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CLINICAL STUDIES ON EXERCISE INDUCED PULMONARY HEMORRHAGE (EIPH) IN HORSES

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ABSTRACT

The present study included 18 Thoroughbred horses with signs of EIPH competing in the 1600 m flat racing discipline. A clinical and endoscopic examination of the trachea (60-90 min after the finish) was performed, which confirmed the presence of blood in all horses examined. Two horses were diagnosed with grade 1 hemorrhage, two horses with grade 2, eight horses with grade 3 and six horses with grade 4. *Klebsiella spp.* and *Streptococcus equi subsp*. *Zooepidemicus* were isolated in Tracheal wash (TW). Tracheoscopy is an extremely valuable method for diagnosing pulmonary hemorrhage in the field.

Key words: Thoroughbred horses, lung, hemorrhage, endoscopy.

Introduction

Pulmonary hemorrhage do to physical exercise (EIPH) is a common condition in sport horses, triggered by intensive workload. It is more often seen in horses competing in disciplines that require maximum muscular effort, such as flat racing, harness racing, eventing, cross-country and polo (Hinchcliff et al, 2015; Leguillette, 2016; Lo Feudo et al, 2022; McGilvray and Cardwell, 2022). For this reason, it mainly affects horses of the breeds Thoroughbred, Standardbred, Paint Horse, Arabian and Quarter Horse, but it can be found also in all horse breeds. No gender or age-related predisposition has been identified. However, older horses are more likely to develop the disease, which is explained not by their age, but by their longer sporting careers and, consequently, a greater number of competitions (Newton *et al.*, 2005; Hinchcliff *et al.*, 2010). The condition was first described in the year 1688 by Markham. An interesting fact is that the ancestor of the most famous Thoroughbred horse Eclipse, named Bleeding Childers (b. 1716), also exhibited bleeding, which proves that horses with such a problem can still compete at the highest level of tournaments (Hinchcliff *et al.*, 2005; Morley *et al.*, 2015; Sullivan *et al.*, 2015; Sullivan and Hinchcliff, 2015). Initially, pulmonary hemorrhage was thought to be a physiological condition that only affects the most dedicated horses that are trained hard in order to win the tournaments (Rohrbach, 1990; Hinchcliff *et al.*, 2005; Hinchcliff *et al.*, 2015; Lo Feudo *et al.*, 2022). In 2015, the American College of Veterinary Internal Medicine (ACVIM) recognized pulmonary hemorrhage due to difficult exercise as a separate lung disease. This decision was supported by studies showing that 60% of sudden death cases during competition were associated with massive pulmonary hemorrhage (Gunson *et al.*, 1988; Boden, 2005; Lyle *et al.*, 2011). The condition was initially recognized by the presence of visible blood in the nostrils of affected horses (epistaxis). However, this sign is not very

certain, as it is only shown in a very small number of horses and ranges from 0.15% (Takahashi, 2001) to 13% (Poole and Erickson, 2016). Furthermore, this symptom is also present in other pathologies, such as nasal trauma, ethmoidal hematomas, or guttural pouches mycosis (aspergillosis) (Archer, 2008; Dobesova, 2012; Hinchcliff *et al.*, 2015). Pascoe *et al.* (1981) first applied endoscopy as a diagnostic method to detect the problem. After the widespread use of the endoscopy method it has been shown that a single check can diagnose the disease in 43% to 75% of racehorses. With two or three checks, the rate increases to 95% (Birks *et al.*, 2002; Hinchcliff *et al.*, 2005; Crispe *et al.*, 2016; Crispe *et al.*, 2018). An additional method to reach diagnosis is the histological examination of samples from tracheal wash (TW) or bronchoalveolar lavage (BAL), which detects the presence of erythrocytes in acute cases and hemosiderophages in subacute and chronic cases (Hoffman, 2008). According to Hinchcliff *et al.* (2005), detection of "hidden" incidents may be facilitated by firstly involving the animal in intense physical activity and subsequent endoscopic examination within 30 to 120 minutes afterwards. Nowadays, the methodology proposed by Hinchcliff *et al.* (2005) is used to assess the severity of bleeding, according to which, depending on the amount of blood poured into the trachea, 4 degrees of bleeding are distinguished. Studies have clearly demonstrated the negative effect of pulmonary hemorrhage on the athletic performance of the affected horses (Dabareiner *et al.*, 2009; Hinchcliff *et al.*, 2015; Morley *et al.*, 2015; Crispe *et al.*, 2017; Crispe *et al.*, 2019). At lower degrees of the condition, this effect is less noticeable, while at the most severe level 4, the heavy impact is the most strongly shown and the chance of such a horse to finish the race in the prize place is about 4 times lower than that of a healthy horse (Hinchcliff *et al.*, 2015; Robinson, 2015; Sullivan, 2015; Crispe, 2017). There are predisposing factors that can contribute to the manifestation of the disease in their own or in combination. These include vigorous physical activity during the winter-spring season and ambient temperatures below 20°C (Hinchcliff *et al.*, 2010), hard terrain (Newton *et al.*, 2005), closed horseshoes (Crispe *et al.*, 2015), and inflammatory processes of the lower respiratory tract (Newton al., 2002). Microbiological studies of respiratory secretions in horses with respiratory problems have most commonly identified the presence of bacteria, such as *Klebsiella spp*. and *Streptococcus equi subsp. zooepidemicus* (Estell *et al.*, 2015; Kasap *et al.*, 2018; Manguin *et al.*, 2020). From the above-mentioned findings, it is clear that EIPH is a widespread disease in sport horses. Despite large amount of studies, there are some uncertainties regarding the causes of its occurrence, its impact on the current sports form and future development prospects of sport horses, as well as the methods and means that lead to its limitation and treatment. Presently, research in Bulgaria on this pathology is quite scarce, which gives us reason to launch a series of studies on this problem. In this article, we present results from studies conducted with Thoroughbred horses suffering from EIPH.

Materials and Methods

The present study was conducted during the active racing season, starting from March to October 2024, at the racetracks in Sandrovo, Ruse, Kriva Bara (Bulgaria) and Racetrack in Ploesti (Romania). It included 18 Thoroughbred horses with signs of EIPH competing in the 1600 m flat racing discipline. All horses have been regularly dewormed and vaccinated against the diseases influenza, tetanus (Proteq Flu-Te, Boehringer Ingelheim Animal Health) and herpes virus infection strains 1,4 (Equip EHV 1,4, Zoetis UK) with accordance to the requirements of the International Equestrian Federation (FEI) and adopted by National federations. In Table 1 is shown the individual age and gender characteristics of the horses.

Table 1: Age and gender of horses

№	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Age (years)	4	3	6	3	4	5	5	4	5	4	6	7	5	4	5	8	6	7
Gender	S	S	S	M	M	G	G	G	M	M	S	M	G	M	S	G	S	M

The clinical examination was performed 60 to 90 minutes after finishing the race and included the following parameters: body temperature, heart rate, respiratory rate, color of visible mucous membranes, and capillary refill time. The endoscopic examination (VED-6000) included visualization of the larynx, trachea, and the beginning of both main bronchi. Horses were examined in a standing position after sedation with Xylazine (Bioveta, Czech Republic) using a dose of 0.5 – 1 mg/kg bw according to their reactivity. The severity of the disease was determined according to the criteria for assessment in four categories (Hinchcliff *et al.*, 2005) as follows:

Grade 0 = no blood detected in the pharynx, larynx, trachea, or mainstem bronchi

Grade 1 = presence of 1 or more flecks of blood or 2 or fewer short (less than one-quarter the length of the trachea) narrow (<10% of the tracheal surface area) streams of blood in the trachea or mainstem bronchi visible from the tracheal bifurcation

Grade 2 = 1 long stream of blood (greater than half the length of the trachea) or more than 2 short streams of blood occupying less than a third of the tracheal circumference

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

Grade 4 = multiple, coalescing streams of blood covering more than 90% of the tracheal surface with blood pooling at the thoracic inlet.

Samples for microbiological examination were obtained after flushing with 100 ml of sterile saline (0.9% NaCl) through the working canal of the endoscope into the most caudal part of the trachea containing a blood. Immediately afterwards, about 30-50 ml of the solution was aspirated and stored in sterile containers.

After centrifugation, the samples were inoculated on blood agar with 5% defibrinated sheep blood and on Mac Conkey agar. The incubation of the cultures was performed at 37ºC for 24-48 hours under aerobic conditions. Microscopic preparations were prepared from the isolated pure cultures and stained according to Gram. The morphological characteristics of the colonial growth, the presence or absence of hemolysis were assessed. Tests for catalase and oxidase activity were performed. For isolates determined as *Klebsiella spp.*, growth in Kligler medium, their attitude to lactose, the type of metabolism using the Hugh Lifeson test were also taken into account, indole and motility tests and the IMViC test were performed. For *Streptococcus spp.*, in addition to Gram staining, catalase and oxidase tests, as well as the type of hemolysis, as the final stage in the phenotypic determination of streptococcal strains, we used STREPTO 24test (Erba Lachema, Czech Republic), which includes 24 substrates. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, with 5% defibrinated sheep blood added to the agar for streptococcal isolates. All culture media used were manufactured by Hi Media, India.

The track characteristics were defined according to the requirements of the "Equibase Codes and Definitions" as follows: hard (3 races), good (2 races), wet and fast (1 race). The air temperature ranged from 18ºC to 31ºC. All horses were shod, with seven of them having steel open horseshoes,

four having steel closed horseshoes, three having open aluminum and four horses having closed aluminum horseshoes.

Results and Discussion

The conducted clinical examination did not reveal any statistically significant deviations in the studied parameters. The body temperature was in the range 37.9±0.25ºC, the heart rate – 48±6 /min, the respiratory rate – 14±5 / min. The color of the visible mucous membranes was the physiological pale pink, and the capillary refill time was less than 2 seconds. The lack of significant deviations in the cited indicators confirms the previously presented results of Morán and Folch (2013), Hinchcliff *et al.* (2015) and Crispe *et al.* (2017) and makes them unreliable factors in the diagnosis of pulmonary hemorrhage. Endoscopic examination revealed the presence of blood in various parts of the upper respiratory tract (nasal cavity, larynx and trachea) in all horses. In 6 horses (33.3%) the hemorrhage was manifested in the most severe 4th degree (Fig. 1), in 8 horses (44.4%) the 3rd degree was established (Fig. 2), in 2 horses (11.1%) the 2nd degree (Fig. 3) and 2 horses (11.1%) the mildest 1st degree (Fig. 4). To improve the diagnostic value of tracheoscopy in order to detect even the mildest degree of hemorrhage (Grade 1), we performed the examination according to the recommendations of Hinchcliff *et al.* (2005) and Stack (2015) within the interval from 60 to 90 minutes after the horses finished racing. We believe that this time window is sufficient to allow even the small amount of blood in the alveoli and small bronchi to enter the trachea and be later visualized. There is evidence that when the examination is performed immediately after the end of the exercise, small hemorrhages may not be detected (Sweeney, 1991) and therefore the problem may not be diagnosed. Four (22%) of the examined horses had a muffled cough after finishing, which can be explained with the presence of blood in the lower respiratory tract. Tracheoscopy data confirmed this assumption and according to the above mentioned criteria we categorized the hemorrhage as grade 4. Three of the horses with cough had clearly visible epistaxis from both nostrils (Fig. 5).

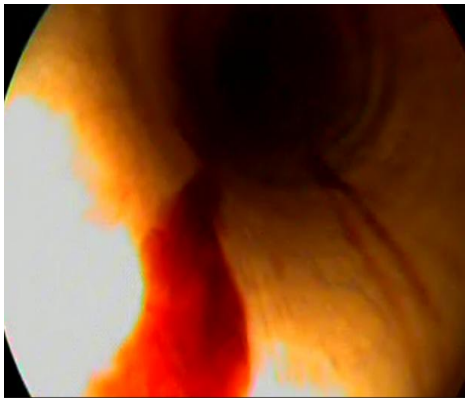

Figure 1: EIPH fourth degree

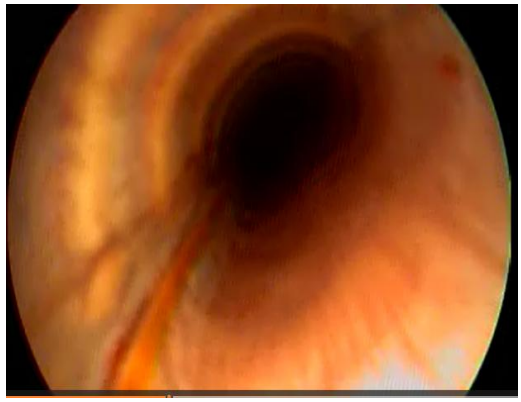

Figure 2: EIPH third degree

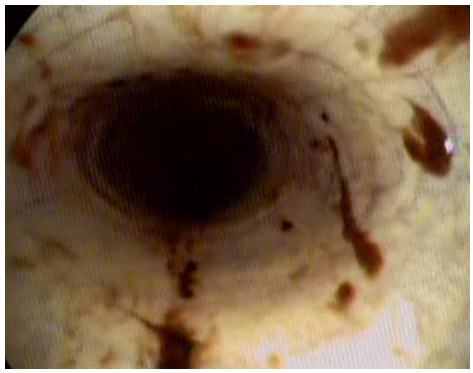

Figure 3: EIPH second degree

Figure 4: EIPH first degree

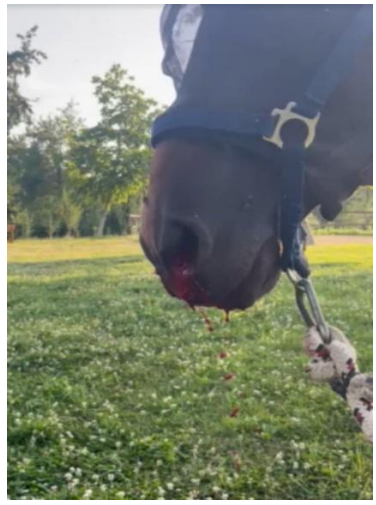

Figure 5: Epistaxis

The manifestation of epistaxis in conditions of heavy physical activity, which is inevitable in flat racing, can be considered a rather significant sign indicating pulmonary hemorrhage. In our study, it was present in five horses (27.7%), which can be a limitation of its practical application. This statement is further strengthened by its non-specificity, as it can also be found in other pathologies such as nasal trauma and guttural pouches aspergillosis (Takahashi *et al.*, 2001; Archer, 2008; Dobesova *et al.*, 2012; Hinchcliff *et al.*, 2015; Poole and Erickson, 2016). Four horses (22%) showed empty chewing movements, which we think is due to the blood reaching the nasopharynx and the subsequent irritation. In our opinion, the appearance of a dull cough and "unexplained" chewing movements towards the end of the distance or immediately after finishing the race can be taken as an indication of the possible presence of blood in the respiratory tract, which requires special attention and confirmation by endoscopic examination. This is an important check because of the presence of a similar coughing as a symptom in some other pathologies of the respiratory system, such as laryngotracheitis, bronchitis, asthmatic syndrome (Couetil *et al.*, 2007; Rettmer *et al.*, 2015). According to Pascoe (1997), the act of coughing is a consequence of the irritation of receptors located in the wall of the trachea by blood coming from the lung. Because of the presence

of blood in the nasopharynx, stridor may also be detected, which additionally leads the examiner to consider a possible hemorrhage of pulmonary origin (Hinchcliff, 2014). Undoubtedly, the presence of blood in the respiratory tract has a negative influence on gas exchange and subsequently worsens the performance of the horse. The conducted studies have established a proportional correlation between the amount of blood and the physical condition of horses. With minimal volumes of effusions, the effect is almost unnoticeable, while with an increase, the performance deteriorates significantly (Hinchcliff *et al.*; 2005; Derksen *et al.*, 2011; Sanchez *et al.*, 2005; Stack, 2015). Studies by Robinson *et al.* (2015) and Sullivan and Hinchcliff (2015) show that horses with no or little amount of hemorrhage are four times more likely to win a race than horses with severe hemorrhage. This is confirmed by the rankings of the horses included in the study. Ranking from 1st to 3rd place – 2 horses with first, 1 horse with second degree of bleeding, from 4th to 6th place – 1 horse with second, 3 horses with third degree of bleeding, out of the ranking after 7th place – 5 horses with third and four horses with fourth degree of bleeding, non-finishers – 2 horses with 4th degree. Regardless of these differences, we believe that in order to draw more reliable conclusions additional studies are necessary. They should involve a larger number of horses while also taking into account other factors influencing the current ranking (training regimen, competitive illnesses). Regarding the future sports career of the horses, the results of these studies establishing progressive damage to the lung parenchyma and the development of venoocclusive disease in the dorsocaudal lobes, which significantly reduces the chances of competitive success, must also be taken into account (Derksen *et al.*, 2011; Stack *et al.*, 2013; Robinson *et al.*, 2015). Track characteristics and weather conditions should also be taken into consideration and in this respect we support the results of Hinchcliff *et al.*, (2010), Newton *et al.* (2005) and Crispe *et al.* (2015). Due to the relatively small number of horses included in the study, we believe that further studies are needed to draw more reliable conclusions about their impact as predisposing factors for the disease. The microorganisms *Klebsiella spp. and Streptococcus equi subsp. Zooepidemicus* isolated in TW we attribute to the presence of a competing infection in the respiratory tract and not to a direct cause of hemorrhage. In this regard, we support the opinion of Estell *et al.* (2015), Kasap *et al.* (2018) and Manguin *et al.* (2020) regarding the role of pathogenic microflora in respiratory pathology in horses. EIPH is an extremely widespread condition found in racehorses, which undoubtedly requires further in-depth research to establish its etiology, treatment options and prevention.

Conclusion and Recommendation

EIPH is a frequently diagnosed condition in sports horses subjected to extreme physical exertion. In severe cases, it undoubtedly deprives sports performance and shortens the sports career. Direct endoscopy and visualization of blood in the trachea is an extremely reliable diagnostic method that also detects mild forms of the disease. The presence of microorganisms in the tracheal wash is in our opinion, a consequence of a parallel respiratory tract infection. Additional studies are needed to assess the impact of other potential factors such as the nature of the track, climatic features and the type of horseshoes.

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