

Scientific Electronic Archives

Issue ID: Sci. Elec. Arch. 9:5 (2016)

November 2016

Article link

<http://www.seasinop.com.br/revista/index.php?journal=SEA&page=article&op=view&path%5B%5D=404&path%5B%5D=pdf>

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Cytology pulmonary in horses (*Equus caballus*) maintained the field or stabled

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Abstract. Tracheobronchial secretion was collected by endoscopy (Welch-Allyn®, 200cm long and 9mm diameter), from equines with different ages, races and sex, for posterior cytological analysis. Thirty-six equines were divided into two groups (Group I = stabled, n = 18; Group II = field regimen, n = 18). Animals of Group I (age = 3.5 years) were lodged in the Tarumã's Jockey Club in Curitiba, Parana State. The equines of Group II always were maintained at field condition (never were stabled), in the farm located in Jacarezinho, Paraná State. Following endoscopic exams, tracheobronchial secretion smears were stained by Pappenheim's method and submitted to cytological analysis. Group I had higher frequency of tracheal discharge than Group II. The cytological analysis showed more presence of macrophages (58.8%), foamy cells (2.3%), body giant cells (0.2%), neutrophils (4.57%), eosinophils (0.7%) and Curshmann's spirals (0.04%) on 3 of the 18 animals. The cytological analysis of Group II showed higher frequency of ciliated epithelial cells than Group I, and no Curshmann's spirals were observed. In conclusion, data showed that horses stabled had more cytological alterations than the animals maintained at field, therefore more tracheobronchial complications. Thus, the care of place where horse are stabled have influence on development of respiratory diseases, may predispose to affections as Chronic Obstructive Pulmonary Disease and Exercise-Induced Pulmonary Hemorrhage, as well athletic horses may be never reach their full respiratory potential.

Keywords: Cytology. Tracheobronchial. Equine.

Introduction

Diseases of the respiratory tract in horses can be caused by a combination of infectious agents and causes predisposing, as unfavorable weather conditions, stress of weaning or transport and unsuitable environments and with poorly ventilated for the horse athlete be housed, each contributing to weaken the defense mechanisms of horses (DEEGEN, 1984).

Clarke (1987) cited that 20.50% of PSI horses in training, are lost due to diseases associated with airway and the athletic ability and longevity are closely linked to respiratory welfare. ARTUR (1990) and Mason *et al* (1990) reported that respiratory diseases are as important and common practice in the hippodrome as to musculoskeletal diseases.

Michelotto (1993) rated 129 PSI foals between 18 and 24 months of age. Employed a questionnaire for evaluating the life of the colts until the moment of examination with respect to stall

handling, feeding, health and membership. Later, foals underwent endoscopic and clinical examination, where it was found that foals kept in stables for longer and those who received into hay alfalfa in the feed showed greater changes in the tracheal aspirate.

O'Callaghan *et al* (1987) stated that the precise causes of EIPH are not yet known, but cited three hypotheses: the theory of capillary rupture, theory of injuries consecutives of the lungs and the theory of chronic inflammation of the lower airways. This last, the author explain that the inflammation of the bronchioles entails alveolar hypoxia, pulmonary arterial constriction, hyperplasia of arterial bronchi and the formation of new bronchial vessels that can rupture during exercise. It combines these assumptions with infectious and allergic problems. Hamilton (1999) described that the causes to occur exercise-induced pulmonary hemorrhage (EIPH) are not simple to explain, but that various diseases of the respiratory airways result in inflammation of lung

tissue and horses with predisposed to EIPH including infections and allergic causes.

It was believed that infections of viral and bacterial origin caused chronic conditions and kept active, but it is known that due to unfavorable environmental conditions, the clinical signs of bronchitis are aggravated (BEECH, 1991; BURCH, 1987; CLARKE, 1987; DERKSEN, 1983; EYRE, 1972; MURRAY, 1989). These negative environmental influences may be, for example, the dusty air of the stables or training pavilions, food with dusty hay, as well as influences of air pollution in thermal inversion times. Furthermore, the air saturated ammonia in poorly ventilated stalls, as well as the worsening of symptoms because of the humid air, cold and cloudy (BEECH, 1991; KOBŁUK, 1995; REED, 2000; ROBINSON, 1992; STRAUSS, 1992; SPEIRS, 1999).

Many authors have tried to prove the etiology of other type of chronic bronchial affections and this effort revealed a range of interesting results of research, but do not lead to a response with general validity (DEEGEN, 1984).

It is increasingly evident that chronic infectious process is of little importance, being responsible for one of only secondary role, and what more imposes itself to view is to be a hypersensitivity reaction type I or III that supports chronic disease airway (DEEGEN, 1984 e REED, 2000).

This study aimed to determine through cytological study possible changes in the respiratory tract of horses, comparing cytological findings with management in stabling and handling as close as possible to their creation environment, the field (animals Group II never entered in a stall or similar buildings throughout their life until the moment of collection), as many researchers relate the possible causes of respiratory diseases of horses with allergic problems caused by inadequate ventilation and stay in stables, health and food with care, among others.

Methods

We conducted the evaluation of the respiratory tract of two groups of horses, each group consisting of 18 animals of various breeds, ages and genders.

The first group was housed horses in the Jockey Club of Paraná, kept in intensive management system, i.e., animals that remained most of the day confined in stalls, independent management and cleaning, clinically healthy and assisted by a veterinarian. This group had an average age of 3 years and 6 months and also received half-yearly vaccinations against equine influenza virus and equine herpesvirus type I and IV. The second group, belonging to a rural property located in the city of Jacarezinho, State of Paraná, it was composed of horse kept in extensive handling system (the field), i.e., animals that since birth were never kept in bay and its creation the closest to the

natural environment. This group of horses had an average age of 8 years and 8 months and never received vaccination or concentrated and hay, the only food supply that have was the existing pastures on the property.

Clinical examination

This study aimed to evaluate the clinical parameters related to the upper and lower respiratory system. The group I were examined in their own stalls and group II were examined in extensive farming property, bringing the animals into the corral where the tests were performed.

The clinical parameters were performed through observation, palpation and the use of a stethoscope and thermometer, always trying to cause minimal stress on animals in both groups, with the aim of selecting the clinically healthy animals to be part of the equine groups that were examined endoscopically and subsequently evaluated by tracheobronchial secretion cytology.

Questionnaire

The targeted questionnaires to obtain as much data about the animals examined, in both systems, in order to obtain information from previous diseases that may have caused possible changes of upper and lower respiratory system and select considered animals clinically healthy to undergo endoscopy and collection of tracheobronchial secretions.

Endoscopy

The examination of the upper and lower respiratory tracts were performed through a Welch-Allyn video-endoscope with 200cm long and 9 mm in diameter. The tests were carried out on their own premises where they were animals.

The structures of the upper and lower respiratory systems were being scanned sequentially during the introduction of the optical fiber. The trachea was evaluated, the tracheal lumen to the bifurcation of the main bronchi.

Analyzing the presence of secretion in the tracheal lumen, we used the following scale described by Eppinger (1990) and Michelotto (1993):

- 1 – CLEAN - no secretion in the trachea;
- 2 – SCARCE ITEMS - some points, the size of a pinhead, distributed randomly in the tracheal lumen;
- 3 – POINTS - several points, the size of a pinhead diffusely throughout the length of the trachea;
- 4 – FLAKES - secretion collections found throughout the length of the trachea;
- 5 – THREAD - continuous branch secretion from the main bronchi to the larynx, the trachea floor;
- 6 – ABUNDANT - large amounts of secretion, occupying the floor and sidewalls of the trachea.

Cytology of tracheobronchial aspiration

During the endoscopic procedure was obtained tracheobronchial secretions, if there were any. Where there was no material for harvest was carried tracheal washings (BAIN, 1997; ROSZEL, 1986 e SPEIRS, 2000). The collection of secretion and/or lavage was performed through a Teflon catheter with 2mm diameter coupled to a 20ml syringe inserted through the working channel of the endoscope or biopsy. The sampling procedure was performed by viewing the endoscope if aspirating materials from different parts of the trachea. After harvesting, held smear slide as soon as possible. After each harvest, the catheter was sanitized to perform new procedure.

The method of the staining of the smears was Pappenhein technique, which consists in the association of dyes with May Grunwald and Giemsa staining as described by Fernandes (1947) and Deconto (1983):

1 – After the smear has recently been dried in air without prior fixation, it was stained with May Grunwald dye undiluted for three minutes;

2 – Without pouring the dye was added an equal amount of distilled water, moving the blade in all directions to mix well and leave to act for a minute;

3 – It was poured dye and, without washing, been poured over the swab one Giemsa solution, which was diluted at time of use at a ratio of four (4) drops of the dye solution for two (2) ml of distilled water, for fifteen (15) minutes;

4 – It was washed abruptly and quickly on a trickle of faucet water;

For the examination of pulmonary cytology, it was used the technique of hematimetric count, with a minimum of was 10 (ten) fields in 100X, 400X and 1000 (immersion) X; totaling 400 (four hundred) cells by slides/animal (DECONTO, 1983).

Cells and structures studied

- 1 Ciliated epithelial cells;
- 2 Goblet cells;
- 3 Macrophages;
- 4 Foamy macrophages;
- 5 Foreign-body giant cell;
- 6 Neutrophil granulocytes;
- 7 Eosinophil granulocytes;
- 8 Curshmann's spirals (structure).

Statistic

It was used the "t" Student for comparison of two independent samples in the statistical calculation of this work.

Results and discussion

Animals examined in the group I (in stable), which were stabled showed a higher presence of tracheobronchial secretions on endoscopy exam

than group II (in the field), which is consistent with literature citations. Clarke et al (1987) found higher incidence of secretion in the trachea of horses kept in facilities with poor ventilation. Derksen (1991) mentioned that inhalation of ambient particles can prolong and to exacerbate the clinical signs of horses affected by chronic respiratory diseases.

The higher frequency of cells such as macrophages (58.8%), foamy macrophages (2.3%) and neutrophils present in the group I, also show that the respiratory tract of stabled horses suffer greater aggression of environmental agents and may lead to chronic diseases.

Beech (1991) described the allergic etiology of groups of horses and ponies with evidence suggestive of chronic respiratory problems after being stabled and fed with dusty hay and subsequently being repeatedly induced by physical effort showed airway obstruction and worsening of gas exchange. The author also noted that several studies confirm epidemiological observations that chronic respiratory diseases are common among horses that are housed in stables and get dusty hay for long periods of time, but that, however, the disease is rare in places where animals are kept field.

Provocation cough through the pressure of the first tracheal rings was not indicative of the presence of secretions in the trachea. Michelotto (1991) also describes non-relation with the cough induction with the presence of secretions in the trachea. Beech (1991) cited the absence of cough does not discard the possibility of the presence of exudate in the airways.

In many cases the presence of secretions in the trachea cannot be evidenced through the tracheal auscultation, confirming the citation of Derkesen (1991) which also found no relationship between tracheal auscultation and presence of secretion in the trachea. Michelotto (1993) described the presence of false-negative results in tracheal auscultation indicates that this procedure should not be considered completely reliable, despite being an important segment in the evaluation of the respiratory system.

A incidência de níveis mais elevados na secreção traqueal são sugestivos de alguma doença respiratória pré-existente. Michelotto (1993) reported that higher levels of secretion in the trachea suggest diseases of the respiratory tract. Deconto (1983), Deegen (1984) e Derksen (1991) described increased production of mucus in the lower respiratory tract of horses with inflammation of the small airways.

Deconto (1983) e Beech (1990) described in their papers that direct tracheal aspirate is a good technique for the diagnosis of inflammation of the small airways.

It was observed ciliated epithelial cells (63.2%) in greater quantities in the aspirate of the animals in group II than in group I. Zinkl (1992) found that the ciliated epithelial cells are the main

finding in aspirate of horses without lower respiratory tract injuries. Deconto (1983) mentioned that ciliated epithelial cells are observed in small quantities in aspirates from horses with chronic respiratory problems. Observation of goblet epithelial cells (19.4%) was higher in group I than in group II and, too showed a higher incidence of tracheal secretions. Michelotto (1993) described that the observation with the highest frequency of goblet cells in horses with higher amounts of discharge suggests that this group of cells is not a normal finding of the endotracheal aspirates. Clarke (1987) suggested that horses with increased number of goblet cells in the tracheobronchial secretions are subject to not achieve their maximum potential athletic.

Alveolar macrophages were seen more frequently in group I, average of 58.8%. Deconto (1983) reported that alveolar macrophages are always present in the tracheobronchial secretions with a tendency to decrease in quantity with the progression of chronic airway obstruction. These cells were present in group II, on average 23.5% which is in agreement with Thomson and McPherson (1989) who cite the presence of these cells in an incidence of 10% to 20% of aspirate from horses considered healthy. There was a higher incidence of foamy macrophages (2.3%) in the aspirate of the animals belonging to group I. Deconto (1984) described that these cells become after phagocytosis of this excess surfactant in the bronchi and alveoli. It is understood as surfactant the phospholipids combined with polysaccharides and proteins produced by type II pneumocytes that lining the alveoli. According Deconto (1985), the presence of foamy macrophages suggests obstruction of small bronchi and bronchioles.

There was the presence of giant cells of foreign body (0.2%) with higher incidence in group I than in the other group (0.1%). Deconto (1983) and (1985) stated that the presence of this cell in the tracheal aspirate indicates prolonged bronchial obstruction or depression of the lung cleaning mechanism. Michelotto (1993), in your works, have associated the incidence of giant cells in the aspirate with inadequate handling practices and supply of food with bad quality of preservation.

The presence of neutrophils (4.5%) in greater quantities in group I, coincides with the account of MAIR (1987) quoting the neutrophil as the predominant inflammatory cells in the aspirate of horses with problems in the lower airways. Michelotto (1993) mentioned that the lower incidence of neutrophils in the tracheobronchial secretion of horses suggests a little intense commitment of these airways, which coincides with the incidence of findings of neutrophils (0.7%) in group II.

Eosinophils (0.5%) were more frequently in the tracheobronchial aspirate of Group I than Group II (0.3%). Deconto (1985) mentioned that an increased number of eosinophils in secretion may be

observed in cases of allergic bronchitis and migration of pulmonary parasites. Michelotto (1993) reported that the presence of these cells in aspirates may be correlated to feeding with alfalfa hay and periods when the horses were housed in stable, suggesting that the incidence of these cells can be influenced by handling and environmental conditions. Beech (1991) commented that the absence of eosinophils does not rule out chronic lung disease of allergic origin.

The Curshmann's spirals (0.04%) were present in three of the animals examined in group I and absent in group II. According to Deconto (1983) these structures are formed from transudates and exudates in the region of small bronchi and, at the same time there is a decrease or stopped of the ciliary movement in the site. He also states that the presence of these structures is a sign of temporary obstruction of the small airways and can be found in large numbers in secretions after treatment with bronchodilators or after massive infusion fluid (NaCl 0.9%).

Final considerations

With the results obtained from this work can be concluded:

1 – The results obtained in group I, with higher amounts of goblet cells (19.4%), alveolar macrophage (58.8%), foamy macrophages (2.3%) and neutrophil granulocytes (4.5%) were statistically significantly with diagnostic value, to show that horses which are kept in stalls and which are considered healthy, show greater cytologic alterations of the lower respiratory tract.

2 – The animals selected for Group I, regardless of sanitary conditions, had major changes in the respiratory tract in cytological evaluation, despite having a lower mean age (3 years and 6 months), receiving veterinary care permanently, having a deworming program annual and every semester receive vaccines against equine influenza virus and equine herpes virus type I and IV.

3 – Horses of Group II, despite never having been kept in stables or similar buildings in your life until the time of collection of the material analyzed for this study and present clinically healthy, showed by the used technique a percentage of 30.35% of alveolar macrophages and 0.75% of neutrophil granulocytes in average of the group.

4 – The animals selected for Group II, regardless of sanitary conditions showed minor changes in the respiratory tract by the cytological evaluation, despite having a higher age (8 years and 8 months) than animals from group I and not receive veterinary care routinely or vaccinations.

5 – The Curshmann's spirals presents in group I (0.04%) were not statistically significant compared to group II (0.0%), but their presence in group I and

their absence in the group II suggests that these structures have diagnostic value for disorders of the small airways.

6 – The importance of further studies in this area, as there is a wide variation in the management system of stable, the food served to the animals, type of bed, sanitary control and care with better conditions of aeration of the same and few values of reference for comparison of cytological findings of traquibronchial secretion through direct aspirated from athletes horses kept at bay and clinically healthy. Moreover, to date, there is no reference by the technique used to healthy horses that never in their lives were confined or semi-confined in stalls or similar buildings.

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