

# Association between exercise-induced pulmonary hemorrhage and inflammatory airway disease in polo ponies

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*The respiratory system is essential for health and high athletic performance in horses. Respiratory diseases have been recognized as having a major impact on training equine animals and are commonly cited as the second most common cause of wasted training time. Inflammatory airway disease (IAD) is an important cause of poor performance in young racehorses. Exercise-induced pulmonary hemorrhage (EIPH) is considered a major issue for the equine industry because of its high prevalence and association with reduced athletic performance. In Brazil, polo is a growing equestrian sport, but studies on it are still scarce. The aim of this study was to evaluate the occurrence of EIPH, the association between EIPH and IAD, and EIPH influence on the tracheal cytological profile of polo ponies. Thirty-seven horses regularly used for polo were included in this study. Endoscopic examination was performed every 30 to 90 min after practice, and tracheal lavage was performed after 18 to 24 hr. Sixteen animals (43.2%) presented a score of 0 for mucus in endoscopy; twelve animals (32.4%) presented a score for 1 and nine animals (24.3%) presented score 2 of mucus. IAD was characterized by tracheal cytology in 12 animals (32.4%). The occurrence of EIPH in this study was 29.7% (11/37). No significant difference was found in the cell types in tracheal cytology when EIPH-positive and EIPH-negative horses were compared. Polo ponies are affected by IAD and EIPH in relevant proportions, but there was no association between EIPH and tracheal cytological profile.*

**Key words:** EIPH, IAD, neutrophils, tracheal wash

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Respiratory diseases are one of the major causes of low performance and economic losses in the equine industry. Despite the growth in the number of animals used for polo activity and the reported high frequency of respiratory diseases in other activities [2, 4, 24, 36], horses used for polo have been poorly studied in clinical and epidemiological studies.

Inflammatory airway disease (IAD) can be defined as a failure in the regulation of inflammatory cell homeostasis in the airways and presents variable clinical signs [6, 9]. Animals with IAD usually do not show clear signs of respi-

ratory or systemic disease but instead show discrete and variable degree of airway inflammation [29]. IAD has been described in animals used for various activities, such as race horses [5, 18, 33] and horses used for policing [19]. For IAD diagnosis, collection of respiratory secretions is considered very important [11, 14]. Although bronchoalveolar lavage (BAL) is the adopted method for collection of respiratory secretions [9], it requires the animal to rest for at least 24 hr afterward [16], which makes the procedure unfeasible for sporting animals. It has been suggested that the presence of tracheal inflammation may not be sufficient to characterize IAD due to the lack of correlation between TW (tracheal wash) and BAL cytology [8, 29]. However, the term IAD has already been used to describe the inflammation based on the presence of increased neutrophils in the tracheal lavage of racehorses. [18, 36].

Exercise-induced pulmonary hemorrhage (EIPH) refers to the presence of pulmonary blood in the airways after intense exercise [7, 26]. Its prevalence varies according to

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the exercise intensity and diagnosis method. In racehorses, the occurrence of EIPH varies from 15 to 75% [3, 7, 33] and can reach 90% of animals [21]. In polo ponies, prevalences of 46% [25] and 34.09% [27] have been reported.

Studies have been carried out to investigate the cause-effect relationship between IAD and EIPH [22], and they describe two mechanisms: inflammation (and consequently bronchoconstriction), which increases intrapleural pressure and leads to bleeding, and the presence of blood in the alveoli and interstitial spaces, which may lead to inflammation and fibrosis [32]. However, EIPH can occur regardless of whether or not the pulmonary inflammation is present [34, 35]. The association between EIPH and airway inflammation has been studied in race animals [1, 12, 15, 26] but the results remain controversial. However, to the best of our knowledge, there are currently no descriptions of an association between EIPH and IAD in polo ponies.

The aim of this study was to evaluate the occurrence of EIPH, the association between EIPH and IAD, and the influence of EIPH on the tracheal cytological profile of polo ponies.

## Materials and Methods

This study was approved by the Committee for Animal Experimentation of Fluminense Federal University (study number 0068-08).

### Methods

We studied 37 horses (21 females and 16 males between 3 and 22 years old and weighting between 380 and 480 kg) regularly used for polo practice in the city of Rio de Janeiro, Brazil. The animals were fed commercial food, oat and alfalfa hay, and supplemented with mineral salt. All animals were kept in stables of 12 m<sup>2</sup>, and under the same bedding conditions. The horses were periodically vaccinated against tetanus, influenza, eastern and western encephalomyelitis, and rabies. All the animals had engaged in regular physical activity, with no physical complaints or signs of apparent disease. No horse was under any treatment or drug effect during the study.

Polo matches last roughly 2 hr and are divided into periods called “chukkas” [31]. Each chukka has a duration of 7.5-min, and there is a 3-min interval between them. All the horses in this study took part for just one period, twice a week. On other days of the week, only light exercises were performed.

An endoscopic evaluation of the respiratory tract was performed between 30 and 90 min after the end of each chukka [28]. The presence of blood and tracheal mucus was scored [7, 13]

TW was collected 12 to 16 hr post exercise as previously

described [37]. TW samples were centrifuged at 110 g for 5 min (model 206R; Fanem, São Paulo, SP, Brazil). Cell pellets were used to prepare slides with the linear smear technique [38]. Smears were fixed in methanol and stained with Giemsa. A differential cell count was obtained by analyzing 300 cells/slide from direct smears, and then mean values were calculated.

Horses with TW containing neutrophils counts <20% and eosinophil counts <1% were considered healthy, and those with neutrophil counts ≥20% and/or eosinophil counts ≥1% were considered to have an inflammatory profile [29]. We assigned the term IAD positive to these animals [18].

Statistical analysis was performed using the IBM SPSS Statistics for Windows software. A Kolmogorov-Smirnov test was used to evaluate data distribution. The cell type quantities of EIPH-positive and EIPH-negative groups were compared by Mann-Whitney test, with the significance level set at 5%.

## Results

### Endoscopic examination

Sixteen animals (43.2%) presented a score of 0 for mucus in endoscopy; twelve animals (32.4%) presented a score of 1 for mucus and nine animals (24.3%) presented a score of 2 for mucus. No mucus rated above a score of 2 was observed.

Endoscopic evidence of EIPH was found in 11 animals (29.7%). Seven animals had an EIPH score of 1, one animal had an EIPH score of 2, and two animals had an EIPH score of 3.

### Tracheal cytology

Twelve (32.4%) animals had IAD characterized by increased neutrophils and/or eosinophils. Nine (24.3%) presented neutrophilia in tracheal cytology and three (8.1%) presented eosinophilia. The mean percentages and standard deviations for the differential counts of healthy and IAD animals are shown in Table 1.

No significant difference was detected between the cell types found in the tracheal wash when comparing EIPH-positive and EIPH-negative animals. The mean cell counts and their standard deviations are shown in Table 2.

## Discussion

The occurrence of IAD in this study was similar to the reported occurrence in horses in military use [30] and lower than that observed in racehorses [9, 36]. Two factors that must be considered are the time period in which the endoscopic examination occurred (at rest or post exercise) and the subjectivity of the observer.

IAD occurrence was also similar to that reported for

**Table 1.** Mean percentages and standard deviations for differential cell counts in TW of polo ponies

n=37	Neutrophils	Lymphocytes	Macrophages	Eosinophils	Epithelial cells
Healthy (n=25)	7.39 ± 6.31	7.80 ± 7.28	36.63 ± 22.59	0.36 ± 0.76	47.68 ± 29.48
IAD (n=12)	57.76 ± 28.82	4.36 ± 1.95	24.71 ± 20.01	0.1 ± 0.21	13.03 ± 18.05

**Table 2.** Mean percentages and standard deviations for cell counts in TW of EIPH-positive and EIPH-negative polo ponies

n=37	Neutrophils <i>P</i> =0.431	Lymphocytes <i>P</i> =0.743	Macrophages <i>P</i> =0.418	Eosinophils <i>P</i> =0.183	Epithelial cells <i>P</i> =0.540
No EIPH (n=26)	19.26 ± 24.61 <sup>a</sup>	7.71 ± 7.35 <sup>a</sup>	31.05 ± 19.25 <sup>a</sup>	0.26 ± 0.64 <sup>a</sup>	41.6 ± 29.71 <sup>a</sup>
EIPH (n=11)	20.50 ± 31.36 <sup>a</sup>	5.16 ± 3.76 <sup>a</sup>	40.05 ± 28.41 <sup>a</sup>	0.38 ± 0.77 <sup>a</sup>	33.7 ± 34.18 <sup>a</sup>

Results are expressed as the mean ± standard deviation (SD). Same letters in the columns indicate no statistically significant differences (Mann-Whitney, *P*<0.05).

army horses [20] and lower than that reported animals in training [1] or used in policing [17]. In racehorses with a history of poor performance, IAD occurrence was higher [18]. Young horses in training often present posterior airways inflammation characterized by cytological evidence in tracheobronchial aspirates [32].

Bronchoalveolar lavage is the chosen method for IAD diagnosis [9]. However, it is important to consider the difficulty of performing this procedure in animals used for sporting activities due to the time period needed for rest and sedation. In these cases, tracheal lavage should be recommended. The term IAD was previously used for animals classified with neutrophilic infiltration in tracheal lavage [18]. We believe that in some situations, the term IAD can and should be employed. All animals in our study had no clinical complaints and had engaged in regular physical activity. Therefore, the proportion of airway inflammation should be considered important. Tracheal lavage was determinant for IAD diagnosis, taking into account the fact that mucus scoring at endoscopy is subject to variation for several reasons such as the time period after exercise and observer interpretation.

Endoscopic evidence of EIPH was found in 11 animals (29.7%). The occurrence of EIPH in polo animals was similar to that reported previously in the same population [27]. These authors did not observe a statistically significant relationship among animals with tracheal secretion and with EIPH. The occurrence of EIPH in our study is also lower than observed in Chile [25] and higher than that observed in Brazil [20] in polo ponies.

#### *Cytological profiles of EIPH horses*

The differential cell counts of EIPH-positive and EIPH-negative animals showed no statistical difference. These findings are similar to those of previous studies [1, 4, 5]. Other studies suggest that IAD may be important for EIPH

evolution, but this does not mean inflammation is a prerequisite for EIPH [22].

An increase in neutrophil counts for EIPH-positive animals was also found [12, 14, 23]. This result may vary due to differences in the tracheal lavage collection period after the exercise, as well as the place where the procedure was performed. In our study, the period between bleeding and lavage collection was between 12 and 16 hr in length, and this may not have been enough to detect a local inflammatory response. It is known that the inflammatory response caused by previous episodes of EIPH has an important role in new and more intense bleeding episodes, especially if horses undergo intense exercise within 3 to 7 days after the first episode of bleeding [22]. An association between EIPH and inflammation has also been demonstrated, and it was demonstrated that previous hemorrhage within a period of one month was a major risk factor for EIPH [26]. The time interval for performing diagnostic tests and the nature of the exercise performed, are factors that should be considered in the study's design. Other studies suggested that standardization of time and characterization of IAD phenotypes are critical parameters for studies of equine lung diseases [9].

Regarding procedural variations, tracheal lavage provides a pool of samples from both lungs, the distal trachea and bronchi. On the other hand, BAL allows for collection directly from the lung site involved in bleeding (caudal lobe) [10]. Thus, TW represents a more diluted sample when compared with BAL. These differences could influence the variability of results among studies. More information about IAD and EIPH pathophysiology is still needed to improve knowledge about possible interactions between these diseases [8]. Our results do not allow us to demonstrate the influence of EIPH on IAD. It should be considered that the etiology of EIPH is related to pulmonary capillary stress and failure and not necessarily related to pulmonary inflammation [32].

It has been demonstrated that polo ponies presented IAD and EIPH in important proportions, even when apparently healthy or physically active. These respiratory diseases should be considered in the physical state evaluation of athlete animals. No significant differences were found in the cytological profiles of tracheal lavage, regardless the presence of EIPH. Thus, there was no influence of EIPH on the cytological profile of tracheal lavage or IAD.

### Conflict of Interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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