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Original article

Prevalence and severity of ultrasonographic pulmonary findings in horses with asthma – a preliminary study

N. Siwinska[1], A. Zak[1], M. Slowikowska[1], P. Krupinska[2], A. Niedzwiedz[1]

[1] Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats,
[1,2] Faculty of Veterinary Medicine,
University of Environmental and Life Sciences, Pl. Grunwaldzki 47, 50-366 Wrocław, Poland

Abstract

Asthma is one of the most common non-infectious respiratory diseases in horses. Ultrasound examination is a widely available non-invasive additional diagnostic tool. To date, there are no studies focusing on ultrasonographic findings in horses with asthma. The aim of this study was to analyse the prevalence and severity of ultrasound lesions in lung tissue in horses with asthma. Lung ultrasonography was carried out on six healthy horses (controls) and 12 horses with asthma (six with mild and six with severe asthma). The sonographic changes in three lung sections were assessed using a scoring system. The most common changes present in all the animals were comet-tail artefacts. More advanced lesions were present in horses with severe asthma. Statistically significant differences in the overall average intensity of the ultrasound changes were seen between the controls and the study group and between the horses with mild and severe asthma. The lesions were usually located in the caudal lung regions, but they were also present in other areas as the disease progressed. Ultrasonography is a useful additional diagnostic tool enabling an assessment of the stage of the asthma progression. It is a very sensitive technique that visualizes minor lesions in the lung tissue even in clinically healthy animals. Due to its low specificity, it cannot replace endoscopy and the bronchoalveolar lavage in horses with asthma.

Key words: comet tail artefact, equine asthma syndrome, horse, lung, ultrasonography

Introduction

Equine asthma is one of the most common non-infectious respiratory disease in horses. According to the latest nomenclature, this disease consists of two forms - a mild (known as IAD - Inflammatory Airway Disease) and an advanced form (known as RAO - Recurrent Airway Obstruction) (Couëtil et al. 2016). Both are a lower respiratory tract inflammation without evidence of systemic signs of infection (Kutasi et al. 2011, Couëtil et al. 2016). Equine asthma is caused by hyperactivity of the respiratory tract to external factors, causing bronchospasm and an increased production of secretions (Marinkovic et al. 2007, Kutasi et al. 2011, Wysocka and Klucinski 2014). If untreated, the disease progresses and remodels' the mucosal and muscular

Correspondence to: N. Siwinska, e-mail: natalia.siwinska@upwr.edu.pl

layers of the bronchi and bronchioles (Marinkovic et al. 2007,Kutasi et al. 2011, Wysocka and Klucinski 2014). Chronic obstructive pneumopathies also cause fibrosis of the lung parenchyma (Barton and Gehlen 2016). Post-mortem examinations of horses with RAO revealed the presence of voluminous and expanded pink pale lungs, often accompanied by rib markings on their surface (Marinkovic et al. 2007). There are no available data on pulmonary lesions in horses with IAD. Horses suspected of asthma are diagnosed based on endoscopy with a cytological evaluation of the bronchioalveolar lavage (BAL) fluid (BALF). Ultrasound examination may be helpful in the assessment of the superficial pathological lesions in the pleural and lung tissue (Marr 1993). Ultrasound was reported to be highly sensitive in detecting the severity of episodic exercise-induced pulmonary haemorrhage (EIPH) (Ferrucci et al. 2009). In the study by Kutasi et. al. (2011), imaging diagnostics were used to reveal the presence of comet-tail artefacts in five horses with RAO although the localisation and severity of these lesions were not provided. The only studies using ultrasonography in horses with RAO focused on the evaluation of the caudal lung border. However, no structural changes were described (Bakos et al. 2003). Currently, there are no studies assessing ultrasonographic findings in horses with asthma (Marr 1993).

The aim of this study was to evaluate ultrasonographic findings in horses with asthma versus healthy horses, assess the prevalence and severity of lesions visible on ultrasound in the lung tissue of horses with asthma, and determine the intensity of the disease (mild and severe asthma).

Materials and Methods

Eighteen horses of various warm-blooded breeds (ten females and eight males), used both for low level sport and as pleasure horses, from five to 24 years old (mean 14.7 SD 5.8 years) were included in the study. None of the horses was a performance horse and did not participate in races, endurance riding, eventing or high-level competitions. All the animals were vaccinated against influenza and tetanus and dewormed regularly. All the procedures on the horses used for teaching purposes were performed with the approval of the Animal Experimentation Local Ethics Committee (Permission No. 84/2015). In the case of privately owned horses, the owners of all animals used in the research gave informed consent prior to diagnostic procedure and to the publication of the results.

Examination protocol

Medical interview data were collected from the owners. All the animals underwent a clinical, upper and lower endoscopic and thoracic ultrasound examination, and a complete blood count was performed.

Endoscopic examination and collection of BALF

The resting endoscopic examination was carried out following combined sedation with xylazine (Nerfasin Vet, Le Vet B.V, Oudewater, Holland) administered at 0.3-0.5 mg/kg bwt and butorphanol (Morphasol, aniMedica GmBH, Senden-Bösensell, Germany) at 0.01-0.03 mg/kg bwt. A 180 cm long, 9 mm wide bronchofiberoscope (Karl Storz GmbH, Tuttlingen, Germany) was used. Bronchoalveolar lavage was collected using a large animal broncho-alveolar lavage catheter (MILA International, Inc., Florence, KY, USA) by instilling 300-350 mL of warm sterile saline, using 60 mL syringes. Approximately 40-60% of the lavage fluid was recovered. The collected material was transported to a veterinary laboratory, where it underwent a cytological analysis by a qualified histologist.

Group classification criteria

Horses were included in the study groups based on an interview with the owner, clinical examination, endoscopic examination and BALF cytological analysis. Prior respiratory tract infections were excluded in all the horses based on the interview. The rectal temperature and complete blood count were within the reference range, and the presence of infectious agents (no degeneration or phagocytic neutrophils) was excluded based on the BALF cytology. Animals with signs of a systemic infection or other respiratory diseases, as well as with diseases comorbid to asthma, were excluded from the study. The classification criterion for groups was based on the American College of Veterinary Internal Medicine consensus statement (Couëtil et al. 2016).

Six healthy mares (aged 6.8 SD 1.3 years) owned by the Wroclaw University of Environmental and Life Sciences were enrolled in the control group. These horses had no history of respiratory tract diseases and were healthy at the time of the study as well as eight months later. Examination of these horses was carried out for teaching purposes. There were no abnormalities in the endoscopic examination and the cytological examination of the BALF was normal.

The study group contained 12 private horses with asthma – six of these horses had mild asthma (the IAD group) and six horses had advanced asthma (the RAO group). The IAD group contained five males and one female with a mean age of 15.3 years SD 5.3, while the RAO group contained three females and three males with a mean age of 21.7 SD 1.9. In the studied horses, the symptoms of the disease developed shortly before the study. None of the horses was treated before and at the time of the study. Horses with coughing symptoms, the presence of nasal discharge, poor performance and no signs of increased respiratory effort at rest were included in the IAD group. An increased amount of non-degenerate neutrophils, which comprised up to 20% of all the cells (a mean value of 13.9 SD 3.5% in the specimen), was observed in the BALF. Horses with increased respiratory effort at rest were included in the RAO group. In these animals, louder breath sounds, wheezing and moving secretions were auscultated. BALF cytology revealed an increased amount of non-degenerate neutrophils, which comprised more than 40% of all the cells (mean 75.9 SD 18.3 % in the specimen).

Thoracic ultrasound examination

A lung ultrasound examination was carried out on all the horses prior to sedation and endoscopy. The thorax was shaved bilaterally using an electric clipper. The skin was washed with warm water and chlorhexidine soap, cleansed with alcohol and ultrasound gel was applied to provide acoustic coupling between the skin and the transducer. The examination was performed uniformly in all the animals starting from the third intercostal space (ICS) to the 17th ICS. Each left and right ICS was examined by placing the transducer parallel to the ribs and moving it from the dorsal to the ventral part of the thorax. The ultrasound examination was carried out during inhalation and exhalation. The ultrasound scanner (Terason t3000, Teratech, Burlington, United States) was used with a linear 5.0-7.5 mHz transducer (Terason, Teratech, Burlington, United States). The image parameters were adjusted to the patient in order to obtain optimal image quality. The appearance of the pleura and pathological changes in the lungs were observed. To be more precise in the description of ultrasound lesion location, the lung area was divided into three parts: cranial – including the 3-6th ICS, middle – the 7-11th ICS and caudal – the 12-16th ICS (Fig. 1). Lung ultrasound lesions were classified according to a specifically created scoring system presented in Table 1, based on a system developed by Ferrucci et al. (2009).

Statistical methods

Descriptive statistics were used to analyse the obtained data. Due to the point order scale of collected data nonparametric methods were used in the statistical analysis. To evaluate the differences between the values of thoracic ultrasonography scoring between the healthy, IAD and RAO groups, a nonparametric alternative to variance analysis in the form of the Kruskal-Wallis test was used. The hypothesis of equality was rejected all ways ($p<0.001$) and statistical significance was demonstrated in the course of multiple paired tests. The Mann-Whitney *U* test was used to evaluate the differences between the thoracic ultrasonography scores on the right and left sides separately for the three groups. The significance level was set at 5%, and all the calculations were carried out using commercially available software (TIBCO Software Inc., California, USA).

Results

In all the studied horses, the pleural surface was easily identifiable on ultrasound. The pleural surface without any lesions was visible as a hyperechogenic line with characteristic equidistant reverberation air artefacts (Fig. 2A).

The lesions visible during the ultrasound examination in individual horses are presented in Table 1.

Comet-tail artefacts were the most common lesions present to varying degrees in horses in all the groups. Single lesions were visible in four healthy horses. In three horses, they were located in the middle and caudal lung area. One horse had lesions located only in the caudal area. There were no lesions in the cranial thorax in the healthy horses. There were only two horses from that group with no lesions visible in ultrasound in any of the examined areas. Ultrasound lesions were present in all the animals from the IAD and RAO groups. In the IAD group, they were assessed as single, minor comet-tail artefacts in the middle and caudal areas (Fig. 2B) of the examined lung fields. In two horses from this group, they were also in the cranial part. The most intense lesions were seen in the RAO group, where they were arranged densely and extended across several ICS. Anechogenic focal lesions with a diameter of 0.5-0.7 cm were also observed in this group. The majority of the lesions in this group were located in the middle and caudal parts of the lungs, with more advanced lesions located in the caudal lung fields (Fig. 2C). Two horses had no lesions in the cranial part of the lungs despite the presence of numerous comet--tail artefacts in the caudal part. There were numerous more advanced lesions identified in the right lung fields. However, the difference in the overall average intensity of the ultrasound changes was not statistically significant between the left and the right side of the thorax (healthy $p=0.402$, IAD $p=0.580$, RAO $p=0.646$).

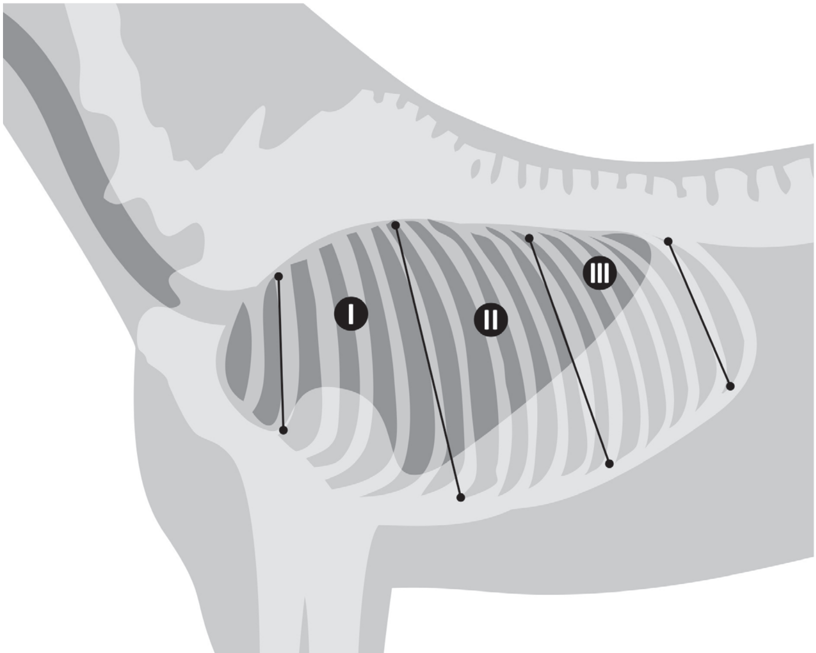

Fig. 1. Schematic illustration of the examined lung area divided into three examination regions: cranial – including the 3-6th ICS, middle – the 7-11th ICS and caudal – the 12-16th ICS.

Table 1. Assessment of the advancement of the lesions in the left and right lungs in individual horses from the control and study groups using a score system.

		Lung area					
		Right side of thorax			Left side of thorax		
Group	Animal	cranial	medial	caudal	cranial	media	caudal
Healthy	I	0	1	1	0	0	1
	II	0	0	0	0	0	0
	III	0	1	1	0	0	1
	IV	0	1	1	0	0	1
	V	0	0	0	0	0	0
	VI	0	0	1	0	0	1
IAD	I	1	0	1	0	1	1
	II	0	0	1	0	0	1
	III	1	1	1	0	1	1
	IV	0	1	1	0	0	1
	V	0	1	1	0	1	1
	VI	0	1	1	0	1	1
RAO	I	2	1/2	2	1	1	2
	II	0	1	2	0	1	2
	III	0	1	2, +	0	1	2
	IV	1	1	2	1	1	2
	V	2	3	3, +	2	2	3
	VI	1	2	3, +	1	2	3

Thoracic ultrasonography scoring system (according to Ferrucci et al.): 0 - no changes in tissue; 1 - single comet-tail artefacts present in individual intercostal spaces; 2- numerous comet-tail artefacts present in individual intercostal spaces; 3 - numerous comet-tail artefacts present in many intercostal spaces; + - larger focal lesions.

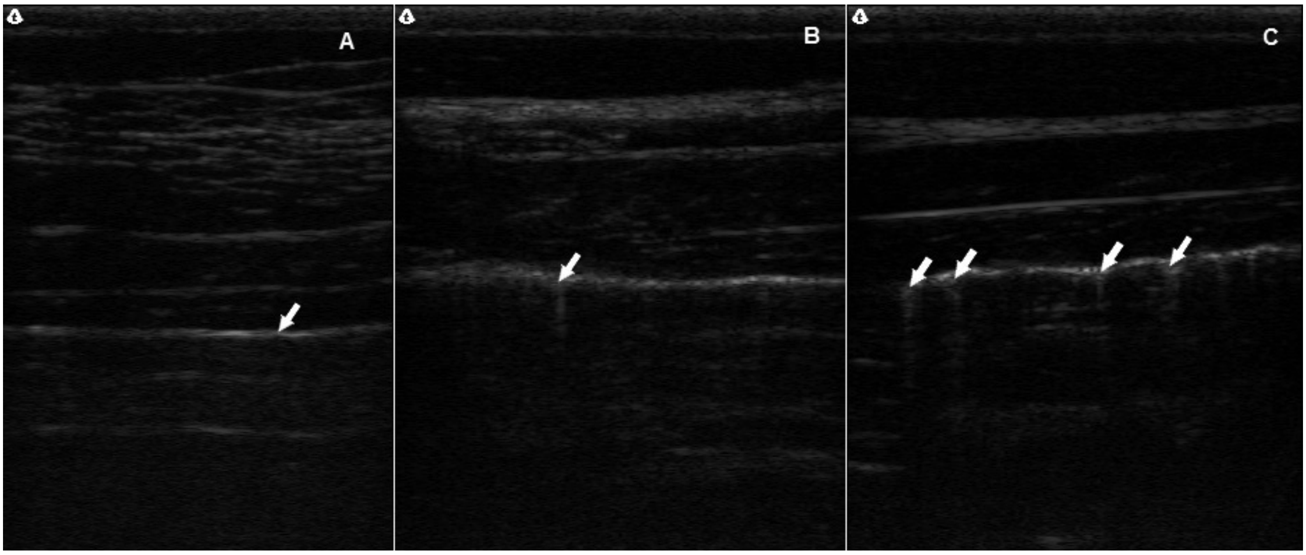

Fig. 2. Ultrasound image of a lung in the right caudal area in 14th ICS: A – normal lungs obtained in healthy horse – the pleura surface forming a straight hyperechogenic, smooth line (arrow) with characteristic equidistant reverberation air artefacts. B - lungs with single comet-tail lesions (arrow) obtained from a horse with mild asthma. C - lungs with numerous comet-tail artefacts (arrows) obtained from a horse with advanced asthma.

Statistically significant differences ($p<0.001$) were seen between the overall average intensity of ultrasound changes in the healthy and the RAO group horses, as well as between the IAD and the RAO group horses ($p<0.001$). However, there were no statistically significant differences between the healthy and the IAD groups ($p=0.206$). More numerous ultrasound pulmonary lesions were seen in the animals with more prominent clinical signs.

Discussion

The current study presents the ultrasound findings in horses with mild and severe asthma in comparison with healthy horses. In healthy animals, the surface of the lungs appears as a very light, smooth line that forms as the reflection of the ultrasound beams from the air filling the lungs (Reef et al. 1991, Roy and Lavoie 2003, Reef 2004). There are parallel, less echogenic lines, which are reverberation artefacts under this light line (Marr 1993). The aerated lung cannot be penetrated by the ultrasound beam (Marr 1993). The ultrasound image of the healthy lungs was seen in all the animals and was also reported by other authors who were examining sites without pathological lesions. Interruption of the pleural echo indicates pulmonary pathology. In the present study, comet-tail artefacts were the most common changes seen in the ultrasound in the horses with asthma. Kutasi et al. (2011) obtained similar results, as they observed comet-tail echoes in five horses with RAO. Similarly, the ultrasound lesions reported in a horse with diffuse lower airway disease presented by Marr (1993) included a dimple at the periphery of the lung with an underlying radiating hyperechoic artefact. Any small irregularities on the surface of the pleura are visible as comet-tail artefacts (Reef et al. 1991, Roy and Lavoie 2003). According to the literature, this type of lesion is one of the most commonly reported ultrasound findings (Reef et al. 1991). Such lesions are formed by small areas of fluid or inflammatory infiltrate in the pulmonary parenchyma (Reef 2004). Numerous pulmonary diseases may produce such artefacts, including: oedema, EIPH, infections, metastasis and fibrosis (Reimer 1990, Reef 1991, Marr 1993, Reef 1998, Gross et al. 2004, Reef 2004). Such lesions were also reported in patients with undifferentiated diseases (Kutasi et al. 2011). The comet-tail artefacts are not specific for any given lung pathology and their presence should be interpreted with the history and the clinical signs taken into consideration. In the current study, other pathologies, such as infectious diseases or EIPH, were excluded based on the history, complete blood count and cytological BALF evaluation.

Single comet-tails artefacts were also found in clinically healthy horses, where the history and presence of lower respiratory tract disease was excluded. Animals from this group did not develop signs of respiratory disease after the study period. Single lesions in healthy animals may be caused by previous asymptomatic infections as well as exposure of the respiratory tract to various non-infectious factors (Marinkovic et al. 2007).

More comet-tail artefacts were found in animals with more advanced clinical symptoms and endoscopy findings, associated with disease severity. However, horses from the RAO group had a much higher mean age than animals in the other groups. This is due to the fact that RAO occurs in older horses, while IAD concerns younger animals. In humans, studies found an age-related decrease in elastic recoil. Lung compliance, which is age related, is thought to be caused by structural changes within the lung tissue, such as modification or loss of elastin and collagen (Turner et al. 1968). However, there are no data that would show ultrasound or radiological changes in the lungs of healthy elderly people. Martin et al. (1977) found no effect of age on elastic recoil in geriatric horses. Histological, radiological and ultrasound changes in the lungs and pleura of older horses have not been studied. Although changes in the form of the comet tails have been described in many pathologies, none of them indicate that they may be caused by the aging of the organism.

Larger lesions not exceeding 0.7 cm were observed in two horses from the RAO group. Unaerated lung areas and consolidations are easily identifiable on ultrasound as different shades of grey (Reef et al. 1991, Roy and Lavoie 2003). Such lesions were observed only in horses with advanced respiratory disease and severe clinical symptoms. Their presence may indicate disease progression or previous asymptomatic respiratory diseases, including previous asthma episodes that were not noticed by the owners (Castelman et al. 1990, Marinkovic et al. 2007).

According to the literature, more pronounced lesions in horses are present in the right thoracic cavity (Rantanen 1986, Reef 1991, Marr 1993, Reef 1998, Reef 2004). In the present study, more numerous and larger lesions were noted on the right side of the thorax. This may be caused by the fact that the right main bronchus is slightly wider and its bifurcation angle is smaller than the left bronchus, allowing easier access for allergens and pathogens. Similarly, the tracheal secretion can move into this bronchus. Studies in humans have also found that these two factors increase the likelihood of foreign body aspiration into the right bronchus (Tahir et al. 2009). The caudal area of the lungs was a permanent site of lung tissue lesions in all the study animals and in some from the control group. The lesions appeared in the middle and cranial lung areas with the disease progression. According to previous studies, consolidations are seen mainly in the caudo-ventral lung areas (Reef 2004). Similarly, lesions in the lung tissue in the course of EIPH visible on ultrasound are mainly visible in this area (Ferrucci et al. 2009). According to O'Callaghan et al. (1987), this lung field is most commonly affected by pathologies. Leguillette (2003) explains that the lesions initially focus in the small bronchi (located peripherally), and a large number of constricted small bronchi is necessary to impede airflow throughout the lower parts of the lung to the point where clinical signs may appear.

Diagnostic imaging is an additional tool used to assess tissue lesions. Ultrasound examination is widely used and provides the differentiation of healthy tissue from tissue with density changes (O'Brien and Biller 1997). It also allows an easy detection of small superficial pleural or parenchymal abnormalities (Reef et al. 1991, Roy and Lavoie 2003). The study carried out by Ferrucci et al. (2009) found a high sensitivity of the diagnostic accuracy of ultrasound in horses with EIPH. The high sensitivity of this examination makes it a useful diagnostic tool for the detection of pulmonary lesions. Despite the advantages of sonography in the detection of lung pathologies, it is a non-specific diagnostic tool (Ferrucci et al. 2009). Disseminated parenchymal lesions are often very similar and cannot be differentiated via an ultrasound examination (Marr 1993). In this study, similar lesions were present in both healthy and sick animals. Ultrasound examination provides information about the extent and severity of the lesions rather than their type and character.

Since this is a preliminary study, the main limitation of this study was the small number of examined animals. A further limitation was using only non-invasive techniques for the analysis of the lung tissue. Biopsy specimens were not collected from the lesions visualized in the ultrasound examination for further specification. Intra-thoracic pressure, measured to assess respiratory resistance, was not evaluated in any of the horses with asthma because of the lack of appropriate equipment. Due to the lack of data concerning changes in the pleural and lung tissue in healthy geriatric horses, it would be interesting to perform additional tests in this group of animals.

Conclusion

Ultrasound examination should be treated as a valuable additional diagnostic tool in asthma enabling the assessment of the advancement of the disease process. This method is sensitive in detecting very small superficial lesions. Due to the low specificity, it cannot replace endoscopic examination with an analysis of the BALF. The results of the ultrasound examination should be correlated with the clinical symptoms and other tests in order to correctly diagnose the disease.

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