Investigation of respiratory disorders in Thoroughbred racehorses training at the Brazilian Jockey Club

Natália Rebouças Pires¹ Maria Fernanda de Mello Costa² Marcia Torres Ramos³
Anna Paula Balesdent Barreira⁴ José Renato Junqueira Borges⁵

¹Faculdade de Veterinária, Universidade Castelo Branco (UCB). Programa de Pós-graduação em Medicina Veterinária - Clínica e Reprodução Animal, Faculdade de Veterinária, Universidade Federal Fluminense (UFF), Rua Vital Brasil Filho, 64, 24230-340, Niterói, RJ, Brasil. E-mail: nreboucas@infolink.com.br. Corresponding author.
³Instituto de Veterinária, Programa de Pós-graduação em Medicina Veterinária, Universidade Federal Rural do Rio de Janeiro (UFRJR), Seropédica, RJ, Brasil. Programa Mestrado em Diagnóstico Clínico e Laboratorial em Medicina Veterinária, Universidade Severino Sombra (USS), Vassouras, RJ, Brasil.
⁴Departamento de Medicina e Cirurgia Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro (UFRJR), Seropédica, RJ, Brasil.
⁵Faculdade de Agronomia e Medicina Veterinária, Universidade de Brasília (UnB), Brasília, DF, Brasil.

ABSTRACT: Athletic horses need to maintain healthy airways for optimal performance. This study investigated the presence of respiratory problems in apparently healthy Thoroughbred racehorses in training. According to the Revised Consensus Statement on Inflammatory Airway Diseases of Horses (2016), determining the prevalence of respiratory disorders in different equine populations is fundamental for understanding these diseases. In total, 72 clinically sound Thoroughbred racehorses, in training at the Brazilian Jockey Club (JCB), were initially examined using interpleural pressure measurement by ventigraphy and respiratory endoscopy. When secretions were present in the airways, transendoscopic tracheal aspiration was performed, and the sample was sent for cytology. The main findings included a combination of bronchospasm and tracheal secretions with 61% of the cytology slides showing neutrophil counts ≥20%. Overall, a significant number of the horses displayed signs suggestive of inflammatory airway disease (mild equine asthma), including 47% with increased DPplmax, 11% with tracheal mucus accumulation [mucus score (MS) ≥2] and 18% with carina edema. This was more pronounced in 2-year-old horses within the population studied. These findings are consistent with the literature and reinforce the importance of routine respiratory examination of athletic horses. There is a high incidence of subclinical respiratory disorders in Thoroughbred racehorses in training at the JCB and a significant association between tracheal MS≥2, carina edema, and elevated DPplmax.

Key words: subclinical airway inflammation, sports medicine, endoscopy, interpleural pressure, cytology.

INTRODUCTION

Optimal airway function is essential for horses performing at high levels. It is common for subclinical respiratory diseases to go undetected on routine clinical examinations, often requiring auxiliary diagnostic methods (WYSOCKA & KLUCINSKI, 2014). According to COÛETIL et
Inflammatory Airway Disease-IAD- (mild to moderate equine asthma) is a nonseptic inflammation, which can affect horses of any age and is prevalent in racehorses. The diagnosis is based on the presence of clinical signs of lower airway disease (poor performance, cough), documentation of lower airway inflammation based on mucus detected during endoscopy [mucus score (MS)≥2], cytology or abnormal lung function, and the exclusion of severe equine asthma [recurrent airway obstruction (RAO)/heaves] along with infectious and other respiratory diseases. There are indications that the frequent observation of this condition in racehorses may be linked to the innate immune response of equine bronchial epithelial cells that is possibly altered by intense physical exercise (FRELLSTEDT et al., 2015).

A study by HOLCOMBE et al. (2006) indicated that only 4% of horses with tracheal mucus observed during endoscopy also had nasal discharge, that pulmonary auscultation of horses with tracheal mucus accumulation was often normal, and that coughing was usually absent in these horses. These observations reinforce the importance of respiratory endoscopy as an effective diagnostic method, especially in horses mildly affected by chronic or sub-acute pathologies (BEECH, 1991). Also, a recent study demonstrated that endoscopic assessment of carina thickness and airway mucus is useful for diagnosing IAD and RAO (WYSOCKA & KLUCINSKI, 2014).

In animals with bronchospasm, there is an increased difference between the lowest inspiratory pressure and the highest expiratory pressure (BOERMA et al., 1995), allowing maximal changes in interpleural pressure ($\Delta P_{pl\text{max}}$) to be measured under field conditions for the purpose of assessing the severity of bronchospasm (HERHOLZ et al., 2002). This diagnostic method, known as ventigraphy, uses an esophageal balloon attached to the distal end of the catheter with a pressure transducer connected to a physiograph and provides readings for $\Delta P_{pl\text{max}}$. Absence of bronchospasm is confirmed by the demonstration of values below 4cm of H$_2$O (KLEIN & DEEGEN, 1987).

Cytology is a well-described auxiliary diagnostic test for detecting inflammation or infection in the airways. The collection of samples via transendoscopic aspiration has especially proven valuable for racehorses in training because it is minimally invasive and does not require sedation or disruption of the training schedule (KUSANO et al., 2008). Despite several studies describing cytological and endoscopic profiles of horses with inflammatory airway conditions, to the authors’ knowledge there are no studies reporting the incidence of subclinical airway disease in racehorses in training at the Brazilian Jockey Club (JCB), which is notably one of the most important racetracks in Latin America. This study investigated the occurrence of airway disorders in asymptomatic Thoroughbred racehorses training at the JCB, while describing the most common abnormalities and the association between them.

MATERIALS AND METHODS

In total, 72 clinically sound and actively racing Thoroughbreds were examined during the Brazilian summer and autumn, between the months of February and June. The study population consisted of equal numbers of males and females aged 2–6 years under the care of three trainers at the JCB, Rio de Janeiro. This sample size represented 5% of all active horses at the JCB during the research period. Horses were selected from a group of 150 animals without the clinical signs of respiratory disease based on history and clinical examination. Tests were performed at the stables where the horses were housed between 8 am and 9 am, 60-90min after morning exercises. Ventigraphy was followed by endoscopy, and when secretions were observed, tracheal samples were collected for cytology. Each examination took 8-10min; the horses were mechanically restrained with a twitch, and the examination was well tolerated. The probe of the ventigraph (Ventigraph®) consisted of a rigid outer covering with a thin internal catheter and a balloon at the end and measured 170cm in length and 8mm in diameter. It was introduced into the esophagus through the right nostril up to the mediastinum, approximately 135-145cm from the nostril, and only then was the balloon tip inflated with 10mL of air pushed through a syringe. According to the technique described by KLEIN & DEEGEN (1984), accurate placement of the probe is confirmed by recording a sequence of 10 regular respiratory cycles. Differences in pressure measured by the balloon were transmitted pneumatically to the ventigraph through the probe and registered graphically on the recording paper provided with the equipment by the manufacturer. The recording paper speed was 2mm/s for 5min. Interpleural pressure differences ≤4cm H$_2$O were considered as physiological, 5-8cm H$_2$O as mildly increased, 9-14cm H$_2$O as moderately increased, and ≥15cm H$_2$O as markedly increased (KUSANO et al., 2008).
increased, and \( \geq 15 \text{cm H}_2\text{O} \) as severely increased according to literature (AINSWORTH et al., 2003; PEARSON et al., 2007).

Endoscopic exams were performed by the same veterinarian (NR) with a Pentax flexible fiberscope, model 38LX, 170cm long, 12.8mm diameter, and with a working channel of 3.8mm. Abnormalities were recorded with the amount of tracheal mucus being scored subjectively (0-5) according to GERBER et al. (2004). Tracheal septum thickness was evaluated according to WYSOCKA & KLUCINSKI (2014) and the severity of exercise-induced pulmonary hemorrhage (EIPH) as described by PASCOE (1981).

Transendoscopic collection of tracheal mucus was achieved by the insertion of a polyethylene tube (ESS Supplies®) through the equipment’s working channel and aspiration of the sample from the caudal portion of the trachea, cranial to the bronchial bifurcation according to the technique described by SWEENEY (1992).

The tracheal samples were placed on glass slides and prepared using the squash technique described by COWELL et al (1992). Slides were immediately labeled, fixed with methanol, stained with Wright-Giemsa, and sent to the Veterinary Anatomic Pathology Laboratory at Universidade Federal Fluminense (UFF) for a differential count of 300 nucleated cells as mentioned in ROBINSON (2003).

Results of the ventigraphy and endoscopy were subjected to statistical analysis with Minitab®17.3.1 software using the Chi-square test, with a P≤0.05 considered statistically significant, to investigate the correlation among increased \( \Delta \text{Pplmax} \), tracheal mucus \( \geq 2 \), and carina edema, and the correlation between tracheal mucus and age. Descriptive statistics were used to assess the incidence of airway disorders in this population of clinically healthy racehorses.

This study was performed according to the ethical standards of the local committee (Colégio Brasileiro de Experimentação Animal - COBEA).

RESULTS AND DISCUSSION

Based on ventigraphy alone, 47% (n=34) of the horses examined presented some degree of bronchospasm evidenced by interpleural pressure variations higher than 4cm H\(_2\)O (Table 1). When only horses with abnormal \( \Delta \text{Pplmax} \) were considered, 94% had pressure variations between 5 and 8cm H\(_2\)O, while only 3% reached 11cm H\(_2\)O (Table 1), demonstrating that bronchospasm was predominantly mild. According to COÜETIL et al. (2007 and 2016) mild variation in \( \Delta \text{Pplmax} \) (5-10cm H\(_2\)O) could be associated with IAD horses.

When endoscopy alone was considered, 50% (n=36) of the horses had at least one airway abnormality. From the 36 animals with endoscopic abnormalities, 56% (n=20) presented one, 36% (n=13) presented two, and 8% (n=3) presented three or more alterations. Presence of tracheal secretions was the main finding in the endoscopy (16/36) followed by pharyngeal lymphoid hyperplasia (14/36), carina edema (13/36), EIPH (9/36), dorsal displacement of soft palate (3/36), recurrent laryngeal neuropathy (1/36), and epiglottic entrapment (1/36).

When 2-year-old horses were compared to older animals using Chi-square, they had a significantly greater likelihood (P=0.001, \( \chi^2=11.15 \)) of presenting a tracheal MS\( \geq 2 \) (Table 2). This may be related to the adaptation to intensive training, which has been reported to alter the innate immune response in the lung and the systemic circulation.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number of horses</th>
<th>Normal ( \Delta \text{Ppl} )</th>
<th>Altered ( \Delta \text{Pplmax} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>11</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>07</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>38</td>
<td>13</td>
</tr>
</tbody>
</table>

\( \Delta \text{Pplmax} \): maximal change in interpleural pressure.

Table 1 - Ventigraphy results from 72 thoroughbred racehorses in training at the Brazilian Jockey Club, in Rio de Janeiro, according to age.
(MICHELOTTO et al., 2010; FRELLSTEDT et al., 2015). Nearly 50% of the 2-year-old horses in our study exhibited MSs of 2/5 (Table 2). In previous reports, up to 73% of 2-year-olds and 28% of 3-year-olds had tracheal mucus accumulation (GERBER et al., 2003; CARDWELL et al., 2011; WYSOCKA & KLUCINSKI, 2014), further supporting the possibility of adaptation. Increased mucus accumulation is likely to impact athletic performance, with tracheal mucus scores of 2 or greater being associated with poor performance (HOLCOMBE et al., 2006).

Of the 72 horses studied, 16 (22%) had visible tracheal secretions, 8 (11%) had MS≥2, which can be considered a typical feature of IAD (HOLCOMBE et al., 2006; COÜETIL et al., 2016). Additionally, 13 (18%) animals presented with carina edema, and 4 (5%) presented simultaneously with MS≥2 and carina edema. All horses with MS≥2 demonstrated a ΔPplmax higher than 4 cm H₂O, indicating bronchospasm. Conversely, none of the 38 horses with normal interpleural pressure exhibited tracheal secretions on endoscopy. According to WYSOCKA & KLUCINSKI (2014), the presence of tracheal mucus and carina edema on endoscopic examination are indicative of lower airway inflammation.

Statistical analysis demonstrated that there was a significant association (P=0.002, χ²=10.01) between elevated ΔPplmax and a tracheal MS≥2. The mean ΔPplmax for horses diagnosed with MS≥2 was 7.00±1.93, while it was 4.88±1.32 for horses with MS<2. Using a two-sample t test, this difference was significant (P=0.019, 95% CI, -3.78 - -0.47). When ΔPplmax values from horses with and without carina edema were compared, the difference was also statistically significant (P=0.033, 95% CI, -1.93 - -0.01).

Overall, when single signs suggestive of IAD were considered, 47% (34/72) of the animals examined had abnormal ΔPplmax, 18% (13/72) had a thickened carina, and 11% (8/72) had tracheal MS≥2. When the combination of results was investigated, 26.5% (8/34) had carina inflammation and abnormal ΔPplmax, 23.5% (9/34) had tracheal MS≥2 and abnormal ΔPplmax, and 50% (4/8) had tracheal MS≥2 and carina edema.

Transendoscopic tracheal aspiration and cytology were performed on the 16 horses that demonstrated tracheobronchial secretions with the purpose of evaluating cytological profiles, which revealed the following average percentages for cell counts: macrophages, 60.89±23.12; neutrophils, 29.39±25.17; lymphocytes, 8.50±7.68; mast cells, 0.00±0.00; and eosinophils, 1.22±0.97. No universally accepted reference intervals exists for tracheal aspirate differential cell counts; however, CIAN et al. (2015) established the following means ± standard deviations in asymptomatic horses: macrophages, 79.6±8.2; neutrophils, 9.3±5.8; lymphocytes, 9.3±4.9; mast cells, 0.2±0.6. Except for neutrophils, the cell populations appeared to be within these ranges (CIAN et al., 2015).

The percentage of neutrophils was greater than 20% in 61% of the slides examined, which could be indicative of airway inflammation (ROBINSON, 2003). Also, 90% of the animals with neutrophilia (≥20%) showed abnormalities in both ventigraphy and endoscopy. Because only horses with tracheal mucus had cytology evaluated, no further analyses were pursued in order to not introduce bias into the analyses.

Although only 9 (12%) of all the examined horses showed signs of EIPH on endoscopy, 94% (n=15) of the horses with tracheal secretions showed

Table 2 - Tracheal secretions score observed in 72 clinical sound thoroughbred racehorses after morning training at Jockey Club Brasileiro, RJ, according to age.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>n</th>
<th>G0</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>11</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>17</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>17</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

G0: grade 0; G1: grade1; G2: grade2; G3: grade 3; G4: grade 4; G5: grade 5, according to Gerber, 2004; n = number of horses.

Ciência Rural, v.47, n.8, 2017.
hemosiderophages. 44% (n=7) showed hemosiderin, 94% (n=15) showed activated macrophages, and 75% (n=12) showed epithelial cells on cytology. As the endoscopy was performed 60-90min after morning exercises (submaximal exercise), a low incidence of EIPH was expected. Presence of hemosiderophages was also expected because all horses were actively racing. Sweeney et al. (1992) demonstrated that 86% of horses presented hemosiderophages on cytological evaluation of tracheal aspirate. According to Dixon (1997), the presence of hemosiderin within macrophages is a post-EIPH finding and rules out the possibility that erythrocytes or hemosiderin resulted from the trauma during the collection of samples.

Results of this study demonstrate high rates of non-infectious respiratory alterations, supporting research conducted in various countries that reported rates of 40%-80% of subclinical cases of respiratory disorders (Gerber et al., 2003; Ramzan et al., 2008; Kusano et al., 2008; Wysocka & Klucinski, 2014).

CONCLUSION

There is a high incidence of subclinical respiratory disorders in Thoroughbred horses trained at the Brazilian Jockey Club, especially in the 2-year-old ones, which reinforces the value of routine respiratory investigation in racehorses. Ancillary diagnostic tools such as ventigraphy, endoscopy, and tracheal aspirate cytology were useful for the diagnosis of subclinical respiratory conditions, including signs suggestive of IAD, and a significant association between a tracheal mucus score ≥2, carina edema, and elevated ΔPplmax exists.

BIOETICS AND BIOSecurity commitTEE aPROval

We authors of the article entitled “Investigation of respiratory disorders in thoroughbred racehorses training at the Brazilian Jockey Club" declared, for all due purposes, the project that gave rise to the present data of the same has not been submitted for evaluation to the Ethics Committee of the Universidade Federal Fluminense (UFF), but we are aware of the content of the Brazilian resolutions of the Conselho Nacional de Controle de Experimentação Animal (CONCEA) <http://www.mct.gov.br/index.php/content/view/310553.html> if it involves animals.

Thus, the authors assume full responsibility for the presented data and are available for possible questions, should they be required by the competent authorities.

REFERENCES


