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Original article

Cytological evaluation of tracheal aspirate and broncho-alveolar lavage fluid in comparison to endoscopic assessment of lower airways in horses with recurrent airways obstruction or inflammatory airway disease

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Abstract

The aim of the present study was to compare the grade of discharge accumulation in the tracheal lumen, area of tracheal bifurcation, main bronchi and the tracheal septum thickness with the cytology of the tracheal aspirate (TA) and broncho-alveolar lavage fluid (BALF) in horses with recurrent airways obstruction and inflammatory airway disease from those horses. This study was conducted on 96 horses with RAO, 139 horses with IAD and 10 control horses. In all the horses, both clinical and endoscopic examinations were performed. During endoscopy, a score of mucus accumulation was estimated in 3/4 lower of the trachea and in the tracheal bifurcation. In addition, thickening of the tracheal septum was also assessed; tracheal aspirates and broncho-alveolar lavage were performed. An estimate of cell percentage was done in TA and BALF samples. In horses suffering from RAO and IAD, there was a positive correlation between the percentage of neutrophils and the accumulation of discharge, and in the IAD group, there was a negative correlation between the percentage of eosinophils and the accumulation of discharge. There was no correlation between tracheal septum thickening and the percentage of neutrophils and/or eosinophils.

Key words: horses, RAO, IAD, endoscopy, TA aspirate, BA lavage, cytology

Introduction

Despite many studies conducted for years in different veterinary clinics all over the world, etiology and pathogenesis of chronic noninfectious diseases of lower airways in horses creates multiple practical problems, both diagnostically and therapeutically. Particular attention is brought to the differential diagnosis of recurrent airways obstruction (RAO) and inflammatory airway disease (IAD).

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Both diseases are characterized by cough, nasal discharge, accumulation of discharge in the airways and poor performance without an elevation of body temperature (Coultil et al. 2007, Gerber et al. 2009, Wysocka and Kluciński 2014).

Introducing endoscopic examination to the routine diagnostics allows for the analysis of a series of new parameters. These parameters are: anatomical structure of the upper and lower airways, accumulation of the discharge, thickness of tracheal septum as well as cytological and microbiological evaluation of TA and BALF (Derksen et al. 1989, Kluciński et al. 1994, Wysocka and Kluciński 2014).

Based on studies performed in 238 horses with clinical signs of RAO and IAD, we concluded that there is purposefulness in grading the accumulation of discharge not only in 3/4 of the lower trachea, but also, in the tracheal bifurcation area. In addition to this, an evaluation of tracheal septum thickness was also proven to be useful (Wysocka and Klucinski 2014).

The aim of this study was a comparative analysis between endoscopic scoring (discharge accumulation in the tracheal lumen, in area of the tracheal bifurcation, and tracheal septum thickening) and cytological evaluation (TA and BALF) in horses with RAO and IAD. This kind of clinical analysis has not yet been published, but it could prove useful in the clinical diagnostics of those diseases.

Materials and Methods

Animals

The study was conducted on 96 horses with recurrent airways obstruction (RAO), 139 horses with inflammatory airway disease (IAD) and 10 horses as a control group.

The patients were of different breeds and sexes, aged from 4 to 23 years, and used for sports, racing and pleasure. The horses in the control group had no history of respiratory disease and no clinical signs of respiratory disease.

The main criteria in this division of horses with RAO or IAD was the presence or absence, in either the clinical history or in the clinical examination, of respiratory distress at rest. The horses with signs of dyspnea were classified into the RAO group, and the horses lacking history and symptoms of respiratory distress at rest, but with other clinical signs, such as, cough, poor performance or nasal discharge, were classified into the IAD group.

Diagnosis of RAO or IAD was based on the anamnesis conducted with owners, riders, and trainers, and clinical examination (heart rate, respiratory rate, body temperature, palpation of superficial lymph nodes, bilateral auscultation of lung fields and heart, palpation and auscultation on the trachea and larynx, determining the presence of dyspnea and cough).

Endoscopy of the upper and lower airways was performed and TA and BALF samples were taken. All procedures were done in the horses at rest.

Endoscopic examination of the upper and lower airways was performed by using colonoscope Fujinon Cd MP2 1,7 m long with a 1 cm outer diameter. Images used for the evaluation of upper and lower airways, the grading of discharge in the lower 3/4 of the trachea and the tracheal bifurcation, and the assessment of tracheal septum thickness were taken with a digital camera adjusted to the endoscope.

The horses were sedated before passing the endoscope with intravascular xylazine dosed a at 0.3-0.5 mg/kg b.w. and butorphanol dosed at 0.01 – 0.05 mg/kg b.w.

The tracheal septum thickness index was calculated based on digital pictures analyzed by a computer using the GIMP ink 2.6 program and it was a ratio of the horizontal diameter of the right main bronchus and the tracheal septum thickness in its mid-height (Wysocka and Klucinski 2014).

Endoscopic scoring of discharge accumulation in the lower 3/4 of trachea, on a 6 grade scale, was performed by visual evaluation (Allen et al. 2007, Bartner et al. 2006, Wysocka and Klucinski 2014).

Endoscopic scoring of discharge accumulation in area of tracheal bifurcation was performed on the basis of a 4 grade scale which was developed during our earlier research (Wysocka and Kluciński 2014).

The method of sampling of tracheal aspirate and broncho-alveolar lavage

The TA and BALF were taken during and immediately after endoscopy, according to the well-known principles (Derksen et al. 1989, Kluciński et al. 1994). The sterile guarded propylene catheter was introduced into the working channel of an endoscope and 20 ml of sterile, warmed (37°C) saline was introduced into the tracheal lumen. Immediately after this introduction, the fluid was withdrawn into the sterile syringe. Afterwards, 0.2% lignocaine solution was applied into the bifurcation area to decrease the cough reflex, and a silicone ballooned catheter was introduced into the bronchi. Through the catheter, 60 ml of warmed (37°C) sterile saline was introduced four times into the bronchi and withdrawn immediately. After recovering the fluid, the volume of liquid was measured.

Methods of cytological and microbiological evaluation

The samples were stored in sterile tubes and transported to the lab within 2 hours.

The tracheal aspirate samples were cultured according to standardized methods. TA and BALF were centrifuged for 9 minutes at 1500 rev/min. The precipitate was suspended in 0.5 ml of saline and the number of cells was counted. From the precipitate, slides were made and stained using the May-Grunwald Giemsa method. The percentage of different cell-types were evaluated under 100x magnification in both samples. The percentage of cells was estimated to the number of 200 cells. During cytological evaluation attention was paid to any signs of infection or parasitic infestation. All the tracheal aspirate samples were submitted for microbiological culture.

Hematology and biochemistry of blood

The basic hematology examination of blood samples was done by using analyzer Abacus Junior Vet and the biochemistry examination (for ALT, ASP, total protein, creatinine, bilirubin) was done by using Pointe Scientific set on analyzer Pointe miura.

Statistical analysis

In the statistical analysis, we included only those patients with RAO and IAD whose culture was negative for pathogenic bacteria, and hematologic and biochemistry values were within normal limits.

Results are shown in mean arithmetic values with standard error. Statistical analysis was done by using statistical pack SPSS 210 with Kolomgrow- Smitrow, Kruskala-Wallis, Manna-Whitney and Pearson's tests.

Results

Clinical and laboratory parameters in sick and control horses

In control horses, the parameters of the clinical examination were within normal limits. Mean body temperature was 37.7±0.5°C, mean breaths per minute was 12.0±3.0, and heart rate was 34.0±8.0. In these animals, there was no cough, nasal discharge, or any other symptoms from the respiratory tract in their clinical examination or history.

Ninety-six patients were included in the RAO group with clinical signs like cough, nasal discharge, mixed dyspnea (predominantly expiratory) and visible heavy line. In these horses, crackles and wheezes were heard in auscultation, and they showed exercise intolerance and nasal discharge. The mean body temperature was 37.2±0.5°C.

In the IAD group, 139 horses were included. The mean body temperature in this group was 37.5±0.7°C. Based on the conducted clinical examination and anamnesis (presence of cough, increased lung sounds, nasal discharge without sings of respiratory distress at rest), these horses were diagnosed as having IAD.

The basic laboratory parameters were within normal limits in both control horses and sick animals.

The mean number/value of erythrocytes was 8.30±2.10 x 10^{12}/l, PCV was 37±1.4%, hemoglobin was 12.8±1.4 g/dl, WBC was 6.1±0.7 x 10^{9}/l, ALT was 24.3±1.3 U/l, AST was 180±20.1 U/l, total protein was 62±6.1 g/l, creatinine was 0.08±0.02 mmol/l and total bilirubin was 0.06±0.02 mmol/l.

The total number of cells from TA and BALF fluid in control horses and horses with IAD and RAO.

In control horses, the total number of recovered cells from TA and BALF after one procedure was statistically comparable ($p>0.05$) and was 1.0±0.8x10^{6} cells for ml of fluid. In horses with diagnosed RAO or IAD, the total number of recovered cells from the trachea and broncho-alveolar space was 10 times higher ($p<0.001$) and the mean number in horses with RAO was 12.3±8.1x10^{6} in TA and 13.4±9.1x10^{6} for BALF. In horses with IAD, the number was 10.1±8.4x10^{6} cells/ml of fluid. There was no statistical difference in the number of recovered cells between groups of sick horses ($p>0.05$) concerning tracheal aspirate as well as broncho-alveolar lavage fluid.

The volume of withdrawn fluid from the broncho-alveolar space was measured and it was 72.0±12% in control horses and 46±10% in RAO and 65±7% in IAD animals.

Proportion of separate cell types recovered from tracheal and broncho-alveolar space

The percentage of cell-types recovered from control horses and sick animals has been summarized in Table 1.

In control horses, the highest proportion (around 60%) of cells were macrophages in both samples (TA, BALF).

The proportion of neutrophils in the control group was highest in TA (27.1±3.2%), when compared to the broncho-alveolar lavage fluid (9.2±2.8%) $p<0.05$.

The proportion of lymphocytes and epithelial cells in control animals was significantly lower in TA,

Table 1. Percentage of cells recovered from tracheal aspirate and bronchoalveolar lavage fluid and tracheal septum thickness index in horses with diagnosed RAO and IAD.

Groups of horses (n)	Percentage of cells					
	neutrophils	eosinophils	macrophages	lymphocytes	Epithelial cells	Tracheal septum thickness index
Control (10)	27.1±3.2	0.0	59.5±5.8	12.4±2.8	1.0±0.5	12.5±0.7
RAO (96)	89.4±2.2[a]	0.3±0.1[a]	6.4±1.8[a]	3.5±0.8[a]	0.6±0.3[a]	4.8±0.2[a]
IAD (139)	66.0 ±1.9[ab]	1.9±0.5[ab]	22.9±1.5[ab]	7.6±0.6[ab]	1.6±0.2[ab]	6.8±0.2[ab]
	Bronchoalveolar lavage fluid					
Control (10)	9.2±2.8[c]	0.0	64.1±9.1	21.9±4.2[c]	4.8±1.1[c]	12.5±0.7
RAO (96)	72.5±2.6[ac]	0.7±0.2[ac]	17.2±2.0[ac]	8.1±1.4[ac]	1.5±0.3[a]	4.8±0.2[a]
IAD (139)	41.0±0.2[abc]	2.7±0.7[abc]	39.7±1.7[abc]	14.3±1.2[abc]	1.8±0.3[ab]	6.8±0.2[ab]

Commentary: results are shows in mean arithmetic values ±SE, (n) = number of animals
a – statisticaly significant differences ($p<0.05$) between the sick horses and control horses.
b – statisticaly significant differences ($p<0.05$) between RAO and IAD
c – statisticaly significant differences ($p<0.05$) between cells recovered from TA and BALF

compared to the proportion of those cells from BALF ($p<0.05$).

In control horses, there were no erythrocytes or eosinophils in either sample.

In horses with RAO and IAD, there was marked increase in proportion of neutrophils and eosinophils in both washings with a simultaneous decrease in percentage of macrophages, epithelial cells and lymphocytes when compared to control horses ($p<0.05$).

In horses with diagnosed IAD, TA and BALF showed a marked increase in the percentage of neutrophils, but the proportion was significantly lower when compared to RAO horses ($p<0.05$). In IAD horses, the percentage of eosinophils and macrophages was significantly higher than in RAO horses, in both washings ($p<0.05$).

There was no correlation between tracheal septum thickening and the proportion of different cell-types from both samples (Table 1).

The proportion of neutrophils and the accumulation of discharge in the 6 and 4 grade scales

The proportion of neutrophils recovered from the trachea and broncho-alveolar space in horses with RAO and IAD, in correlation with the accumulation of discharge in the trachea and area of tracheal bifur cation, is summarized in Table 2 and 3. Based on conducted statistical analysis, we found a positive correlation between the grade of accumulation of discharge in the trachea in the 6 grade scale, and in area of the tracheal bifurcation in 4 grade scale and the percentage of recovered neutrophils in horses with IAD (Table 2 and 3).

In horses with RAO, there was a positive correlation between the accumulation of discharge in the area of the tracheal bifurcation in the 4 grade scale, with the proportion of neutrophils in both washings (Table 3). There was no statistically significant correlation between the accumulation of discharge in the trachea in the 6 grade scale and the percentage of neutrophils in both washings (Table 2).

The proportion of eosinophils and grade of discharge accumulation in the 6 and 4 grade scale

The results of the percentage of eosinophils and grade of accumulation of discharge, in the trachea and in the area of the tracheal bifurcation in horses with RAO and IAD are summarized in Tables 4 and 5.

In horses suffering from RAO or IAD there was a negative correlation between the accumulation of discharge and the percentage of eosinophils. The highest proportion of eosinophils was found in tracheal aspirates from horses without accumulation of discharge in the trachea or tracheal bifurcation (Table 4 and 5).

Table 2. The correlation between percentage of neutrophils recovered from TA and BALF and accumulation of discharge in the tracheal lumen in 6 grade scale in the sick animals.

Groups of horses (n)	The accumulation of discharge in 6 grade scale						Correlation*	p*
	0°	1°	2°	3°	4°	5°		
Tracheal aspirate								
RAO (96)	86.0±0.0 (1)	89.5±2.8 (8)	89.7±1.5 (32)	88.4±1.6 (30)	89.7±2.4 (20)	93.0±1.8 (5)	0.046	0.656
IAD (139)	45.6±4.7 (30)	62.0±4.5[a] (37)	70.2±3.9[b] (46)	87.5±1.9[b] (23)	90.7±1.2[b] (3)	none (0)	0.493	0.000
Bronchoalveolar lavage fluid								
RAO (96)	70.0±0 (1)	66.4±9.8 (8)	74.0±3.6 (32)	68.1±3.9 (30)	76.0±4.7 (20)	86.2±3.1 (5)	0.123	0.233
IAD (139)	23.7±3.8 (30)	33.8±4.0 (37)	45.7±4.2[a] (46)	60.5±4.7[ab] (23)	80.7±7.4[ab] (3)	none (0)	0.484	0.000

Commentary: the mean percentage of neutrophils ±SE, (n) = number of animals
a – statisticaly significant differences ($p<0.05$) between the sick horses and control horses.
b – statisticaly significant differences ($p<0.05$) compared to 0° and 1° accumulation of discharge
c – statisticaly significant differences ($p<0.05$) compared to 0°,1°,2° accumulation of discharge
* – correlation between grade of discharge accumulation and percentage of neutrophils

Table 3. Correlation between percentage of neutrophils recovered from TA and BALF and accumulation of discharge in the area of the tracheal bifurcation in 4 grade scale in sick animals.

Grups of horses (n)	The accumulation of discharge in 4 grade scale				Correlation*	p*
	0°	1°	2°	3°		
Tracheal aspirate						
RAO (96)	85.3±1.9 (19)	87.8±2.0 (28)	91.4±1.3[a] (28)	92.5±1.3[a] (21)	0.313	0.002
IAD (139)	58.0±3.0 (92)	80.5±3.3[a] (30)	83.6±4.2[a] (16)	89.00±0.0 (1)	0.385	0.000
Bronchoalveolar lavage fluid						
RAO (96)	65.2±5.4 (19)	69.5±4.4 (28)	78.4±3.3 (28)	75.6±4.1 (21)	0.200	0.046
IAD (139)	34.5±2.6 (92)	55.2±5.3[a] (30)	48.4±7.5[a] (16)	88.0±0.0 (1)	0.290	0.001

Commentary: the mean percentage of neutrophils ±SE, (n) number of animals
a – statisticaly significant differences ($p<0.05$) compared to percentage of neutrophils in 0° grade of discharge accumulation
* – correlation between grade of discharge accumulation and percentage of neutrophils

Table 4. The correlation between percentage of eosinophils recovered from TA and BALF and accumulation of discharge in the tracheal lumen in 6 grade scale in the sick animals.

Groups of horses (n)	The accumulation of discharge in 6 grade scale						Correlation*	p*
	0°	1°	2°	3°	4°	5°		
Tracheal aspirate								
RAO (96)	0.0±0.0 (1)	0.0±0.0 (8)	0.16±0.1 (32)	0.5±0.4 (30)	0.2±0.2 (20)	0.0±0.0 (5)	0.035	0.738
IAD (139)	5.7±3.0 (30)	1.6±0.8[a] (37)	0.4±0.2[a] (46)	0.4±0.2[a] (23)	0.0[a] (3)	none (0)	-0.219	0.010
Bronchoalveolar lavage fluid								
RAO (96)	2.0±0 (1)	0.1±0.1[a] (8)	0.5±0.4[a] (32)	0.4±0.3[a] (30)	1.3±0.7 (20)	0.6±0.2[a] (5)	0.093	0.368
IAD (139)	7.0±3.5 (30)	0.4±0.1[a] (37)	2.4±0.9[a] (46)	1.8±1.0[a] (23)	0.0 (3)	none (0)	-0.140	0.100

Commentary: the mean percentage of eosinophils ±SE, (n) number of animals
a – statisticaly significant differences ($p<0.05$) compared to percentage of eosinophils in 0° grade of discharge accumulation
* – correlation between grade of discharge accumulation and percentage of eosinophils

Table 5. The correlation between percentage of eosinophils recovered from TA and BALF and accumulation of discharge in the tracheal bifurcation in 4 grade scale in the sick animals.

Grups of horses (n)	The accumulation of discharge in 4 grade scale				Correlation*	p*
	0°	1°	2°	3°		
Tracheal aspirate						
RAO (96)	0.8±0.5 (19)	0.3±0.3[a] (28)	0.0[a] (28)	0.0[a] (21)	-0.231	0.023
IAD (139)	2.6±1.0 (92)	0.2±0.1[a] (30)	0.8±0.4[a] (16)	0.0 (1)	-0.110	0.197
Bronchoalveolar lavage fluid						
RAO (96)	0.4±02 (19)	1.4±0.7[a] (28)	0.4±0.3 (28)	0.1±0.1[a] (21)	-0.114	0.269
IAD (139)	3.4±1.2 (92)	1.7±0.9 (30)	0.8±0.4 (16)	0.0 (1)	-0.100	0.240

Commentary: the mean percentage of eosinophils ±SE, (n) number of animals
a – statisticaly significant differences ($p<0.05$) compared to percentage of eosinophils in 0v grade of discharge accumulation
* – correlation between grade of discharge accumulation and percentage of eosinophils

Table 6. The correlation between percentage of macrophages recovered from TA and BALF and accumulation of discharge in the tracheal lumen in 6 grade cale in the sick animals.

Groups of horses (n)	The accumulation of discharge in 6 grade scale						Correlation*	p*
	0°	1°	2°	3°	4°	5°		
				Tracheal aspirate				
RAO (96)	12.0±0.0 (1)	3.9±0.9 (8)	7.1±1.3 (32)	6.9±1.2 (30)	6.1±1.6 (20)	2.6±0.9 (5)	-0.068	0.509
IAD (139)	37.4±4.1 (30)	24.2±3.5[a] (37)	19.2±2.9[a] (46)	11.4±3.3[a] (23)	5.0±2.0[a] (3)	none (0)	-0.401	0.000
				Bronchoalveolar lavage fluid				
RAO (96)	20.0±0.0 (1)	26.4±9.6 (8)	16.3±2.5[a] (32)	18.6±2.5[a] (30)	15.4±3.8[a] (20)	6.4±1.8[a] (5)	-0.169	0.100
IAD (139)	46.8±4.1 (30)	43.0±3.5 (37)	39.4±3.4 (46)	29.0±3.6[a] (23)	14.3±5.4[a] (3)	none (0)	-0.289	0.001

Commentary: the mean percentage of macrophages ±SE, (n) number of animals
a – statisticaly significant differences ($p<0.05$) compared to percentage of macrophages in lower grades of discharge accumulation
* – correlation between grade of discharge accumulation and percentage of macrophages

Table 7. The correlation between percentage of macrophages recovered from TA and BALF and accumulation of discharge in the tracheal bifurcation in 4 grade scale in the sick animals.

Grups of horses (n)	The accumulation of discharge in 4 grade scale				Correlation*	p*
	0°	1°	2°	3°		
			Tracheal aspirate			
RAO (96)	9.8±1,9 (19)	7.6±1.5 (28)	5.3±0.8[a] (28)	3.1±0.6[a] (21)	-0.363	0.000
IAD (139)	27.8±2.4 (92)	15.1±3.3[a] (30)	10.3±2.8[a] (16)	8.0±0.0 (1)	-0.0313	0.000
			Bronchoalveolar lavage fluid			
RAO (96)	22.5±4.2 (19)	17.8±3.3[a] (28)	13.5±2.1[a] (28)	16.6±3.6[a] (21)	-0.146	0.156
IAD (139)	43.5±2.2 (92)	30.1±3.7[a] (30)	38.1±6.4 (16)	8.0±0.0 (1)	-0.197	0.020

Commentary: the mean percentage of macrophages ±SE, (n) number of animals
a – statisticaly significant differences ($p<0.05$) compared to percentage of macrophages in lower grades of discharge accumulation
* – correlation between grade of discharge accumulation and percentage of macrophages

Table 8. The correlation between percentage of lymphocytes recovered from TA and BALF and accumulation of discharge in the tracheal lumen in 6 grade scale in the sick animals.

Groups of horses (n)	The accumulation of discharge in 6 grade scale						Correlation*	p*
	0°	1°	2°	3°	4°	5°		
				Tracheal aspirate				
RAO (96)	2.0±0.0 (1)	5.8±2.6 (8)	2.8±0.5 (32)	3.6±0.6 (30)	3.6±1.4 (20)	2.8±1.7 (5)	-0.039	0.707
IAD (139)	8.9±1.8 (30)	9.4±1.6 (37)	7.5±1.4 (46)	3.9±1.1[a] (23)	2.0±1.0[a] (3)	none (0)	-0.196	0.021
				Bronchoalveolar lavage fluid				
RAO (96)	8.0±0.00 (1)	6.0±2.2 (8)	7.3±1.6 (32)	11.6±2.7 (30)	5.6±2.1 (20)	5.0±1.9 (5)	-0.019	0.857
IAD (139)	18.4±3.5 (30)	20.6±3.1 (37)	10.8±1.6[a] (46)	7.1±1.6[a] (23)	4.0±2.7[a] (3)	none (0)	-0.301	0.000

Commentary: the mean percentage of lymphoctes ±SE, (n) number of animals
a – statisticaly significant differences ($p<0.05$) compared to percentage of lymphocytes in lower grades of discharge accumulation
* – correlation between grade of discharge accumulation and percentage of lymphocytes

Table 9. The correlation between percentage of macrophages recovered from TA and BALF and accumulation of discharge in the tracheal bifurcation in 4 grade scale in the sick animals.

Grups of horses (n)	The accumulation of discharge in 4 grade scale				Correlation*	p*
	0°	1°	2°	3°		
			Tracheal aspirate			
RAO (96)	3.1±0.7 (19)	4.0±1.1 (28)	2.5±0.6 (28)	4.3±1.1 (21)	0.042	0.686
IAD (139)	8.5±1.0 (92)	6.6±1.6 (30)	4.7±1.8 (16)	3.0±0.0 (1)	-0.148	0.081
			Bronchoalveolar lavage fluid			
RAO (96)	10.6±3.4 (19)	9.0±2.3 (28)	7.0±2.0 (28)	5.8±1.1 (21)	-0.156	0.128
IAD (139)	15.9±1.7 (92)	11.6±3.2[a] (30)	10.9±2.5[a] (16)	3.0±0.0[a] (1)	-0.141	0.098

Commentary: the mean percentage of lymphoctes ±SE, (n) number of animals
a – statisticaly significant differences ($p<0.05$) compared to percentage of lymphocytes in grade 0° in 4 grade scale accumulation of discharge in area of tracheal bifurcation
* – correlation between grade of discharge accumulation and percentage of lymphocytes

The percentage of macrophages and accumulation of discharge in the 6 and 4 grade scale

The percentage of macrophages in TA and BALF in correlation with the accumulation of discharge in the trachea and tracheal bifurcation is summarized in Tables 6 and 7.

Based on conducted studies we found that in horses with IAD, with an increasing accumulation of discharge in the tracheal lumen (3/4 of lower length) and tracheal bifurcation, the proportion of macrophages was significantly decreased ($p<0.05$). In horses with diagnosed RAO, there was the same negative correlation, but there was a statistically important correlation between the accumulation of discharge in the area of the tracheal bifurcation and the proportion of macrophages in tracheal aspirates (Table 7).

The percentage of lymphocytes and the accumulation of discharge in the 6 and 4 grade scale

The percentage of lymphocytes recovered from TA and from BALF, in correlation with the grade of accumulation of discharge in the tracheal lumen and in the area of tracheal bifurcation, is summarized in Tables 8 and 9.

Based on the obtained results we found a statistically significant negative correlation between the proportion of lymphocytes and the accumulation of discharge in the tracheal lumen. In IAD horses, the proportion of lymphocytes in TA and in BALF decreased with an increase in accumulation of discharge in the tracheal lumen, but not in the area of the tracheal bifurcation (Table 9).

There was no correlation between the proportion of lymphocytes in the washings, and the grade of discharge accumulation in the tracheal lumen and in the area of tracheal bifurcation in horses with diagnosed RAO.

Discussion

As we are still lacking a plain definition describing the phenotype of horses suffering from RAO and IAD this study was an attempt to approach such description. There is a need for large field studies to collect information about noninfectious diseases of the lower airways in horses (Pirie 2014, Robinson et al. 2016). As long as methods of molecular and genetic diagnostics are unavailable for the diagnosis of these diseases, we will need to rely on simple diagnostic techniques like endoscopic examination and and cytology (Robinson et al.2006). Nowadays, only data-collection can be helpful in diagnosing and providing a correct prognosis in noninfectious respiratory diseases.

Noninfectious inflammation of the trachea and lower airways, accompanying RAO and IAD, is causing a dramatic influx of cells, particularly neutrophils. Their significant increase is detectable in TA as well as in BALF. The present results confirm previous findings (Beech 1975, Derksen 1989, Kluciński et al. 1994). The undisputed fact is that noninfectious inflammation leads to a 10 fold increase in the number of cells in TA and BALF and among these cells, the highest proportion are neutrophils. Similar phenomena can be observed in noninfectious metritis and mastitis. The neutrophils play an important role in local non-specific and cell-mediated immunity.

When comparing the percentage of neutrophils recovered from the TA and from the BALF, in horses with RAO and IAD, there is a higher influx of neutrophils in RAO, despite the accumulation of discharge in tracheal 3/4 lower length and in the area of the tracheal bifurcation.

In the horses with diagnosed IAD, the proportion of neutrophils increased with an increase in discharge accumulation in the 4 and 6 grade scale.

It should be highlighted that in some horses there was a marked increase in the number of neutrophils without an accumulation of discharge in the tracheal lumen and in the area of the tracheal bifurcation. Similar results were obtained by Gerber et al. (2004), in a study where healthy horses were exposed to dusty hay. According to our study, as well as other results, it can be implicated that determining the number of neutrophils in both washings is a more sensitive method than the evaluation of discharge accumulation. In our study, the diagnostic value of TA and BALF was comparable.

The study done by Robinson et al. (2006) showed a similar correlation between the proportion of neutrophils and the accumulation of discharge in the tracheal lumen, but this study was done without grading the accumulation of discharge and without comparing the proportion of cell-types in TA and BALF.

The study performed by Kluciński et al. (1994) showed that noninfectious inflammation in the airways of RAO affected animals is proceed with changed, by neutrophils presence, mechanism initiating phagocytosis. This study concluded that phagocytosis, initiated by neutrophils, is carried out without immunologically specific receptors, so there is no need for opsonized particles. In this process, there is less oxygen consumption, which is a positive phenomenon because there is less production of free radicals.

In this study we found a characteristic phenomenon, which was an eosinophilia in the tracheal aspirate and broncho-alveolar lavage fluid in both the RAO and IAD horses. The proportion of these cells was decreasing with the increase in the grade of accumulation of discharge in the tracheal lumen and bifurcation of the trachea. In comparing the two diseases we found that in the horses diagnosed as IAD, the proportion of eosinophils was much higher than in thehorses with clinical signs of RAO.

The horses with a higher proportion of eosinophils had less accumulation of discharge. In the case of these animals, there was a better response to treatment and a decreased risk for developing RAO (non-published data, Wysocka) when compared to the horses with an increased proportion of neutrophils. In available literature, there was no description of such a correlation. Because eosinophilia accompanies lung helminthiasis, attention was paid to any signs of parasitic infestation in the cytological evaluation. *Dictiocaoulus arnfieldi,* according to the literature, is very rarely encountered parasite in Poland (Kamińska et al. 2008) as the population of donkeys is very small in Poland. The results obtained in this study are original, as there are no publications describing similar findings.

As it is difficult to establish a reliable proportion of mast cells in tracheal aspirates and in broncho-alveolar lavage fluid using the MCG stain method (Hansen 2014), and there is marked influx of these cells into the bronchial wall (Kunzle et al. 2007), the number of the cells was not included in the study, but there was a higher percentage of mast cells in IAD horses when compared to RAO horses. A similar cell count determination, without a mast cell population, was done by Zang et al. (2012).

The proportion of epithelial cells was not statistically different between the groups of horses. To this day, there is no data about the correlation between the percentage of these cells and inflammation in IAD or RAO, but there is some information about an increase of cytokines detected in washings produced by epithelial cells in the inflamed airways (Riihimaki et al. 2008). This study, revealed there was a higher proportion of epithelial cells in chronically sick horses compared to the control horses in which there was higher activity of these cells.

When determining the proportion of different cell types recovered from TA and BALF, it must be remembered that the determination of neutrophils and eosinophils is most important because the proportion of other cells (macrophages and lymphocytes) is dependent from the number of those granulocytes. The interesting correlation was that there was an accumulation of discharge accompanying an increased percentage of neutrophils, despite of group affiliation. Similar results were published by Robinson et al 2006, but in that study there was no division of horses into different groups.

To conclude, diagnostics of noninfectious lower airways diseases should include as many parameters of endoscopic examination as possible and a cytological evaluation of TA and BALF.

There is a high proportion of horses which do not show respiratory distress at rest, which is the main division criteria between RAO and IAD, but these horses have an increased proportion of neutrophils and a similar accumulation of discharge like that observed in RAO horses. If there is no possibility of a very precise diagnosis, all efforts must still be made to make a precise-as-possible prognosis of the examined animal using all accessible diagnostic tools and cytological profiles of the lower airways. It is very probable that IAD and RAO are only different stages of the same disease which is very similar to human asthma.

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