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A new option for treating EMPF in horses (Equine Multinodular Equine Fibrosis) – report of four cases with a good outcome

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Summary: EMPF (Equine Multinodular Equine Fibrosis) is a newly recognized disease in horses and has been first described in 2007. It is associated with gamma herpes virus infection, especially EHV-5. Most of the cases described in the literature show a poor prognosis despite specific and intensive supportive therapy and often the diagnosis is made post mortem. The current report describes four cases of EMPF diagnosed in Germany. The horses were first presented for unspecific symptoms like recurrent fever, cough, loss of condition but developed in the further course of the disease typical findings of EMPF: severe nodular interstitial pattern on lung X-rays, pulmonary finding associated with numerous comet tail artefacts, nodules or consolidation at sonographical examination as well as the presence of EHV-5 assessed by PCR on nasal swabs, tracheal wash (TW) or BAL samples. Lung biopsy was only realised in one case because of the risks of this procedure and confirmed the suspicion of EMPF. Because of deterioration of clinical and diagnostic imaging findings with conventional treatment including acyclovir, several antibiotics and later prednisolone, these four horses were treated as the last option before euthanasia with tulathromycin i.v., a macrolide antibiotic, because of the immunomodulatory effect of that group of drugs described in human medicine. They all presented after several weeks of therapy a positive outcome, are still alive to date (or died for another reason) and those ridden before the disease returned to sport. Macrolide antibiotics are well known in human and veterinary medicine for their antimicrobial effect but also for their anti-inflammatory and immunomodulatory effects. Several studies in human medicine show that their use in interstitial pulmonary disease clearly improves the survival rate of the patients. The positive results obtained on these cases with tulathromycin might be a new option as a potential treatment for EMPF in horses.

Keywords: horse, interstitial pneumonia, pulmonary fibrosis, EMPF, EHV-5, treatment, macrolide

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Introduction

Interstitial lung disease and interstitial fibrosis are poorly understood in horses. They affect adult horses but the cause of such conditions is rarely identified. EMPF has been recognized as one of these fibrotic lung diseases and has been first described in the USA by *Williams* et al. (2005, 2007). These authors proposed an association with an infection with the Equine γ-herpes Virus EHV-5. Since then, several clinical reports are available from USA (*Wong* et al. 2008) and Europe (Germany and Austria; *Poth* et al. 2009, *Schwarz* et al. 2012, *Niedermaier* et al. 2010, *Scharner* 2012, *Back* et al. 2012, UK, *Soare* et al. 2013, *Scheurer* et al. 2020). *Scheurer* et al. 2020 identified worldwide altogether 74 cases in the literature between 2007 and 2020.

There is apparently neither breed nor sex predilections for developing the disease. However, affected horses are adult horses with mean age from 13 to 17,6 years according to the different studies (*Wong* et al. 2008, *Poth* et al. 2009, *Williams* et al. 2007, *Niedermaier* et al. 2010, *Ainsworth* 2012, *Scheurer* et al. 2020). There is no evidence of natural transmission of EMPF; the majority of the reported cases were housed with horses that remained sound (*Marenzoni* et al. 2015).

Most of the horses are first presented because of one or more of the following symptoms: decreased appetite, weight loss, recurrent fever, cough, exercise intolerance, nasal discharge, tachycardia, tachypnea, and later respiratory distress (*Wong* et al. 2008, *Soare* 2011, *Scheurer* et al. 2020), and did not respond to usual therapy (*Belgrave* 2009, *Schwarz* et al. 2012, *Scheurer* et al. 2020) such as broad-spectrum antibiotics, NSAID, inhalation.

Clinical examination reveals often a reduced or poor body condition, fever (intermittent, constant), moderately increased bronchovesicular sounds bilaterally at auscultation of the thorax, increased abdominal expiration (*Belgrave* 2009) or normal costoabdominal pattern (*Scheurer* et al. 2020), tachycardia, tachypnea (*Schwarz* et al. 2012).

Laboratory findings typically reflect a systemic inflammation and include anaemia, neutrophilic leucocytosis, lymphopenia, hyperfibrinogenaemia, hypoproteineamia and hypoal-

buminaemia (*Niedermaier* et al. 2010, *Back* et al. 2012), hyperglobulinemia, an elevated SAA (*Scheurer* et al. 2020). A reduced albumin:globulin ratio is common and supportive of a chronic inflammatory process (*Belgrave* 2009). Lymphopenia is a common finding in the acute stages of viral infection. It has not been reported in most cases of other equine interstitial pneumonia in the literature (*Wong* and al 2008). Electrophoresis was rarely worked-up and mostly unremarkable excepts for a moderate polyclonal hypergammaglobulinaemia (*Schwarz* et al. 2012). Blood gas analysis reveals hypoxemia (*Scheurer* et al. 2020).

Radiographic examinations of the lower respiratory tract usually show a severely increased interstitial lung pattern with diffuse, multifocal nodular interstitial pattern and later increased density in the mid-ventral to cranio-ventral lung lobes (*Marenzoni* et al. 2015). At ultrasonographic examination different findings are described: multiple well-defined 1 to 7 cm nodular lesions on the surface of the lungs, diffuse pleural roughening producing numerous comet tail artefacts (*Scheurer* et al. 2020), severe cranioventral lung consolidation (*Wong* et al. 2008, *Belgrave* 2009, *Back* et al. 2012, *Schwarz* et al. 2012).

Findings of endoscopic examination of the airways are mostly a mild accumulation of mucus in the distal aspect of the trachea (*Wong* et al. 2009, *Niedermaier* et al. 2010, *Back* et al. 2012, *Schwarz* et al. 2012), in few cases endoscopy appears normal (*Scheurer* et al. 2020). Cytologic examination of broncho-alveolar lavage (BAL) samples shows mild neutrophilic inflammation characterised by 60 to 80% nondegenerative to slightly degenerative neutrophils, 20 to 40% vacuolated macrophages and lymphocytes with eosinophilic intranuclear inclusion bodies, and scattered mast cells. Bacterial and fungal culture under aerobic and anaerobic condition are negative in almost all cases (*Wong* et al. 2009, *Niedermaier* et al. 2010, *Back* et al. 2012, *Schwarz* et al. 2012). Confirmation of the presence of EHV-5 is often made by PCR analysis of either BAL or transtracheal wash (TTW) (*Belgrave* 2009) and a quantitative association has been found between this type of sample and EHV-5 load (*Marenzoni* et al. 2015).

Lung biopsy or post mortem histologic examination of the lung often reveals a severe chronic interstitial and fibrosing pneumonia with acute inflammatory components, which differs significantly from other fibrosing lung diseases due to its nodular pattern, the remodelling of the lung architecture, the presence of luminal neutrophils and macrophages within the alveolar-like architecture (*Williams* et al. 2007), and eosinophilic intranuclear viral inclusion bodies within intraluminal macrophages (*Poth* et al. 2009, *Schwarz* et al. 2012, *Soare* et al. 2011). Macroscopically, the lung parenchyma is interspersed with multiple large, bulging, coalescent, tan-white and firm nodules of fibrosis. The size of individual lung nodules is described from 1 cm to 5 cm in diameter. The areas of fibrosis show predominantly distinct borders to the adjacent relatively normal lung tissue (*Poth* et al. 2009, *Back* et al. 2012). Generally, two kinds of fibrosis can occur: (1) deposition of unstructured loose and interlacing collagen bundles, which expanded alveolar septae and (2) deposition of well-organised mature collagen fibres resulting in the formation of abnormal cystic airspaces of various size (honeycombing), which are lined by a cuboidal epithelium (*Poth* et al. 2009).

EHV-5 DNA is detected in all cases on PCR analysis of affected lung tissue (*Williams* et al. 2007, *Soare* et al. 2011, *Scheurer* et al. 2020), sometimes associated to EHV-2 DNA (*Wong* et al. 2008, *Poth* et al. 2009, *Niedermaier* et al. 2010) or AHV-5 (*Back* et al. 2012). In some cases, the whole virus EHV-5 was isolated (*Williams* et al. 2007).

Differential diagnosis for interstitial lung disease includes fungal pneumonia (*Ainsworth* 2012), interstitial pneumonia, pulmonary neoplasia (*Wong* and al 2008), granulomatous pneumonia (*Poth* et al. 2009), idiopathic chronic eosinophilic pneumonia, interstitial pneumonia due to toxins (Pyrrolizidine Alkaloids) or inhalation of pneumotoxic chemicals (silicates, mineral oil), silicosis, hypersensitivity reactions, idiopathic pneumonia (Walston et al. 2009).

Treatment is largely symptomatic and has until now been performed with antiviral molecules (acyclovir, valacyclovir), corticoids, AINS, broad spectrum antibiotics, and tetracycline without long term success (*Wong* et al. 2008, *Marenzoni* et al. 2015, *Scheurer* et al. 2020). Some horses seem to react positively to treatment with antiviral drugs combined with corticosteroids and survive (*Wong* et al. 2008).

The prognosis is guarded (*Poth* et al. 2009) and the clinical suspicion is often confirmed at post-mortem examination. Horses have to be euthanized due to severe respiratory disease or because they did not respond to therapy. In the cases reported in the literature, the survival rate is therefore very low: *Wong* et al. (2009) reported three from five horses died, *Poth* et al. (2009) all five horses died, *Niedermaier* et al. (2010) reported two of two horses died (the diagnosis of EMPF was made post-mortem and maybe the treatment before was not well chosen), *Back* et al. (2012) one horse died, *Soare* et al. (2011) two of two horses died, *Scheurer* et al. (2020) all three horses were euthanized due to continuing deterioration.

The current case series highlights the different clinical features of EMPF and offers a new option of treating EMPF.

Case 1

A 12 years old Holsteiner CSO mare was presented in August 2017 at the Equine Clinic Destedt after an episode of recurrent mild fever following a show. At examination, the horse showed only a slightly elevated rectal temperature (38,6 °C) without other findings. The lung was clear at auscultation. The horse received a paraimmunization. After a couple of days, the auscultation revealed increased respiratory noise in the trachea and the lower respiratory tract. A treatment was started with marbocyl (Marbox Injektionslösung 10%) and Acetylcystein (Equimucin Beutel 2 g). After 5 days of treatment, the horse showed no improvement of the lung auscultation or the intermittent pyrexia and further examinations were performed. Haematology revealed a neutrophilic leucocytosis, lymphopenia, and an eosinophilia. A nasal swab was examined for Equine Infectious Anaemia, EHV-1 and 4, Influenza, Streptococcus equi equi and zooepidemicus. All these tests were negative.

About 4 weeks later, the horse was presented again for tachypnea: respiratory rate 20/min. At admission the horse

was alert, had a normal body score condition (BCS 4/9) and showed no respiratory distress, a normal rectal temperature (37,8 °C), the respiratory rate was increased (24 breath/min), without cough. Auscultation revealed increased respiratory noise in the trachea and the lower respiratory tract. Further examinations included ultrasound of the abdomen and lung, radiography of the lung, blood work up, endoscopy of the airways with TW samples (cytology, PCR for EHV-5, bacterial culture), as well as blood gas analysis. Haematology revealed a neutrophilic leucocytosis and lymphopenia. Ultrasound examination of the abdomen showed a slightly enlarged stomach, the lung presented on both sides numerous comet artefacts and a consolidation of the lung tissue localised ventrally (Fig. 1). Blood gas analysis showed a moderate hypoxemia with a PaO_2: 88 mmHg (Reference Value 100 ± 5 mmHg), $PaCO_2$: 35 mmHg (Reference Value 40 ± 3 mmHg). Endoscopic examination of the airways showed a mild accumulation of thick mucus on the distal aspect of the trachea. Cytologic examination of the airway secretions showed a moderately increased number of neutrophils and neither identification of bacteria nor fungi. The EHV-5 PCR revealed a high load of EHV-5.

A diagnosis of interstitial pneumonia was made and a treatment was started with TMPS (Synutrim-Pulver 72 %, PO, q 12 h). The qPCR showed a positive result for EHV-5 Screening and the diagnosis was therefore EMPF caused by EHV-5. According to the knowledge in the literature at this time, the treatment was started with acyclovir infusion (20 mg/kg BW, IV) and then valacyclovir for 10 days (20 mg/kg BW, PO, q 12 h). After a week of therapy, the horse showed a clear improvement of general condition, the respiratory rate was slightly reduced (18 breath/min), and auscultation was improved especially in the lung area.

15 days after beginning therapy with acyclovir, the horse was reevaluated. At this time, it was clinically sound. The ultrasound examination revealed especially on the left side no improvement with clearly visible wide comet artefacts as well as a consolidation of the lung tissue ventrally. The hypoxemia was still present (PaO_2: 86 mmHg). Mucus could still be seen on the distal aspect of the trachea, and in the airway sample the EHV-5 PCR was negative.

According to these results, it was decided to change TMPS for doxycycline (Pulmodox: 10 mg/kg BW, PO, q 12 h) which would be given for over 1 month. An ultrasound was then performed and showed again no improvement, the hypoxia was slightly more severe (PaO_2: 81 mmHg). A corticosteroid (Equisolon, Prednisolon) was therefore added to the doxycycline for another 5 weeks. Once again ultrasound after 3 weeks revealed no improvement, the respiratory rate was increased again (24 breath/min) and the horse was depressed. After 5 weeks of therapy, the ultrasound even worsened with increased consolidation over a large area; on endoscopy, the quantity of mucus increased and qPCR for EHV-5 was performed on TW secretions and was positive again.

Radiography of the lung revealed an increased homogenous mixed density of the lung parenchyma in the post cardiac ventral area (Fig. 2a). At sonography a very large area of consolidation especially on the right side was observed (Fig. 2b). A lung biopsy was performed and histology confirmed the diagnosis of EMPF.

Previous treatment was stopped and changed for Draxxin® (Tulathromycin 50 mg/mL, Zoetis) at a dose of 1,5 mg/kg BW administered through slow IV injection once a week over eleven weeks. After two injections the owner noticed clinical improvement and after three injections, first improvements were detectable at ultrasonography: consolidations were smaller; the horse was alert again and respiratory rate reduced (16 breath/min). Improvements continued further on over time clinically as well as ultrasonographically. After seven weeks of treatment, a blood screening (haematology, biochemistry, SAA) revealed no abnormality. The horse was clinically sound. The ultrasound had further improved with comet artefacts still present but reduced, small consolidation superficially in the middle of the lung area, no more consolidation on the ventral aspect of the lung (Fig. 3).

After a last check eleven weeks after beginning of Draxxin® therapy, the horse was clinically sound, the blood gas analysis revealed no abnormality and the mare returned to training and performed well during two years until retirement for breeding.

Case 2

A 7 years old Appaloosa gelding had a history of recurrent fever since 2 weeks without associated symptoms. During these 2 weeks, haematology had been run and showed at first a leucocytosis, lymphopenia, anemia, hyperproteinemia; SAA values clearly increased (1140 mg/dL – ref 1–50 mg/dL); a control haematology after a week showed a reduced leucocytosis, as well as reduced SAA value (309 mg/dL). A treatment had been started with flunixin, meloxicam, Gastrogard®, marbocyl (doses unknown).

The horse was presented in March 2019 at the Pferdeklinik an der Rennbahn due to recurrent fever. At examination, the horse showed only a slightly elevated rectal temperature (38,8 °C), and slightly elevated respiratory rate (16 breath/min). Further examinations included a nasal swab, endoscopy of the airways

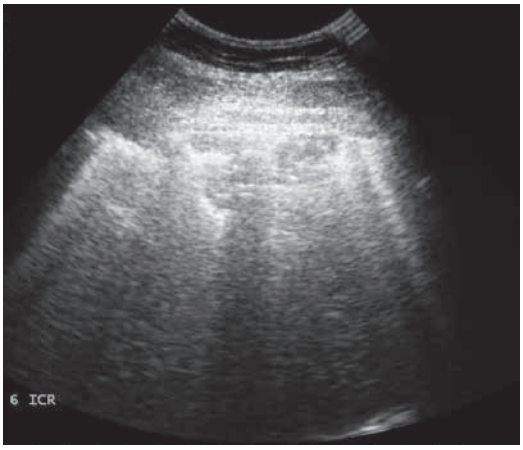


Fig. 1 Case 1: six weeks after first presentation for fever. Ultrasonographic image of the left side of the thorax, 6th intercostal space. Notice the sub pleural area of consolidation with heterogenous echogenicity and a penetration of 3cm and the neighbouring comet tail artefacts. | *Fall 1: Ultraschallbilder der Lunge, linke Seite. Zu beobachten ist eine Konsolidierung der Lunge, heterogene Bilddarstellung und zahlreiche Kometenschweifechos.*

inclusive tracheal wash sample, ultrasound and X-ray of the lung. The nasal swab was positive for EHV-2, 4 and 5. The endoscopy showed a mild accumulation of mucus, the fungal culture was negative and the bacterial culture detected a slow amount of Enterococcus. Cytological examination showed an increased amount of neutrophils and PCR was as positive for EHV-2, 4 and 5. Ultrasonography of the lung showed high number of comet tail artefacts on both sides, and the X-rays an increased density with a nodular and severe interstitial pattern (Fig. 4).

A diagnosis of interstitial pneumonia, EMPF, was made and the antimicrobial treatment (marbocyl IV, q 24 h) of the referring veterinarian was pursued; in addition, the horse received AINS (flunixin-meglumin PO, q 24 h.), low dose omeprazol PO, q 24 h and was inhaled with budesonid twice daily. The horse was kept in the clinic for further treatment and observation. After 4 days, the fever appeared again (39,1 °C) without any additional signs and it was decided to adapt the treatment: marbocyl medication was stopped, and doxycyclin (10 mg/kg BW, PO, q 12 h) therapy started.

Over the next 5 days the fever as well as respiratory rate were fluctuating and the ultrasound and X-rays findings didn't change. The horse was discharged and received further therapy at home. A treatment with Draxxin® (Tulathromycin) was at this time negated by the owner (due to hospitalisation and a lack of clinical studies on Tulathromycin in treatment of EMPF).

During the following 2 weeks, the gelding showed a deterioration of his breathing and was brought back to the clinic for recheck and therapy. At examination the horse had a normal rectal temperature (37,0 °C), the respiratory rate was elevated (24 breath/min) and blood gas analysis revealed a decreased O_2 partial pressure (PaO_2: 84 mmHg). Ultrasonography and X-ray findings were unchanged. The nasal swab at this time was still positive for EHV-2 and 5.

A therapy with Draxxin® (Tulathromycin 50 mg/mL, Labor Zoetis) at a dose of 1,5 mg/kg BW administered as slow IV injection once a week was started for a duration of 7 weeks and the horse was monitored daily. At first the rectal temperature as well as the respiratory rate remained slightly elevated and returned to normal on day 18 after the first injection. After the 2 first weeks of therapy with Draxxin® the ultrasound and X-ray findings showed a mild improvement.

The following recheck occurred 2 months after the first administration of Draxxin®. The clinical examination was normal. The ultrasound and X-ray findings showed further improvement (Fig. 5). The nasal swab was still positive for EHV-5.

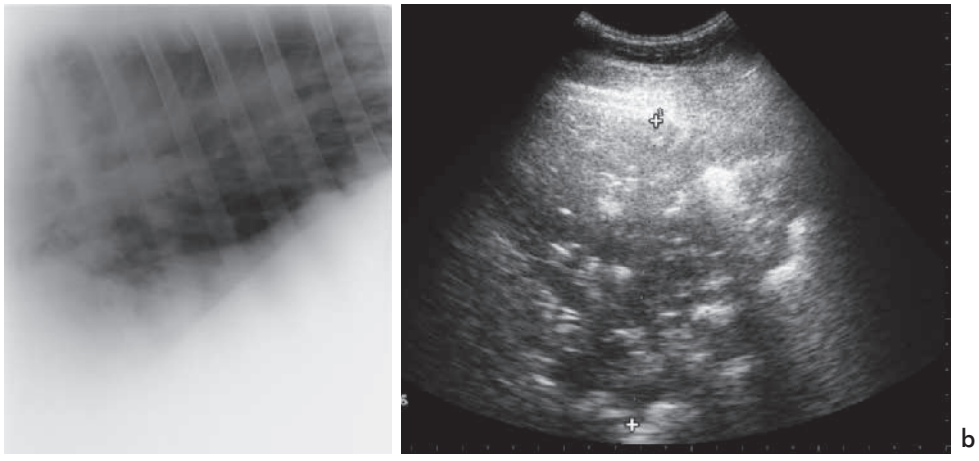
Further 3 months later the horse was still clinically sound, ultrasound and X-ray findings showed a clear improvement and the gelding returned to training.

Case 3

A 17 years old Holsteiner pregnant mare was first presented the 19th of July 2021 at the Neuberg Equine Practice for decreased appetite, weight loss, exercise intolerance, intermittent coughing lasting for a couple of weeks. At clinical examination, the horse was depressed, reluctant to move, showed a decreased body condition score (BCS 2/9) associated to anorexia. The rectal temperature was slightly elevated (38,5 °C) as well as the respiratory rate (18 breath/min); auscultation of the lung revealed increased bronchovesicular sounds bilaterally and increased abdominal component at expiration. The transrectal examination of the foetus and associated structures showed no abnormality and the foetus was alive. Haematology showed at this time a moderate leucocytopenia and a clear lymphopenia.

Based on anamnesis, clinical examination and blood work, the mare was first treated for a bacterial pneumonia with antibiotics (trimethoprim sulfonamid first IV then PO over 5 days) complemented with omeprazole.

The horse was rechecked clinically after 5 days of treatment and showed no improvement except for slightly improved appetite. Auscultation of the lung worsened as well as rectal



a b

Fig. 2 2a: Case 1: Chest radiograph on the day of diagnosis EMPF. Notice the quite clear parenchyma in the caudal area and the increase mixed density in the ventral caudal area. 2b: Case 1: Ultrasonographic image on the day of diagnosis EMPF. Right side of the thorax, 6th intercostal space. A large area of consolidation with heterogenous echogenicity and a penetration of 12 cm was observed. | *2a: Fall 1: Röntgenbilder der Lunge am Tag der EMPF Diagnosestellung. Im kranio-ventralen Bereich ist eine Verdichtung sichtbar. 2b: Fall 1: Ultraschallbilder der Lunge, rechts, am Tag der Diagnosestellung. Eine breite Konsolidierungszone von 12 cm Tiefe ist sichtbar.*

temperature (39,5 °C). Submandibular lymph nodes were enlarged. Haematology showed no changes. A nasal swab was obtained and a PCR for EHV-1 and 4 was negative. Simultaneously the treatment was changed to penicillin, gentamicin and flunixin meglumin in order to control the fever, all administrated IV for 5 days. Again the mare did not show any improvement but maintained intermittent pyrexia (38,1 to 39,5 °C).

Because of the lack of clinical improvement and the pyrexia, further examinations were performed. Chest radiographs revealed a severe, diffuse, uniformly distributed nodular interstitial pattern (Fig. 6a). Ultrasonography of the lung revealed multiple well-defined 1 to 5 cm nodular lesions on the surface of the lungs as well as diffuse pleural roughening on both sides of the thorax (Fig. 6b and c). These findings speaking for an interstitial fibrosis, it was decided to search for EHV-5 on TW-PCR to lead eventually to the EMPF diagnosis.

Endoscopic examination of the airways revealed a mild accumulation of mucus on the ventral aspect of the trachea,

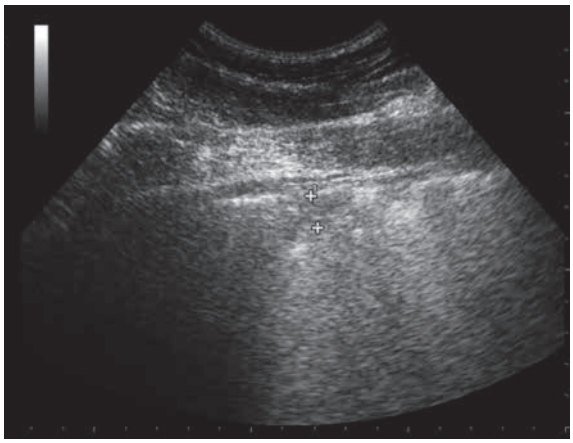


Fig. 3 Case 1: Ultrasonographic image five weeks after starting Tulathromycin treatment. Right side of the thorax, 6th intercostal space. Small area of consolidation (1.4 cm). | *Fall 1: Ultraschallbilder fünf Wochen nach Beginn der Tulathromycin Therapie. An der rechten Seite des Thorax ist ein kleiner Bereich mit Konsolidierung sichtbar (1,4 cm).*

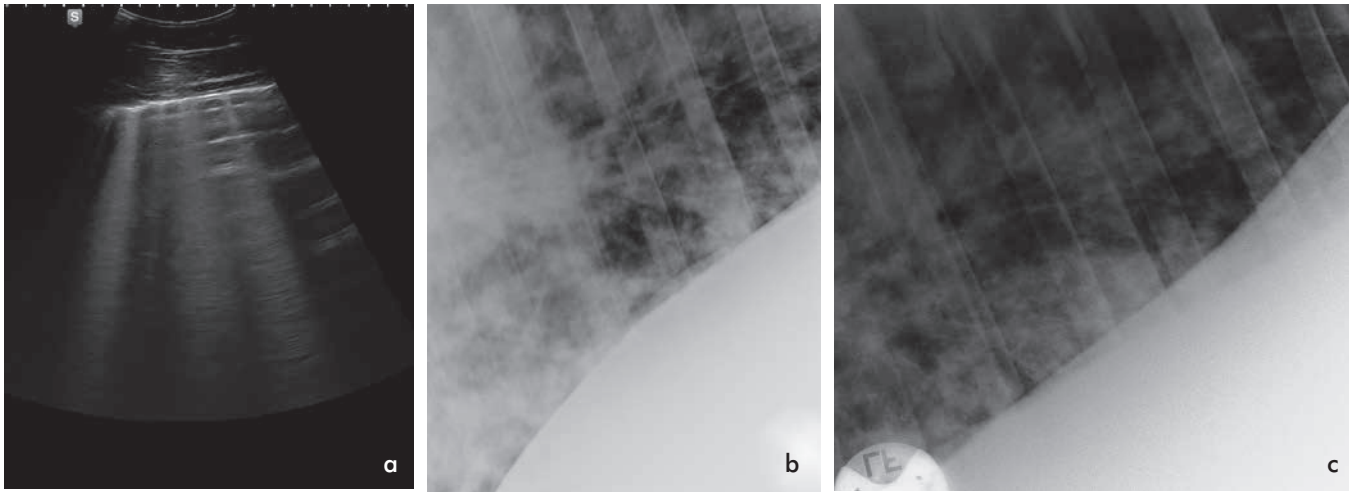


Fig. 4 4a: Case 2: Ultrasonographic image of the right side of the thorax at diagnosis of EMPF. Notice the numerous comet tail artefacts. 4b and c: Case 2: Radiography of the chest at day of diagnosis revealed increased density of lung tissue with a nodular and severe interstitial pattern. | *4a: Fall 2: Ultraschallbilder am Tag der EMPF Diagnose Stellung: viele Kometenschweifechos sind sichtbar. 4b und c: Fall 2: Röntgenaufnahmen am Tag der Diagnosestellung. Das Lungengewebe ist verdichtet im Sinne eines nodularen und interstitiellen Musters.*

Table 1 Findings at diagnosis of EMPF. | *Klinische Befunde bei der EMPF Diagnosestellung.*

	RR	clinical signs	Temp.	Radiologic findings	Sonographical findings	Bacteriology on airway sample	EHV-5-PCR
Case 1	20	none	37.8 °C	increased homogenous mixed density of the lung parenchyma in the post cardiac ventral area	numerous comet artefacts and a consolidation of the lung tissue localised ventrally	negativ	pos.
Case 2	16	none	38.8 °C	increased density with a nodular pattern	high number of comet tail artefacts on both sides	low amount of Enterococcus	pos.
Case 3	18	Intermittent coughing, weight loss, depression	39.5 °C	severe, diffuse, uniformly distributed nodular interstitial pattern	on both sides of the thorax - multiple well-defined 1 to 5 cm nodular lesions - diffuse pleural roughening	Not done	Pos. qPCR: 27,53
Case 4	28–40	tachypnea, weight loss, depression	40.0 °C	moderate, diffuse, nodular interstitial pattern	some well-defined nodular lesions on the surface of the lung, comet tails artefacts, as well as consolidation of the lung	negative	pos.

RR: Respiratory rate, pos.: positive

as well as prominent submandibular lymph nodes bulging in the guttural pouches. A tracheal wash sample was collected and qPCR examination performed for *Rhodococcus equi* and EHV-5. *Rhodococcus equi* was negative whereas EHV-5 was positive with a high load of DNA (q.PCR: 27,53). The diagnosis of EMPF was made.

Further examination (blood biochemistry, SAA or fibrinogen, lung biopsy) were at this point not desired by the owner due to the bad prognosis of the disease, the risks encountered for the lung biopsy and the costs already engaged.

A treatment with Draxxin® (Tulathromycin 50 mg/mL, Zoetis) at a dose of 1,5 mg/kg BW administered through slow IV injection once a week was started (31th of July) for a duration of 11 weeks. Fever was still observed with fluctuation over 10 days. After two weeks of treatment, the owner noticed an improvement of the general condition. After three weeks of therapy, auscultation of the lung showed improvement with only slightly increased bronchovesicular sounds bilaterally. The mare was eating normally again and was alert. The clinical condition improved progressively over time to reach a fully normal state after eight weeks of treatment. At this time a blood count was normal.

The lung was reevaluated at ultrasonography: three weeks after the therapy was started no changes were noticed; it seemed at this time that nodular lesions were even bigger and sometimes coalescent. It was possible to count about four to five nodules in each intercostal space. Only after 7 weeks of treatment ultrasound examination showed a progressive improvement: the nodules became smaller and the roughening of the pleura resolved. It could clearly be seen that the distinct borders of the nodules progressively disappeared and the nodules were somehow filled up with echogenic content (Fig. 7).

After eleven weeks of therapy, the clinical examination was normal with a clear improvement of the body condition at

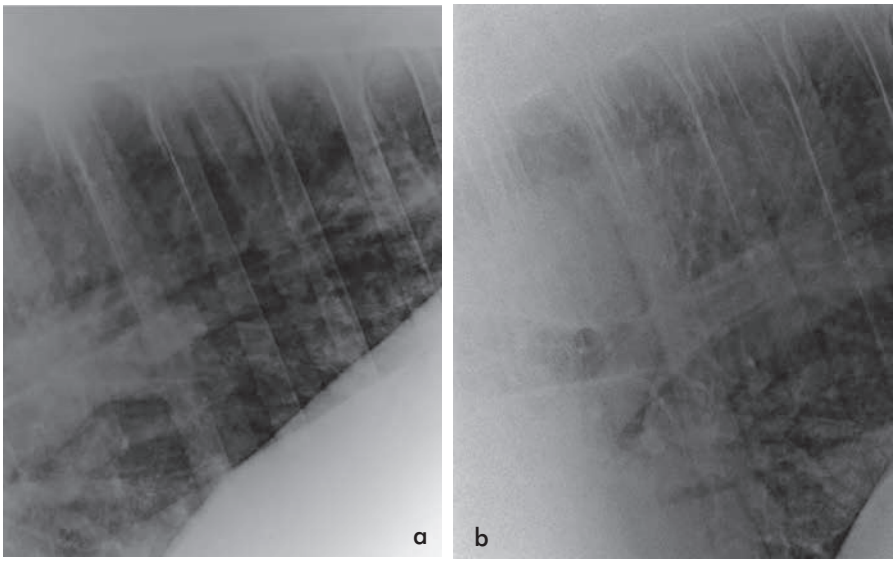


Fig. 5 5a and b: Case 2: Lung radiograph 8 weeks after starting Tulathromycin treatment. The nodular pattern has nearly disappeared. | *5a und b: Fall 2: Röntgenaufnahme der Lunge 8 Wochen nach Therapiebeginn mit Tulathromycin. Das nodulare Muster ist fast verschwunden.*

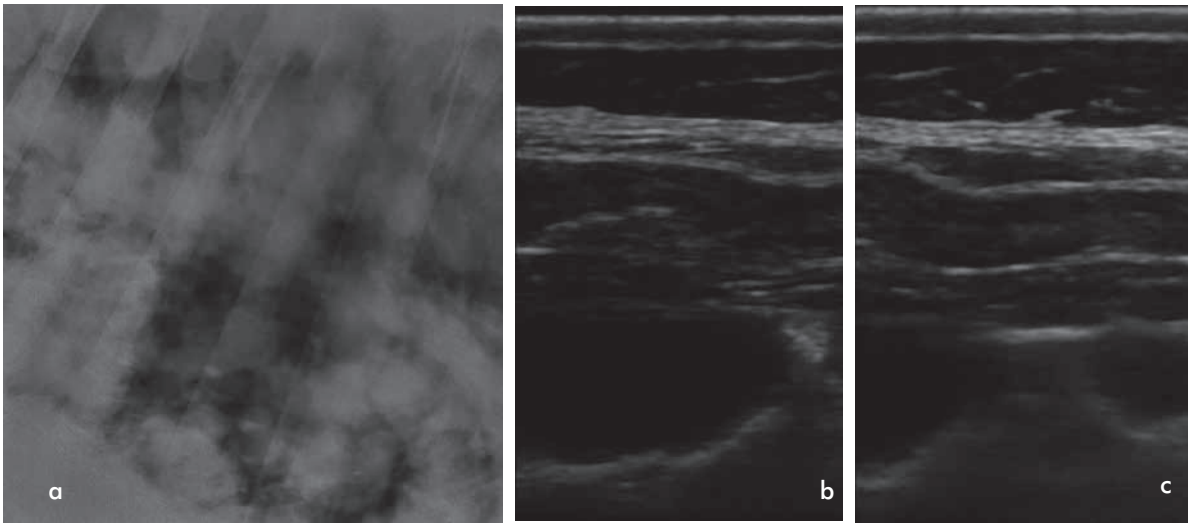


Fig. 6 6a: Case 3: Lung radiograph at diagnosis of EMPF. Notice the severe diffuse nodular interstitial pattern. 6b and c: Case 3: Ultrasonographic finding at diagnosis of EMPF. Subpleural consolidations marked with the line. | *6a: Fall 3: Röntgenaufnahme der Lunge bei der EMPF Diagnosestellung. Zu beobachten ist ein deutlich nodulares interstitelles Muster. 6b und c: Fall 3: Ultraschallbilder am Tag der EMPF Diagnosestellung. Oberflächliche Konsolidierung der Lunge gezeichnet mit dem Pfeil.*

this time (Fig. 8), the ultrasound examination of the lung was normal, and the mare was still pregnant without abnormal findings of the foetus or associated structures.

Five months after the end of therapy, the owner reported the mare to be healthy. On the 19.03.22 the foal was born without complications, was healthy and showed a normal development.

Case 4

A 13 years old Hanoverian gelding was presented at the Clinic for horses in Telgte in October 2021 with a history of intermittent fever and episodes of tachypnea/dyspnea for 8 weeks. He had been treated for equine asthma complicated by an unknown infectious agent with trimethoprim sulfonamid, penicillin, flunixin, clenbuterol and dembrexin and a course of oral prednisolone (1 mg/kg BW) for 10 days which improved the situation only temporarily. The day before admission the horse had a rectal temperature of 40 °C and antibiotic treatment was switched to marbofloxacin.

On the day of admission the horse was alert, had a normal rectal temperature (37,7 °C) and a slightly elevated respiratory rate (16 breath/min). The auscultation of the lung revealed slightly increased bronchovesicular sounds bilaterally. Haematology showed at this time a moderate leucocytosis. Because of the history of respiratory disease, radiographic und ultrasonographic examinations of the lung were performed: the X-rays showed a moderate, diffuse, nodular interstitial pattern, and on ultrasound some well-defined nodular lesions at the surface of the lung, comet tails artefacts, as well as some areas of consolidation were detectable (Fig. 9). During the day, the horse developed fever again (up to 40 °C) and became tachypneic (respiratory rate 32 breath/min).

The antimicrobial treatment (marbofloxacin) was pursued for three more days; in addition, the horse received AINS (flunixin meglumin) to control the fever and dembrexin PO. Over these days, the fever was fluctuating but remained, as well as the tachypnoea and the leucocytosis. The treatment was changed to doxycyclin (10 mg/kg BW, PO, q 12 h) and flunixin meglumin (PO, q 12 h). Again, the clinical examination showed no improvement.

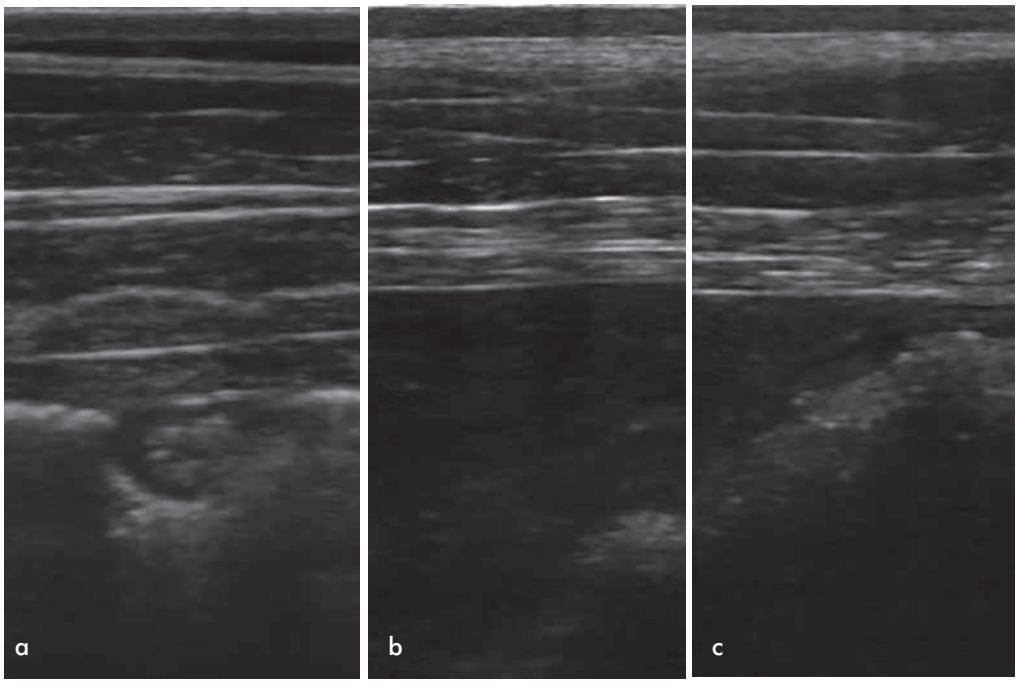


Fig. 7 Case 3: Ultrasonographic finding at 7 weeks of treatment (13.9.21), 7a: smaller nodule, echogenic content, not well defined contour, 7b and c: consolidation of the lung still visible. | *Fall 3: Ultraschallbilder sieben Wochen nach Therapiebeginn. 7a: kleineres Nodule, gefüllt, unscharf abgegrenzt. 7b und c: Konsolidierung der Lunge noch sichtbar*

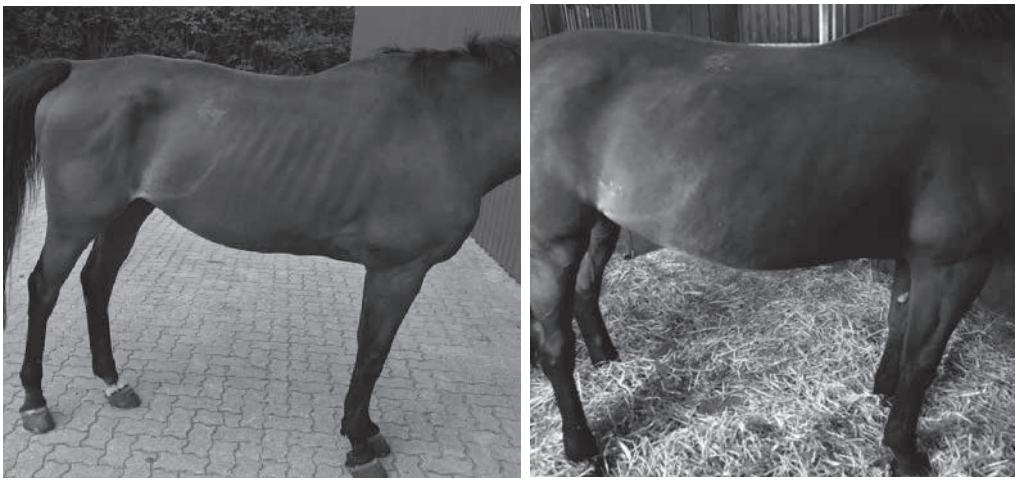


Fig. 8 Case 3: Body condition. 8a: 19th of July 2021, clearly reduced body score (BCS: 2/9). 8b: 13th of September 2021, improved bodyscore after therapy (BCS: 4/9): the mare regained muscle and fat. The abdomen slowly shows signs of a pregnancy. | *Fall 3: Körperzustand der Stute. 8a: 19 Juli 2021: deutlich reduzierter Körperzustand (Bodyscore condition, BCS: 2/9). 8b: 13 September 2021: Körperzustand gebessert nach Therapie (BCS: 4/9): die Stute hat wieder Fett und Muskelmasse gebildet. Der Bauch zeigt langsam eine Trächtigkeit.*

Further examinations included a blood work and endoscopy of the airway inclusive BAL. The blood work showed a persistent leucocytosis with neutrophilia and a midly elevated SAA (250 mg/L – ref: < 10 mg/L). The endoscopy showed a mild accumulation of a thick mucus in the trachea, the BAL sample had a dark orange stain. Cytological examination showed a moderately increased number of neutrophils. The PCR for EHV-5 was positive.

Based on the clinical signs, the radiological and sonographic findings and the positive PCR result for EHV-5, a diagnosis of EMPF was made. The owners did not give their consent to lung biopsy.

A treatment with Draxxin® (Tulathromycin 50 mg/mL, Zoetis) was started at a dose of 1,5 mg/kg BW administered through slow IV injection once a week over 8 weeks.

After 4 weeks of therapy a clear clinical improvement could be seen. The gelding gained weight, the breathing pattern improved, the respiratory rate was around 24 breath/min or lower, the rectal temperature within the physiological range. The blood gas analysis was completely normal. SAA was at that point < 10 mg/L. However significant leucocytosis with neutrophilia was still present. At that point X-rays showed only moderate improvement: the nodular interstitial pattern is still visible. On ultrasound the thickening of the pleura was reduced, less comet tail artefacts were seen and the nodular and consolidated area were significantly reduced in size (Fig. 10).

After eight weeks of treatment the horse was clinically sound, haematology within the physiological range. The gelding left the clinic without further treatment.

He was rechecked 14 weeks after starting therapy with Draxxin®. He remained at examination clinically sound. The ultrasound showed a clear improvement: the roughening of the pleura and the comet tail artefacts were reduced, the nodular lesions were significantly smaller (Fig.11).

The horse died due to colic 4 months later.

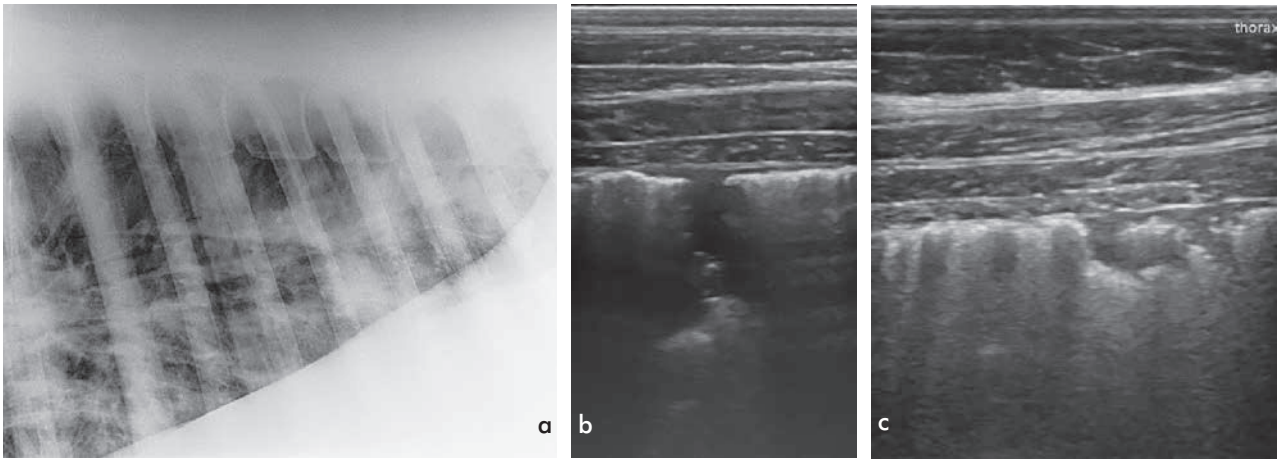


Fig. 9 9a: Case 4: Lung radiograph at diagnosis of EMPF. Notice the diffuse nodular interstitial pattern. 9b, c: Case 4: Ultrasonographic finding at diagnosis of EMPF. Notice the roughening of the pleura, the comet tail artefacts (c), and the subpleural consolidation (b). | *9 a: Fall 4: Röntgenaufnahme der Lunge bei der EMPF Diagnosestellung. Zu beobachten ist ein deutlich nodulares interstitielles Muster. 9b, c: Fall 4: Ultraschallbilder bei der EMPF Diagnosestellung. Zu beobachten sind Kometenschweifechos (c), und die Konsolidierung der Lunge (b))*

Table 2 Course of treatment before and after diagnosis of EMPF. | *Behandlungsverlauf vor und nach der Diagnose EMPF.*

	Airways therapy before EMPF diagnosis	Duration of disease for EMPF diagnosis	Miscellaneous therapies according to published EMPF treatment options	Duration of therapies before starting TUL treatment	Number of TUL injections	Time from starting treatment with TUL to full recovery
Case 1	*paraimmunization *AB: marbocyl *ACC	About 4 weeks	*AB: TMPS *AV: acyclovir + valacyclovir *AB: doxycyclin *AIS: prednisolon	11 weeks	11	11 weeks
Case 2	*AB: marbocyl *AINS (flunixin meglumin/ meloxicam)	2 weeks	* AB: marbocyl, * AB: doxycyclin * AINS (flunixin meglumin) *Inhalation with budesonid	2.5 weeks	7	20 weeks
Case 3	*AB: TMPS *AB: penicillin + gentamicin	several weeks	/	10 days	11	11 weeks
Case 4	*AB: TMPS, penicillin, marbocyl *AINS (flunixin feglumin) *clenbuterol + dembrexin *AIS (prednisolon) *AB: doxycyclin	11 weeks	(already used as therapy before diagnosis of EMPF)	11 weeks	8	9 weeks

AB: Antibiotic, AINS: non-steroidal anti-inflammatory drug, AIS: steroidal anti-inflammatory drug, AV: anti-viral drug, TUL: Tulathromycin, TMPS: Trimetoprim Sulfadiazin

Discussion

Implication of EHV-5

A constant finding in all 4 cases was the detection of EHV-5 to support the diagnosis of EMPF. Over the past years the implication of EHV-5 in the disease has been controversial in some studies. The latest one confirms however the implication of the virus, and therefore the importance of its detection in diagnosing the disease.

There is little information available about the distribution and pathogenicity of EHV-5 in horses. *Poth* et al. (2009) mentioned the idea that the chronic interstitial pneumonia could possibly represent an initial condition that facilitates a secondary EHV-5 infection. Another doubtful finding was the detection of EHV-5 in nasal swabs or BAL in many young horses or foals (*Wang* et al. 2007, *Bell* et al. 2006), sick horses without EMPF or healthy horses (*Fortier* et al. 2009, *Wong* et al. 2008).

More recently *Williams* et. al. 2013 demonstrated the pathogenicity of EHV-5 in horses and provided the evidence that EHV-5 causes EMPF in horses. The exact pathogenicity of EHV-5 remains still speculative, but three hypotheses are currently existing: 1) the route of infection (inoculation in the accessory lung lobe) could play a role in determining the pathogenicity of a virus considered non pathologic in its natural host (*Marenzoni* et al. 2015). 2) only particular strains of EHV-5 are pathogenic, 3) it is postulated that there is a relation between the load of EHV-5 and the pathogenicity (or severity of EMPF).

A recent study (*Van Cleemput* et al. 2019) explains for the first time the steps of EHV-5 infection. It is assumed that foals become infected through the upper respiratory tract around the age of 1–6 months. Following primary infection, EHV-5 establishes latency in peripheral blood mononuclear cells to persist in its hosts (*Williams* et al. 2007). Upon inhalation in a healthy horse, infectious EHV-5 particles do not infect the ciliated respiratory epithelium, but are rather propelled by the mucociliary escalator towards the tonsillar crypts, embedded in the nasopharynx. EHV-5 then directly infects lymphocytes situated in the lymphoid follicles. The virus spreads by cell to cell transfer to adjacent lymphocytes in the lymphoid follicles or draining lymph nodes. Via blood flow or lymphocyte-homing, EHV-5 infected lymphocytes (re)routes to different parts of the respiratory tract, e.g. lungs. The onset of fibrosis may be triggered by viral replication and host-specific predisposing factors (*Scheurer* et al. 2020)

Diagnostic of the disease

As the clinical signs of EMPF are most of the time unspecific, sometimes not even suggesting an airway disease, further

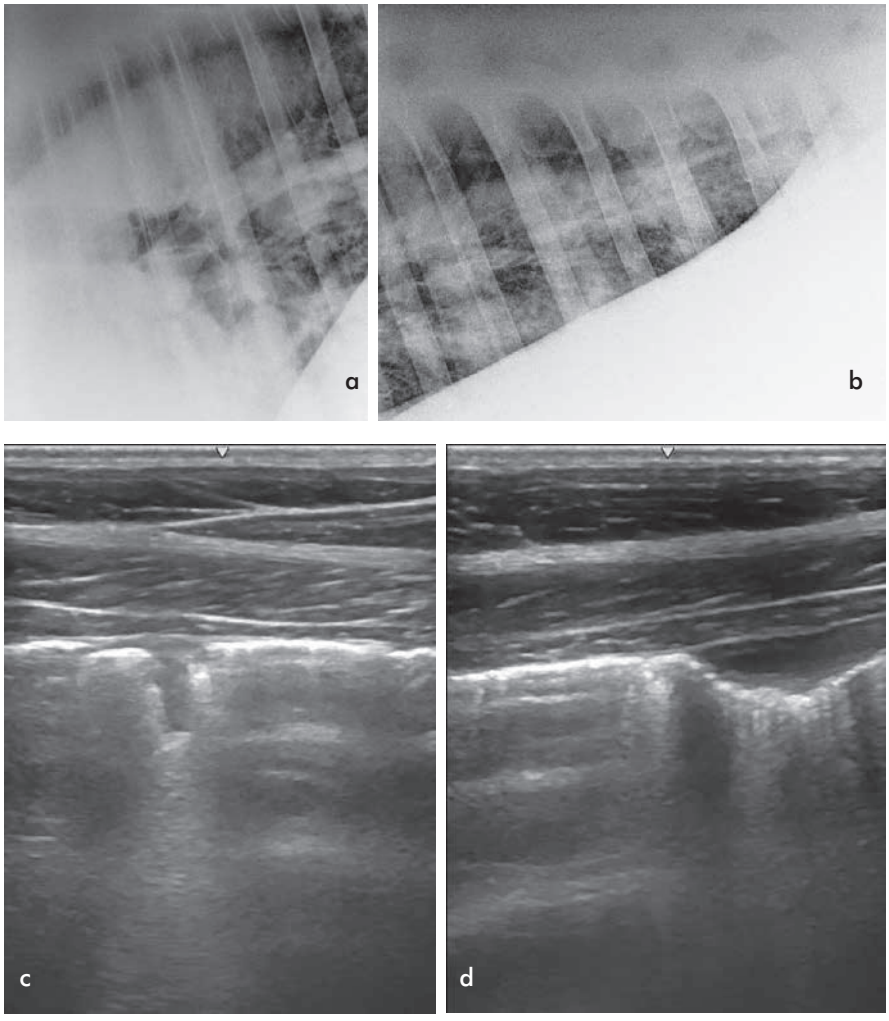


Fig. 10 10a, b: Case 4: Lung Radiography four weeks after beginning of Tulathromycin treatment. The clear interstitial nodular pattern is improved but still present. 10c, d: Case 4: Ultrasonographic examination four weeks after beginning of Tulathromaycin treatment. The nodular lesions are reduced. Notice the roughening of the pleura and the comet tail artefacts which are still detectable. | *10a, b: Fall 4: Röntgenaufnahme der Lunge 4 Wochen nach Therapiebeginn mit Tulathromycin. Das Interstitielle Muster ist noch vorhanden. 10c, d: Fall 4: Ultraschalluntersuchung 4 Wochen nach Therapiebeginn mit Tulathromycin: weniger nodulare Läsionen sind darstellbar; die Kometenschweifechos sind noch vorhanden.*

examinations have to be performed to establish the diagnosis.

The blood screening reveals in all cases findings which are not specific to EMPF: signs of chronic infection, viral infection, reduced PaO_2, SAA and fibrinogen elevated to highly elevated. Not all these parameters have been checked in each of the presented cases, depending on the reason for first or further examination, budget of the owner, as well as habits of the treating vet.

The striking radiographic and ultrasonographic appearance of the lower respiratory tract are the most prominent diagnostic features (*Soare* et al. 2011) and these findings have been clearly observed in all 4 cases presented here. Thoracic radiography and ultrasonographic examination of the lung may allow evaluation of the progression of the disease (*Schwarz* et al. 2012). Case 1 and 3 have been rechecked on regular basis with ultrasound and showed a continuously improvement of the findings, case 2 has been followed up with X-rays and showed a slow improvement of the interstitial patterns over time. Both methods can be recommended for a follow up of the disease during the therapy.

The endoscopic examination of the airways includes cytologic examination and PCR (or q-PCR) assays on TW or BAL. In horses with EMPF a positive PCR for EHV-5 is a consistent finding (*Pusterla* et al. 2017). *Marenzoni* et al. 2011 reported that viral load can be assessed using quantitative PCR. All 4 horses of the report showed positive results for EHV-5 either after PCR on BAL, TW samples, or on nasal swabs (case 2); quantitative PCR was performed in 3 of 4 cases. It seems that some horses with a positive PCR-negative outcome after therapy still remain positive for EHV-5 at PCR examination (*Schwarz* et al. 2012).

Only few authors reported a PCR-negative horse, when diagnosed with EMPF (*Wong* et al. 2008). In that case the BALF was collected well after treatment with corticoids and acyclovir. The prior treatment could have negatively affected the EHV-5 PCR results. *Scheurer* et al. 2020 detected EHV-2 and AHD-5 on one horse but no EHV-5, and a fully negative horse for Gamma viruses; according to the authors, detection technique and virus load may be responsible for the inconsistent results from the BAL; as post-mortem examination of these two cases show the presence of Gamma viruses, a lack of EHV-5 in BAL fluid doesn't rule out EMPF.

As suggested by *Schwarz* et al. (2012), in all cases of EMPF with respiratory signs, where BAL or TW cytology is performed, careful examination of the cells may identify eosinophilic intranuclear inclusion bodies in macrophages and hence to help to diagnose EMPF early in the course of the disease. This was not specifically performed in the current four cases.

The gold-standard examination for the diagnosis is still the histopathological examination of lung tissue of lung biopsy or post-mortem, which also reveals the presence of EHV-5. The ultrasound-guided lung biopsy procedure is especially challenging in the tachypnoeic horse as increased breathing frequency increases the risk of lung laceration and pulmonary haemorrhage (*Ainsworth* 2012). Due to the risk of the biopsy procedure on live horses (bleeding, infection, lung collapse, respiratory distress and coughing, tachypnoea etc.) (*Pusterla* et al. 2017), some clinicians/owners may decline this procedure as part of the examinations to confirm the diagnosis. This is one of the reasons why histopathological findings of the lung are only available for case 1 in the current report.
According to other studies, in which EMPF cases did not undergo lung biopsy, it is nowadays accepted that combined results of clinical signs, US and X-rays typical pattern findings, blood trials, quantitative PCR for EHV-5 on BAL or TW samples are sufficient to make the diagnosis of EMPF (*Pusterla* et al. 2017, *Scheurer* et al. 2020).

Treatment

The therapeutic goals in interstitial pneumonia are suppression of inflammation, maintenance of oxygen delivery to the tissues, relief of associated bronchoconstriction and treatment of underlying or secondary infection (*Wilkins* 2003). The conventional therapy for EMPF includes antiviral drugs such as acyclovir or valacyclovir, corticosteroids and antibiotics.

Wong et al. 2008 report in 2 of 5 cases of their study a successful outcome after therapy with acyclovir 20 mg/kg BW PO q 8 h combined with prednisolone. Until now, the use of acyclovir remains entirely speculative because the susceptibility of equine γ-EHV to acyclovir is unknown. The oral absorption of acyclovir is inconsistent because of poor bioavailability (*Ainsworth* 2012); a better way of administration could be an infusion of acyclovir (10 mg/kg BW) in 1L of saline over 60 min (*Wilkins* et al. 2005).

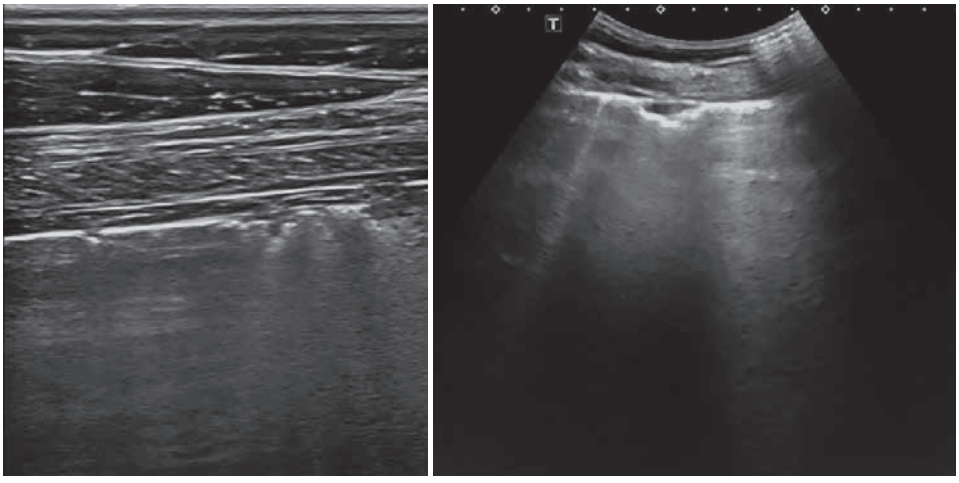


Fig. 11 11a, b: Case 4: Ultrasonographic examination 14 weeks after starting treatment. Small nodules and comet tail artefacts still detectable. | *11a, b: Fall 4: Ultraschall Untersuchung 14 Wochen nach Therapiebeginn. Kometenschweifechos und Konsolidierung der Lunge sind noch sichtbar.*

Valacyclovir (a prodrug of acyclovir) has a much greater bioavailability when administered orally. *Schwarz* et al. (2012) reported one case successfully treated with valacyclovir (40 mg/kg BW PO q 8 h) combined with phenylbutazone administrated for 7 days. The dose rate of 40 mg/kg BW PO q 8 h appears to procure a sufficient plasma concentration (Garré et al. 2007, 2009). Other authors have suggested a loading dose of 27 mg/kg BW PO q 8 h for the first two days followed by a maintenance dose of 18 mg/kg BW PO q 12 h (*Schwarz* et al. 2012, *Scheurer* et al. 2020). A treatment period of a few weeks has been suggested by *Wong* et al. 2008. According to the cost of valacyclovir it is sometimes no therapeutical option for the owners. However, its effectiveness against EHV-5 in vitro has not been demonstrated (*Ainsworth* 2012). A report of *Easton-Jones* et al. (2018) aim to determine if valacyclovir has an effect on EHV-5 viral load and no efficacy could be statistically measured. In the case report of Scheurer et al. (2020) two horses were treated with valacyclovir in combination with other drugs (dexamethasone, antibiotics, inhalation). Both horses showed a progressive deterioration over time and had to be euthanized. These results seem to be consistent with the fact that fibrosis is induced during the latency of the virus, not during the active replicating phase when valacyclovir exerts its effect (*Scheurer* et al. 2020).

Corticosteroids are the mainstay of treatment for interstitial pneumonia. They do have a potent anti-inflammatory action and could control fibrosis (*Marenzoni* et al. 2015); they have been used in some studies with beneficial effects (*Wong* et al. 2008). On the other hand, the side effects of the use of corticoids can lead to the decision not to use them in the therapy plan. Risk for laminitis, immunosuppressive action with the risk of reactivation of EHV latent infection (*Schwarz* et al. 2012), profound reduction in lymphocyte counts. In human with idiopathic pulmonary fibrosis (IPF) associated with γ-herpesvirus infection (Epstein-Barr virus), treatment with corticosteroids does not seem to be beneficial (*Schwarz* et al. 2012).

Antimicrobial therapy is generally recommended to avoid bacterial superinfection. Doxycycline can be chosen and has been until now recommended mainly for its potential anti-inflammatory effect; in murine models of pulmonary disease, this drug interacts with bound zinc or calcium ions required for metalloproteinase activities (*Ainsworth* 2012, *Easton-Jones* et al. 2018). The cases presented above have been initially treated for suspected bacterial airway diseases and received antibiotics commonly used for this purpose (TMPS or marbofloxacin). The four horses did not improve during these treatment protocols. This is the reason why the antibiotic in case 1, 2 and 4 was switched to doxycyclin even without antibiogram, to benefit of the anti-inflammatory effect of the drug.

Because of the lack of improvement with above mentioned conventional treatment, the four horses of the report were treated with Tulathromycin with the focus on the immunomodulatory effect. Until now, there exists no case in the literature of horses with EMPF which received this kind of therapy. This unconventional/controversial choice to treat a viral disease with an antibiotic was based on results and experience in human medicine where macrolide antibiotics are described since at least 1998 as having a strong anti-inflammatory effect and a function as immunomodulatory drug. Clinical studies have proved better outcomes in human with IPF when treated with macrolides (*Kuse* et al. 2016, *Kawamura* et al. 2017).

Macrolides antibiotics are widely used in the treatment of infection. They show a broad spectrum antibacterial activity against Gram positive bacteria and intracellular bacteria and combine this with good tissue penetration (*Wales* et al. 1999). They have been shown to alter the structure and architecture of the bacterial biofilm which enhanced phagocytosis and clearance of bacteria by alveolar macrophages, as well as mucus clearance itself (*Altenburg* et al. 2011). On top of that, it seems that they are able to disturb bacterial metabolism (*Altenburg* et al. 2011, *Kanoh* et al. 2010). The 14 and 15-membered macrolides (e.g. erythromycin, clarithromycin, azithromycin) show apart their antibacterial activity other properties. Immunomodulation is the term used to describe the downregulation of a hyperimmunity or hyperinflammation without impairing the normal immune or inflammatory response to defend against infection (*Kanoh* et al. 2010).

Wales et al. (1999) describe the macrolide as drug having anti-inflammatory and anti-oedema action, able to reduce the neutrophils counts in BAL examination, the oxidative response of polymorphonuclear neutrophils (PMN), the production of inflammatory cytokines. Erythromycin and clarithromycin seem to have an inhibitory effect on Endothelin-1 (*Takizawa* et al. 1998). In addition macrolides act at the level of gene transcription and reduce positively the inflammation in modulating the production of pro-inflammatory cytokines in monocytes, T-cell line, pulmonary epithelial cells as well as the production of NO (Nitric oxide) a strong inflammatory mediator (*Tamaoki* 2004). Furthermore, the macrolides can reduce the quantity of mucus by down regulating the production and activity of cytokines such as LPS (lipopolysaccharides) or TGF-α (Transforming Growth Factor). Still, they do not appear to affect normal physiologic secretion (*Kanoh* et al. 2010).

More recent studies show the positive effects of macrolides in human IPF patients. IPF being a similar disease in human than EMPF in horses, it may allow an extrapolation of the results. The in vitro effect of macrolides on IPF-fibroblasts and control fibroblast was compared and the results strengthen the role of azithromycin (AZT) as a potential anti-fibrotic compound in the therapy of IPF patients (*Krempaska* et al. 2020).

Tulathromycin is a semi-synthetic macrolide antibiotic, a mixture of 13-membered and 15-membered molecules. Through its high positive charge, tulathromycin has an enhanced cell penetration.

Tulathromycin is successfully used in combination in treatment of abscessing pneumonia of foals caused by *Rhodococcus equi* (*Rutenberg* et al. 2017). Because concentration of tulathromycin in plasma, pulmonary epithelial lining fluid and bronchoalveolar cells are more than 30-fold lower than the MIC50 of Tulathromycin against *R. equi*, the efficacy of tulathromycin could be only related to its anti-inflammatoy and immunomodulatory effect as macrolides (*Rutenberg* et al. 2017).

Lung pharmacokinetic studies in cattle, pigs, horses, and mice show that tulathromycin accumulates in lung tissue and persists there for a long time after a single administration. The

mechanism and processes of accumulation remain unknown. The accumulation of tulathromycin in bronchoalveolar cells (BACs) was reported in foals (*Venner* et al. 2010, *Villarino* et al. 2012). Tulathromycin cumulates also in leukocytes and macrophages to concentrations several times above the serum level (*Scheuch* et al. 2006).

Effects of tulathromycin on the immune system has mainly been studied in cows. It significantly increased leukocyte apoptosis and reduced levels of proinflammatory cytokines produced by mononuclear and epithelial cells (*Villarino* et al. 2013); In vivo tulathromycin induces apoptosis of BAL fluid leukocytes 3 h after its administration. Tulathromycin did not significantly alter BAL neutrophil numbers at 24 h of infection. In a study on rats, *Er* et al. (2012), showed that tulathromycin has more pronounced anti-inflammatory effects than other macrolides, via depressing the production of inflammatory mediators in the lung (especially CRP-C reactive protein, and PGM – 13,14-dihydro-15-keto-prostaglandine F2α).

The positive results obtained on these cases with tulathromycin might be a new option as a potential treatment for EMPF in horses.

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