Case report

CLINICAL ANALYSIS OF RECURRENT AIRWAY OBSTRUCTION IN HORSES

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Summary: Our research included 13 adult horses of different ages and breeds that had been referred to our clinic and suspected of having a pulmonary disease. The criteria for diagnosing recurrent airway obstruction (RAO) included having had, for a period longer than two months, clinical signs of a pulmonary disease likely to have been associated with the presence of hay and/or straw in the affected horse's environment. It also included finding pathological changes during a clinical examination, the presence of excessive respiratory secretions in the trachea during a bronchoscopic examination, a reduction in the partial pressure of the arterial oxygen and neutrophilia in the bronchoalveolar lavage fluid. All the horses used in our study met this criteria.

The most common clinical signs of RAO include high breathing frequency, a white serous or mucous nasal discharge, coughing and dyspnoea. We find that measuring the partial pressure of oxygen in the arterial blood is a reliable means of determining that a pulmonary disease is present and that a bronchoscopic examinationis is a reliable diagnostic method for examination of airways. We established that neutrophilic inflammation was in the bronchoalveolar lavage fluid and that two thirds of the examined horses were stabled most of the time.

Key words: veterinary medicine; obstructive lung diseases; diagnosis; horses

Introduction

Recurrent airway obstruction (RAO) is an inflammatory, obstructive airway disease that becomes clinically evident in middle-aged horses (1).

Attacks of airway obstruction are induced by the exposure of susceptible animals to organic dust (typically from hay or bedding). Following the exposure there is a massive influx of neutrophils into the airways, which is accompanied by bronchospasm and accumulation of mucus (2). The obstruction and inflammation are usually resolved when the source of the dust is eliminated (3).

In the early phases of the disease horses are alert and afebrile, although they usually cough during activity, feeding and/or the cleaning of the stable. There is reduced exercise tolerance and delayed recover from it. The frequency of coughing increases as the disease progresses. The nostrils may be flared and the horse may have a milky, serous or mucous nasal discharge. The horse may also use its abdominal muscles for exhalation to an exaggerated degree, and if the animal has had respiratory distress for some time, a heave line due to hypertrophy of the external abdominal oblique muscle may be obvious. Abnormal lung sounds are heard in varying degrees depending on the severity of the airway obstruction. Usually, the breathing sounds are louder throughout the airways, particularly over the peripheral lung fields, and wheezing is quite commonly heard. Percussion reveals an increase in the size of the lung field in severely affected animals (4).

In our research we wanted to determine which criteria are important for a clinical diagnosis of RAO. The research included horses that had come to our clinic during a one-year period with clinical signs and a history of having had a respiratory disease. All the horses were clinically examined, blood was taken for haematological and arterial gas analyses, a bronchoscopy was performed

and the bronchoalveolar lavage fluid was cytologically analysed. The control group consisted of clinically healthy horses of different ages and breeds.

Material and methods

Animals

Thirteen horses of different breeds and ages were included in our research. They were referred to our clinic during a one-year period with a history and some clinical signs (coughing, nasal discharge, dyspnoea, reduced exercise performance) of lung disease. The control group consisted of 11 healthy horses of different ages and breeds.

Examination protocol

Each animal's history included details of its age, breed, sex, duration of disease, cough, nasal discharge (unilateral or bilateral), dyspnoea at rest, exercise, types of bedding and food used as well as the animal's general routine.

During the clinical examinations we collected data on submandibular lymphadenitis, rate of breathing, dyspnoea at rest, abnormal lung sounds both at rest and after a 30-second manual bilateral-nasal occlusion, abnormal sounds heard during a tracheal auscultation and any lung-field percussion abnormalities.

Bronchoscopy

The bronchoscopic examinations were performed after sedating the horses with detomidine and, if needed, with the use of a nose twitch. The tip of the endoscope was covered with a lidocaine-hydrochloride based local-anaesthetic gel. The examinations involved looking for the presence of erythema, checking the normally sharp carina for signs of bluntness and examining the tracheal and bronchial respiratory mucosae for evidence of inflammation. A local anaesthetic (lidocaine hydrochloride) was applied to the bronchial bifurcation and a fibroscope was inserted, distally, until it was felt to wedge. A plastic catheter was then passed through the fibroscope, through which 300 ml of buffered saline, warmed to 37 °C, was instilled and, immediately afterwards, gently aspirated using 60-ml syringes.

The respiratory secretions (RS) present in the cranial thoracic trachea were described according to volume, colour and nature. Some samples were bacteriologically and mycologically examined to confirm the absence of an infectious airway disease.

The bronchoalveolar lavage fluid was centrifuged. After the supernatant was poured off, smears were prepared from the remaining cell pellet and then air-dried. The dry smears were then stained following the method described by May–Grünwald-Giemsa and a differential count of 200 non-epithelial cells was performed using a light-microscope (40×). The results were given as a percentage of non-epithelial cells. The cells as well as any other non-cellular structures, micro-organisms and/or foreign particles were described.

Haematology

An automatic haematological analyser was used to give a white-cell differential count and a complete blood count of blood taken from the jugular vein.

Arterial blood samples for a blood-gas analysis were collected from a. transversa faciei puncture and analysed with a gas analyser within 5 minutes of collection.

Statistics

All the data collected from the group of ill horses were compared to those from the group of healthy horses. The significance of differences between the two groups was analysed using a t–test for parametric data and a Mann–Whitney U test or an x^2 test for nonparametric data. The statistical analysis was performed using the SPSS (v.11) package for Windows. Differences of $p<0.05$ were considered statistically significant.

Results

Diagnostic criteria

According to the horses' owners, the horses had displayed clinical signs of a respiratory disease from between 3 to 60 months, or 19.54 months on average (SD = 5.4).

The owners also indicated that a cough was present in 12 of the horses (92.3 %) and that all

Table 1: Frequency of breathing, arterial blood gas measurement, bronchoalveolar lavage fluid analysis and haematology (arithmetic middle value ± SD; t-test)

	Control group	Ill horses	P
Breathing frequency	14.0 ± 0.8	23.1 ± 1.3	< 0.001
PaO_2 (mmHg)	106.03 ± 1.75	89.81 ± 2.96	< 0.001
$PaCO_2$ (mmHg)	39.72 ± 0.73	41.75 ± 0.59	0.040
pH	7.4322 ± 0.0044	7.4298 ± 0.0040	0.700
Macrophages (BAL) (%)	57.1 ± 6.0	18.1 ± 3.7	< 0.001
Lymphocytes (BAL) (%)	14.3 ± 2.0	7.2 ± 1.0	0.007
Neutrophils (BAL) (%)	25.1 ± 6.4	73.5 ± 4.8	< 0.001
Eosinophils (BAL) (%)	3.4 ± 1.0	1.0 ± 0.4	0.042
Basophils (BAL) (%)	0.2 ± 0.1	0.2 ± 0.1	0.863
Leukocytes (blood) ($*10^9$/L)	8.12 ± 0.53	10.87 ± 0.66	0.004
Erythrocytes (blood) ($*10^{12}$/L)	7.81 ± 0.30	7.80 ± 0.215	0.990
Haemoglobin (blood) (g/L)	128.5 ± 3.2	133.8 ± 2.95	0.233
Neutrophils (blood) (%)	50.17 ± 2.22	63.16 ± 2.00	< 0.001
Lymphocytes (blood) (%)	36.70 ± 2.18	27.68 ± 1.95	0.006
Monocytes (blood) (%)	6.14 ± 0.43	4.98 ± 0.37	0.054
Eosinophils (blood) (%)	4.52 ± 1.23	2.18 ± 0.53	0.103
Basophils (blood) (%)	0.68 ± 0.09	0.47 ± 0.06	0.056

Legend: PaO_2 - partial pressure of oxygen in arterial blood; $PaCO_2$ - partial pressure of carbon dioxide in arterial blood; pH - concentration of hydrogen ions in arterial blood; BAL - bronchoalveolar lavage fluid

horses had a nasal discharge, which we classified as being constant (15.4 %), frequent (69.2 %) or occasional (15.4 %).

Ten horses (76.9 %) displayed dyspnoea and were less tolerant of exercise according to their owners.

Age, breed and gender

The median age of the ill horses was 10.1 years (SD = 1.65), although most of the horses were aged between 7 and 8 years. The median age of horses in the control group was 9.7 years (SD = 1.24). There was no statistically significant difference between the two groups (t-test, p = 0.85).

There were five breeds represented in our study: Lipizzaner, Standardbred, Icelandic, Warmblood and Thoroughbred. There were no statistically significant differences among the ill and healthy groups of horses (x^2 test, p = 0.46).

There were no significant differences in the gender mix of the two groups (x^2 test, p = 0.48). The group of ill horses had 77 % females and 23 % males, while the control group had 63.6 % females and 36.4 % males.

Feeding, bedding, living in / outdoors

According to the owners 12 of the ill horses (92.3 %) and the entire control group were fed hay. One ill horse was fed grass silage. Straw was the most common bedding and was used by 46.2 % of the ill and 45.5 % of the control horses. The rest of the horses either had shavings for bedding or lived and slept outdoors. There were no statistically significant differences between the groups (x^2 test, p = 0.97).

About one third of the horses were kept outdoors (30.7 % of the ill horses and 36.3 % of the controls). The rest of the horses spent most of the time indoors, 38.5 % of the ill and 45.5 % of the control horses had only a few hours per day outside, while the remaining horses (30.8 % ill; 18.2 % control) spent less than 2 hours a day outside. There were no statistically significant differences between the groups (Mann-Whitney, p = 0.58).

Nasal discharge, dyspnoea, trachea auscultation, thoracic auscultation and percussion

Nearly half of the ill horses (46.2 %) displayed a nasal discharge during the examinations.

Generally it was bilateral, a milky white colour and its composition serous to mucous.

Dyspnoea at rest was evident in 30.7 % of the ill horses and 15.4 % of the ill horses had submandibular lymphadenitis, which was presenting as a bilateral enlargement of the submandibular lymph nodes.

Abnormal lung sounds were heard during the auscultation of each of the ill horses. In the two most serious cases there were no lung sounds at all during auscultation and biphasic expiration was evident.

Thoracic percussions revealed enlarged lung-field borders in 38.4 % of the ill horses and after a 30-second bilateral nose-closure 76.9 % of the ill horses developed abnormal tracheal sounds. None of these clinical sounds was heard in the control group.

Bronchoscopy

All the ill horses had some respiratory secretions (RS) present during the bronchoscopic examinations. There were a few droplets in 23.1 % of the horses, there was a small pool in 30.7 %, a large pool in 30.7 % and a very large pool in 15.5 % of the horses. Three of the control horses (27.2 %) had a small pool of respiratory secretions present in the trachea. The control group had far less respiratory secretions present in the trachea and this was statistically significant (Mann-Whitney, p < 0.001). The secretions of the ill horses were serous (53.8 %), mucous (30.8 %) and mucopurulent (15.4 %). The secretions of the control group were mucous. There was no statistically significant difference in the quality of the secretions of the groups of horses (Mann-Whitney, p < 0.001).

Frequency of breathing, arterial blood gas measurement, bronchoalveolar lavage fluid analysis and haematology

A t-test was used to analyse the results of comparisons between the medians of the two groups (Table 1). The differences between the average data were significant at p < 0.001.

There was a statistically significant difference between the breathing rate of the ill horses and that of the control group (Table 1).

The PaO_2 value of the ill horses, where 61.5 % had an Hg value lower than 95 mm, was statistically significantly lower than that of the control group (Table 1).

Statistically there were no significant differences between the $PaCO_2$ and pH of the ill horses and those of the control group.

The bronchoalveolar lavage fluid of the ill horses was a milky serous fluid, while the BAL fluid of the healthy horses was white and serous. There was a foamy surfactant present in both groups. Approximately one half of the instilled fluid was aspirated. The BAL fluid of the ill horses contained a significantly higher percentage of neutrophils and a lower percentage of alveolar macrophages than the group of healthy horses (Table 1). The percentages of lymphocytes, eosinophils and basophils did not differ between the two groups. There were also foreign structures such as plant particles present in the BAL fluid. Most of the BAL fluid contained Curschmann's spirals and macrophages.

There were no significant haematological differences between the two groups of horses (Table 1). While there was a statistically higher percentage of neutrophils in the group of ill horses, both groups fell within the limits of the normal range (Table 1).

Discussion

RAO as an inflammatory, obstructive airway disease becomes clinically relevant in middle-aged horses (1). It is one of the most common reasons for the retirement of sport and pleasure horses. In RAO there is an inflammatory reaction present in the airways that is responsible for bronchospasms, mucus accumulation, airway wall thickening and bronchial hyperreactivity. Clinical signs of the disease become evident at around 8 years of age (5, 6, 7, 8), which accords with our own experience and that of other authors. Most of the ill horses included in our research were aged between 7 and 9 years.

There was a significant difference in the breathing rates of the two groups of horses; a rapid breathing rate is a classic symptom of a respiratory disease. Lower airway obstructions in RAO-affected horses lead to changes in ventilation and blood flow, which in turn leads to inefficient blood-gas exchange and hypoxaemia. The body compensates for the insufficient gas exchange by breathing more rapidly (9).

During our research we found that measuring the level of PaO PaO_2 was useful in confirming the

presence of the respiratory disease. However, the PaO_2 value can be misleading. In RAO-affected horses that do not have an inflammation of the airways present at the time of examination, the PaO_2 value is likely to be within the normal limits (10, 11, 12), and we encountered this problem in 38.5 % of the ill horses. While we suspect that measuring the PaO_2 level of horse with RAO before and after treatment would be a good indicator of the efficacy of a treatment, we must rely on other tools to make a diagnosis of RAO.

The haematological values of the RAO-affected horses in our research group were within the normal ranges, which corresponds to the results from other published studies (5, 6, 13, 14).

The RAO-affected horses had significantly higher levels of respiratory secretions than the healthy horses. Three horses in the control group had a small pool of respiratory secretions present in their trachea. After consulting with their owners we discovered that these horses had had a viral respiratory infection during the previous month. The respiratory secretions of the ill horses in our study were mostly serous to mucous, which accords with reports from other authors (5, 8, 11). We found no signs of tracheal or bronchial respiratory mucosa inflammations in any of the horses. We consider a bronchoscopy to be an efficient method for the visualisation of airways and for confirming the presence of an airway disease. Some of the horses that had significant amounts of respiratory secretions present during the bronchoscopic examination, showed no signs of a respiratory disease during the clinical examination.

The average number of neutrophils was higher (25.1 %) in the control group than that reported in other studies (10, 15, 16). This was probably on account of the three horses that were in the recovery phase of a viral respiratory infection and the only way to distinguish between the two afflictions is through BAL cytology (11, 17). While the percentage of alveolar macrophages in our study was the same as that published by other authors, the lymphocyte count was lower, which was most probably due to the higher than normal neutrophil level. The most significant finding in the RAO-affected horses was the presence of neutrophilic inflammations (8, 15, 16, 17), where neutrophils represented 50 to 70 % of all cells. The percentages of neutrophils in our study was slightly higher than those reported in other stud-

ies. That could be due to the BAL-fluid aspiration technique. The length of the endoscope (2 m) didn't allow complete wedging of the endoscopic tube in bigger horses, so the samples were "contaminated" with cells from the upper airways, which contain higher concentrations of neutrophils (10, 15).

By the end of our study we were able to diagnose RAO in all the ill horses we examined. This could be because most of the horses in our ill population were adult Warmbloods (older than 5 years of age). The criteria we used for our diagnoses were as follows: the presence, for more than two months, of clinical signs of a respiratory disease together with the presence of hay and/or straw in the horses surroundings, pathological changes detected during the clinical examinations, the presence of respiratory secretions during the bronchoscopy, a lowered PaO_2 level and neutrophilia in the BAL fluid (50 to 70 % of neutrophils) (8, 15, 18). Other literature describes different sets of criteria for the diagnosis of RAO. These include the clinical presence of a respiratory disease (1, 7, 19, 20), neutrophilic airway disease – more than 10 % of neutrophils in the BAL fluid - connected to the presence of hay and/or straw in the horses' surroundings as well as a lowered PaO_2 level (1, 7, 17, 19, 20).

We found that coughing and a nasal discharge were reliable indicators that a respiratory disease was present. The coughing of RAO-affected horses is typically connected to higher concentrations of dust that are caused by feeding, the cleaning of the stable or through the activity of the horse (20, 21). An owner may also notice that the horse is performing poorly and that it has a nasal discharge and dyspnoea, which manifests as a bigger abdominal effort and flared nostrils during breathing (9, 20). In our research group 76.9 % of the ill horses were either performing poorly or were displaying signs of dyspnoea.

Most of the ill horses and all those in the control group were fed hay. One ill horse was fed grass forage as it had a history of RAO attacks whenever it was fed hay. Half of the horses had straw present in their surroundings, which was a second source of organic dust.

The manner in which a horse is kept is another factor influencing attacks of RAO. Two thirds of the ill horses were mainly kept indoors. The presence of RAO in Slovenia is probably due in part to climatic circumstances and to the tradition of

keeping horses indoors, especially during the wintertime. It is also partly due to a lack of awareness amongst horse owners and handlers of the correct ways to keep, feed and handle susceptible horses and of the causes and the manner of controlling the disease.

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KLINIČNA ANALIZA KONJ S PONAVLJAJOČO SE OBSTRUKCIJO DIHAL

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Povzetek: V klinično analizo smo vključili 13 konj različnih pasem in starosti, ki so na našo kliniko prihajali z anamnezo bolezni dihal. Merila za postavitev diagnoze ponavljajoče se obstrukcije dihal (POD) so bila: klinični znaki bolezni dihal dlje kot dva meseca v povezavi s prisotnostjo sena oziroma slame v okolici konja, patološke spremembe pri kliničnem pregledu, prisotnost respiratornega izločka pri bronhoskopiji, znižan parcialni tlak kisika v arterijski krvi in nevtrofilija v bronhoalveolarnem izpirku. Tem merilom so ustrezali vsi pregledani bolni konji.

Povišana frekvenca dihanja, belkast voden ali sluzast nosni izcedek, kašelj in oteženo dihanje so najznačilnejši klinični znaki POD. Ugotovili smo, da je parcialni tlak kisika dokaj zanesljiv kazalec obolelosti pljuč. Bronhoskopijo ocenjujemo kot učinkovito diagnostično metodo za pregled dihalnih poti in zanesljivo ugotavljanje bolezni dihal. V bronhoalveolarnem izpirku konj s POD smo ugotovili nevtrofilno vnetje. Kar dve tretjini bolnih konj je živelo pretežno v hlevu.

Ključne besede: veterinarska medicina; pljučne bolezni, obstruktivne – diagnostika; konji