide in attenuating the severity or reducing the incidence of EIPH in racehorses under field conditions has not been demonstrated.

Furosemide is administered to the majority of Thoroughbred horses racing in the United States. It was reported in 1999 that of 22,589 Thoroughbred horses racing once over a 16 day period in July 1997, 74.2% received furosemide before racing and 84.5% had received furosemide at least once in their career (Gross *et al.* 1999; Hinchcliff 1999). More recent but less formal studies demonstrate that the proportion of Thoroughbred horses administered furosemide before racing has risen to over 92%. Rates of administration of furosemide to Standardbred racehorses, while varying regionally in the United States, are 50-70%. These data demonstrate that furosemide is the standard form of pharmacologic prophylaxis of EIPH in Thoroughbred and Standardbred horses racing in the United States. This widespread acceptance has occurred without conclusive scientific evidence of the efficacy of furosemide in reducing the incidence or severity of EIPH in horses under racing conditions.

There are few studies that have addressed, either directly or indirectly, the effect of furosemide on incidence or severity of EIPH in horses under field conditions and only 2 that examined the effect of furosemide on EIPH during racing. Taken in composite, these studies do not

provide a clear indication of the efficacy of furosemide in reducing the incidence or severity of EIPH. Direct comparison of the studies is hindered by the differing populations of horses examined, the methods of examination (endoscopy, grading of EIPH, and measurement of red cell count in bronchoalveolar fluid), and exercise (actual race versus breezing or galloping).

Studies of horses involved in racing have not demonstrated an effect of furosemide on the incidence or severity of EIPH. In a study addressing the effect of furosemide on performance, it was concluded that there was no evidence that furosemide administration reduced the incidence of EIPH in Thoroughbred racehorses (Sweeney *et al.* 1990). The severity of haemorrhage was not assessed in this study. Similarly, the study by Birks *et al.* (2002) did not detect an effect of furosemide on either the incidence or severity of EIPH in Standardbred or Thoroughbred horses (Birks *et al.* 2002). Experimental studies of the efficacy of furosemide in reducing EIPH of Thoroughbred horses galloping over ground (breezing) have produced equivocal results. Administration of furosemide before galloping reduced the severity of EIPH, assessed by endoscopic examination of the proximal trachea, compared to no treatment. However, subsequent re-analysis of the data by another researcher revealed that administration of isotonic saline (a placebo control) also was associated with a reduction in the severity of EIPH when compared to no treatment, thereby questioning the reliability of the observed effect of furosemide (Clarke 1989). This explanation may be explained by the phenomenon that, for any disease that varies in intensity over time such as does EIPH, the likelihood is that when only severely diseased animals are selected for testing of a treatment, some of these animals can be expected to have a reduction in severity of the disease purely by chance. This is likely to have occurred in this study in which both furosemide and saline appeared to reduce the severity of EIPH. Alternatively, there could have been a beneficial response to furosemide. Clearly, the results of the study are inconclusive.

Because of the logistical problems associated with studying horses galloping over ground, the effect of furosemide on EIPH has also been examined in small numbers (n=8 or fewer) of horses running on a treadmill. However, while some of these studies demonstrated an effect of

furosemide in reducing red cell count in bronchoalveolar lavage fluid collected after exercise, their relevance to horses racing over ground is uncertain. Under the guidelines for evidence based medicine, results of these studies would not be considered level one evidence of efficacy, but do provide justification for further study of the drug and testing of its efficacy in the target population.

Exercise-induced pulmonary haemorrhage is a common cause of poor performance among racehorses. Currently, the accepted treatment for EIPH in horses in the United States is administration of furosemide before racing. However, there is no conclusive evidence of the efficacy, or lack thereof, of furosemide in reducing the incidence or severity of EIPH in racehorses. Level one evidence-based trials are needed to determine the efficacy of furosemide in decreasing the incidence and/or attenuating severity of EIPH in racehorses. Appropriately designed and statistically powered studies of horses exercising in conditions pertinent to racehorses are required to determine whether or not furosemide is efficacious in the treatment of EIPH.

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REGULATORY ISSUES REGARDING DRUG TREATMENT OF EIPH

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ABSTRACT

Furosemide is the drug most commonly used in the treatment of exercise-induced pulmonary haemorrhage (EIPH) with approximately 70–80% of Thoroughbred horses in some US racing jurisdictions receiving pre-race injections of this drug. Furosemide is a high-ceiling loop diuretic that increases urine flow soon after intravenous administration with urine production reaching values that are as much as 40–50 times the normal rate. Diuresis persists for approximately 3 hours with the rate of urine production returning to near or below normal values. The increased rate of urine production causes the urinary concentrations of substances that are not normally reabsorbed in the renal tubules to decrease and thereby interferes with methods to detect them. Analysts recognised this diluting effect of furosemide soon after its introduction and called for restrictions on the time of its administration so that post race urine samples would not be diluted. Regulators responded in 1985 by limiting the dose of furosemide to no more than 250 mg, requiring that it be administered intravenously, and prohibiting its administration within 4 h of the scheduled post time of the race in which the horse was entered. Various regulatory measures were taken to ensure that these requirements were followed. These measures have included a requirement for furosemide to be administered in secure areas by regulatory veterinarians and by imposition of regulatory thresholds for furosemide in plasma. Use of secure areas has been abandoned by most regulatory bodies because of the expense of maintaining the areas. Most regulators now rely on the use of regulatory thresholds and a requirement that the urine specific gravity exceed some limit. The Racing Medication and Testing Consortium (RMTC) and the

Association of Racing Commissioners International, Inc (ARCI) recommended regulations establishing a urine specific gravity less than 1.010 as a violation of the rules of racing if the corresponding plasma (serum) concentration is greater than 100 ng/ml. These limits have been challenged by some investigators. One group has demonstrated that urine specific gravity is a poor predictor of time since dose administration and hence is not useful in detecting administration of furosemide within 4 h of race time. Others have pointed out that the purpose of measuring specific gravity is not to detect administration within 4 h of race time but to detect administration that results in collection of a dilute sample. Others have suggested that a plasma concentration in excess of 100 ng/ml is too high and that plasma concentrations 4 h after administration are substantially lower.

INTRODUCTION

The use of furosemide in the prophylaxis of exercise-induced pulmonary haemorrhage is often attributed to veterinarian Alex Harthill. It was suggested that furosemide administration would reduce pressures in pulmonary blood vessels through its antihypertensive effects and that the reduced pressures would result in decreased severity or incidence of pulmonary haemorrhage. Subsequently pre-race administration of furosemide was permitted by a number of racing commissions in the United States as a result of efforts by horse trainers to allow use of the drug to treat their horses.

REGULATORY HISTORY

Pre-race administration of furosemide and certain other therapeutic drugs became widespread in the

1970s (Gabel *et al.* 1977). Analysts working in regulatory laboratories quickly noted that the administration of furosemide to racehorses resulted in the submission of urine samples that were dilute and they suspected that detection of prohibited substances was compromised. Several groups of investigators demonstrated that pre-race administration of furosemide under the conditions that were permitted by the rules of racing at the time caused interference with the detection of certain drugs or metabolites but not others (Tobin *et al.* 1979; Soma *et al.* 1984). These scientific results and pressure from analysts to restrict or curtail the use of furosemide in racing led National Association of State Racing Commissioners (NASRC) to amend its model rules and to recommend that all racing commissions prohibit pre-race administration of furosemide. Several racing commissions soon adopted these recommendations. Horse trainers demanded that the use of furosemide be permitted and threatened to withhold their horses from racing unless the use of furosemide was permitted. Commissioners responded that they would reconsider the ban on furosemide if it could be demonstrated that it would not interfere with drug detection by commission laboratory analysts. After the American Association of Equine Practitioners declared that they would recommend a maximum intravenous dose of 250 mg administered 4 h or more before race time, it was possible to conduct a series of rather basic studies that demonstrated that administration under these conditions results in no appreciable effects on the detection of the analytes that were studied (Sams and Maylin, unpublished observations, 1984). The NASRC responded by recommending that state racing commissions permit the use of furosemide under the following conditions:

1. The maximum permissible dose is not greater than 250 mg;
2. the route of administration is intravenous;
3. the dose is administered 4 or more hours before the scheduled post time of the race in which the horse is entered.

These recommendations were not incorporated into the model rules of the NASRC but were quickly adopted (with some minor modifications) by most racing jurisdictions in the United States. The New York Racing and Wagering Board

resisted efforts to allow furosemide use in New York racing until the mid-1990s.

Considerable laboratory efforts were directed toward identifying violations of the rules restricting the use of furosemide (Singh and McArdle 1992; Uboh *et al.* 1992; Soma and Uboh, 1998). These studies revealed that it was difficult, if not impossible, to control the dose, route, and time of administration of furosemide by laboratory analysis. However, it is possible to identify a urine sample that is characterised by a specific gravity that is lower than that produced post race by a horse that has not been administered furosemide and to identify the cause of the dilute urine sample if the corresponding plasma furosemide concentration is elevated above those found 4 h after intravenous administration of permitted doses.

As a result of findings from these studies and based on recommendations from various experts, the Racing Medication and Testing Consortium (RMTC) recommended in 2004 to control the use of furosemide by measuring the specific gravity of the urine to determine whether it is dilute and to measure the corresponding plasma (serum) concentration of furosemide if the urine specific gravity is less than 1.010 and to report a violation if the plasma concentration is greater than 100 ng/ml of plasma. Furthermore, the RMTC recommended testing of the plasma sample if a urine sample was not collected and that any such sample containing furosemide at a concentration greater than 100 ng/mL be considered a violation of the rules. The RMTC also recommended increasing the maximum permitted dose to 500 mg. Many racing commissions have subsequently adopted these recommendations.

PHARMACOLOGY AND PHARMACODYNAMICS

Furosemide (4-chloro-N-furfuryl-5-sulfamoyl-anthranilic acid; Fig 1) is a high-ceiling loop diuretic that inhibits reabsorption of chloride and sodium ions in the proximal and distal tubules as well as the loop of Henle. Inhibition of the reabsorption of sodium and chloride ions decreases the reabsorption of water from the nephrons thereby increasing the volume of urine produced and causing a period of intense diuresis that begins within 15–30 min and continues for 2–3 h after intravenous administration of

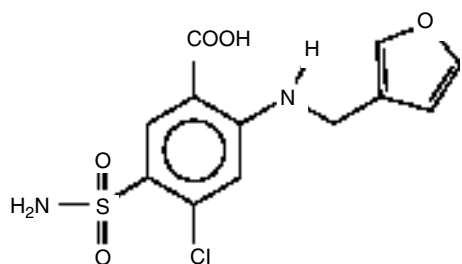


Fig 1: Chemical structure of furosemide.

permitted doses in racehorses (Soma and Uboh 1998).

The disposition of furosemide in horses is characterised by a relatively small apparent volume of distribution, rapid clearance, and short terminal elimination half-life (Chay *et al.* 1983; Dyke *et al.* 1996; Johansson *et al.* 2004). The period of diuresis corresponds to that period during which plasma furosemide concentrations are above approximately 20–40 ng/ml (Chay *et al.* 1983).

The effects of furosemide-induced diuresis on the pharmacokinetics and urinary excretion of other drugs have been investigated and are reasonably well understood. The renal clearances of drugs (metabolites) that are not appreciably reabsorbed from the renal tubules are not affected by diuresis. Consequently, the plasma concentrations of these substances are not altered but their urine concentrations are decreased (Miller *et al.* 1977; Combie *et al.* 1981; Soma *et al.* 1984; Stevenson *et al.* 1990). The horse metabolises many xenobiotics and endogenous substances by conjugation with glucuronic acid and sulfuric acid. These metabolites are highly hydrophilic and are therefore not appreciably reabsorbed. Many of the drugs that are of great interest to racing regulators are drugs that are excreted as conjugated. The effects of furosemide-induced diuresis on their detection in test samples is therefore of great interest. Some of the drugs that are in this group include morphine, etorphine, oxymorphone, hydromorphone, lidocaine, mepivacaine, pyrilamine, and others.

The renal clearances of drugs that are reabsorbed in the renal tubules are increased because these substances are not as extensively reabsorbed during diuresis because the concentration difference between the tubular fluid and the renal blood is diminished thereby reducing

the driving force for reabsorption. The concentrations of these substances in urine are not decreased as extensively as those substances that are not normally reabsorbed. Furthermore, the detection of these substances is often enhanced because the concentrations of other substances, including endogenous substances and those of dietary origin, that have the potential to interfere with their detection are reduced.

REGULATORY ISSUES

Advances in analytical methods since the early 1980s have lowered the limits of detection for most analytes, particularly for those that are excreted in urine. Therefore, the significance of decreased urinary concentrations of many analytes as a result of furosemide-induced diuresis has been reduced. However, there are still some analytes that cannot be detected adequately and any dilution by diuresis could render them undetectable. Therefore, rules and procedures designed to prevent or detect furosemide-induced diuresis around the time of racing are still necessary and appropriate.

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EIPH AND HORSERACING IN THE USA - SCALE OF THE PROBLEM, MANAGEMENT, REGULATION AND UNIQUE ASPECTS

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SCALE OF PROBLEM

There is little question that the perception among racing participants in the United States is that exercise-induced pulmonary haemorrhage (EIPH) is a widespread problem and a significant cause of decreased performance. Because furosemide administration is allowed in North American racing and its administration is denoted on post race charts there is some empirical evidence of this perception. In 2005, there were 66,692 unique starters in Thoroughbred races in the North America and 62,017 (or 92.99%) of them started at least once on furosemide. From this group, there were 426,756 total starts and 398,900 (or 93.47%) of those listed furosemide. While there may be other motivations for the administration of furosemide other than for the management of EIPH these statistics indicate the feeling among veterinarians and trainers that EIPH is a significant problem. The economic impact of these statistics for owners is staggering. At an average cost of \$20 per injection, owners spent almost \$8 million on furosemide alone in 2005. Given the fact that many horses received treatment beyond just furosemide combined with adjunctive diagnostic procedures such as endoscopic examination it is conceivable that EIPH cost owners close to \$20 million in 2005.

Further evidence of the problem of EIPH creates for participants came from a survey conducted by the University of Arizona Race Track Industry Programme in 2001. The survey was designed to be a comprehensive look at the medication issue in the United States and involved licensed trainers, veterinarians and owners. When veterinarians were asked what their greatest concerns were regarding the medication issue in the United States, many voiced frustration at only

being able to administer furosemide in many jurisdictions to manage EIPH and indicated that in a number of horses, furosemide was simply not sufficient to control bleeding. The following was a typical response from the survey:

“Allow a few antibleeder medications raceday, which are allowed in Kentucky and Louisiana. Other states you must give this medication in violation of the rules. It really concerns one when you see a horse stop badly in a race and he comes back coughing and you do an endoscopic exam of the trachea and it is full of blood. I feel I am being inhumane and violating my Hippocratic Oath by not being able to prevent EIPH with therapeutic, nonperformance enhancing medication other than Lasix.”

Trainers also voiced the need for additional raceday medications to combat EIPH. Of the 34 respondents that indicated that more medications were necessary on raceday, 18 specifically mentioned ‘bleeder’ drugs either by specific name or generically. The following is a typical verbatim response from the survey:

“States are too tough on treating bad bleeders. Lasix will not do the job alone”.

MANAGEMENT OF EIPH IN THE USA

The medical management of EIPH in the United States involves medication for the vast majority of racing animals. In fact, cottage industries have been formed around developing products that claim to effectively treat bleeders. Many of these products are totally unproven. There are also a number of substances available on the black market that are given colorful names such as ‘Black Ice’ in which

the active ingredient is a true unknown. One of the more effective treatments for horses with performance reducing levels of EIPH, rest, is generally not advocated or agreed to until it is the only option remaining. The desire to manage this condition pharmaceutically is shown by the message board on the website racehorsesdrugs.com. Posts to this message board, which was designed with education in mind, are made mostly by active Standardbred trainers in the United States and Canada. Using the word ‘bleeder’ as a keyword, a search of the website turns up 4 pages of topics, usually identifying a specific medication and asking others whether they have had success with the product in managing EIPH. Numerous drugs and other substances are discussed ranging from known pharmaceuticals such as diuretics to herbal remedies to the aforementioned unknown substances. This provides some evidence of the general desperation level to keep horses in training and not lose purse opportunities to EIPH alone and the reliance on combinations of drugs and ‘voodoo’ science.

REGULATION OF EIPH

In the United States, racing is regulated by those states which conduct pari-mutuel wagering. Because of this, there is no central rule making authority and the regulations differ sometimes substantially when state lines are crossed. The closest the United States has had to a uniform rule since the early 1990s however, is the legal raceday administration of furosemide to assist in managing EIPH. All 38 racing jurisdictions currently permit the administration of furosemide although there are many state-to-state differences in terms of withdrawal time before a race, maximum and minimum dosages and route of administration. There are also differences between states in how a horse qualifies for the administration of furosemide. Some states allow the trainer or veterinarian to voluntarily place the horse on the furosemide list while other require endoscopic proof that the horse suffers from EIPH. In addition, several jurisdictions have recently allowed the raceday administration of so called ‘adjunct’ bleeder medications in response to veterinary and horsemen association pressure. The most common adjunct medications allowed are aminocaproic acid, carbazochrome, tranexamic acid and the conjugated oestrogens.

The Racing Medication and Testing Consortium (RMTC) was formed in 2001 to address some of the inconsistencies in rules between states by providing model rules for adoption. The RMTC is a broad based coalition of racing industry stakeholder groups and has achieved remarkable success given the fractious political nature of racing in the United States. As a result, some of the state-to-state differences regarding furosemide administration have been resolved and true uniformity as it relates to the regulation and management of EIPH will be achieved in the near future. The model rule language relative to the administration of furosemide is attached to this report as an appendix. In addition, the RMTC has taken a strong stance regarding the use of adjunct bleeder medications by requiring scientific proof that these medications are efficacious and not a threat to the integrity of the race before condoning them as raceday medications. Currently there are 2 research projects funded by the RMTC to ascertain the efficacy of these adjunct medications. Ultimately it is the hope of the RMTC that a more effective medication for controlling EIPH can be developed to replace furosemide due to the negative perception of the drug among segments of the scientific community and racing stakeholders and fans.

APPENDIX

Model Rule Section on Furosemide

A. Furosemide

1. Furosemide may be administered intravenously to a horse, which is entered to compete in a race. Except under the instructions of the official veterinarian or the racing veterinarian for the purpose of removing a horse from the Veterinarian’s List or to facilitate the collection of a post race urine sample, furosemide shall be permitted only after the official veterinarian has placed the horse on the Furosemide List. In order for a horse to be placed on the Furosemide List the following process must be followed.

- a) After the horse’s licensed trainer and licensed veterinarian determine that it would be in the horse’s best interests to race with furosemide they shall notify the official veterinarian or his/her designee, using the prescribed form, that they wish the horse to be put on the Furosemide List.

- b) The form must be received by the official veterinarian or his/her designee by the proper time deadlines so as to ensure public notification.
 - c) A horse placed on the official Furosemide List must remain on that list unless the licensed trainer and licensed veterinarian submit a written request to remove the horse from the list. The request must be made to the official veterinarian or his/her designee, on the proper form, no later than the time of entry.
 - d) After a horse has been removed from the Furosemide List, the horse may not be placed back on the list for a period of 60 calendar days unless it is determined to be detrimental to the welfare of the horse, in consultation with the official veterinarian. If a horse is removed from the official Furosemide List a second time in a 365-day period, the horse may not be placed back on the list for a period of 90 calendar days.
 - e) Furosemide shall only be administered on association grounds.
 - f) Upon request of the regulatory agency designee, the veterinarian administering the authorised bleeder medication shall surrender the syringe used to administer such medication which then may be submitted for testing.
2. The use of furosemide shall be permitted under the following circumstances on association grounds where a detention barn is utilised:
- a) Furosemide shall be administered at the direction of the official veterinarian no less than 4 h prior to post time for the race for which the horse is entered.
 - b) A horse qualified for furosemide administration must be brought to the detention barn within time to comply with the 4 h administration requirement specified above.
 - c) The dose administered shall not exceed 500 mg nor be less than 150 mg.
 - d) Furosemide shall be administered by a single, intravenous injection.
 - e) After treatment, the horse shall be required by the Commission to remain in the detention barn in the care, custody and control of its

trainer or the trainer's designated representative under association and/or Commission security supervision until called to the saddling paddock.

3. The use of furosemide shall be permitted under the following circumstances on association grounds where a detention barn is not utilised:
- a) Furosemide shall be administered no less than 4 h prior to post time for the race for which the horse is entered.
 - b) The furosemide dosage administered shall not exceed 500 mg nor be less than 150 mg.
 - c) Furosemide shall be administered by a single, intravenous injection.
 - d) The trainer of the treated horse shall cause to be delivered to the official veterinarian no later than 1 h prior to post time for the race for which the horse is entered the following information under oath on a form provided by the Commission:
 - i) The name of the horse, racetrack name, the date and time the furosemide was administered to the entered horse;
 - ii) The dosage amount of furosemide administered to the entered horse; and
 - iii) The printed name and signature of the attending licensed veterinarian who administered the furosemide.
4. Test results must show a detectable concentration of the drug in the post race serum, plasma or urine sample.
- a) The specific gravity of post race urine samples may be measured to ensure that samples are sufficiently concentrated for proper chemical analysis. The specific gravity shall not be below 1.010. If the specific gravity of the urine is found to be below 1.010 or if a urine sample is unavailable for testing, quantitation of furosemide in serum or plasma shall be performed;
 - b) Quantitation of furosemide in serum or plasma shall be performed when the specific gravity of the corresponding urine sample is not measured or if measured below 1.010. Concentrations may not exceed 100 ng/ml of furosemide per of serum or plasma.

B. Bleeder List

1. The official veterinarian shall maintain a Bleeder List of all horses, which have demonstrated external evidence of exercise induced pulmonary haemorrhage from one or both nostrils during or after a race or workout as observed by the official veterinarian.

2. Every confirmed bleeder, regardless of age, shall be placed on the Bleeder List and be ineligible to race for the following time periods:

- a) First incident – 14 days;
- b) Second incident within 365 day period – 30 days;
- c) Third incident within 365 day period –180 days;

d) Fourth incident within 365-day period – barred for racing lifetime.

3. For the purposes of counting the number of days a horse is ineligible to run, the day the horse bled externally is the first day of the recovery period.

4. The voluntary administration of furosemide without an external bleeding incident shall not subject the horse to the initial period of ineligibility as defined by this policy.

5. A horse may be removed from the Bleeder List only upon the direction of the official veterinarian, who shall certify in writing to the stewards the recommendation for removal.

6. A horse which has been placed on a Bleeder List in another jurisdiction pursuant to these rules shall be placed on a Bleeder List in this jurisdiction.

EIPH AND HORSERACING IN HONG KONG: SCALE OF THE PROBLEM, MANAGEMENT, REGULATION AND UNIQUE ASPECTS

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INTRODUCTION

The Hong Kong Jockey Club has around 1,100 Thoroughbreds in flat-race training at any one time which are imported mainly from Australia, New Zealand and Europe and they are stabled with 25 trainers at a centralised stabling, training and racing complex at Sha Tin Racecourse in the New Territories of Hong Kong. There are 20 blocks of air-conditioned concrete stables either 2 or 3 storey. Trainers may have a maximum of 60 horses and for the 2004/2005 racing season, the average was 44. During the same season the average age of the horses was 5.2 years with a range of 2–10 years and average number of races per horse was 7.7. The horses are fed a wide range of traditional imported grain feeds and compound feeds as well as a variety of hays and most would receive some proprietary feed supplements. The horses are bedded on shredded paper (local) and imported, straw (wheat or rice) or wood shavings. The horses train mainly on dirt tracks.

The climate in Hong Kong is sub-tropical with generally cool/mild and dry winters and (occasionally humid) springs, hot, wet and humid summers and warm, dry less humid autumns.

The Hong Kong Jockey Club has well documented and published the incidence of both external and internal EIPH cases in a number of International Conferences and Journals since 1982 (Mason *et al.* 1983, 1984; O'Callaghan *et al.* 1987 a–h; Mason 1992).

During the 2004/2005 Hong Kong horse-racing season which ran from early September to early July, there were 78 race meetings comprising 710 races and 9,153 starters. The races were held over distances varying from 1,000–2,400 metres, 89.4% of which were on sand-mesh turf and 10.6% on all-weather dirt tracks.

EIPH STATISTICS

Of the total population of 1,358 horses in the 2004/2005 season, there were 50 'bleeding' records (49 individual horses) during racing (3.68%), 5 'bleeding' records during barrier trials (0.37%) and 23 'bleeding' records during track work (1.69%). Therefore in training and racing combined, there were a total of 78 'bleeding' records (5.74%). Despite over 150,000 recorded events of swimming exercise over the whole season, which accounted for 1,155 individual horses, there was no 'bleeding' horse recorded post swimming (Fig 1).

During the season the average percentage of 'bleeders' per trainer as a percentage of starters was 0.58 with a range of 0.00%–2.10%. Only 2 trainers exceeded 1.00% at 1.65% and 2.10% finishing 11th and 22nd respectively in the trainers' table based upon number of winners. The leading trainer stood at 0.44% and the bottom trainer at 0.40%.

During racing in 2004/2005 racing season, the 50 'bleeding' incidents with 49 individual horses represented 0.55% of the starters. In recent years 'bleeding' as a percentage of starters during racing has fluctuated between a low of 0.46% (41/8,844 starters) in 2000/01 and a high of 0.79% (68/8,653 starters) in 1999/00 (ie 4.6–7.9 'bleeds' per 1,000 starts respectively).

For the 12-year period from the 1993/94 to the 2004/05 racing seasons, there were a total of 798 'bleeding' records (combined training and racing). On average almost a third (30.6%) of horses, which 'bled' the first time (611 records), had a second 'bleeding' incident (187 records). Many horses would have been voluntarily retired following the first 'bleeding' incident, either immediately or anytime afterwards.

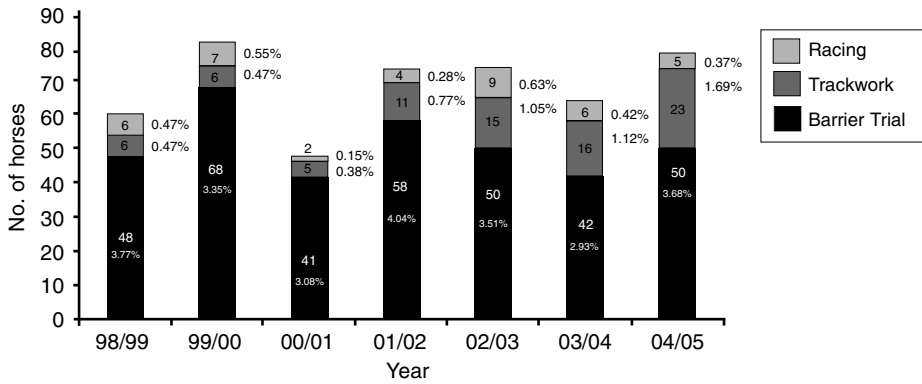


Fig 1: Number and percentage of 'bleeders' per season by type.

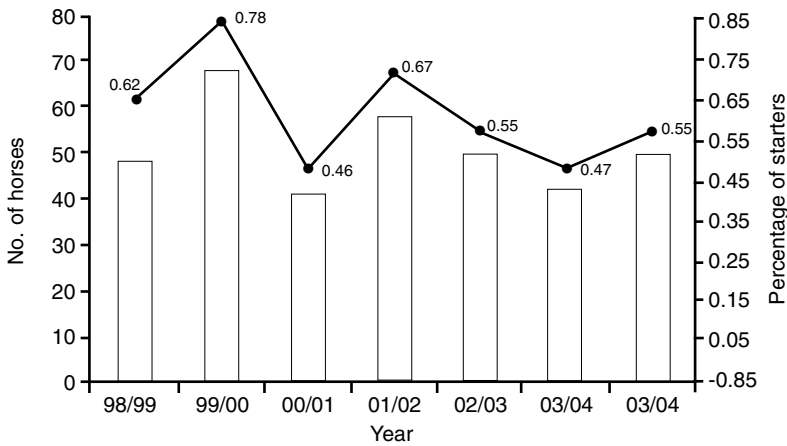


Fig 2: Number of 'bleeders' (during race) as a percentage of total starters by season.

Five and 6-year-old horses appeared to be at the highest risk with a 'bleeding' incidence rate of 7.2 and 7.4 per 1,000 starts as recorded over 4 racing seasons (2001/02 to 2004/05 season), whereas 4- and 7-year-olds were 5.9 and 5.1 respectively and 3- and 8-year-olds were 3.3 and 2.3 (Figs 2 and 3).

Totally 276 horses 'bled' (including 1st and 2nd time 'bleeders') between 1 July 2000 and 11 February 2006. Three of these were also noted to have a heart irregularity (atrial fibrillation). Six of the horses collapsed and died as a result of 'bleeding'. One of these horses had 'bled' officially 5 months previously, however the other 5 horses had no previous official 'bleeding' record. It may be speculated that these could have suffered both a heart irregularity and EIPH.

Seasonal and track trends appear to occur and these are being looked at for statistical significance (Figs 4 and 5).

In the case of incidence rate of internal EIPH with moderate to severe amount of blood noted on endoscopic examination in the official post race clinical examination, a gradual increase from 4.6% (29 of 626 examinations) in 2002/03 season to 5.8% (50 of 851 examinations) in 2004/05 has been observed.

REGULATION

The Hong Kong Jockey Club has the following specific rules regarding the official management of 'bleeders':

THE HONG KONG JOCKEY CLUB RULES OF RACING (EXTRACTS) REVISED TO 1ST SEPTEMBER 2005

Definitions

Rule 7. (2) 'Bleeding' means exercise-induced pulmonary haemorrhage (EIPH) and is evidenced

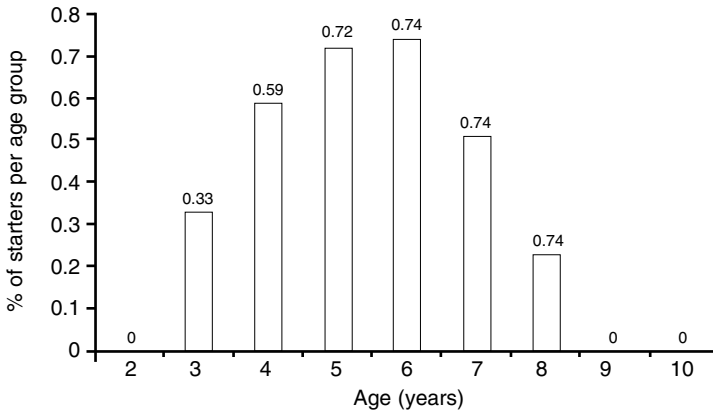


Fig 3: Raceday 'Bleeders' as a average percentage of starters per age group for the 4 seasons 2001/02 to 2004/05.

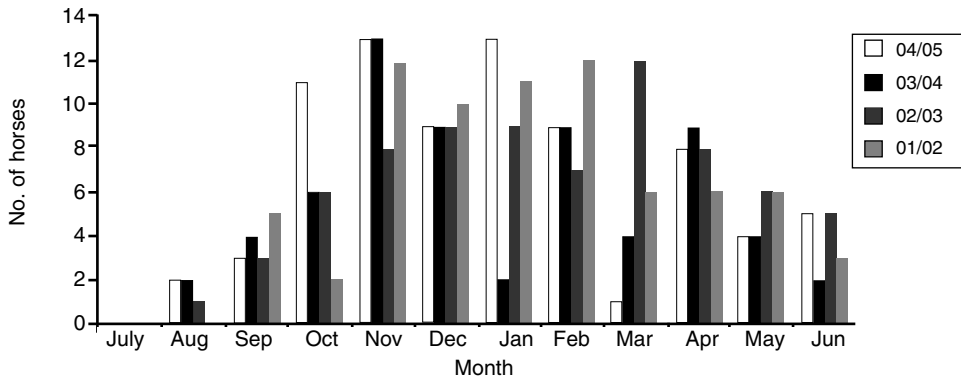


Fig 4: Distribution of 'Bleeders' per month by season.

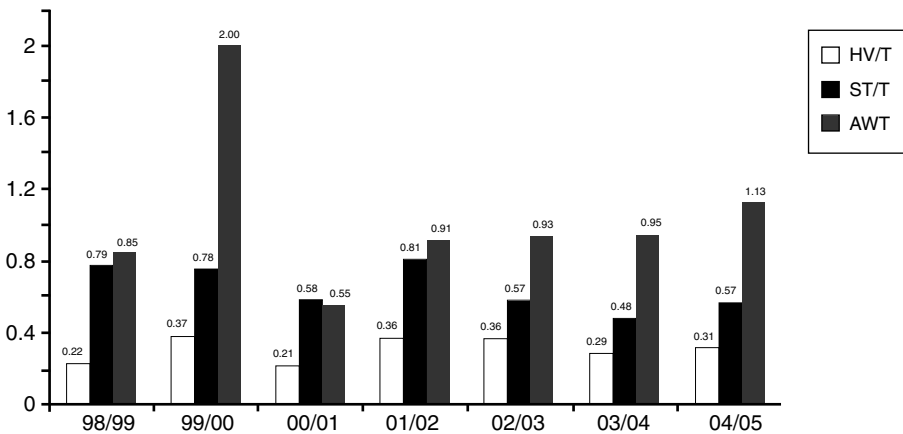


Fig 5: Raceday 'Bleeders' as a percentage of starters by track.

by the appearance of blood at one or both nostrils of a horse, originating from the lungs.

INSTRUCTIONS MADE BY THE STEWARDS OF THE JOCKEY CLUB (EXTRACTS)

Inst. 8. Bleeding

- 1) i) Any horse which bleeds, either from one or both nostrils, must be reported by the Trainer, or his authorised staff, to the Stewards and to a Veterinary Surgeon of the Club, as soon as possible on the same day that the horse bleeds.
- ii) The Veterinary Surgeon of the Club will examine any horse which is reported to have bled from the nostril(s) on the day that it bleeds and may confirm a horse as subject to bleeding without resort to endoscopic examination.
- 2) i) On the first occasion the horse will be barred from racing for a period of three months. During the first 2 months of the ban, the horse will be restricted to using the horse walkers, lungeing ring, and the trotting rings. During the third month of the ban, the horse will be allowed to use all the above facilities, plus the swimming pool, the aquatred, grass tracks, the small all weather track and the large all weather track.
- ii) On the second occasion the horse will be permanently barred from any further racing.
- iii) Any horse which has bled may not be entered until the full ban has expired and may not be declared to race again until it has passed such test or trial as directed by the Stewards and to the satisfaction of the Club's Veterinary Surgeon.
- iv) Notwithstanding the foregoing provisions, the Stewards of the Jockey Club on receiving a report from the Veterinary Surgeon endorsed by the Stewards reserve the right to refuse entries permanently or for any recommended length of time for any horse they may consider to be a serious risk to racing.

TRAINER'S HANDBOOK (EXTRACTS) REVISED 21ST SEPTEMBER 2005

Horse 'Bleeding' [Exercise Induced Pulmonary Haemorrhage (EIPH)]

36. Any horse which bleeds, either from one or both nostrils, must be reported to the duty

Steward and to a Veterinary Surgeon of the Club as soon as possible.

'Bleeders' - Exercise

37. Horses which have been banned from racing following a 'bleeding' (exercise induced pulmonary haemorrhage or EIPH) attack may use the following Club training facilities, but cannot be entered until the full ban has expired and may only be declared to run in a race after they have passed an official veterinary examination.
 - a. Months 1 and 2
 - Horse walkers
 - Lungeing ring
 - Both trotting rings
 - b. Month 3
 - All the above
 - The swimming pool
 - The aquatred
 - The grass, small all weather track and large all weather track
38. As the objective of the enforced rest period following a 'bleeding' attack is to allow the lung tissue to heal, trainers are advised that during the first 2 months exercise must be limited to walking and trotting.
39. The Club's Veterinary Surgeon may issue such further individual advice as he considers necessary.

Bleeders

98. A bleeder may not be entered to run until the full ban has expired and may not be declared to race again until it has passed such trial or test as directed by the Stewards.

In summary, the Hong Kong Jockey Club (HKJC) Rules of Racing define a 'bleeder' as a horse, which exhibits blood at one or both nostrils, which has originated from the lungs. On the first occasion a horse 'bleeds' it will be barred from racing for a period of 3 months and on the second occasion the horse will be permanently barred from any further racing.

If the haemorrhage in a first time 'bleeder' is severe and/or the horse pulls up severely

distressed or collapsing, then a recommendation may be made for either a prolongation of the rest period or, more usually, compulsory retirement of the horse in the interests of race track safety and horse welfare.

Although not required under the rules, horses with unilateral epistaxis are invariably subjected to an endoscopic examination of their respiratory tracts to confirm that the blood originated from the lungs and if it did not, then the horse is not confirmed as an official 'bleeder'. Such horses usually have an immediate history of suffering head trauma, eg rearing and hitting their heads in the Starting Stalls, causing bleeding from the nasal passages and/or sinuses.

During the 3.5 year period from 2002–2006, there were 31 cases which had unilateral/bilateral epistaxis, which were confirmed not to be official 'bleeders' after endoscopic examination of the airway.

Hong Kong conducts 'prohibited substances free racing' and in addition horses do not train on frusemide or equivalent diuretics.

SUMMARY

The Hong Kong Jockey Club has had a 'working rule' in place for very many years in order to manage and regulate 'bleeders'. Although not perfect by any means, the rule does however provide for an objective means of managing, what otherwise would be, in many cases, a subjective decision making process, which inevitably would lead, on occasion, to accusations of inconsistency and unfairness. Racetrack safety (for both jockeys and horses), horse welfare and racing integrity must be safeguarded at all cost. If 'science' can be used to formulate a better EIPH rule, then the regulators of the sport will, no doubt, be prepared to listen.

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EIPH AND HORSERACING IN AUSTRALIA – SCALE OF THE PROBLEM, MANAGEMENT, REGULATION AND UNIQUE ASPECTS

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Prior to 1972, there were no regulatory controls in Victoria over horses identified as having ‘bled’ following racing, save for the refusal of nominations of horses which had bled 3 times. Detailed records for bleeders had been kept since 1969. However, these indicated that the numbers of horses displaying uni- or bilateral epistaxis following racing seemed small relative to the numbers of starters and individual horses racing. Nevertheless, authorities considered this condition to be of such significance that they felt it necessary to exercise some form of control over its incidence. This prompted the introduction of Australian Rule of Racing 53A on the 1st August 1972 which read as follows:

“If a horse suffers an attack of bleeding at any time, the fact of such bleeding shall be reported by the Trainer without delay to the Stewards.

A horse which has in the opinion of the Stewards suffered an attack of bleeding shall not without permission of the Stewards:

- a) Be trained, exercised or galloped on any racecourse for a period of 2 months thereafter.
- b) Start in any race for a period of 3 months, and then only after a satisfactory gallop of at least 1,000 m in the presence of a Steward.

If a horse shall suffer more than one attack of bleeding such horse shall be ineligible to start in any race.

If any Principal Club shall advise in writing that any horse has suffered an attack or attacks of bleeding, such advice shall be prima facie evidence that such horse has suffered an attack or attacks of bleeding”.

A similar rule had been in place in some other Australian states since the early 1960s but not in

Victoria. The definition of a ‘bleeder’ was one which had the appearance of blood at one or both nostrils following exercise (racing or training). This definition was altered in 1983 by adding the following clause to the rule.

“An attack of bleeding shall be the appearance of blood at both nostrils irrespective of quantity”.

This amendment was in recognition of the fact that some uni-lateral epistaxes were the result of external trauma, not EIPH. This was supported by the records which showed that these horses rarely if ever bled a second time.

The advent of the flexible fibre-optic endoscope in the early 1980s enabled investigations of the horse’s respiratory tract beyond the larynx/pharynx for the first time. These demonstrated that in general, the post exercise epistaxis identified following racing was pulmonary in origin, not nasal as had been previously presumed (Pascoe and Wheat 1980; Mason *et al.* 1982; Spiers *et al.* 1982). This finding caused regulators to question a system of control based entirely on the appearance of blood at a horse’s nostrils. It also had to be balanced against the need to be able to apply the rules practically and equitably in the context of the way in which Australian racing is conducted. For this reason, the somewhat pragmatic approach to the control of this condition as defined in AR53A above has been maintained in more or less the same form since 1972.

It is worth detailing some of the statistics recorded in relation to bleeders in Victoria up until 1984. Bourke (1987), reported that whilst there appeared to be no correlation with sex, the incidence of the condition did increase with age. Whilst approximately 40% of first time bleeders

did not seek reinstatement, 25% of those which did bled a second time. Age, sex, length of absence from racing and number of starts after the first episode of bleeding were not predictors of the likelihood of a second episode. Whilst 2-year-olds were under represented as first-time bleeders relative to their percentage of the general racehorse population (<3% cf 16%), the high incidence of repeat episodes in 2-year-olds was an interesting, if unexpected finding. No explanation could be offered for this finding. Prior to 1972, the records showed that second-time bleeders were more likely to bleed a third time when compared to first time bleeders (50% cf 25%), irrespective of the length of spell.

The same author (Hinchcliff 2005) also showed that prior to 1972, the incidence of bleeders was 1% of the racing population. This fell to 0.5% (approximately) of individual horses racing following the introduction of AR53A. Compulsory retirement of horses after the second episode along with the decision by owners to voluntarily retire many horses after the first episode (46%), were the 2 most significant factors in the reduction of the rate of bleeders. There was a trend for 2- and 3-year-olds to be under represented as bleeders relative to their distribution in the total horse population, whereas horses of 4 years of age and older were over represented. In a 5 year period (1985–1990) there were 249 bleeders identified, representing 0.59% and 0.11% of individual horses and starters respectively.

DISCUSSION

Over the intervening years the Rules have been the subject of numerous reviews and modification. The following sub-rules have been added to AR53A:

- 1) An attack of bleeding shall be the appearance of blood at both nostrils, irrespective of quantity, unless in the opinion of the Stewards such bleeding was caused by external trauma.
- 6) If a horse displays blood at one nostril, the trainer shall without delay report such occurrence to the Stewards.
- 7) Unless the Stewards are satisfied that the presence of blood provided for in subrule (6) was attributable to external trauma, the horse shall before racing again be required to

undergo a satisfactory gallop of at least 1,000 metres in the presence of a Steward. [subrules (6) and (7) added 1.12.05]”

The rest of the relevant Rules are essentially as they were when introduced in 1972. These additions were designed generally to deal with uni-lateral epistaxis, whether of pulmonary or nasal origin, the latter being provided for in circumstances where it could be established that external trauma in some form was the cause.

Australia has used the appearance of blood at both nostrils as its preferred form of regulation over horses suffering EIPH. This parameter clearly and significantly understates the incidence of EIPH6 (predictive value of 0.8%), a fact regulators in this country have recognised (Bourke 1987) for many years. Using Hinchcliff’s (2005) finding that the incidence of EIPH in the Victorian racing population is 55.3%, our data suggests that epistaxis as a predictor of EIPH is 1.44% rather than the 0.8% quoted in that paper. This figure is still very low by any standards.

The ‘epistaxis’ approach has been the subject of numerous reviews by the Australian Racing Board, the latest having been conducted in 1997 (Suann 1999). The current rules reflect the need to have a uniformly applicable approach suitable to the somewhat unique conditions encountered across the entire spectrum of Australian racing. Due to the size and scope of the Australian (and Victorian) racing industry, its somewhat unique nature and the level of resources and facilities currently available, it would be impossible in a practical sense to apply any other form of control across the entire industry, especially one based on the use of the fibre-optic endoscope.

Table 1 compares the original statistical data compiled by Bourke (1987) in 1986 to that for the last 5 racing seasons. Whilst there has been a slight increase in the numbers of horses being presented for reinstatement, the incidence of second time bleeders has remained relatively static at 26% (of reinstated horses) over the last 20 years. Table 2 however, shows a significant increase in the percentage of individual horses bleeding for the first time (0.79%). Whilst it still remains well below the 1% experienced prior to 1972, it has risen by 33% between the 2 survey periods. This increase can be explained in part at least by enhanced surveillance and reporting measures instituted across the state in the 2000/01 racing season.

TABLE 1: Comparative reinstatement percentages

5 Year periods	Bled 1st time	Re-instated	% vs F/T	Bled 2nd time	% vs R/I
2000-01/2004-05	374	220	59%	57	26%
1985-86/1990-91	274	134	54%	34	25%

TABLE 2: Comparative bleeder percentages

5 Year periods	Bled 1st time	Individual horses	% vs bled F/T	Starters	% vs Bled F/T	Average starters per horse
2000-01/2004-05	374	47,000	0.79%	242,373	0.15%	5.12
1985-86/1990-91	274	45,874	0.59%	253,722	0.11%	5.53

TABLE 3: Age distribution of bleeders

Age 00-01/04-05	Bled 1st time	% total horse pop.	% total bleeders F/T	R/I	% R/I	Bled 2nd time	% vs R/I
2	8	12.1%	2.10%	7	87.5%	1	14.3%
3	71	27.7%	19.0%	44	62.0%	15	34.1%
4	103	25.1%	27.50%	63	61.2%	27	42.9%
5	90	16.5%	24.1%	57	63.3%	7	12.3%
6	56	9.7%	15.0%	27	48.2%	5	18.5%
>6	46	8.8%	12.3%	22	47.8%	2	9.1%

The percentage of first-time bleeders relative to starters (0.15%) has increased by 36% over the previous survey period. Having said this, the relative incidence of horses suffering regulatory disabilities as a consequence of AR53A remains relatively small when compared to the total Victorian horse population. Perhaps of more significance is an apparent failure to see any further decline in the incidence of bleeders. Whilst the reasons for the increase seen in first-time bleeders could be many and varied, it might also suggest that any 'advances' in veterinary medicine in relation to the management and/or treatment of this condition have failed to deliver any measurable improvements. It is also interesting to note that in New South Wales the incidence of first-time bleeders relative to starters is of the order of 0.21% and 1.12% of individual horses. The reasons for these figures being greater than those experienced in Victoria are unknown, but might include factors such as climate which is significantly different between NSW and Victoria.

As identified by Bourke (1992), the data in Table 3 again demonstrates that 2- and 3-year-olds are under represented, and horses 4 years of age and older are over represented as first -time

bleeders. Horses were not presented for reinstatement for an average of 5.7 months (range 2–24 months), and second-time bleeders occurred 4.14 months on average after reinstatement (range 0–28 months). Length of spell appears to be of no relevance to the incidence of second-time bleeders (as was found previously). The disproportionately high incidence of second time bleeders amongst 3- and 4-year-olds relative to older horses is difficult to readily explain.

One interesting finding is the under representation of 2, and to a lesser extent 3-year-olds as first-time bleeders relative to their percentage of the total racehorse population. Hinchcliff *et al.* (2004) found that age was not an important risk factor for EIPH as determined by endoscopy (rather than epistaxis).

The 2 findings appear to be at odds, especially if it is accepted that epistaxis is an indicator of the only most severe forms of EIPH and, that incidence and severity are not age related (Hinchcliff *et al.* 2005). The instillation of autologous blood into the trachea as a model for the residual effects of EIPH on normal lung tissue has been investigated (Slocombe, personal communication). It was found that 200 ml of

blood or greater had significant implications for lung pathology and that this effect was found to continue for up to 3 weeks. It could be that in some horses, repeated episodes of EIPH may result in ongoing cumulative damage to lung tissue, especially if horses were to be raced and/or trained with sufficient intensity within the 3 week timeframe referred to above. In turn, this may increase the likelihood of more severe episodes of EIPH and, as a consequence, might increase the chance of epistaxis in such horses in the future. This is a possible explanation for the apparently contradictory findings between the 2 data sets. Factors such as frequency of racing, type of training regimes and length of spells may influence this process, especially if the lungs do in fact have some capacity for recovery after each episode of EIPH.

Given the apparent inability of research into the causes and prevention of EIPH to have any measurable affect on the incidence and severity of this condition over the last 20 years, perhaps it is time to consider a change in research directions. As it appears that grades of EIPH less than 2 have little or no influence on performance, perhaps an increased effort in investigating possible treatment modalities to mitigate the effects of EIPH on lung tissue would provide a more rational approach to the management of a condition which to some degree can be considered physiological. Reducing the residual lung pathology following appropriately severe episodes of EIPH may represent, *inter alia*, a logical albeit alternative approach to the problem. In any event, the current directions of research into EIPH need to be critically reassessed. The possible influences of the environment and/or the equine genome are other potentially relevant areas that have yet to be investigated.

In relation to control and/or management of EIPH from a regulatory perspective, the means adopted must be able to be practical to implement and applied with equanimity. The issue of where the regulatory line should be drawn has been and always will be the subject of questioning by industry stakeholders. Interestingly, the range of views on this matter in Australia at least, encompasses the full spectrum of possible opinions on the matter.

The topic will and should be the subject of regular review by administrators. At the present time, the Australian regulatory approach is the only one which meets the aims outlined above given the, in some ways, unique circumstances under which Australian racing is conducted. On current evidence, it is effectively removing from the racing population the most severe manifestations of EIPH (and therefore perhaps, the most at risk horses) without impacting significantly on the total available racehorse population. Research which is meaningful, relevant and applicable to the regulation of EIPH may provide better approaches in the future.

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EIPH AND HORSERACING IN SOUTH AFRICA - SCALE OF THE PROBLEM, MANAGEMENT, REGULATION AND UNIQUE ASPECTS

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Horseracing in South Africa is limited to flat racing of Thoroughbred horses. There are approximately 10,000 horses in training and approximately 45,000 starters per annum. Racing takes place year-round at 12 race courses and under various environmental and climatic conditions.

A number of studies have quantified the prevalence of epistaxis in Thoroughbred horses in South Africa. A study in 1948 and 1949 (Pfaff 1950) recorded a total of 62 cases of epistaxis in a population of 4,015 horses (1.2%). This study included a total of 3,883 races in which each horse competed in an average of 19.5 races (ie approximately 78,300 race starts). The prevalence of epistaxis in this study was thus 0.8 cases per 1,000 race starts. The author of this study suggested that prevalence of epistaxis was higher in: 1) geldings, 2) older horses, 3) horses competing in sprints, 4) the months of August to October, and 5) horses competing in races at sea level.

The same author performed a second study on the prevalence of epistaxis in South Africa over the 14 year period from January 1962 to December 1975 (Pfaff 1976). During this time a total of 460 cases of epistaxis were recorded (2.41% of horses). The prevalence of epistaxis in this study was also approximately 0.8 cases per 1,000 race starts. The author of this study suggested that prevalence of epistaxis was higher in: 1) geldings; 2) older horses; 3) the months of April–July; and 4) horses competing in races at sea level.

A study published in 2003 reported a total of 1,287 cases of EIPH from a total of 788,111 starts between 1986 and 2001 (ie 1.65 cases per 1,000 starts) (Weidman *et al.* 2003). This study also reported that the prevalence of epistaxis was higher in horses racing at sea level than at an altitude of greater than 1,500 m. These data also suggested

that epistaxis could be heritable in Thoroughbreds in southern Africa (Weidman *et al.* 2003).

The rules of racing in South Africa stipulate that horses are suspended from racing for a period of 3 months following the first occurrence of epistaxis, 6 months following the second occurrence and indefinitely following the third episode. Race day medication, including the use of furosemide, is prohibited in South Africa. The National Horse Racing Authority enforces this policy strictly through stringent drug testing. However, furosemide is widely used in horses in South Africa during training for racing.

A study has recently been performed where tracheobronchoscopic examinations were performed on over 1,000 Thoroughbred racehorses within 2 h of racing at 5 different racetracks in 28 race meets. The overall severity of EIPH was: Grade 0 (44.8%); Grade 1 (31.6%); Grade 2 (11.4%); Grade 3 (8.7%); and Grade 4 (3.4%). Preliminary analysis of the data indicates that the prevalence and severity of EIPH may be higher at sea level than at higher altitudes. The possible causes of this warrants further investigation.

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