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Equine Multinodular Pulmonary Fibrosis in association with an EHV-5 infection in 5 horses

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Summary

Between 2006 and 2008, 5 adult horses hailing from South Germany (4/5 cases) and Austria (1/5) with different case histories but similar lung lesions were investigated at the Faculty of Veterinary Medicine of the Ludwig-Maximilians-University in Munich. Anamnesis included amongst others ongoing respiratory symptoms, weight loss, and poor body condition. Pathological examination of the lungs revealed 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, and the presence of eosinophilic intranuclear viral inclusion bodies within intraluminal macrophages. Neither bacteria nor fungi could be detected using special staining methods. In 4 horses, PCR analysis of paraffin-embedded lung tissue was initiated and yielded positive results for equine herpesvirus type 5 (EHV-5) DNA in all cases. Additionally, EHV-2 DNA was detected in 3 horses. Based on histopathological findings and PCR results, the diagnosis of equine multinodular pulmonary fibrosis (EMPF) associated with an infection of EHV-5 was established. EMPF was first recognised in 2005 in the USA. To the best of our knowledge, this is the first report on the occurrence of EMPF in Europe.

Abbreviations: B = Bavaria; DNA = deoxyribonucleic acid; DNS = Desoxyribonukleinäure; EMPF = equine multinodular pulmonary fibrosis; EHV-2 = equine herpesvirus type 2; EHV-5 = equine herpesvirus type 5; f = female; GMA = 2-hydroxyethyl methacrylate; H&E = hematoxylin and eosin; mc = male castrated; MMA = methyl methacrylate; PAS = periodic acid Schiff's reaction; PCR = polymerase chain reaction; y = years

Schlüsselwörter: Pferd, Lunge, Equine Multinoduläre Pulmonale Fibrose, EMPF, Equines Herpesvirus, EHV-5, EHV-2.

Zusammenfassung

Equine Multinoduläre Pulmonale Fibrose in Assoziation mit einer EHV-5-Infektion bei 5 Pferden

In den Jahren 2006 bis 2008 wurden in der Tiermedizinischen Fakultät der Ludwig-Maximilians-Universität insgesamt 5 aus Bayern (4/5 Fälle) und Österreich (1/5) stammende Pferde mit unterschiedlicher Krankheitsgeschichte aber gleichartigen Lungenveränderungen untersucht. Die vorberichtlich mitgeteilten klinischen Befunde enthielten unter anderem andauernde respiratorische Symptome, Gewichtsverlust und schlechten Allgemeinzustand. Die pathologische Untersuchung der Lungen erbrachte in allen Fällen eine gleichartige chronische, interstitielle und fibrosierende Pneumonie mit akuter Komponente. Diese unterschied sich deutlich von anderen fibrosierenden Lungenerkrankungen insbesondere durch ihr überwiegend noduläres Muster, den Umbau der Lungenarchitektur und das Vorhandensein von eosinophilen intranukleären Viruseinschlusskörperchen in einzelnen Alveolarmakrophagen. Anhand von Spezialfärbungen waren keine Bakterien oder Pilze zu erkennen. Mittels PCR, die an Lungengewebe von 4 Pferden durchgeführt wurde, konnte in allen Fällen DNS von Equinem Herpesvirus (EHV)-5 nachgewiesen werden. Bei 3 Pferden gelang zusätzlich der Nachweis von EHV-2. Basierend auf den histopathologischen Befunden und den molekularbiologischen Ergebnissen wurde die Diagnose Equine Multinoduläre Pulmonale Fibrose (EMPF) in Assoziation mit einer EHV-5- Infektion gestellt. EMPF wurde erstmals 2005 in den USA beschrieben, aber soweit uns bekannt ist, liegen bislang keine Berichte über das Auftreten dieser Erkrankung in Europa vor.

Introduction

Equine multinodular pulmonary fibrosis (EMPF) is a chronic and fibrosing interstitial lung disease in adult horses. EMPF results in characteristic lung lesions and was first recognized in 2005 by WILLIAMS et al. At present only 2 studies about the occurrence of EMPF in the USA have been published (WILLIAMS et al., 2005, 2007; WONG et al., 2008). In the reported cases, the disease is associated with infection of the equine herpesvirus type 5 (EHV-5), which belongs to the genus percavirus within in the gammaherpesvirus subfamily (MCGEOCH et al., 2006).

This paper describes the investigation of 5 horses with an uncommon form of pneumonia accompanied by a severe interstitial fibrosis. In 4 horses, EHV-5 virus DNA was detected in the lungs using polymerase chain reaction (PCR). Gross pathological and histological examination of all lungs revealed lesions characteristic for EMPF. To the best of our knowledge, this is the first report on EMPF in Europe.

Casuistics

The study includes 3 warmblood and 2 Icelandic horses hailing from South Germany (4/5 cases) and Austria (1/5) with different case histories and from different holdings

(Tab. 1). 3 horses were geldings and 2 horses were mares, and their age ranged from 10 to 25 years with a mean age of 17.2 years. Anamnesis included amongst others weight loss (4/5 cases), apathy (3/5) and respiratory symptoms (3/5). Based on thoracic radiography findings (3/5), tentative diagnoses of lung tumour and granulomatous pneumonia were established. The animals were finally euthanised due to unsuccessful treatment.

Detailed information on the clinical course were only available from horse 1 and horse 2 that were initially investigated in the Horse Clinic of the Ludwig-Maximilians-University Munich.

Horse 1

The 16 years old Holsteiner gelding was admitted to the Horse Clinic in March 2006 with a history of weight loss and fever up to 40 °C for several weeks. Physical examination revealed tachycardia and tachypnoea. Thoracic radiography showed a diffuse nodular interstitial and bronchiolar pattern. Multiple lung biopsy specimens were collected and submitted for histopathological examination. For animal welfare reasons, the horse was eventually euthanised.

Horse 2

The 18-year-old Mecklenburger mare was presented to the Horse Clinic in January of 2008 due to lethargy, oral ulceration and inappetence. Clinical findings included depression, ulceration of oral mucous membranes, and intermittent cough. Radiographic examination revealed a widespread diffuse interstitial pattern and large radiodense opacity in the caudodorsal area of the lung. Differential diagnosis included interstitial pneumonia, pulmonary neoplasia, and fungal pneumonia. Because of the poor prognosis, the owner declined further investigation and opted for euthanasia.

Material and methods

Lung biopsy specimens (2/5 cases) and carcasses (3/5), respectively, were submitted by veterinary practitioners and horse hospitals for pathological examination (Tab. 1). Representative lung specimens of all horses as well as further organ samples of necropsied horses were fixed in 7 % buffered formaldehyde, routinely embedded in paraffin (Paraplast®), cut into 4 µm-thick sections, and stained with haematoxylin and eosin (H&E) and according to Giemsa. Special stains from lung sections of horse 1 and 5 included periodic acid Schiff's (PAS) reaction to detect fungi and mucosubstances, Fite-Faraco stain to visualise acid-fast rods, and Masson's trichrome stain to demonstrate collagen.

Additionally, selected lung specimens of horse 1 were embedded in a mixture of 2-hydroxyethyl methacrylate (GMA) and methyl methacrylate (MMA) using standard protocols. The plastic-embedded lung tissue was cut into 2 µm-thick sections and stained with H&E and according to Giemsa.

Paraffin-embedded lung tissue of 4 horses was investigated for the presence of EHV-5 and EHV-2 DNA using standard PCR method (performed by IDEXX Laboratories GmbH, Ludwigsburg, Germany).

Results

The lungs of all 5 horses showed severe gross and histopathological findings of similar type. Therefore, they are conjointly described in the following and summarised in Tab. 2. In carcasses, all organs underwent autolysis in different degrees at the time of necropsy.

Macroscopically, the lung parenchyma was interspersed with multiple large, bulging, coalescent, tan-white and firm nodules of fibrosis. The cut surface was homogeneously coloured and the patchy areas of fibrosis showed predominantly distinct borders to the adjacent relatively normal lung tissue (Fig. 1). The bronchial lymph nodes in horse 2 and 5 were noticeably enlarged.

The histological examination of the lungs revealed a primary affection of the interstitial tissue and alveoli. The findings were characterised by the coexistence of both, acute and chronic stages of inflammation and a severe interstitial fibrosis. The fibrosis resulted in a vast interstitial expansion and eventually led to a complete remodelling of the lung architecture and honeycombing. According to the gross findings, the nodular lesions were histologically sharply demarcated from normal parenchyma (Fig. 2), except for some locations showing a diffuse distribution pattern, which merged indistinctly with unaffected parenchyma or locations consisting completely of consolidated parenchyma (Fig. 3).

Generally, 2 kinds of interstitial fibrosis occurred: (i) deposition of unstructured loose and interlacing collagen bundles, which expanded alveolar septae (Fig. 4) and (ii) deposition of well-organised mature collagen fibres resulting in the formation of abnormal cystic airspaces of various size (honeycombing), which were lined by a cuboidal epithelium (Fig. 5, 7, 8). The latter type of fibrosis represented the main form in all cases.

The interstitial tissue was infiltrated by numerous inflammatory cells including lymphocytes, plasma cells, and neutrophils. Some eosinophils, mast cells, and multinucleated giant cells were scattered throughout the interstitial parenchyma. The bronchioli, alveoli and abnormal cystic airspaces contained numerous enlarged, vacuolated macrophages and neutrophils (Figs. 5 - 8), and occasionally multinucleated giant cells (Fig. 8). In several alveoli, the accumulated inflammatory cells underwent degeneration (Fig. 7) and subsequent dystrophic calcification. Some lung areas revealed a marked alveolar oedema and hyperplasia of type II pneumocytes (Fig. 6). Furthermore, a few enlarged intraluminal macrophages with eosinophilic intranuclear inclusion bodies (Cowdry type A, Fig. 8) were detected only within lung areas, which showed chronic inflammatory lesions. Special stains for bacteria and fungi yielded negative results.

The bronchial lymph nodes of horse 2 and 5 revealed a reactive hyperplasia with scattered multinucleated giant cells within sinuses.

The presence of EHV-5 DNA was detected in paraffin-embedded lung tissue of 4 cases using PCR analysis. Additionally, 3 horses were positive for EHV-2 DNA (Tab. 1).

Necropsy of 3 horses revealed that the inflammatory lesions were restricted to the lungs, and affected all lung lobes. Following additional findings were present and interpreted as secondary findings: a severe ulcerative glossitis and moderate chronic glomerulonephritis in horse 2 and a

Tab.1: Equine multinodular pulmonary fibrosis (EMPF) in 5 horses

Horse No.	Breed	Age (y)	Sex	Habitation	Date of investigation	Method	PCR results EHV-5	EHV-2
1	Holsteiner	16	mc	Germany (B)	3/2006	lung biopsy	+	+
2	Mecklenburger	18	f	Germany (B)	1/2008	necropsy	+	–
3	Icelandic horse	10	f	Germany (B)	10/2008	lung biopsy	+	+
4	Icelandic horse	25	mc	Austria	11/2008	necropsy	not done	
5	Westfalian	17	mc	Germany (B)	12/2008	necropsy	+	+

B = Bavaria; f = female; mc = male castrated; PCR = polymerase chain reaction; y = years

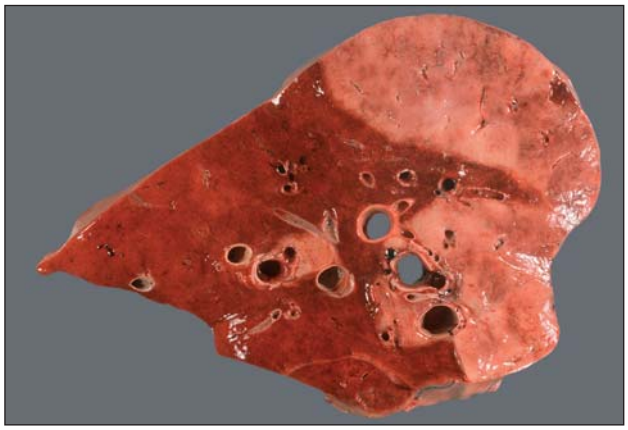

Fig. 1: Lung, horse 2, EMPF, gross pathology, cut section: discrete nodular form with disseminated large nodules of fibrosis, which are sharply demarcated from adjacent normal appearing lung tissue

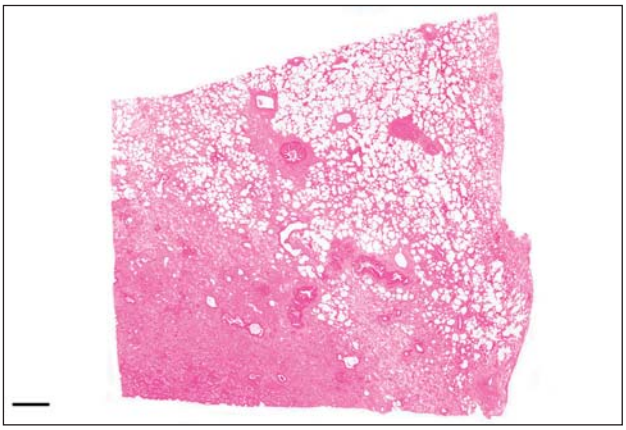

Fig. 2: Lung, horse 1, EMPF, histopathology, overview; discrete borders between foci of affected lung tissue and normal parenchyma; bar = 1mm; H&E

Fig. 3: Lung, horse 1, EMPF, histopathology, overview; area with severe pneumonic consolidation, no normal lung parenchyma present; bar = 1 mm; H&E

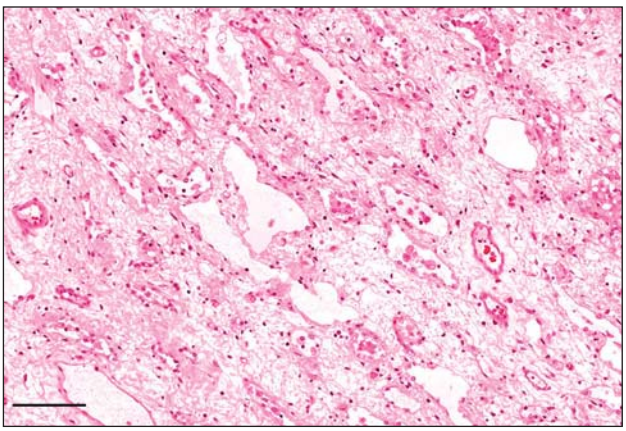

Fig. 4: Lung, horse 1, EMPF, histopathology; deposition of unstructured loose and interlacing collagen bundles resulting in a severe thickening of alveolar septae; bar = 100 µm; H&E

mild chronic hepatitis and interstitial nephritis in horse 5. Horse 4 suffered concurrently from liver cirrhosis of unknown cause, but ragwort intoxication was suspected.

Discussion

In the present study, all 5 horses revealed similar findings in the lungs, i.e. severe interstitial pneumonia with prominent interstitial fibrosis. Based on the characteristic pathomorphological lung findings and PCR results, the diagnosis of equine multinodular pulmonary fibrosis (EMPF) in association with an infection of EHV-5 was established. The lesions differ significantly from other interstitial fibrosing lung diseases in horses due to their nodular pattern, the loss of normal lung architecture with replacement by abnormal cystic airspaces (honeycomb-

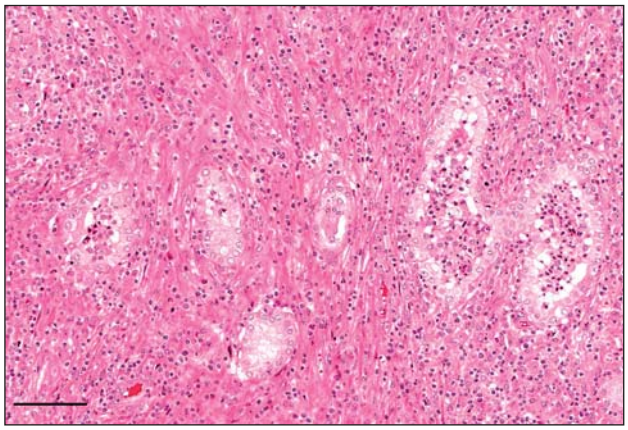

Fig. 5: Lung, horse 1, EMPF, histopathology; remodelling of lung architecture due to deposition of well-organised mature collagen fibres with formation of abnormal airspaces (honeycombing); bar = 100 µm; H&E

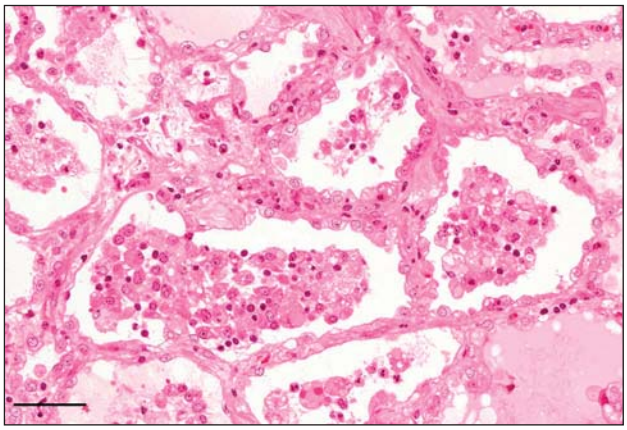

Fig. 6: Lung, horse 1, EMPF, histopathology; marked alveolar oedema, hyperplasia of type II pneumocytes, and intraluminal accumulation of enlarged, vacuolated macrophages and neutrophils; bar = 50 µm; H&E

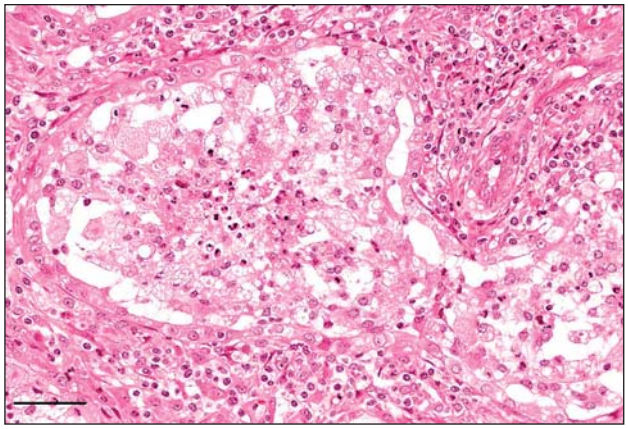

Fig. 7: Lung, horse 1, EMPF, histopathology; abnormal cystic airspace lined by a cuboidal epithelium, and filled with vacuolated macrophages, neutrophil granulocytes, and cellular detritus; bar = 50 µm; H&E

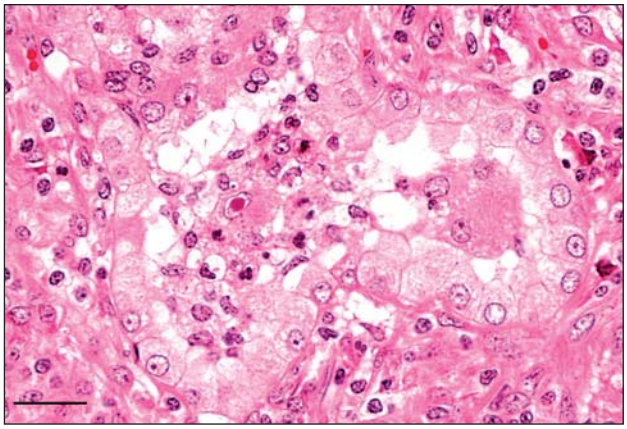

Fig. 8: Lung, horse 1, EMPF, histopathology; detection of an eosinophilic intranuclear inclusion body of Cowdry type A within an enlarged intraalveolar macrophage and presence of an intraalveolar multinucleated giant cell in an abnormal cystic airspace; bar = 25 µm; H&E

ing), and the presence of intranuclear viral inclusion bodies within intraluminal macrophages. Thermal and chemical injury, toxic gases, ingested toxins, endotoxin, pneumoconiosis, uremia, chronic left heart failure, and equine influenza for example are considered as causes for interstitial pneumonia (SCHWARTZ et al., 1981; TURK et al., 1981; DUNGWORTH, 1982; CASWELL and WILLIAMS, 2007), all of which could be excluded in the presented 5 horses by the case histories and the clinical and pathological examination.

The lesions described in our 5 cases match exactly the ones described and diagnosed as EMPF in literature (WILLIAMS et al., 2005, 2007; CASWELL and WILLIAMS, 2007; WONG et al., 2008). In the study of WILLIAMS et al. (2007), a series of 24 horses were investigated and compared with 23 age-matched control animals. The affected horses were predominantly Thoroughbreds with an average age of 14.5 years. Lung tissue of all horses was positive for EHV-5 and 33 % were positive for EHV-2.

WONG et al. (2008) report about the clinical course of 5 horses suffering from EMPF. The study included 4 Thoroughbreds and 1 Oldenburg horse with an average age of 13 years. In all cases, EHV-5 DNA was detected in lung specimens using PCR.

In the present study, 5 horses were investigated including 3 geldings and 2 mares with a mean age of 17.2 years. The histological lesions are identical to those described by WILLIAMS et al. (2007). Additionally, we detected the presence of multinucleated giant cells within the lungs and the bronchial lymph node of 2 horses, whereas WILLIAMS et al. (2007) reported multinucleated giant cells only within the lymph nodes.

Based on the detection of viral inclusion bodies and the PCR results, the lung lesions in the present study are in all probability associated with an infection by EHV-5. Although PCR analysis was not performed in horse 4, viral aetiology seems also to be likely in this case due to identical pathomorphological lesions. Our assumption of a viral aetiology is supported by results of other authors, who expanded their examinations to include transmission electron microscopy and in-situ-hybridisation (WILLIAMS et al., 2005, 2007; CASWELL and WILLIAMS, 2007; WONG et al. 2008).

Tab. 2: Summary of significant pathomorphological findings in lungs with equine multinodular pulmonary fibrosis (EMPF) based on the present study of 5 horses as well as on a study of 24 horses investigated by WILLIAMS et al. (2007)

Gross findings	• lesions are restricted to the lungs and the bronchiolar lymph nodes • pulmonary induration, involving all lung lobes • large tan-white and firm nodules of fibrosis in 2 distribution patterns: (i) diffuse nodular form: coalescent nodules, up to 5 cm in diameter, distinctly separated from little unaffected lung parenchyma, representing the more common pattern (ii) discrete nodular form: disseminated large nodules, up to 10 cm in diameter, with discrete borders and larger areas of normal parenchyma (resembling a tumour)
Histological findings	• fibrosis and inflammation in different stages and degrees • 2 distribution patterns: (i) lesions are demarcated from normal parenchyma (predominant form) (ii) lesions with indistinct borders merge with normal parenchyma
Interstitium	• expanded by fibrosis and inflammation • fibrosis occurs in 2 forms: (i) predominant form: deposition of mature connective tissue with loss of alveolar architecture and replacement by abnormal cystic airspaces of various size (honeycombing) (ii) rare form: deposition of immature and loose fibres, which causes destruction of alveoli • infiltrated mostly by lymphocytes, plasma cells, neutrophils; several eosinophils and mast cells • multinucleated giant cells
Alveoli	• accumulation of neutrophils, and enlarged, vacuolated macrophages; several multinucleated giant cells • viral eosinophilic intranuclear inclusion bodies in intraluminal macrophages • hypertrophy of type II pneumocytes • formation of abnormal cystic airspaces, which are lined by a cuboidal epithelium (honeycombing)

There is little information available about the distribution and pathogenicity of EHV-5. It seems to have a low natural prevalence and can be infrequently detected within peripheral blood mononuclear cells and nasal swabs in clinically healthy horses and within lungs showing EMPF (BORCHERS et al., 1999; NORDENGRAHN et al., 2002, WILLIAMS et al., 2005, 2007; BELL et al., 2006). EHV-5 belongs to the genus percavirus within the gammaherpesvirus subfamily (MCGEOCH et al., 2006) and shows uncommon biological characteristics compared with other members of the gammaherpesvirus subfamily. The affinity for macrophages instead of B and T lymphocytes is unusual for a gammaherpesvirus and the formation of inclusion bodies is normally a main feature of the alpha- and betaherpesviruses. EHV-5 was initially placed in the betaherpesvirus subfamily (BROWNING and STUDDERT, 1987). Based on nucleotide sequence analyses, EHV-5 was reclassified in the meantime as a member of the gammaherpesvirus subfamily (TELFORD et al., 1993; MCGEOCH et al., 2006).

In the present work, EHV-2 DNA was additionally detected in lung tissue specimens. This virus is also grouped into the genus percavirus of the gammaherpesvirus subfamily (MCGEOCH et al., 2006), and is widespread throughout the horse population in Europe (BORCHERS et al., 1997; NORDENGRAHN et al., 2002). It has been involved amongst others in upper respiratory tract diseases and general malaise, most frequently in foals, but can also be isolated from clinically healthy horses (BORCHERS et al., 1997; NORDENGRAHN et al., 2002). In the present study, similar lung lesions were found in all animals, but EHV-2 DNA was detected in lung tissue of 3 cases of a total of 4 horses investigated by PCR analysis. Our results correspond with the experiences of WILLIAMS et al. (2007), who found a co-infection with EHV-2 in only 33 % of all horses suffering from EMPF. A further study reported about the sole detection of EHV-5 in horses with EMPF (WONG et al., 2008). The absence of EHV-2 in some horses could be interpreted as further evidence for the assumption that EMPF is not associated with EHV-2 infection. Comparing the lung findings in horses suffering from EMPF with those resulting from interstitial pneumonia induced by asinine herpesviruses that are related to gammaherpesviruses in donkeys (KLEIBOEKER et al., 2002), EMPF seems to be confined to horses (WILLIAMS et al., 2007).

Although EMPF was first reported in 2005 (WILLIAMS et al., 2005), it seems likely, that this kind of interstitial lung disease has been occurring for years in the horse population. There are previous reports about interstitial fibrosing pneumonia of uncertain aetiology in adult horses (BUERGELT, 1995; DONALDSON et al., 1998) that resembles morphologically EMPF. It would be interesting to reinvestigate these cases in regard to the presence of EHV-5 and EHV-2.

The pathogenesis of EMPF is still unclear. EMPF has

only been diagnosed in adult animals (WILLIAMS et al., 2005, 2007; WONG et al., 2008), despite infections with EHV-5 also occurring in young horses (NORDENGRAHN et al., 2002). Thus, a prolonged lag period with induction of first lung lesions in adult animals is suggested (WILLIAMS et al., 2007).

Interestingly, the interstitial pneumonia in all presented cases ranges from acute to chronic stages, which are located side by side, suggesting a chronic but active inflammation with a progressive behaviour. The final stage seems to be represented by the deposition of abundant organised mature collagen bundles resulting in the loss of lung architecture and replacement by cystic alveolar spaces of various size (honeycombing, Fig. 5, 7, 8). There is no explanation yet, why inclusion bodies are only found in chronic lesions of the lungs. Furthermore, it is also still unclear, whether the interstitial fibrosis is exclusively induced by the inflammatory cell infiltration as known for other forms of interstitial pneumonia (DUNGWORTH, 1982) or additionally by the virus itself via other pathways of fibrosis induction.

Although EHV-5 DNA could be detected in lung tissue of horses suffering from EMPF, the Henle-Koch postulates are not completely fulfilled at the moment. It is not definitely demonstrated, that the lung lesions are actually the sequelae of the herpesvirus infection. The chronic interstitial pneumonia could possibly represent an initial condition of other unknown causes that facilitates a secondary EHV-5 infection. Further studies are necessary to prove that EHV-5 induces EMPF in horses, to get an overview about the distribution of EHV-5 in the European horse population and to contribute to a better understanding in pathogenesis of EMPF.

Conclusion

EMPF is a newly recognised form of interstitial pneumonia in horses, which is characterised by a marked interstitial fibrosis mostly in a multinodular pattern. It is associated with an EHV-5 infection and should be considered as an important differential diagnosis within the group of fibrosing lung diseases in horses. Due to the progressive course of disease and the consecutively arising poor body condition, the prognosis of EMPF is tentative to unfavourable. Despite the fact that only few reports exist until now, it seems to be likely that EMPF has been occurring for years. In the present study, 3 of 5 cases were examined in the last quarter of 2008 (Tab. 1) indicating an increase of horses suffering from EMPF for the future.

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