EQUINE MULTINODULAR PULMONARY FIBROSIS (EMPF) IN 5 HORSES

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Abstract

Equine multinodular pulmonary fibrosis (EMPF), a progressive fibrosing interstitial lung disease has been associated with γ-herpesviruses. This case series describes five horses with EMPF. Three horses were diagnosed with EMPF *ante mortem* with the typical clinical presentation including dyspnoea and weight loss. All died or had to be euthanised despite treatment with corticosteroids. Two horses were diagnosed with EMPF *post mortem*. They not only had EMPF but also concurrent other diseases. EHV-5 DNA was identified in all horses by PCR.

Keywords: Horse, EMPF, γ-herpesvirus, immunosuppression

Introduction

Since 2007, when the pathology of equine multinodular pulmonary fibrosis (EMPF) was first described by Williams et al. (2007) multiple cases have been reported (Hart et al., 2008, Wong et al., 2008, Poth et al., 2009, Niedermayer et al., 2010, Verryken et al., 2010, Lehmbecker et al., 2011, Marenzoni et al., 2011, Schwarz et al., in press, Soare et al., 2011). All of these cases have been associated with the equine γ-herpesvirus EHV-5 and in some cases additionally EHV-2 was present (Williams et al., 2007). Wong et al. (2009) have been the first to describe the typical history and predominant clinical signs of horses with EMPF. All of the reported cases had a history of either lethargy, weight loss, fever, cough, tachypnoea, respiratory distress or a combination of these symptoms

(Kubiski et al., 2008, Wong et al., 2008, Niedermayer et al., 2010, Verryken et al., 2010, Marenzoni et al., 2011, Soare et al., 2011, Schwarz et al., in press). The main clinical signs are tachycardia, tachypnoea, increased respiratory effort, lethargy, fever and nasal discharge (Kubiski et al., 2008, Wong et al., 2008, Niedermayer et al., 2010, Verryken et al., 2010). However, Niedermayer et al. (2010) reported two cases, in which additionally ulcerative keratopathy and oral ulcers, potentially γ-herpesvirus related symptoms were observed. Furthermore, cases, where EHV-5 seems to induce bone marrow pathology are described. Hart et al. (2008) report a case with pancytopenia and two cases have been described linking EMPF with leukaemia/lymphoma via EHV-5 (Schwarz et al., in press, Van der Werf et al., 2011). An induction of lymphoproliferative disease by EHV-5 in horses might occur similar to what is reported for Epstein-Barr Virus (EBV) in humans (Kawa, 2000). Main laboratory changes described with EMPF are hypoxaemia, leukocytosis due to mature neutrophilia, lymphopenia, anaemia and hyperfibrinogenaemia (Wong et al., 2008). Some horses also showed hypoalbuminaemia (Wong et al., 2008, Niedermayer et al., 2010). Typical lesions on thoracic radiographs and ultrasound compass a nodular interstitial pattern and a general roughening of the pleura with nodular lesions, respectively (Hart et al., 2008, Kubiski et al., 2008, Wong et al., 2008, Niedermayer et al., 2010, Verryken et al., 2010, Marenzoni et al., 2011, Soare et al., 2011). Ante mortem diagnosis is based on these results in combination with a positive EHV-5 PCR (from BALF) and exclusion of other pathological agents (Wong et al., 2008, Verryken et al., 2010) and can be proven by molecular and histopathological investigations of lung biopsies (Wong et al., 2008, Niedermayer et al., 2010). Macroscopically the discrete nodular form can be differentiated from the diffuse nodular

form, which is reported to be the most common (Williams et al., 2007, Poth et al., 2009). In the diffuse nodular form the tan-white and firm nodules are small (< 5cm), but numerous and coalescing with discrete borders to generally rare unaffected lung tissue (Williams et al., 2007). The discrete nodular form is dominated by several similarly large nodules (up to 10 cm), embedded in grossly not affected lung parenchyma (Williams et al., 2007, Poth et al., 2009). In some horses enlargement of lung lymph nodes was found additionally (Williams et al., 2007, Poth et al., 2009), however generally pathological lesions are restricted to the lung (Williams et al., 2007).

Histologically the disease is characterised by different stages of interstitial fibrosis and inflammation (Williams et al., 2007, Poth et al., 2009). Mature collagen deposition can be found in the interstitium (Williams et al., 2007, Poth et al., 2009). Alveoli are destroyed and replaced by alveolar-like structures lined by cuboidal cells (honeycombing) (Williams et al., 2007, Poth et al., 2009), which contain numerous inflammatory cells, predominantly neutrophils and macrophages (Williams et al., 2007). In some of these macrophages eosinophilic intranuclear inclusion bodies (Cowdry Type A) can be found (Williams et al., 2007, Poth et al., 2009). Furthermore, hypertrophy of type II pneumocytes is apparent (Williams et al., 2007, Poth et al., 2009). Rarely there is a deposition of immature collagen only causing destruction of the alveolar architecture, without the typical "honeycomb-formation" (Williams et al., 2007, Poth et al., 2009). Inflammatory components in the interstitium consist of lymphocytes, plasma cells, and neutrophils (Williams et al., 2007, Poth et al., 2009) but also multinucleated giant cells can be seen (Poth et al., 2009, Marenzoni et al., 2011). PCR for EHV-5 revealed positive results in 100 % of reported cases (Williams et al., 2007, Wong et al., 2008, Hart et al.,

2008, Poth et al., 2009, Verryken et al., 2010, Marenzoni et al., 2011, Soare et al., 2011, Schwarz et al., in press). Williams et al. (2007) observed 33.3 % of horses additionally to be positive for EHV-2 by PCR.

This case series describes three cases with the typical clinical presentation of EMPF described in the literature: dyspnoea and weight loss (Wong et al., 2008, Niedermayer et al., 2010, Verryken et al., 2010). All three were treated with corticosteroids but did not survive. Furthermore two cases were diagnosed with EMPF *post mortem* after they were euthanized/died due to an other than respiratory disease.

Case reports

Between November 2008 and August 2011 the three Equine Hospitals of the University of Veterinary Medicine Vienna (Austria), the University of Veterinary and Pharmaceutical Sciences, Brno (Czech Republic) and the Szent István University (Hungary) diagnosed seven cases with EMPF, five of which are presented in this case series.

EMPF cases with typical clinical presentation

Case 1

History: An 8-year old Holsteiner mare which was used for show jumping started to show dyspnoea and nasal discharge and was treated with clenbuterol (Ventipulmin)[1] (0.008 µg/kg bwt *per os*, q. 12 h) and mucolytic mediation with bromhexidine (Bisolvon)[1] (0.025 mg/kg bwt *per os*, q. 12 h) for suspected bronchitis. The horse seemed to improve but started to be mildly pyrexic (38.5°C) after 2 weeks. Oxytetracyclin (Engemycine)[2] (10 mg/kg bwt i.v. q. 24 h) and gentamicin (Vetrigent 5)[3] (6.6 mg/kg bwt i.v. q. 24 h) were added to the treatment regime. But over the next 3 weeks further symptoms, such as anorexia, weight loss, tachypnoe and dyspnoea were noted and the horse was referred.

Clinical examination: At admission the horse was in a poor body condition with normal temperature (37.8°C), a heart rate of 40 beats/min and tachypnoea with 32 breaths/min

(see Fig. 1). Furthermore the horse showed a mild expiratory dyspnoea and was mildly dehydrated with pale mucous membranes and a CRT of 3 sec. Slight mucous, bilateral nasal discharge was observed. Thoracic auscultation revealed increased lung sounds and on the right side wheezes could be heard ventrally.

Further examinations: Arterial blood gas analysis revealed hypoxaemia (pO_2 75 mmHg, rr 95-105 mmHg). Haematology and blood biochemistry showed a leucocytosis (23.1 x 10^9/l, rr 5.0-10.0 x 10^9/l) with mature neutrophilia (21.8 x 10^9/l, rr 3.0-7.0 x 10^9/l) and lymphopenia (0.73 x 10^9/l, rr 1.0-4.5 x 10^9/l) and hyperfibrinogenaemia (3.74 g/l, rr 1.50-2.20 g/l). Endoscopy revealed moderate amounts of brownish mucus in the trachea, mildly injected mucous membranes and a mildly thickened septum. Tracheal wash cytology showed neutrophilic inflammation (85% neutrophils) and bacterial culture revealed *Actinobacillus equuli*. Therefore, a diagnosis of bacterial bronchitis was made and the horse treated with penicillin-streptomycin (Tardomyocel Comp. III.)[4] (22,000 IU/kg bwt bwt q. 48 h) and enrofloxacin (Baytril 10%)[4] (5 mg/kg bwt i.v. q. 24 h) for 13 days. Additionally, the horse received sucralphate (Alusulin tabl.)[5] (1 g/kg bwt *per os* q. 6 h), Vitamin C (Acidum ascorbicum)[6] (10 g *per os* q. 24 h) and different NSAIDs (flunixin-meglumine, phenylbutazone, ibuprofen) as needed. As no improvement was noticed treatment was changed to trimethoprim-sulfadiazine (Equibactin vet.)[7] (30 mg/kg bwt *per os* q. 12 h) and metronidazole (Supplin)[8] (20 mg/kg bwt *per os* q. 12 h) for ten days.

Clinical outcome: After 23 days of treatment the horse remained hypoxaemic (pO_2 77 mmHg, rr 95-105 mmHg). Leucocytosis (12.4 x 10^9/l, rr 5.0-10.0 x 10^9/l) had improved but the horse still showed a mature neutrophilia (11.1 x 10^9/l, rr 3.0-7.0 x 10^9/l). Bacterial

and fungal culture of a repeat tracheal wash was negative as was a PCR for *Mycoplasma spp*. Bronchoalveolar lavage was performed and showed 62% neutrophils. Erythrocytes and haemosiderophages indicated both, fresh and old bleeding. Furthermore, reactive macrophages were noted. Radiographs of the thorax showed a mixed bronchointerstitial pattern with 5-6 nodular densities with a diameter of up to 1.5 cm (see Fig. 2). Ultrasonography of the thorax showed a generalised pleural roughening with multiple comet tails and one subpleural hypoechogenic nodule with a diameter of 3.5 cm. An ultrasound-assisted Tru-cut lung biopsy was taken and confirmed the diagnosis of EMPF. Furthermore, EHV-5 PCR of BALF and lung biopsy was positive. The treatment regime was changed to acyclovir (Telviran)[9] (20 mg/kg bwt *per os* q. 8 h) and pentoxifylline (Pentoxyl-EP)[10] (8 mg/kg bwt *per os* q. 8 h) for 14 days. For the first four days the horse also received dexamethasone (CP-Dexamethason)[11] (0.1 mg/kg bwt i.v. q. 24 h). After one week of antiviral therapy euthanasia was advised due to total anorexia and severe dyspnoea. The horse was fed via nasogastric tube and received oxygen supplementation intranasally, but over the next seven days the horse`s condition worsened until it died.

Case 2

History: A 6 year old Oldenburg stallion, used for show jumping started to show the first symptoms directly after a competition and 4 weeks before admission. The horse was anorexic and rapidly lost weight (approx. 100-150 kg). The last 2 weeks before admission he also had intermittent cough.

Clinical examination: On presentation the stallion was in a poor body condition (410 kg) and was depressed. The heart rate was 44 beats/min, respiratory rate was 24 breaths/min and rectal temperature 38.7°C. The horse showed bilateral serous nasal discharge, cough and a mild mixed dyspnoea. Thoracic auscultation revealed moderately increased lung sounds, but no wheezes or crackles.

Further examinations: Arterial blood gas analysis showed a mild hypoxaemia with a pO_2 of 94 mmHg (rr 95-105 mmHg). Haematology revealed a leucocytosis (16.6 x 10^9/l, rr 5.0-10.0 x 10^9/l) with mature neutrophilia (12.6 x 10^9/l, rr 3.0-7.0 x 10^9/l). Blood biochemistry showed a hypoalbuminaemia (23 g/l, rr 25-40 g/l) and hyperfibrinogenaemia (5.49 g/l, rr: 0.5 – 4 g/l). Endoscopy of upper and lower airways showed moderate amounts of mucus in the trachea, injected mucus membranes and a mildly thickened tracheal septum. Tracheal wash cytology showed a neutrophilic inflammation. Bacterial and fungal culture was negative. Cytological evaluation of BALF revealed 24% mainly non-degenerate neutrophils, 74% mononuclear cells, some mast cells (2%) and very few eosinophils. Erythrophagocytosis and haemosiderophages indicated pulmonary haemorrhage. EHV5 PCR was positive, mycoplasma PCR negative. Thoracic radiographs showed a nodular interstitial pattern. Ultrasonography of the thorax revealed a generalised roughening of the pleura with multiple comet tail artefacts and multiple small nodular hypoechoic subpleural lesions.

Clinical outcome: During hospitalisation the horse had no appetite and had to be fed via nasogastric tube. For seven days the horse was treated with flunixin-meglumine (Flunixin)[12] (1,1mg/kg bwt i.v. q. 24 h), acetylcystein (ACC200 granulatum)[8] (10 mg/kg bwt *per os* q. 12 h) and trimethoprim-sulfadiazine (Equibactin vet.)[7] (30mg/kg bwt *per os*

q. 12 h). Clinically no significant changes were noted and the horse remained intermittently pyrexic up to 38.9°C. Additional thorax radiographs two days after admission and another 10 days later showed a change of the initially nodular interstitial pattern to a rather diffuse interstitial pattern. Haematology at that point still showed a mild leucocytosis (14.6 x 10^9/l, rr 5.0-10.0 x 10^9/l) with mature neutrophila (11.0 x 10^9/l, rr 3.0-7.0 x 10^9/l). Fibrinogen had returned to normal, but the horse remained hypoalbuminaemic (22 g/l, rr 25-40 g/l). When the results of bacteriological culture from the tracheal wash fluid and the positive EHV-5 PCR result from the BALF were received a suspected diagnosis of EMPF was made and the horse treated with acyclovir (Telviran)[9] (20 mg/kg bwt *per os* q. 8 h) and dexamethasone (CP-dexamethason)[11] (0.05mg/kg bwt i.v. q. 24 h). But 2 days after the start of acyclovir therapy the horse was euthanized on the owner`s request.

Case 3

History: A 16-year old Furioso mare from Slovakia used for pleasure riding had shown intermittent chronic cough and dyspnoea with an increased abdominal effort for at least four years, which was suspected to be an RAO. Two weeks before admission the horse acutely showed severe dyspnoea with tachycardia (60/min) and tachypnoea (60/min) and fever (39.8°C) and was treated with dexamethasone (Colvasone)[12] (0.1 mg/kg bwt i.v. once) and potentiated sulphonamides (Trimazine)[13] (25 mg/kg bwt per os q. 12 h) for seven days. The horse`s condition only temporarily improved and it was therefore referred.

Clinical examination: At admission the horse was in a poor body condition (463 kg). Temperature was 37.5°C, heart rate and respiratory rate were 60 beats/min and 66 breaths/min, respectively. The horse was in severe respiratory distress with nasal discharge. Markedly increased lung sounds with crackles and wheezes were auscultated on both sides of the thorax.

Further examinations: Oxygen saturation on the standing, non-sedated horse measured by pulse oxymetry was 60%. Haematology revealed leucocytosis (18.9 x 10^9/l, rr 5.0-10.0 x 10^9/l) due to mature neutrophilia (14.3 x 10^9/l, rr 3.0-7.0 x 10^9/l). The horse was dehydrated with a haematocrit of 50%. Blood biochemistry showed an elevated fibrinogen concentration (5.56 g/l, rr 0.5 – 4 g/l). On endoscopic examination large amounts of viscous white mucus could be found in the entire airways, especially in the trachea. The tracheal septum was markedly thickened. Cytological evaluation of tracheal wash fluid predominantly showed neutrophils. Thoracic radiographs showed a marked bronchointerstitial pattern with many peribronchial cuffings. Ultrasonographic examination of the thorax revealed diffuse irregularities of the pleura and one hypoechogenic nodule (1.5 cm diameter) and echocardiographic findings consistent with a *cor pulmonale*. Differential diagnoses were acute exacerbation of RAO or severe lung disease of unknown aetiology. Due to the findings on blood work, ultrasound and radiography EMPF was considered likely.

Clinical outcome: The horse received emergency treatment with dexamethasone (Dexadreson a.u.v. inj.)[14] (0.1 mg/kg bwt i.v.), clenbuterol (Ventipulmin)[1] (0.8 mg/kg bwt *per os*), bromhexin (Eres)[15] (40 mg/kg bwt *per os*), continuous intranasal oxygen (9 l/min) and finally atropine (Atropin Biotika)[16] (2.5 mg i.v.). During the non-invasive

examination the status of the horse progressively worsened and approximately 90 minutes after atropine administration the horse died.

EMPF diagnosed at post mortem

Case 4

A 14 month old Arabian filly was admitted for evaluation of lethargy and severe anaemia, but died on the way to the clinic. The owner did not report any respiratory signs.

Case 5

A 2 year old Warmblood filly was admitted as an emergency case with neurological signs. The horse had no history of previous medical problems and was found recumbent in the field. After transport to the clinic in sternal recumbency the filly was not able to stand at admission and was placed in an Anderson sling. Neurological examination revealed an abnormal mentation and severe ataxia (5/5). Due to financial restraint no further examinations were performed. A traumatic brain injury was suspected and treatment started with fluid therapy, DMSO (1 g/kg bwt i.v. as 10% solution in 0.9% saline) and dexamethasone (Dexavana)[17] (0.1 mg/kg bwt i.v.). After approximately 6 hours the filly lost the menace response bilaterally and developed bilateral mydriasis, not responding to light. The filly was euthanized on the owner`s request.

Post mortem of all cases

Gross pathology showed the discrete form of EMPF in three horses (cases 1, 4, 5). The lungs showed numerous, variably sized, homogeneously, grey-white coloured, dense, sharply demarcated nodules up to 6.5 cm and lungs did not collapse on opening of the thoracic cavity. Mediastinal and tracheobronchial lymph nodes were diffusely enlarged. Case 1 also showed signs of diffuse chronic pleuritis on the visceral pleura with short fibrinous tags in some areas and small amounts of purulent exsudate were present in some of the small airways. Furthermore the heart showed signs of right ventricular hypertrophy.

Histopathologically the lung areas affected with interstitial fibrosis were sharply demarcated from unaffected parenchyma. Examination of the nodules revealed an extensive thickening and fibrosis of the interstitial tissue due to deposition of diffuse mature collagen and infiltrations by inflammatory cells, predominantly lymphocytes, which were most prominent in perivascular and interlobular spaces (see Fig. 4). "Alveolar-like" structures ("honeycombing"), lined by flat to cuboidal epithelial cells, contained a mixed inflammatory infiltrate. Within airways abundant detritus with numerous macrophages and neutrophil granulocytes, some multinucleated giant cells and rarely cells with intranuclear eosinophilic inclusion bodies (see Fig. 5) could be found additionally, except in case 1. In some areas a proliferation of type II pneumocytes was prominent within alveoli. EHV-5 PCR was positive in lung specimen of all three cases.

In case 1 pathological lesions were restricted to the lung and regional lymph nodes, whereas in the other two horses (case 4 and 5) additional pathology was found.

In case 4 a severe anaemia was evident beside a haemorrhagic diathesis with petechial bleedings and subcutaneous oedema. Lymphatic organs such as lymph nodes, GALT, bone marrow and spleen revealed severe depletion of lymphocytes. Additionally larval cyathostominosis could be diagnosed and both parotid glands showed disseminated necrotic lesions. Necropsy of the spleen revealed abscesses up to 10 mm diameter and with bacteriological culture S*treptococcus equi subsp. zooepidemicus* could be isolated besides other bacteria. Furthermore two chondroids up to 10 mm within the guttural pouches were suggestive for strangles infection in the past. A severe combined immunodeficiency seemed rather unlikely with regard to the age of the filly, but an immune-mediated process, responsible for multifactorial disease, could not be ruled out and seemed likely. Equine infectious anaemia was excluded by Coggins test and PCR. EHV-5 could not be detected in bone marrow by PCR.

Case 5 revealed a lymphoplasmohistiocytic panencephalomyelitis with accompanying leptomeningitis and foci of demyelination in the cerebellar white matter. Focal bleedings could be found oligofocally within medulla oblongata and lumbar spinal cord. Immunohistochemical examination of paraffin-embedded brain tissue against Rabiesvirus-, Suid Herpes-Virus-1, Borna-Disease-Virus, West-Nile-Virus-, Equine Herpes-Virus-1- and Tick-borne Encephalitis-Virus-antigen revealed negative results and aetiology of the panencephalomyelitis remained unclear.

In two horses (case 2 and 3) the diffuse form of EMPF was found with confluent areas of smaller nodules. In both cases an EHV-5 PCR of the lung tissue was positive, but inclusion bodies were not found in case 3.

Discussion

This case series describes five cases with EMPF, which occured in Austria, Hungary and Slovakia. EMPF therefore has to be included as differential diagnosis for horses with respiratory symptoms but also in cases with weight loss in this area.

Horses with EMPF, which survived have been treated with steroids and acyclovir (Wong et al., 2008). Therefore in cases 1 and 2 the treatment regime was changed to acyclovir and corticosteroids once a diagnosis of EMPF was made. Case 1 finally was euthanized after the horse was not-responding to antiviral therapy after one week of medication. There are different possible reasons why this horse was not responding to antiviral therapy. First of all the pulmonary fibrosis probably already was in an advanced stage. Treatment might have been not effective due to the reported poor bioavailability of acyclovir (3-8%) (Wilkins et al., 2005, Bentz et al., 2006, Garré et al. 2007). Furthermore it is still not known if EHV-5 is susceptible to acyclovir.

Although case 2 seemed to be quite early in the course of disease the owner opted for euthanasia, because of the poor prognosis of EMPF, high costs of antiviral medication and parenteral feeding due to complete anorexia. In cases like this a poor prognosis might therefore lead to a self-fulfilling prophecy.

Case 3 presented with acute respiratory distress and cardiovascular decompensation due to severe lung disease. Decompensated pulmonary hypertension with *cor pulmonale* is the end stage of disease and seems to be a poor prognostic indicator (Hart et al., 2008, Wong et al., 2008). This horse had a history of suspected RAO. Likely respiratory symptoms in the early stages of EMPF were attributed to RAO as reported in other cases with EMPF (Lehmbecker et al., 2011). A further difficulty diagnosing EMPF early might be the diagnosis of a bacterial bronchitis as in case 2 or other cases in the past (Wong et al., 2002, Marenzoni et al., 2011, Schwarz et al., in press). The horses are treated for what is thought the reason for the respiratory problems but in fact is a secondary bacterial infection. In the meantime progression of EMPF leads to worsening of clinical signs and therefore reevaluation of the patient with further diagnostics, like ultrasound, radiographs, bronchoalveolar lavage, EHV-5 PCR, etc. Once a diagnosis is made the disease might be already in an advanced stage and more difficult to treat successfully.

Corticosteroids have been used to treat EMPF given their anti-inflammatory and anti-fibrotic effect. But their immunosuppressive properties and hence possible promotion of viral replication are controversially discussed (Hart et al., 2008, Wong et al., 2008, Marenzoni et al., 2011). In idiopathic pulmonary fibrosis (IPF), a human disease associated with Epstein-Barr Virus (also a γ-herpesvirus), treatment with corticosteroids does not seem to have an advantage (Doran and Egan, 2005).

Interestingly cases 4 and 5, even in an advanced stage of disease did not show respiratory symptoms noticed by the owners. Case 4 predominantly showed depression and anaemia, which led to spontaneous death. Case 5 was euthanized with acute neurological symptoms due to an idiopathic panencephalitis. Therefore even in cases where clear

335 respiratory signs are lacking EMPF could be an ongoing problem. It is questionable if the
336 other diseases in these horses developed concurrently with EMPF or EHV-5 infection as
337 a result of immunosuppression. Immunosuppression or an immunological predisposition
338 with a different host response have been discussed in the aetiopathogenesis of EMPF
339 (Hart et al., 2008) and in some cases – as case 5 – an association between EMPF and
340 immunosuppression seems to be obvious.

341 EHV-5 appears to induce pathology in the bone marrow (Hart et al., 2008, Schwarz et al.,
342 in press). Case 4 died of anaemia and haemorrhagic diathesis (thrombocytopenia) and
343 lymphoid organs were noticed to be depleted of lymphocytes (lymphopenia). Although
344 EHV-5 PCR in the bone marrow was negative in this horse, it seems to resemble the case
345 described by Hart et al. (2008). However in that case bone marrow was EHV-5 PCR
346 positive and viral inclusion bodies were found in the bone marrow. Similar to EBV
347 inducing lymphoproliferative disease in humans, EHV-5 might be responsible for cases
348 of leukaemia and lymphoma in horses (Schwarz et al., in press, van der Werf et al.,
349 2011).

350 Similar to EBV, EHV-5 is also found in healthy individuals (Torfason et al., 2008) and
351 therefore further research is needed to clarify the role of EHV-5 in both,
352 lymphoproliferative diseases and EMPF.

353

354 **Conclusions**

355 EMPF also should be considered as a differential diagnosis for respiratory symptoms and
356 weight loss in Eastern European countries. Further research on the role of

immunosuppression in the pathogenesis of EHV-5 infection and EMPF is necessary to assess the benefit of corticosteroids in treatment of the disease.

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Manufacturers` addresses

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[6]Hungaropharma, Budapest, Hungary

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[16]Hoechst Biotika, Slovakia

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456

457 **Figure legends**

458 **Fig. 1:** 8 year old Holsteiner mare with EMPF (case 1).

459 **Fig. 2:** Lateral radiographs of caudodorsal lung field of case 1. The radiograph displays a
460 severe diffuse interstitial pattern of increased pulmonary density and nodular opacities.

461 **Fig. 3:** Gross pathology of case 2: diffuse form of EMPF with small nodular areas of
462 fibrosis

463 **Fig. 4:** Histology of lung tissue: thickening of alveolar interstitial tissue due to fibrosis.
464 Large amounts of detritus in airways.

465 **Fig. 5:** Histology of lung tissue: intranuclear eosinophilic inclusion body.