

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

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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 $\geq 20\%$. 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.*

Investigação de doenças respiratórias em cavalos de corrida em treinamento no Jockey Club Brasileiro

RESUMO: *Cavalos atletas devem apresentar higidez das vias respiratórias para que obtenham bom desempenho esportivo. Este estudo buscou verificar a incidência de alterações respiratórias em cavalos da raça Puro Sangue Inglês (PSI) de corrida em treinamento, aparentemente sadios. De acordo com o Estatuto do Consenso de Doenças Inflamatórias das Vias Aéreas revisado em 2016, o levantamento das alterações respiratórias em diferentes populações de cavalos é fundamental para o entendimento dessas enfermidades. Nesse sentido, foram examinados 72 equinos PSI em treinamento no Jockey Club Brasileiro (JCB), por meio de mensuração da pressão interpleural através da ventigrafia e endoscopia respiratória. Quando presentes nas vias aéreas, foram coletadas secreções por aspirado traqueal transendoscópico para realização de citologia. Os achados prevalentes foram a combinação de broncoespasmo e secreções traqueais onde, em 61% das lâminas, houve contagem de neutrófilos $\geq 20\%$. De um modo geral, um percentual significativo de cavalos apresentou sinais sugestivos de Doença Inflamatória das Vias Aéreas (DIVA), incluindo 47% com elevação na Δ DPplmax, 11% com aumento no muco traqueal (score de muco ≥ 2) e 18% de edema de carina. Dentro da população estudada, essas alterações foram mais pronunciadas em animais de 2 anos. Esses achados reforçam a importância da investigação respiratória rotineira nos cavalos atletas indo ao encontro do que foi descrito em outras publicações. As alterações respiratórias subclínicas têm alta incidência em cavalos PSI em treinamento no JCB e existe uma associação significativa entre o muco traqueal (score ≥ 2), edema de carina, e elevação na DPplmax.*

Palavras-chave: *inflamação subclínica das vias aéreas, medicina esportiva, endoscopia, pressão interpleural, citologia.*

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

al. (2016), 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) \geq 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 (Δ Pplmax) 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 Δ Pplmax. Absence of bronchospasm is confirmed by the demonstration of values below 4cm of H₂O (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 \leq 4cm H₂O were considered as physiological, 5-8cm H₂O as mildly increased, 9-14cm H₂O as moderately

increased, and ≥ 15 cm H₂O 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, 170 cm long, 12.8 mm diameter, and with a working channel of 3.8 mm. 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 $P \leq 0.05$ considered statistically significant, to investigate the correlation among increased ΔP_{plmax} , tracheal mucus ≥ 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 4 cm H₂O (Table 1). When only horses with abnormal ΔP_{plmax} were considered, 94% had pressure variations between 5 and 8 cm H₂O, while only 3% reached 11 cm H₂O (Table 1), demonstrating that bronchospasm was predominantly mild. According to COÛETIL et al. (2007 and 2016) mild variation in ΔP_{plmax} (5-10 cm H₂O) 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 1 - Ventigraphy results from 72 thoroughbred racehorses in training at the Brazilian Jockey Club, in Rio de Janeiro, according to age.

Age (years)	Number of horses	Normal ΔP_{pl} ----- Altered ΔP_{plmax} -----							
		≤ 4	5	6	7	8	9	11	%/age
2	11	3	1	1	5	-	-	1	72%
3	22	12	5	3	-	1	1	-	45%
4	19	12	6	1	-	-	-	-	36%
5	13	6	1	1	2	3	-	-	53%
6	07	5	-	-	2	-	-	-	28%
Total	72	38	13	6	9	4	1	1	

ΔP_{plmax} : maximal change in interpleural pressure.

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

Age (years)	n	-----Tracheal Secretion Grade (n) -----					
		G0	G1	G2	G3	G4	G5
2	11	6	0	5	0	0	0
3	22	17	4	1	0	0	0
4	19	17	2	0	0	0	0
5	13	10	2	1	0	0	0
6	7	6	0	1	0	0	0
	72	56	8	8	0	0	0

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

(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 $MS \geq 2$, which can be considered a typical feature of IAD (HOLCOMBE et al., 2006; COÛETIL et al., 2016). Additionally, 13 (18%) animals presented with carina edema, and 4 (5%) presented simultaneously with $MS \geq 2$ and carina edema. All horses with $MS \geq 2$ demonstrated a ΔPpl_{max} higher than 4cm H_2O , 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 ($P=0.002$, $\chi^2=10.01$) between elevated ΔPpl_{max} and a tracheal $MS \geq 2$. The mean ΔPpl_{max} for horses diagnosed with $MS \geq 2$ 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 ΔPpl_{max} 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 ΔPpl_{max} , 18% (13/72) had a thickened carina, and 11% (8/72) had tracheal $MS \geq 2$. When the combination of results was investigated, 26.5% (8/34) had carina inflammation and abnormal ΔPpl_{max} , 23.5% (9/34) had tracheal $MS \geq 2$ and abnormal ΔPpl_{max} , and 50% (4/8) had tracheal $MS \geq 2$ 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 \pm standard deviations in asymptomatic horses: macrophages, 79.6 ± 8.2 ; neutrophils, 9.3 ± 5.8 ; lymphocytes, 9.3 ± 4.9 ; mast cells, 0.0 ± 0.0 ; and eosinophils, 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 ($\geq 20\%$) 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

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 ΔP_{plmax} exists.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

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.

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