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Diagnostic methods for exercise-induced pulmonary haemorrhage (EIPH)

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Respiratory conditions have been identified as one main cause of training disruption and interruption of racing competitions in Thoroughbred horses (Wilsher et al., 2006). Exercise-induced pulmonary haemorrhage (EIPH) is a common problem affecting the lower airways of racehorses. The cost of EIPH to the Thoroughbred and Standardbred racing industries in the United States is estimated to be $115,000,000 - $225,000,000 annually, not including the cost of racing and training days missed and the shortened racing career of affected horses (Marlin and Hinchcliff, 2006).

Multiple techniques are available for determining the occurrence of a previous episode of EIPH, including visualisation of epistaxis, tracheobronchic endoscopy or cytological investigation of either tracheal wash (TW) or bronchoalveolar lavage fluid (BALF) for detection/quantification of red blood cells (RBC) or haemosiderophages. The utility of these diagnostic tests, and then the methodological choice, mainly depends on the time between racing and the examination as well as the required sensitivity of the employed test. Tracheobronchoscopy is indeed most appropriate within 30min – 2h post-exercise. Cytology of respiratory fluids is a more sensitive method, since previous episodes of haemorrhage might be detected up to several weeks later. Radiography, scintigraphy and pulmonary function tests, while useful for other respiratory conditions, are largely of minimal relevance in confirming a diagnosis of EIPH or in determining its severity.

The prevalence of EIPH varies with the diagnostic methods being used, and the frequency with which horses are examined. In the Thoroughbred population indeed, the reported prevalence for a single investigation ranges 43.8–75.4% (Pascoe et al., 1981; Newton and Wood, 2002). It is also clear that increased frequency of examination will lead to an increased prevalence. Thus, for a single examination, around 40-60% of horses examined may have blood in the trachea following racing, while the prevalence will reach about 100% when horses are examined on 3 separate occasions (Birks et al., 2002). Epistaxis associated with exercise is almost always attributable to EIPH and occurs with a prevalence of 0.2-9.2% in racehorses (Takahashi et al., 2001; Williams et al., 2001). Using epistaxis as the sole criterion for detecting EIPH might then result in a severe underestimation of the syndrome frequency. Horses that have experienced one episode of epistaxis are however more likely to suffer another one. The recurrence rate of epistaxis in Thoroughbred horses in Japan was approximately 13.5%, despite affected horse not being permitted to race for 1 month after the initial episode (Takahashi et al., 2001). Above all, it is worth mentioning that EIPH is very common in racehorse and should be considered the cause of poor-performance only after other causes have been eliminated (Hinchcliff, 2007).

Tracheobronchoscopic diagnosis of EIPH, according to the previously published gravity score (Hinchcliff et al., 2005a) was however significantly associated with performance in racehorses (Hinchcliff et al., 2005b). Indeed, results from a cross-sectional study of Thoroughbred racehorses in Victoria, Australia, revealed that horses with EIPH grades \( \leq 1 \) were 4.0 times as likely to win and 1.8 times as likely to finish in the first 3 positions as were horses with grades \( \geq 2 \). Similarly, horse with EIPH grades \( \geq 1 \) finished significantly farther
behind the winner than did horses without EIPH (Hinchcliff et al., 2005b). To date, there is still no clinical or laboratory ‘gold standard’ method to provide an accurate or reproducible means of quantifying the severity of EIPH. Indeed, even if it is assumed that a higher tracheal endoscopic score represents more severe haemorrhage, the relationship between the amount of blood in the large airways and the actual amount of haemorrhage has still not been established.

Airway inflammation in horses with EIPH may be pre-existing or may develop as a consequence of pulmonary haemorrhage. 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 airway inflammation, as assessed by TW cytology. Horses with mild to moderate IAD had also higher odds (2.3 - 4.4) of having increased proportions of TW haemosiderophages. Conversely, no significant correlation has been reported between BALF haemosiderophage and neutrophil counts (Sanchez et al. 2005). Such causative or associative relationship has to date not been clearly been elucidated in experimental conditions. Instillation of autologous blood into the airways indeed resulted in controversial results in term of airway inflammation (Art et al. 2002; Derksen et al., 2007).

Since bronchoalveolar lavage using fibrotic endoscopy was first described in horses in the late 80’s, cytological evaluation of respiratory fluids have become important tools in the diagnosis of respiratory disease affecting performance in sport horses alongside clinical and functional examinations. Some studies included haemosiderophage counts in their published differential cells of both TW and BALF from clinically healthy horses, but there are no published reference values for these cells or for RBC counts in respiratory fluids.

Quantification of EIPH based on the cytological evaluation of respiratory fluids remains a big challenge. The detection of RBCs in BALF may simply be due to the BAL procedure (Langsetmo et al., 2000), and very little is known as to whether the segment being examined by lavage is representative of the whole region of the lung that may have bleeding. Erythrocyte detection in BALF, even though representative of acute bleeding, is not sufficient to make a diagnosis of EIPH. Furthermore, there is no direct association between RBC count and exercise performance, despite reports that administration of furosemide or application of a nasal strip either improved performance or reduced RBC count in horses during exercise on both treadmill and racetrack (Geor et al., 2001; Valdez et al., 2004). The problem with these studies was that it was uncertain whether the improvement in performance was due to the reduced RBC count or to a specific effect of the treatment used. This difficulty was compounded by the fact that tracheal endoscopic evaluations were not undertaken.

On the other hand, an ‘alternative’ cytological definition of EIPH has been proposed (Newton and Wood, 2002). Horses in which >50% of the TW macrophages were haemosiderophages were classified as EIPH positive. These authors examined 1614 horses and reported a prevalence of EIPH in 51% compared to only 4% when observing tracheal blood during endoscopic examination. When investigating haemosiderophages/macrophages ratio in BALF samples (May-Grünwald Giemsa [MGG] coloration), a cut-off has also been suggested at 20% for cytological detection of EIPH (Richard et al., 2010); with 10/38 (26.3%) poorly-performing horses exhibiting evidence of previous episodes of pulmonary haemorrhages. Similarly, an alveolar macrophage graded haemosiderin score (Perls coloration) was found to be significantly greater in horses with a history of EIPH, compared to control horses, and to be strongly associated with the presence of large amounts of erythrocytes in BALF (Doucet
and Viel, 2002). To date, no published study investigated haemosiderophage proportions in BALF or haemosiderin scores in relation to race performances.

Both cytological diagnostic methods (MGG vs. Perls) have recently been compared in a recent field study, in which BALF have been obtained from left and right lung in 123 Standardbred racehorses (Depecker et al., 2013). The results being obtained confirmed that both lungs should not be considered equivalent, with regard to EIPH diagnosis. When using MGG coloration, haemorrhage indeed occurred in both lungs for 19 (15.4%) horses, in one lung only for 22 (17.9%) horses, and 82 (66.7%) were considered as controls. While a highly significant correlation (R= 0.816; p< 0.001) was found between haemosiderophages/macrophages ratio (MGG coloration) and haemosiderin score (Perls coloration), a moderate agreement only (κ= 0.65; CI 0.54 – 0.77) was found between both diagnostic methods. Haemosiderin score indeed represents a highly sensitive tool for detecting EIPH, even for a limited period of time (several days) following the previous haemorrhagic episode. Using ‘general’ stains (like MGG), erythrophages may be observable within a few hours or days following haemorrhage, while haemosiderophages may persist in the airways up to several weeks or even months. A subjective timing of the previous haemorrhage may then be proposed to the practitioner, and particularly the occurrence of repetitive episodes of EIPH, with several maturity levels of hematoidin being found within haemosiderophages.

In conclusion, the interpretation of respiratory fluid analysis has, in recent times, considerably improved the diagnosis and understanding of underlying pathophysiological mechanisms in a variety of airway diseases. Indeed, BALF cytology allows the detection and subjective dating of previous haemorrhagic episodes, as well as characterising the possibly concomitant lower airway inflammation. No current data is however currently available for objectively correlate haemosiderophage proportions, severity of pulmonary haemorrhage and performances. Multiple methodological investigations are then of paramount importance, by combining both tracheal endoscopy and BALF cytology. Ongoing work is also continually identifying potential biomarkers of pulmonary haemorrhages, which although currently limited to research applications, may provide valuable clinical tools in the future.

References


