

EVIDENCE OF LOWER RESPIRATORY AIRWAY INFLAMMATION IN HEALTHY THOROUGHBRED YEARLINGS BEFORE STARTING TRAINING

(Evidências de inflamação das vias aéreas respiratórias inferiores, em potros PSI saudáveis, antes do início dos treinamentos)

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ABSTRACT – Lower respiratory airway obstruction has a major impact in racehorse athletic potential. Inflammatory Airway Disease (IAD) is a common finding in young racehorses. This study tested the hypothesis that lower airway inflammation could initiate in farm life. Seven different breeding farms were visited, and 119 yearlings were investigated. Lower airways were evaluated by clinical examination, endoscopy and cytology of the tracheal aspirate. From those yearlings considered clinically healthy, 90,76% evidenced tracheobronchial mucus and some showing cytological profile of inflammatory reaction. Then, the present investigation confirms the occurrence of inflammatory condition of the lower respiratory airways in thoroughbred yearlings before training has commenced, representing a potential risk for the future athletic performance in young racehorses.

Key-words: airways; horses; endoscopy; cytology; tracheal aspirate.

RESUMO – Obstruções do trato respiratório inferior apresentam grande impacto no potencial atlético de cavalos de corrida. Doença Inflamatória das Vias Aéreas (DIVA) constitui achado comum em cavalos de corrida jovens. Esse estudo testou a hipótese de que a inflamação do trato respiratório inferior poderia iniciar ainda na vida no haras. Sete diferentes haras foram visitados, e 119 potros sobreano foram investigados. As vias aéreas inferiores foram avaliadas através de exame clínico e endoscópico, e citologia do aspirado traqueal. Entre os potros considerados clinicamente saudáveis, 90,76% evidenciaram muco traqueo-bronquial e os perfis citológicos evidenciaram reação inflamatória presente. A presente investigação confirma a presença de inflamação pulmonar em potros PSI antes do início do treinamento, representando um risco potencial à capacidade atlética futura.

Palavras-chave: vias aéreas; cavalos; endoscopia; citologia; aspirado traqueal.

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INTRODUCTION

Lower respiratory airway inflammation and obstruction have a major significance in the impairment of athletic performance (GERBER, 2001; HODGSON *et al.*, 2003; ROBINSON *et al.*, 2003; HOLCOMBE *et al.*, 2005; ROBINSON, 2005). Young athletic horses evidence a condition of a non-septic airway inflammation, characterized by an increase in airway secretion with a predominant neutrophil population and alteration of the pulmonary function, recognized as Inflammatory Airway Disease (IAD) (RUSH, 2003; ROBINSON, 2005). IAD can be characterized cytologically by different inflammatory profiles as a mixed inflammation, with an increase in total nucleated cells, mild neutrophilia (>15% of total cells), lymphocytosis, monocytosis, or an increase in metachromatic cells (mast cells >2% of total cells), or eosinophilic inflammation (5 – 40% of total cells) (RUSH, 2003).

The presence of activated neutrophils in the airways initiates mucus production, and the presence of visible mucus may be a clinical tool in order to point out if the increased neutrophil number is causing lung functional effects. Increased mucus in airways implies in functional consequences to the IAD horse, resulting in obstruction, leading to ventilation/perfusion mismatching and hypoxemia (ROBINSON, 2005; HOLCOMBE *et al.*, 2006).

Moderate to increased amount of mucus in the trachea, evidenced by bronchoscopic evaluation, represents a risk factor to the athletic performance (HOLCOMBE *et al.*, 2005). This can be confirmed by other reports that associated increased amount of mucus in the airways to hypoxemia, compromised lung function, cough, increased heart rates, increased blood lactate and reduced athletic capacity (GERBER, 2001; CHRISTLEY *et al.*, 2001; HOLCOMBE *et al.*, 2005).

The etiopathogenesis of IAD is ample, but inhalation of organic dust, particularly environmental endotoxins and molds, is one of the most implicated causes in horses maintained in stalls (McGORUM and PIRIE, 2003). In fact, it was demonstrated that, in a population of young thoroughbred horses with healthy appearance, more than 40% developed neutrophilic inflammation of the airways in the first two weeks after being stabled, giving evidence of the participation of respirable aeroallergens at the horse environment in IAD etiopathogenesis (HODGSON *et al.*, 2005).

The prevalence of IAD in the general race horse populations is around 12%, but the annual incidence in two year-old race horses can be as high as 80% (HOLCOMBE *et al.*, 2005). It has been reported that

40% of young thoroughbred racehorses can develop neutrophilic airway inflammation in the first two weeks after being stabled (HODGSON *et al.*, 2005), but it was not possible to find a report on thoroughbreds airway condition before being entered to an intensive stable management.

Then, it has been considered the hypothesis that lower airway inflammation could be present before training is initiated. Therefore, the aim of our work was to investigate a population of thoroughbred yearlings before initiating their training program, in order to seek for any possible evidence of lower respiratory airway inflammation and related causes, still in the farm life conditions.

MATERIAL AND METHODS

1. SUBJECTS –

The respiratory tract of 119 thoroughbred yearlings, 59 male and 70 female, between 18 and 24 months old, from 7 different breeding farms from Paraná State, Brazil were evaluated. The complete data collection was conducted in each farm and comprehended: questionnaire, clinical examination, endoscopic evaluation and cytological evaluation of the tracheobronchial secretion.

2. QUESTIONNAIRE –

The questionnaire included questions about the life of each individual, including the stable (time spent inside the barn) and feeding management, bed type, vaccination schedule, deworming, and previous respiratory diseases.

3. CLINICAL EXAMINATION –

A complete clinical examination was conducted, evaluating heart and respiratory rates, nasal discharge, cough, pharyngeal lymphnodes and tracheal and respiratory sounds (McGORUM and DIXON, 2007).

4. ENDOSCOPIC EVALUATION –

The airways were evaluated endoscopically using a Welch-Allyn video-endoscope (200cm x 9mm). The airways examination included upper and lower respiratory tract through the tracheal bifurcation (carina). The presence of tracheobronchial mucus received a score of 0-5 according to HOLCOMBE *et al.* (2005). When present, the secretion was collected by tracheal aspirate from the most distal region of

the trachea near the carina, using a 2m polyethylene catheter passed by the endoscope biopsy channel. The smears were prepared by slide extension and air fixation.

5. CYTOLOGICAL EVALUATION –

The stain technique used was May Grunwald/Giemsa proceeded as follows:

- a.) After the air fixation, slides were covered with May-Grunwald stain for 3 minutes;
- b.) After this, distilled water was added to the May-Grunwald, mixing well for 1 minute;
- c.) The stain was taken off the slide without washing it; the smear was covered with a prepared Giemsa stain solution (3 drops of Giemsa stain for each 2 milliliters of distilled water) for 15 minutes;
- d.) Slides were washed.

The cytological evaluation of each prepared slide was proceeded under 100x, 400x and 1000x (immersion), and the slides were examined entirely. The different cell groups were graduated separately according to the frequency of appearance in the magnification 1000x, according to EPPINGER (1990) as follows:

Grade 0 – cell absence

Grade I – one cell or isolated cells, or one or two cells on isolated fields;

Grade II – one or two cells on many fields;

Grade III – three to five cells observed on many fields;

Grade IV – more than five cells observed on many fields (diffuse distribution).

RESULTS

There were four variations for the time spent inside the stall: 0 hours, 1 to 2 hours, 3 to 4 hours and 12 hours, and two kind of bed used, being hay and wood shavings. For the time spent inside the stall, 42.90%, 24.60%, 15.90% and 16.70% of the yearlings stayed 0 hours, 1-2 hours, 3-4 hours and 12 hours inside the barn, respectively. The feeding management varied between smashed oats, smashed oats plus alfalfa hay and whole oats plus alfalfa hay. For the feeding management, 43.4%, 36.4% and 20.2% received whole oats plus alfalfa hay, only smashed oats and

smashed plus alfalfa hay, respectively.

The tracheal auscultation as well as pulmonary auscultation was considered normal in 112 (94,12%) yearlings.

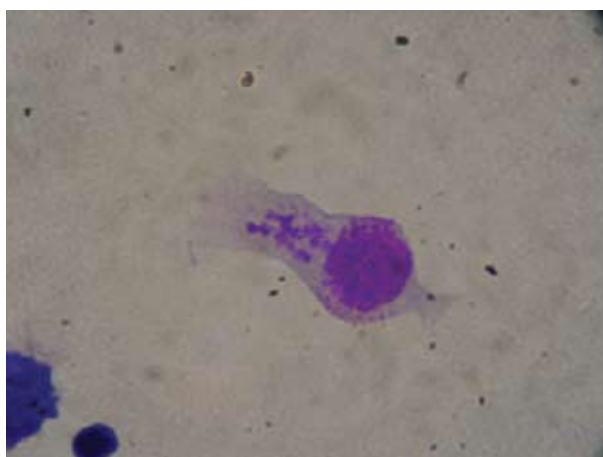
Although all the yearlings were being considered healthy individuals by the time of their examination, 108 (90.76%) evidenced tracheobronchial mucous secretion (TABLE 1). From these, 105 yearlings had never evidenced any respiratory disease in their history, but 96 (91,43%) of them had tracheobronchial secretion during endoscopic evaluation.

TABLE 1 – PRESENCE OF TRACHEAL MUCUS IN 119 THOROUGHbred YEARLINGS EVALUATED BY BRONCHOSCOPIC EXAMINATION.

	Number of Yearlings	(%)
Grade 0	11	9,2
Grade 1	39	32,8
Grade 2	31	26,1
Grade 3	23	19,3
Grade 4	10	8,4
Grade 5	5	4,2

The cytological evaluation of the tracheobronchial aspirates evidenced the epithelial cells as the most prevalent cell population. Globet cells (FIGURE 1) were also observed.

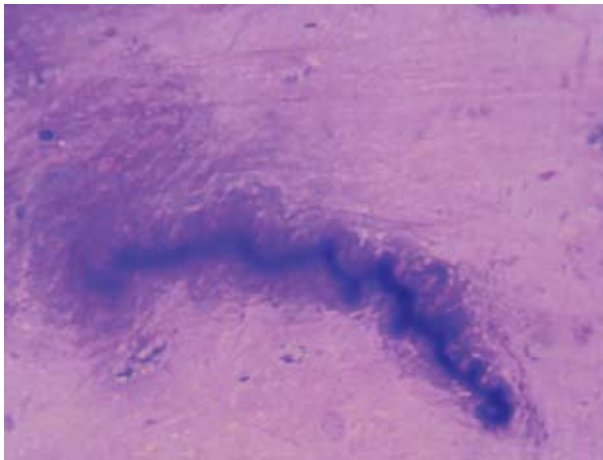
FIGURE 1 – GLOBET CELL IN THE TRACHEAL ASPIRATE OF A THOROUGHbred YEARLING. MAY-GRUNWALD/GIEMSA STAIN, MAGNIFICATION OF 0X.



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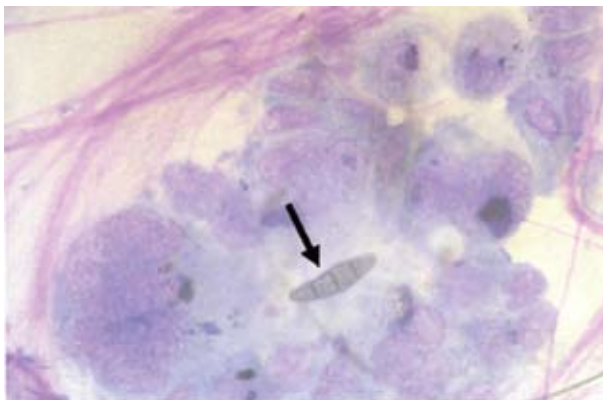
Curshmann's spirals (FIGURE 2) were found in the tracheal aspirates from 16 (13.45%) yearlings, occurring in grades 1, 2, 3 and 4 of tracheal mucus. Fourteen of these yearlings did not present respiratory disease before, being considered completely healthy.

FIGURE 2 – CURSHMANN'S SPIRAL IN THE TRACHEAL ASPIRATE OF A THOROUGHRED YEARLING. MAY–GRUNWALD/GIEMSA STAIN, MAGNIFICATION OF 1000X.



Giant cells (FIGURE 3) were found in the aspirate of 4 yearlings, being three with grade 3 and one with grade 4 of tracheal mucus score.

FIGURE 3 – FORMATION OF A GIANT CELL OBSERVED IN THE TRACHEAL ASPIRATE OF A THOROUGHRED YEARLING. A FUNGI SPORE CAN BE SEEN INSIDE (ARROW). MAY–GRUNWALD/GIEMSA STAIN, MAGNIFICATION OF 1000X.



Eosinophils were present in the secretion of 61 (51.26%) individuals, and appeared in grade II or higher in 20 yearlings.

Neutrophils were observed in the tracheobronchial aspirate of 8 (8.1%) individuals.

DISCUSSION AND CONCLUSION

This work could demonstrate the presence of tracheobronchial mucous secretion in the lower respiratory airways of thoroughbred yearlings, before start training, as evidence of lower airway inflammation. The presence of increased amounts of mucous secretion in the tracheobronchial tree is being considered airway inflammation by ROBINSON *et al.*, 2003; ROBINSON, 2005; ROBINSON *et al.*, 2006; HOLCOMBE *et al.*, 2006. Recently ROBINSON *et al.* (2006) demonstrated that the observation of mucus amount greater than grade 1 (0-5) in the trachea could be considered a reliable evidence and measure of airway inflammation in horses.

The incidence of a high number of yearlings that had not experienced respiratory disease before, showed that a prevalence of subclinical respiratory diseases, or a failure in respiratory diseases diagnosis might have occurred (DERKSEN, 1991).

The importance of the feeding and stable management in the etiology of inflammatory airway diseases was reported in horses with recurrent airway obstruction (RAO) and inflammatory airway disease (IAD) (McGORUM and PIRIE, 2003; GHIO *et al.*, 2006; HOLCOMBE *et al.*, 2006). Hay is a potential source of respirable dust and aeroallergens, and it causes an increase in inflammatory cells number in tracheal secretion of horses (ROBINSON *et al.*, 2006). It was demonstrated that horse barns are rich in endotoxins and mold spores, capable of inducing lower airway inflammation (McGORUM and PIRIE, 2003; HODGSON *et al.*, 2005; GHIO *et al.*, 2006; HOLCOMBE *et al.*, 2006).

Globet cells were observed in the tracheal aspirate of the evaluated yearlings in this study. This cell type is found in the surface epithelium of the lower respiratory tract, where they secrete mucins to protect the epithelial lining from respirable aggressors, but the excess of mucus in certain lung diseases compromise both the airway defenses and the pulmonary function (ROGERS, 2003; THORNTON and SHEEHAN, 2004). The Globet cells were described in horses with RAO (ZINKL, 2002). In fact, it was reported that an increase in the number of globet cells is associated with airway inflammation (VOYNOW *et al.*, 2004; LUGO *et al.*, 2005).

The alveolar macrophages are the most abundant inflammatory cell type in the tracheal aspirate of normal horses (HODGSON and HODGSON, 2003) and were a common finding in the tracheal aspirate of the majority of the yearlings studied.

Curshmann's spirals appearance signifies the occurrence of increased mucous production from the smaller bronchioles (ZINKL, 2002). They represent a mucoid matrix that plugs the small airways, particularly in advanced cases of pulmonary inflammation (VIEL and HEWSON, 2003). The observation of Curshmann's Spiral in tracheobronchial secretion could be related to lower airway inflammation, as the correlation between increased mucus production and airway inflammation is already recognized (ROBINSON *et al.*, 2003; ROBINSON, 2005; ROBINSON *et al.*, 2006; HOLCOMBE *et al.*, 2006).

Although the importance of the physical examination is emphasized (DERKSEN, 1991), the findings in the respiratory tract did not have correlation with the data obtained during the physical examination. The tracheal and pulmonary auscultation results could not be correlated with the findings of tracheal mucous secretion. The poor correlation between the physical findings and the endoscopic findings showed that the physical examination of resting yearlings is not elucidative, and highlighted the importance of the endoscopic and cytological examination in lower respiratory airway evaluation.

The importance of the cytological evaluation of the tracheobronchial aspirate could be demonstrated. This technique proved to be very practical as well as capable to demonstrate airway inflammation in various degrees, being of utility in routine respiratory evaluation (HODGSON and HODGSON, 2003).

In conclusion, the present results displayed the existence of increased tracheobronchial mucus production, evidencing airway inflammation in thoroughbred yearlings at an age for initiating training, suggesting that a possible impairment for their racing potential expression can occur. The findings of this study suggests that lower airway inflammation in horses could be initiating in earlier life times, being necessary that more investigation be conducted to thoroughly study the pulmonary immune system of young horses.

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