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Pulmonary fibrosis in a donkey

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Summary: A 26-year old donkey gelding was referred to the University Equine Hospital of the Vetmeduni Vienna for evaluation of increased respiratory rate and dyspnoea of 2 weeks duration as well as chronic weight loss. Radiography and ultrasonography of the thorax revealed an interstitial lung pattern, pulmonary oedema and a large number of comet tail artefacts. Echocardiography detected severe aortic valve regurgitation and a dilated pulmonary artery indicating pulmonary hypertension. A diagnosis of interstitial fibrosis was made on histopathology of a lung biopsy. The donkey moderately improved with dexamethasone and furosemide treatment. It subsequently developed ventricular tachycardia followed by multifocal ventricular premature beats. Antiarrhythmic therapy was unsuccessful and the donkey was euthanised when in an agonal state. Necropsy and histopathology confirmed severe lung fibrosis similar to previously reported cases affected by Donkey Pulmonary Fibrosis (DPF) as well as dilatation and degeneration of the pulmonary arterial wall consistent with pulmonary hypertension. PCR analysis of bronchoalveolar fluid sampled post-mortem revealed asinine herpesvirus (AHV) 5 DNA. This report aims at highlighting the clinical signs, diagnostic workup and histopathologic features of DPF.

Keywords: donkey, fibrosis, lung, herpesvirus, arrhythmia

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Introduction

Donkey pulmonary fibrosis (DPF) is a chronic and currently untreatable idiopathic condition, which is characterised by excessive and irreversible deposition of extracellular matrix in the pulmonary parenchyma (*Miele* et al. 2014). It is a relatively common finding in elderly donkeys, 35% of 1444 donkeys examined at necropsy were found to have chronic pulmonary fibrosis (*Morrow* et al. 2011).

DPF shares common histopathologic features with human pleuroparenchymal fibroelastosis, a rare idiopathic interstitial pneumonia (*Miele* et al. 2014, *Reddy* et al. 2012, *Travis* et al. 2013, *Von der Thüsen* 2013). These similarities include pleural and subpleural fibrosis with accompanying elastosis detected in more than 50% of donkey lung specimens affected by DPF (*Miele* et al. 2014).

In addition to genetic and environmental factors, viral pathogens, especially human herpes virus infections, might play a role in the development of human interstitial fibrosis (*Barratt* et al. 2018).

Analogous viral DNA of AHV 4 and 5, which belong to the gamma herpesvirus family, has been detected by PCR in the lungs of donkeys suffering from interstitial pneumonia and lung fibrosis (*Kleiboeker* et al. 2002) and in a mare with pyogranulomatous pneumonia (*Witte* et al. 2012). The severity and appearance of lesions in the study from *Kleiboeker* et al. (2002) suggests an active clinical infection, although latent infection of the animals could not be ruled out completely. Another study detected AHV 4 and 5 not only in lung tissue of DPF affected animals, but also in a control group of healthy donkeys, therefore, the role and influence of AHV 4/5 on DPF remains unclear (*Miele* et al. 2014).

Clinical diagnosis of interstitial pneumonia and pulmonary fibrosis is currently based on clinical signs, as well as radiography and ultrasonography of the thorax (*Thiemann* 2012, *Thiemann* and *Bell* 2001, *Wilkins* and *Lascola* 2015). Early stages of the disease may remain undetected until clinical signs progress. Computed tomography (CT) could be an additional useful diagnostic tool, although the size of the animal is a limiting factor. So far, only post-mortem CT examinations of inflated donkey lungs have been reported (*Miele* et al. 2014). For a definitive ante-mortem diagnosis, transthoracic lung tissue biopsy is needed (*Thiemann* and *Bell* 2001, *Wilkins* and *Lascola* 2015).

Treatment of DPF is palliative, including application of oral or inhaled corticosteroids, bronchodilators, mucolytics and antibiotics if secondary bacterial infection is present (*Thiemann* 2012). Corticosteroids may suppress pulmonary inflammation and prevent ongoing fibrosis (*Wilkins* 2013), but there is no evidence of their efficacy in DPF affected animals.

In human interstitial fibrosis patients, treatment with the anti-fibrotic drugs pirfenidone and nintedanib is the current standard (*Ogura* et al. 2015, *Vancheri* et al. 2018). Data on the efficacy of these drugs in donkeys are lacking and due to their high cost they are unlikely to become an option in veterinary medicine. Another promising substance is pentoxiffyline,

which reduced pulmonary fibrosis in rats when given in combination with tocopherol (*Bese* et al. 2007, *Kaya* et al. 2014). The use of pentoxiffyline has been reported for the treatment of equine multinodular pulmonary fibrosis (*Hart* et al. 2008, *Schwarz* et al. 2013).

The prognosis of DPF is considered poor. The majority of affected animals die or have to be euthanised within 2 weeks of the first onset of clinical signs. If cor pulmonale develops secondary to extensive pulmonary fibrosis, the prognosis is grave due to the progressive nature of this disease (*Thiemann* and *Bell* 2001).

Case history

A 26-year old, 111 kg, middle-sized donkey gelding (Equus asinus asinus) was referred to the University Equine Hospital of the Vetmeduni Vienna for evaluation of dyspnoea and tachypnoea of 2 weeks duration, suspected cardiac disease and chronic weight loss. The donkey was treated with prednisolone, dembrexine and clenbuterol by the referring veterinarian the day prior to presentation. Clinical signs did not improve within the short treatment period.

Clinical findings, diagnostic procedures and treatment

On initial examination, the donkey was lethargic. It had a poor to moderate body condition score, was normothermic (36.9 °C) and tachycardic (96 beats per minute) with a regular, but weak pulse and tachypnoeic (68 breaths per minute) with moderate inspiratory and expiratory dyspnoea. Harsh lung sounds and crackles were detected bilaterally over the entire lung field. Moderate amounts of foamy nasal discharge were present in both nostrils. Cardiac auscultation revealed a holodiastolic heart murmur with an intensity of 6/6 and a point of maximum intensity over the left 4th intercostal space. Standard base-apex electrocardiography detected sinus tachycardia.

Thoracic ultrasound performed during emergency hours on the day of admission identified multiple comet tail artefacts consistent with pleural or subpleural lesions diffusely spread on both sides of the thorax. Mild bilateral anechoic pleural effusion could be detected cranioventrally. No abnormalities were found on abdominal ultrasound. On admission, detailed echocardiography was not performed, on a shortened ultrasonographic evaluation of the heart following an emergency protocol severe aortic valve regurgitation, moderate pulmonic valve regurgitation, mild tricuspid valve regurgitation and a severely dilated pulmonary artery were detected.

Haematology revealed a leucocytosis of 16,540/µl, (reference range according to *Svendsen* 2008: 6,100–16,100/µl), cardiac troponin I, serum amyloid A (SAA), creatinine and triglyceride concentrations were within normal limits. Electrolyte concentrations could not be measured for technical reasons. Pulmonary oedema was the initial diagnosis, congestive heart failure, pulmonary hypertension and interstitial pneumonia were suspected. The very poor prognosis for survival was discussed with the owners considering the life threatening conditions of the donkey. Due to financial constraints and their refusal of intravenous treatment, the decision was made to start per os (PO) treatment with furosemide 1 mg/kg bid (day 1–4) in an attempt to reduce pulmonary oedema despite the reported poor bioavailability of per os furosemide described in horses. To the best of the authors' knowledge, there are no studies available on the efficacy of per os furosemide in donkeys.

As the donkey was admitted on a Friday during emergency hours, further diagnostic investigations were performed on the following Monday, day 4 of hospitalisation. Lateral radiographs of the thorax showed a decreased overall lung transparency with focal areas of higher density and an interstitial pattern. Radiographic findings indicated mild pulmonary oedema and interstitial pneumonia (Fig. 1).

Echocardiography using standardised 2D, M-mode and Doppler imaging techniques revealed a subjectively hypocontractile and enlarged left ventricle when compared to the right ventricle, as well as a hyperechoic and thickened aortic valve leaflet and a moderately increased left atrium to aorta ratio (LA/Ao = 1.4). Furthermore, severe enlargement of the pulmonary artery with an arterial diameter that exceeded the aortic root diameter in the right parasternal long axis view indicated probable pulmonary hypertension (Fig. 2). Neither changes in right ventricular size nor abnormal movements of the interventricular septum were noted on 2D echocardiography, which could be indicative of the presence of cor pulmonale.

M-mode evaluation of left ventricular dimensions at the chordal level were within normal limits according to current literature on echocardiographic dimensions of the donkey (*Amory* et al. 2004, *Hassan* and *Torad* 2015, *Roberts* and *Dukes-McEwan* 2016).

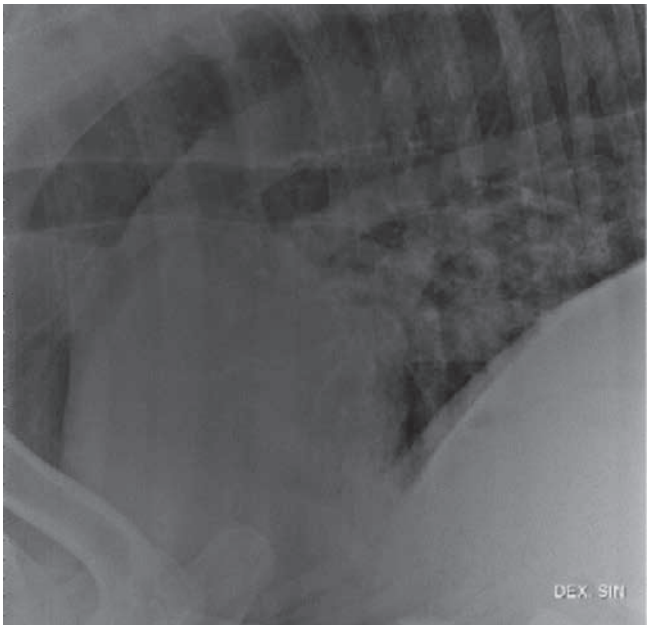

Fig. 1 Right cranio-lateral thoracic radiography shows a decreased overall lung transparency and an interstitial pattern. The image was provided by the Clinical Unit of Diagnostic Imaging of Vetmeduni Vienna. | *Die cranio-laterale Röntgenaufnahme des Thorax von rechts zeigt eine generalisierte Verminderung der Lungentransparenz sowie eine interstitielle Lungenzeichnung. Die Aufnahme wurde von der Abteilung der Bildgebenden Diagnostik der Vetmeduni Vienna zur Verfügung gestellt.*

Doppler echocardiography revealed severe aortic valve regurgitation, moderate pulmonic valve regurgitation, mild to moderate tricuspid valve regurgitation and mild mitral valve regurgitation.

Standard base-apex electrocardiography was recorded concurrently. Two ventricular premature depolarizations during 60 minutes of recording time were present. Mean heart rate was 56 beats per minute (beats/min).

A presumptive diagnosis of interstitial pneumonia, pulmonary oedema, severe aortic valve regurgitation and suspected pulmonary hypertension was made. Due to severe pulmonary and cardiovascular disease, all diagnostic procedures that required sedation were postponed until the donkey showed clinical improvement in order to avoid the risks associated with sedative administration.

The dosage of furosemide was increased to 2 mg/kg bid on day 4 and glucocorticoid treatment was initiated with intramuscular (i.m.) dexamethasone at a dosage of 0.1 mg/kg sid (day 4–day 8).

Arterial blood gas analysis of a blood sample collected from the carotid artery on day 5 following admission revealed arterial hypoxaemia, hypercapnia (PaO_2: 62.9 mmHg, pH 7.41, $PaCO_2$: 54.7 mmHg) and an estimated alveolar-arterial PO_2 gradient of 29.7 mmHg (reference range < 10 mmHg). Blood electrolyte concentrations including sodium, potassium and calcium were within normal limits.

Over the following days, the donkey significantly improved in alertness, demeanour and had a normal appetite. Body temperature was within normal limits at all times, average heart rate was 56 beats/min and average respiratory rate went down to 26 breaths/min. Lung auscultation revealed mildly increased respiratory sounds with the absence of crackles. No cough, nasal discharge or other clinical signs of persisting lung oedema were present.

Due to the clinical improvement, which was believed to be caused by the administration of dexamethasone rather than furosemide and the lack of evidence of the efficacy of per os furosemide in donkeys, diuretic therapy was terminated on day 6.

On day 7, arterial blood gas analysis showed improvement compared to the previous results (PaO_2: 87 mmHg, pH: 7.41, $PaCO_2$: 41.1 mmHg, alveolar-arterial PO_2 gradient: 19.4 mmHg).

On day 9, therapy was changed to PO dexamethasone sid (0.1 mg/kg). As the donkey became more lethargic again within the next two days, therapy was then changed to PO dexamethasone twice daily (0.05 mg/kg) on day 12.

Alertness and demeanour of the donkey improved again following the treatment change. Still no signs of recurrent pulmonary oedema were present. Further diagnostic procedures under sedation including ultrasound guided transcutaneous lung biopsy and bronchoscopy, were performed on day 12, following owner permission. The donkey was sedated with a single dose of 0.035 mg/kg (IV) butorphanol, which resulted in short-term, deep sedation.

Ultrasound guided transcutaneous lung biopsy was performed after clipping and aseptic preparation of the left thorax between the 5th and 9th intercostal spaces. After local anaesthesia and skin incision, the biopsy was taken in the 7th intercostal space of the mid-thorax using a 14 gauge automatic biopsy needle (CareFusion, France). One tissue sample was collected and fixed in 4% formalin. No complications were observed.

Histopathologic examination of the lung tissue revealed severe interstitial fibrosis causing thickening of the alveolar septa. Scant inflammation within septa and alveolar spaces was present with low numbers of alveolar macrophages, lymphocytes, neutrophils and the focal appearance of multinucleated giant cells. Viral inclusions were not detected.

Bronchoscopy was performed during the same sedation. Viscous white mucus along the ventral aspect of the trachea was detected, the caudal part of the trachea showed dorso-ventral flattening of the tracheal rings. The carina was moderately oedematous and mucus was present up to the main bronchi. Tracheobronchial aspirate (TBA) samples and bronchoalveolar lavage fluid (BALF) were taken. Following standard university protocol, 10 ml of sterile saline were instilled transendoscopically via a sterile endoscopic aspiration catheter for TBA collection and re-aspirated at the lowest point of the trachea. As antitussive, 20 ml of a 0.4% lidocaine dilution was instilled via a BALF catheter and BALF collection followed lavage with 120 ml of sterile saline. Cytological examination of TBA revealed inflammatory cells with a mixed cell population containing 92% neutrophils, 8% mononucleated cells as well as sporadic multinucleated giant cells, ciliated epithelial

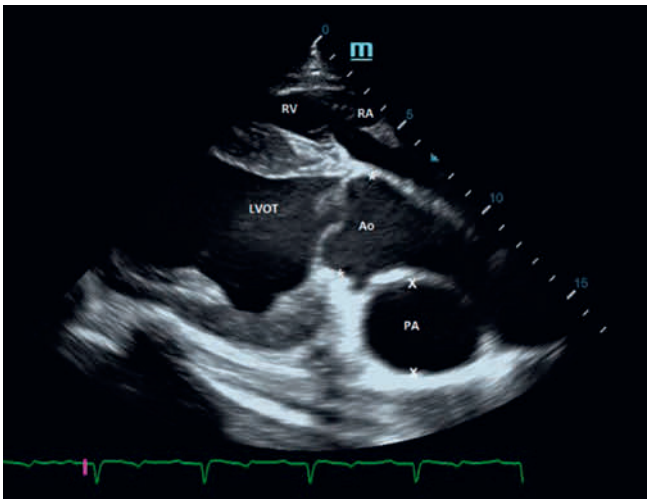

Fig. 2 Right parasternal long axis view of the left ventricular outflow tract and aorta. Pulmonary artery diameter (x) exceeds the aortic root diameter (*). Echoic area at the aortic valve indicates turbulent blood flow due to severe aortic valve regurgitation. Aortic valve leaflets are mildly thickened. Ao: aorta; PA: pulmonary artery; LVOT: left ventricular outflow tract; RV: right ventricle; RA: right atrium. | *Rechter parasternaler Längsachsenschnitt des linksventrikulären Ausflusstraktes und der Aorta. Der Durchmesser der Pulmonalarterie (x) überschreitet den Durchmesser der Aortenwurzel (*). Das echoreiche Areal an der Aortenklappe weist auf einen turbulenten Blutfluss aufgrund der hochgradigen Aortenklappeninsuffizienz hin. Die Aortenklappen erscheinen geringgradig verdickt. Ao: Aorta; PA: Pulmonalarterie; LVOT: linksventrikulärer Ausflusstrakt; RV: rechter Ventrikel; RA: rechtes Atrium.*

cells and epithelial cells of the upper respiratory tract. Cytological examination of BALF revealed an increased population of small lymphocytes (76%), 11% neutrophils and 13% macrophages.

Bacterial culture of the TBA revealed the presence of *Staphylococcus aureus* in moderate amounts and some *Kosakonia cowanii*. As the results of bacterial culture were received only on day 13, just prior to euthanasia, the therapy was not changed and no further investigations were made to rule out bacterial lower respiratory tract infection. No pathogens were detected on cytology of either TBA or BALF, and neither were endoscopic findings indicative of bacterial infection of the respiratory tract. Furthermore, the clinical presentation of the donkey was not characteristic of bacterial pneumonia, as he was normothermic and had no cough, nasal discharge or pleurodynia and since arterial blood gas analysis values had improved with corticosteroid treatment. Therefore, the organisms detected on bacterial culture of TBA were considered to be contaminants rather than causative pathogens. It is noteworthy to mention that donkeys have a relatively insensitive cough reflex, therefore, the lack of cough does not rule out respiratory disease.

Outcome

On day 13, tachycardia with heart rates exceeding 100 beats/min was present. A 3-hour Holter ECG demonstrated sinus tachycardia followed by ventricular premature beats, ending up in uniform ventricular tachycardia (Fig. 3). At this time, the donkey was again mildly lethargic, but maintained a normal appetite.

Due to financial constraints, blood work was reduced to a minimum database including blood electrolytes, cardiac troponin I and serum amyloid A (SAA) concentrations. Calcium, phosphorus, magnesium and cardiac troponin I were within normal range. The SAA level was markedly increased to 2751.3 mg/L (reference < 10 mg/L).
Antiarrhythmic therapy was initiated with IV magnesium sulphate at a dosage of 2.2 mg/kg/min diluted in 500 ml of NaCl, resulting in a total amount of 6.1 g of magnesium sulphate administered over 30 minutes. As no publications on the use, efficacy and safety of antiarrhythmic drugs in donkeys are currently available, the lowest recommended dose for horses was used in order to avoid side effects (*Mitchell* 2017). Ventricular tachycardia converted to sinus tachycardia at a heart rate of 70–80 beats/min (Fig. 4), but soon after receiving almost half of the calculated amount, approximately 12 minutes after initiation of infusion, the donkey started swaying and suddenly collapsed. ECG recorded sinus tachycardia at a rate of 130 beats/min with one supraventricular premature beat, subsequently a 2nd degree AV block occurred followed by ventricular standstill and asystole. The owners decided against resuscitation and so for ethical reasons the donkey received 2 g thiopental followed by 50 ml of T61 IV (10 g embutramide, 2.5 g mebezonium iodide and 0.25 g tetracaine hydrochloride) to shorten the agonal state.

Necropsy and histopathological findings

Necropsy was performed immediately following euthanasia.

The major gross finding was extensive fibrosis of the lung parenchyma infiltrating the entire organ as shown in Figure 5A and 5B. Histopathological investigation of several lung sections confirmed excessive collagen deposition in the alveolar septa as well as in the interstitial lung tissue (Fig. 5C) that was highlighted by Azan Blue staining visualising the tremendously increased amount of connective tissue within the lung parenchyma (Fig. 5D). A few focal areas of calcifications were present as confirmed by Kossa-stain. The lung fibrosis was accompanied by severe alveolar oedema and hyperaemia (Fig. 6A) as well as by extended areas of alveolar emphysema (Fig. 6B). Additionally, oligofocal moderate, mainly lymphoplasmacytic infiltration of the lung interstitium and moderate numbers of erythrocytes, mucus and heart failure cells were located in the alveolar lumina (Fig. 7A). Ferric iron of heart-failure cells was further visualised by Prussian blue staining (Fig. 7B). Macroscopically, the four chambers of the heart were neither dilated nor constricted. The thickness of the ventricular walls as well as the dimensions of the papillary muscles were within the normal range. In the left ventricle acute necrosis of solitary cardiac muscle cells as well as multiple extended myocardial scars (Fig. 7C) combined with mild endocardiosis (Fig. 7D) were determined. Moreover, the aortic valves where highly fibrotic including areas of mucoid degeneration. The truncus pulmonalis was severely dilated at double the diameter of the aortic vessel. Mucoid degenera-

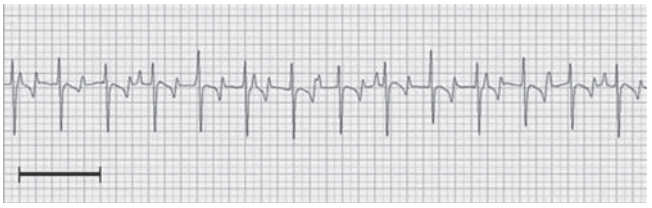

Fig. 3 ECG shows uniform ventricular tachycardia at a rate of 110 bpm. The line bar indicates 1 second. | *Das EKG zeigt eine uniforme ventrikuläre Tachykardie bei einer Herzfrequenz von 110 Schlägen pro Minute. Der Balken markiert eine Sekunde.*

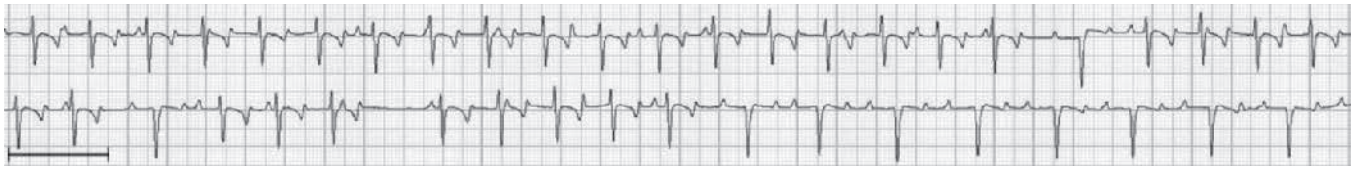

Fig. 4 ECG obtained during magnesium sulphate infusion. Ventricular tachycardia at a rate of 110 beats/min converts to sinus tachycardia at a rate of 70 beats/min. The line bar indicates 1 second. | *EKG Aufzeichnung während der Infusion mit Magnesiumsulfat. Konversion der ventrikulären Tachykardie bei einer Herzfrequenz von 110 Schlägen pro Minute zur Sinustachykardie bei einer Herzfrequenz von 70 Schlägen pro Minute. Der Balken markiert eine Sekunde.*

tion was also found in the vessel walls of the aorta and the pulmonary artery.

Additionally, multiple infarct scars in the kidney, a moderate fatty liver, multiple chronic gastric ulcerations adjacent to the Margo plicatus as well as moderate hyperplasia of lymphatic follicles in the intestinal mucosa were found. Furthermore, dental pathology including hooks, step and wave formation of the upper and lower jaw combined with a chronic gingivitis and mucosal ulcerations were noted.

Virological investigation of a bronchial wash sample taken post-mortem revealed the presence of herpes virus DNA. Subsequent PCR analysis of the specific DNA fragment showed 100 % homology to AsHV 5 according to GenBank AY054993.

Discussion

Severe pulmonary disease as well as serious cardiac abnormalities including severe aortic regurgitation and suspected pulmonary hypertension were present in this donkey. Diagnostic imaging and transthoracic lung biopsy confirmed the suspicion of Donkey Pulmonary Fibrosis (DPF) in this case that was subsequently confirmed on necropsy.

Pulmonary fibrosis is characterised by excessive and irreversible deposition of extracellular matrix in the lung parenchyma, leading to compromised ventilation and organ dysfunction (*Miele* et al. 2014). Although the frequency of occurrence seems high (*Morrow* et al. 2011), little is known about this disease and DPF is currently untreatable.

Clinical signs of DPF include tachypnoea, increased respiratory effort and variable adventitious lung sounds in mild cases (*Thiemann* and *Bell* 2001, *Wilkins* and *Lascola* 2015). More severely affected animals show marked inspiratory dyspnoea with a lengthening of the inspiratory phase to counteract the restriction caused by lung fibrosis. Pyrexia and nasal discharge can occur secondary to bacterial lower airway infection (*Thiemann* 2012). Radiography may detect an interstitial pattern as well as a hypertrophic cardiac silhouette (*Thiemann*

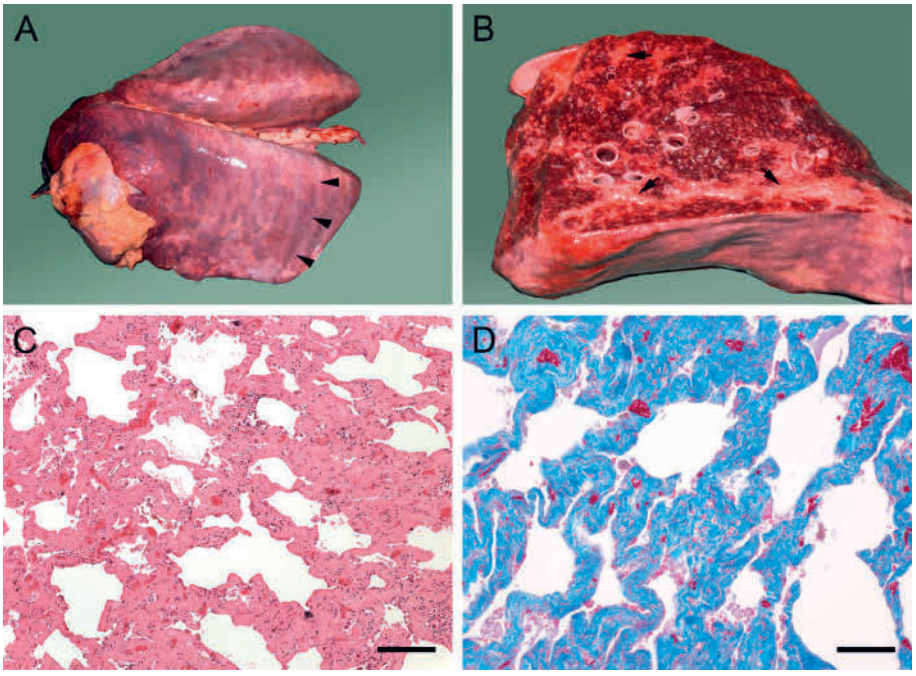

Fig. 5 Extensive pulmonary fibrosis of a donkey lung. A: As a sign of a loss of elasticity, rib impressions are visible on lung surface. Black arrows highlight an impression line. B: Extensive lung fibrosis evident as whitish areas in the lung parenchyma (black arrows). C: Haematoxylin and eosin staining of lung tissue showing massive thickening of the alveolar septa and interstitial tissue in response to extensive deposition of collagen fibers; scale bar = 160 µm. D: Azan blue staining of lung tissue visualising collagen fibers in massively thickened alveolar septa; scale bar = 80 µm. | *Ausgedehnte Fibrose der Lunge. A: Abdrücke der Rippen an der Lungenoberfläche als Zeichen des Elastizitätsverlustes der Lunge. Die Pfeile markieren den Abdruck einer Rippe. B: Die ausgedehnte Lungenfibrose zeigt sich als weißliche Areale im Lungenparenchym (Pfeile). C: In der Hämatoxylin- und Eosin-Färbung von Lungengewebe lässt sich die massive Verdickung der Alveolarsepten und des interstitiellen Gewebes als Folge der ausgedehnten Ablagerung von Kollagenfasern darstellen; Balken: 160 µm. D: Die Azan-Färbung von Lungengewebe zeigt Kollagenfasern in den hochgradig verdickten Alveolarsepten; Balken: 80 µm.*

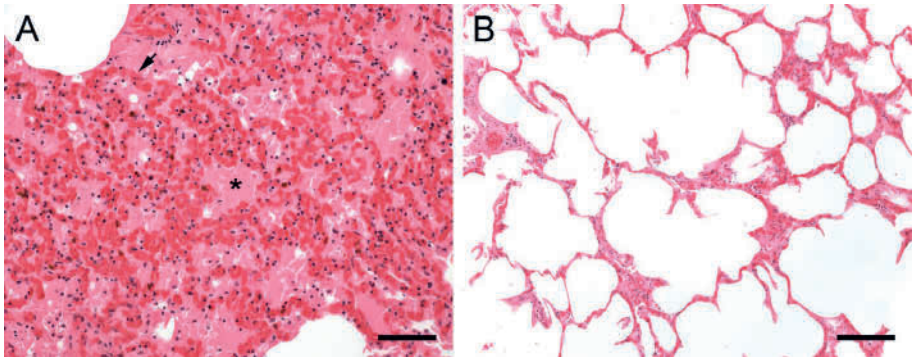

Fig. 6 Hyperaemia, alveolar oedema and emphysema of lung tissue on hematoxylin and eosin stained sections of lung tissue. A: Massive hyperaemia of alveolar septa indicated by rows of eosinophilic red blood cells as shown by arrow; alveolar oedema characterised by alveolar lumina filled with homogeneous eosinophilic exudate marked by asterisk; scale bar = 80 µm. B: Disruption of alveolar septa reducing the alveolar surface area showing severe alveolar emphysema; scale bar = 160 µm. | *Hyperämie, Alveolarödem und Emphysem des Lungengewebes in der Hämatoxylin- und Eosin-Färbung. A: Die eosinophil gefärbten Erythrozyten (Pfeil) weisen auf eine hochgradige Hyperämie der Alveolarsepten hin. Die Lumen der Alveolen sind mit homogenem eosinophilen Exsudat gefüllt (Asterisk), charakteristisch für ein Lungenödem; Balken = 80 µm. B: Das hochgradige Lungenemphysem zeigt sich durch Unterbrechungen der Alveolarsepten mit einer daraus resultierenden Reduktion der Alveolaroberfläche; Balken = 160 µm.*

2012). Further diagnostic imaging such as CT or MRI would be helpful in confirming the diagnosis and in evaluating the extent and degree of severity, however, only post-mortem CT examinations have been described in the current literature (*Miele* et al. 2014). In our case, radiography contributed to the diagnosis of interstitial pneumonia and mild lung oedema, however Equine Multinodular Pulmonary Fibrosis (EMPF) like lesions were not detected. CT examination was rejected due to the risk of general anaesthesia.

DPF can be misinterpreted as equine asthma syndrome or other lower airway diseases, therefore other conditions leading to similar signs must be ruled out before initiating treatment. Lower airway endoscopy should be performed and tracheal/bronchoalveolar washes should be taken to rule out other conditions causing dyspnoea, but these diagnostic tests cannot confirm interstitial pulmonary fibrosis (*Thiemann* 2012). A slightly higher percentage of macrophages in BALF samples (*Campi* et al. 2008) and higher neutrophil and eosinophil counts in tracheal wash samples were reported in healthy donkeys compared to values in horses (*Delvaux* 2001), but no data on BALF and tracheal wash cytology in DPF affected animals exist. Our case showed an increased lymphocyte count in BALF, which can be associated with nonspecific interstitial pneumonia in humans (*Meyer* et al. 2012). Lymphocyte differential counts above 15 % in BALF were associated with a more favourable outcome in idiopathic interstitial pneumonia patients (*Takei* et al. 2017).

In our patient, cytology of the TBA revealed a mixed population of inflammatory cells, not indicative of any particular pathogenesis. Furthermore, although *Staphylococcus aureus* and *Kosakonia cowanii* were detected on bacterial culture of the TBA the clinical significance of these findings is unclear. *Staphylococcus aureus* is an uncommon cause of bacterial pneumonia or pleuropneumonia in horses, but opportunistic bacteria can colonise the lungs especially in animals suffering from a primary disease, leading to secondary bacterial airway infection (*Reuss* and *Giguère* 2015). This fact makes interpretation of the bacterial culture findings difficult. Neither fever nor anorexia, cough nor nasal discharge were present in the donkey. Respiratory distress and increased respiratory rate were thought to be caused by pulmonary fibrosis rather than secondary bacterial respiratory infection. Furthermore, values of arterial blood gas analysis improved with dexamethasone treatment, which would have been unlikely in the case of ongoing bacterial infection. *Kosakonia cowanii* was only present in very small amounts and was not even considered as a causative pathogen for airway infection. No further investigations to rule out or confirm bacterial airway infection were undertaken, as the donkey developed fatal cardiac arrhythmia shortly after the results of bacterial culture and SAA values were received. The donkey did not receive any antimicrobial therapy during hospitalisation. The reason for the increase of SAA concentration to 2751.3 mg/L on day 13 remains unknown as there was no evidence of bacterial pneumonia at necropsy. The preceding lung biopsy could have been a reason for an increase in SAA, although the value seemed too high for the very localised tissue damage resulting from this procedure.

AHV 5 was detected via PCR from a bronchial wash sample which was taken at post-mortem. Pulmonary fibrosis has been associated with gamma herpesvirus infection, but the pathophysiological mechanism has not been completely elucidated. Gamma herpesviruses are widespread in the equine population. Viral DNA of AHV 4 and 5 has been detected by PCR in the lungs of donkeys suffering from interstitial pneumonia and lung fibrosis (*Kleiboeker* et al. 2002) and in a mare with pyogranulomatous pneumonia (*Witte* et

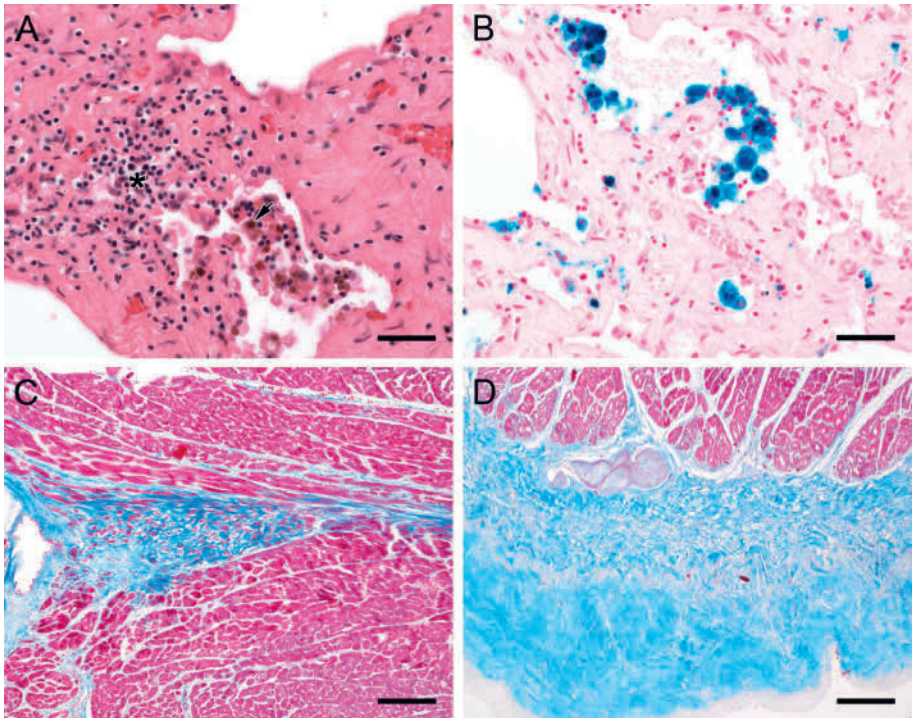

Fig. 7 Lymphoplasmacytic inflammation of the lung tissue and signs of chronic heart failure. A: Focal infiltration of interstitial lung tissue with lymphocytes and plasma cells (asterisk) in hematoxylin and eosin stained sections of lung tissue. Brown granules of hemosiderin from phagocytosed erythrocytes appear in the macrophage cytoplasm of heart-failure cells (indicated by arrow); scale bar = 40 µm. B: Prussian blue stain highlighting ferric iron (blue) in heart-failure cells; scale bar = 40 µm. C: Extended myocardial scars visualized by Azan blue staining; scale bar = 160 µm. D: Thickening of endocardium by deposition of collagen fibers (blue); Azan Blue Staining; scale bar = 160 µm. | *Lymphoplasmozytische Entzündung des Lungengewebes und Anzeichen einer chronischen Herzinsuffizienz. A: Fokale Infiltration des Lungengewebes mit Lymphozyten und Plasmazellen (Asterisk) in der Hämatoxylin- und Eosin-Färbung. Die Herzfehlerzellen (Pfeil) enthalten braunes Granula in ihrem Zytoplasma, welches aus Hämosiderin von phagozytierten Erythrozyten stammt; Balken = 40 µm. B: Berliner-Blau-Färbung von Lungengewebe zum Nachweis von Eisen (blau) in Herzfehlerzellen; Balken = 40 µm. C: Ausgedehnte Myokardnarben dargestellt durch die Azan-Färbung; Balken = 160 µm. D: Ablagerungen von Kollagenfasern (blau) führen zu einer Verdickung des Endokards, dargestellt durch die Azan-Färbung; Balken = 160 µm.*

al. 2012). Another study detected AHV 4 and 5 not only in lung tissue of DPF affected animals, but also in the control group, so the role and influence of AHV 4/5 on DPF remains unclear, although no other possible causative agents could be found (*Miele* et al. 2014). Histopathological findings of the lungs in this case included severe interstitial fibrosis causing thickening of the alveolar septa and the presence of multinucleated giant cells, which are similar features to those previously published associating DPF to AHV 5 (*Kleiboeker* et al. 2002). The clinical relevance of the detection of AHV 5 in our case is debatable. Whether AHV 5 initiated the onset of pulmonary fibrosis or was an incidental finding cannot be determined. In horses experimentally inoculated with the gammaherpesvirus equine herpesvirus 5 (EHV 5), nodular pulmonary fibrosis and induction of myofibroblasts occurred, making it a possible causative agent for the onset of EMPF (*Williams* et al. 2013).

Treatment of DPF is palliative as it is associated with irreversible fibrotic pulmonary remodelling. Corticosteroids, bronchodilators, mucolytics and antibiotics if secondary bacterial infection is present might be helpful (*Thiemann* 2012). As AHV 5 may play a role in the disease, antiviral drugs could be another option, but there are no clinical studies available to support this assumption in DPF cases. Valacyclovir, though, did not reduce EHV 5 viral loads in horses diagnosed with EMPF sufficiently over a treatment period of 10 days (*Easton-Jones* et al. 2018).

To reduce pulmonary oedema, our patient was treated with oral furosemide. Even though no clinical data on the effect of oral furosemide in donkeys exist, poor oral bioavailability is reported in horses (*Johansson* et al. 2004), which made the choice of this treatment arguable and which may have been a major limitation of the clinical management of this case. The decision was made due to the owners' refusal of IV treatment and the lack of other practical treatment options as mentioned above. Furosemide treatment was terminated on day 6, as the benefit of the therapy was doubtful.

The clinical improvement of this patient was most likely caused by the dexamethasone i.m. which was later changed to p.o.. Dexamethasone was administered p.o. due to the clinical improvement during i.m. therapy and to avoid a change of the medical compound. The treating veterinarians were aware of cascade regulations and reclassifications according to the Austrian laws on medical drugs. The donkey showed an acceptable progression and response to the treatment. His appetite returned to normal and he even gained weight during hospitalisation, therefore, a discharge for palliative treatment at home was discussed. Triglyceride concentrations were only measured once and were within normal limits. In contrast to that, the donkey was in poor body condition and histopathology detected fatty infiltrations of the liver, which can be indicative of an ongoing catabolic state or preceding hyperlipaemia. In addition to chronic disease, massive dental pathologies might have led to inadequate feed intake and the weight loss reported by the owner.

Severe cases of DPF have a very poor prognosis, as there is only sparse residual unaffected lung tissue and no effective treatment is currently available. The majority of animals die or have to be euthanised within 2 weeks of the onset of clinical signs (*Thiemann* and *Bell* 2001).

Apart from respiratory disease, the donkey demonstrated various cardiac abnormalities. Based on current literature of echocardiographic cardiac dimensions of the donkey (*Amory* et al. 2004, *Hassan* and *Torad* 2015, *Roberts* and *Dukes-McEwan* 2016), all measured variables were within normal limits except LA/AO ratio and the size of the pulmonary artery.

The pulmonary artery showed dramatic dilatation, but signs of right heart adaptation to pulmonary hypertension, such as cardiac remodelling of the ventricles, atrial dilatation or muscle hypertrophy, were not detectable by echocardiography at the time of examination (*Vonk-Noordegraaf* et al. 2013), indicating either cardiac compensation or acute onset of increasing lung pressure.

Various cardiac abnormalities can result in pulmonary hypertension including mitral valve disease or chronic left-sided heart failure, an increase in pulmonary blood flow due to congenital left-to right shunts or an increase in pulmonary vascular resistance secondary to pulmonary vascular or parenchymal disease (*Schwarzwald* et al. 2006). In the present case, an increase in pulmonary vascular resistance secondary to pulmonary fibrosis seems to be the most likely reason for pulmonary hypertension, leading to severe enlargement of the pulmonary artery, exceeding the aortic root diameter. Right heart catheterisation to measure pulmonary artery pressure and confirm the suspected diagnosis of pulmonary hypertension was not performed.

Tissue scars and fibrosis of the myocardium were detected on histopathology, which is indicative of a chronic process.

Initially performed ECG examination did not detect any abnormalities other than sinus tachycardia, though, the patient developed life threatening arrhythmias during hospitalisation. Antiarrhythmic therapy with magnesium sulphate was initiated and heart rhythm converted to sinus tachycardia, followed by a 2nd degree AV block and ventricular asystole. The donkey was euthanised after acute syncopal collapse. Adverse effects associated with the administration of magnesium sulphate seem to be rare. Overdosing may lead to neuromuscular blockade with respiratory depression and cardiac arrest (*Mitchell* 2017). No studies on the efficacy and safety of magnesium sulphate in donkeys are available, so the lowest recommended dosage for horses of 2.2 mg/kg was used. It cannot be excluded that collapse and asystole might have been initiated by the administration of magnesium sulphate, as hypotension, respiratory depression and cardiac arrest are associated with hypermagnesemia.

Post-mortem histology of the heart revealed acute muscle cell necrosis, multiple extended tissue scars and a mild endocardiosis, likely as a result of hypoxia. Troponin I was within the reference range, though. Histopathological findings can explain the onset of arrhythmias detected in this case, as a result of a suspected disruption of the cardiac conduction system. Furthermore, the change from intramuscular to per os dexamethasone might have contributed to the worsening of the arrhythmias.

In summary, this case describes DPF, an often unrecognised lung disease in donkeys. Beside severe respiratory signs, DPF can also affect the heart due to increased pulmonary vascular resistance secondary to pulmonary fibrosis. This condition can lead to severe and life threatening enlargement of the pulmonary artery, as happened in our case.

Conclusion

In conclusion, our patient was presented in end-stage DPF complicated by severe cardiac disease. The association of DPF with the presence of AHV-5 warrants further investigations. Further research on DPF needs to be done for a better understanding of the pathological mechanisms and for the development of treatment options.

Conflict of interest

No conflicts of interest to declare.

Ethical animal research

Ethical review is not necessary for a retrospective case report.

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Authorship

V. Zehetner managed the clinical case and was responsible for the primary preparation of the manuscript. P. De Heus, S. Berger and W. Fröhlich managed the clinical case and edited the manuscript. K. Lipnik and A. Url were responsible for post-mortem and histopathological investigations and contributed figures. J.-M. V. Cavalleri edited the manuscript. All authors approved the final manuscript.

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