Short Communications

Equine multinodular pulmonary fibrosis in horses in the UK

T. Soare, G. Leeming, R. Morgan, R. Papoula-Pereira, A. Kipar, J. Stewart, U. Hetzel

EQUINE multinodular pulmonary fibrosis (EMPF) is a relatively recently described condition in horses, characterised by a loss of functional pulmonary parenchyma due to extensive nodular to coalescing interstitial fibrosis; the remaining alveoli are lined by cuboidal epithelium and are filled with inflammatory cells (Williams and others 2007). EMPF was described first in the USA (Williams and others 2007, Hart and others 2008, Wong and others 2008), but more recently has been reported in Europe, in Germany and Austria (Poth and others 2009, Niedermaier and others 2010). A strong association between EMPF and equine herpesvirus type 5 (EHV-5) has been established, although the pathogenesis of the condition is still unclear (Williams and others 2007). EHV-5 is a DNA gammaherpesvirus, which has been detected in the USA, Australia, New Zealand and Europe in nasal swabs and peripheral blood lymphocytes (PBLs) from both healthy horses and those with respiratory signs, at highly variable rates (Agius and Studdert 1994, Reubel and others 1995, Franchini and others 1997, Borchers and others 1999, Dunowska and others 1999, Nordengrahn and others 2002, Bell and others 2006, Wang and others 2007, Torfason and others 2008, Fortier and others 2009). EHV-5 has also been identified in horses in the UK, where PCR yielded a positive result in PBLs from five of 21 healthy adult horses (Nordengrahn and others 2002).

This short communication describes two cases of EMPF in the UK, and their association with EHV-5.

The first case, an 11-year-old thoroughbred gelding, was presented in February 2010 with a two-week history of lethargy, listlessness and an increased respiratory rate. Radiographic and ultrasonographic examinations (Fig 1a, b) revealed nodular lesions throughout both lungs. The animal was euthanased. At postmortem examination, gross findings in the lungs were consistent with those described for EMPF (Williams and others 2007). All lung lobes exhibited multifocal to coalescing, white to pale tan, firm, well-demarcated nodules throughout the entire parenchyma. Nodules ranged from 1 cm in diameter to large confluent lesions, resulting in an uneven pleural surface with severely engorged vessels and a distinct superficial vascular branching pattern (Fig 1c, d). Histological and ultrastructural examinations showed that the nodules were represented by areas of moderate to focally severe interstitial fibrosis, associated with a variably intense multifocal interstitial inflammatory infiltrate, composed of lymphocytes with variable proportions of macrophages, neutrophils and eosinophils. Alveoli varied in size and were lined by cuboidal epithelial cells (Fig 2a, b), which were confirmed by the presence of cytoplasmic lamellar bodies to be hyperplastic and hypertrophied type II pneumocytes (Fig 2c). The alveolar spaces contained degenerate type II pneumocytes as well as degenerate neutrophils and macrophages. Occasionally, alveolar macrophages within the alveolar space exhibited eosinophilic to amphophilic intranuclear inclusion bodies with chromatin margination (Fig 2b). The remaining parenchyma exhibited atelectasis or alveolar emphysema and focal acute haemorrhage.

EHV-5 was amplified by PCR from DNA extracted from fresh tissue using previously published primers (Nordengrahn and others 2002). The PCR products were of the expected size and the DNA sequence was determined to confirm their origin (data not shown). The PCR products were cloned into PCRII (Invitrogen) and served as templates to produce digoxigenin (DIG)-labelled RNA probes using a commercially available kit (DIG RNA labelling kit; Roche). These were used to label EHV-5 DNA in formalin-fixed, paraffin-embedded (FFPE) lung sections by in situ hybridisation, using a published protocol (Kipar and others 2005). This revealed EHV-5 DNA in occasional macrophages within alveolar lumina and also rarely in type II pneumocytes within areas of fibrosis (Fig 2d). The gross and histological appearance of the lesions, together with the amplification of EHV-5 DNA, which was shown by in situ hybridisation to be directly associated with the lesions, led to the diagnosis of EMPF.

The diagnosis of EMPF in the first case led to a retrospective review of a second case, a 14-year-old thoroughbred cross gelding examined in August 2007, which had a history of chronic weight loss. Ultrasonography had revealed pulmonary consolidation and radiography had revealed multiple lung masses. At postmortem examination, gross and histological pulmonary changes were similar to those described for the first case. EHV-5 PCR using DNA extracted from FFPE lung sections yielded a PCR product, which, when sequenced, confirmed the involvement of EHV-5.

Pulmonary interstitial fibrosis (PIF) is an uncommon condition in horses, which has been reported in silicate pneumoconiosis, chronic manifestation of interstitial pneumonia (with various potential infectious or non-infectious aetiologies) or as an idiopathic condition (Schwartz and others 1981, Buergelt and others 1986, Donaldson and others 1998, Wilkins 2003). It is likely, however, that cases of EMPF have previously been under-reported due to the paucity of evidence of a viral aetiology (Williams and others 2007). The association between gammaherpesvirus infection and PIF has also been postulated in human beings (with Epstein-Barr virus) and in murine models of pulmonary fibrosis (with murid herpesvirus type 4, also known as MHV-68) (Stewart and others 1999, Kelly and others 2002, Lok and others 2002, Doran and Egan 2005, Mora and others 2005). Repeatedly injured type II pneumocytes and macrophages are a source of transforming growth factor β (TGF-β), which induces interstitial fibrosis (Caswell and Williams 2007). In murine models in which the cytokine response is biased towards a T helper 2 response, MHV-68 infection was associated with increased TGF-β production and PIF (Mora and others 2005); furthermore, it has been shown that latently infected alveolar epithelial cells produce higher levels of TGF-β in vitro, leading to increased production of the chemokine CCL2 , which promotes collagen synthesis in fibrocytes (McMillan and others 2008,

Veterinary Record doi: 10.1136/vr.d4348

T. Soare, DVM, MSc, PhD,
G. Leeming, BVetMed, MPhil, PhD, DipRCPath, MRCVS,
R. Papoula-Pereira, LVM, MRCVS,
A. Kipar, DrMedVetHabil, DiplECVP, FTAPathologie, MRCVS,
U. Hetzel, DrRerNat, DrMedVet, FTAPathologie, MRCVS,
Veterinary Pathology, University of Liverpool, School of Veterinary Science, Crown Street, Liverpool L69 7ZJ, UK
R. Morgan, MA, VetMB, MRCVS, Philip Leverhulme Equine Hospital,
J. P. Stewart, PhD, National Centre for Zoonosis Research, University of Liverpool, Leahurst, Chester High Road, Neston, Cheshire CH64 7TE, UK

Dr Soare is also at the University of Agriculture Sciences and Veterinary Medicine Bucharest, Bucharest, Romania

Correspondence to Dr. Gail Leeming, e-mail: gail.leeming@liverpool.ac.uk

Provenance: not commissioned; externally peer reviewed

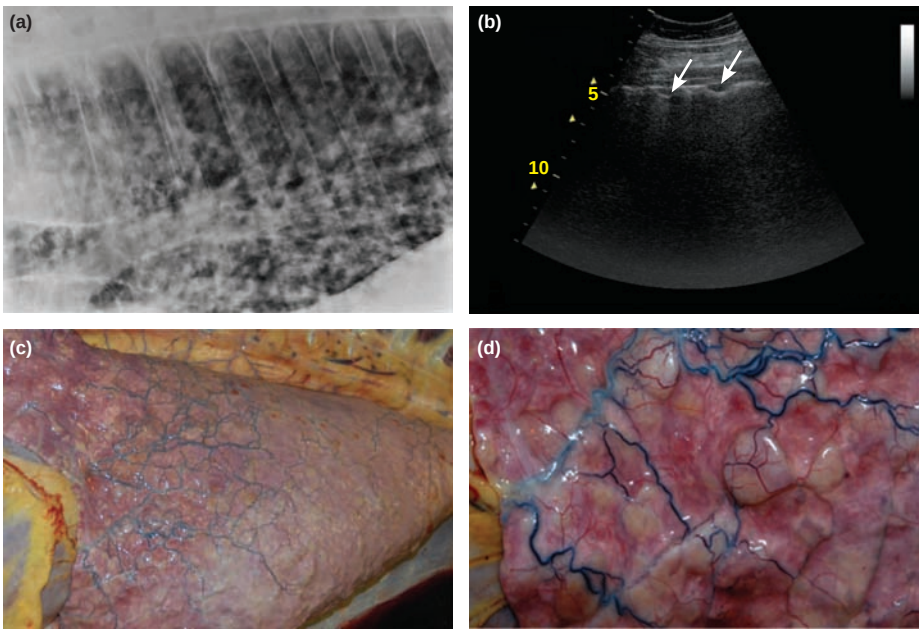

FIG 1: (a) Lateral radiograph of the caudodorsal lung field of a horse, showing a nodular interstitial pattern. (b) Sonographic image of the thorax, showing disruption of the pleural surface. Semicircular hypoechoic regions along the pleural surface correspond to the surface nodules (arrows). Comet tail artefacts are also present. (c) Left lung, showing multifocal to coalescing, well demarcated white to pale tan nodules, with moderate subpleural fibrosis and congestion. (d) Enlargement of part of (c), showing nodular fibrosis

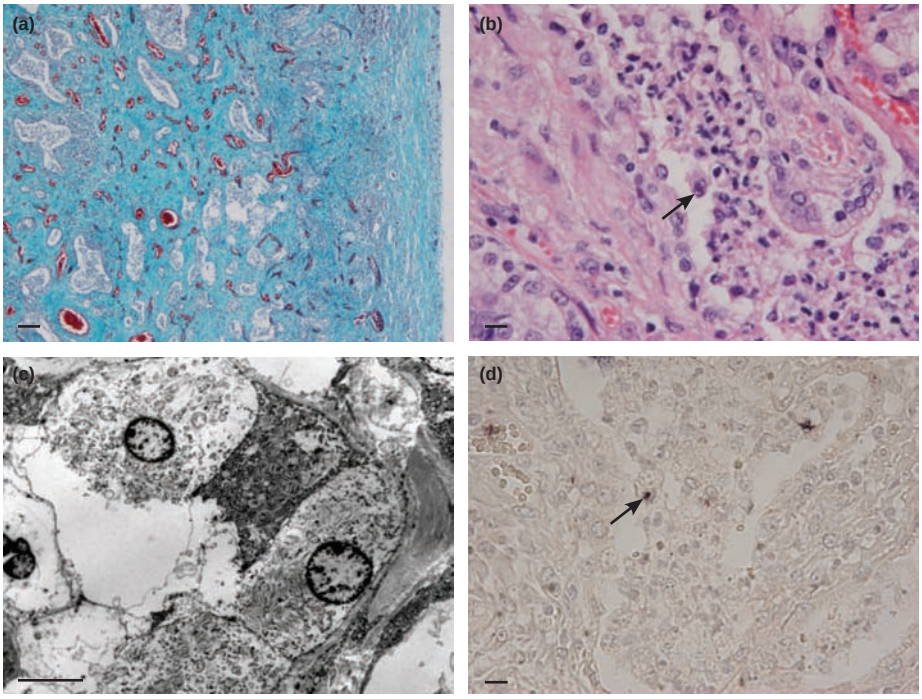

FIG 2: (a) Lung of a horse, showing a focal area of severe interstitial fibrosis. The remaining alveolar spaces are lined by cuboidal epithelium and contain cells and cellular debris. In addition, there is a lymphocyte-dominated infiltrate and moderate diffuse hyperaemia. Gomori's trichrome. Bar=40 μm. (b) Alveolus lined by cuboidal type II pneumocytes within a fibrous connective tissue stroma. A macrophage within the alveolar lumen exhibits an intranuclear inclusion body (arrow). Haematoxylin and eosin, Bar=10 μm. (c) Ultrastructure of hypertrophic cuboidal type II pneumocytes, lining the alveolar lumen. The cells exhibit intracytoplasmic lamellar bodies, typical of type II pneumocytes. Bar=10 μm. (d) In situ hybridisation for EHV-5 DNA, which shows a positive signal within the nucleus of a cell within the alveolar lumen (arrow). BCIP/NBT detection of probe, with Papanicolau's haematoxylin counterstain. Bar=10 μm

Vannella and others 2010). These studies further support the causal link between gammaherpesvirus infection and PIF. However, these findings do not explain the type II pneumocyte hyperplasia seen in EMPF and why gammaherpesviruses, which are associated with a high prevalence in the form of latent infection in their natural hosts, cause disease only in relatively few individuals. Generally, herpesvirus infection in the natural host is associated with low morbidity; serious disease is seen when an alternative species is infected, for example, malignant catarrhal fever of cattle caused by ovine herpesvirus type 2 (Russell and others 2009). In addition, it should be considered that *Mus musculus*, the species of origin of laboratory mice, is not a natural host for MHV-68 (Ehlers and others 2007). Infection of MHV-68 in a natural host, the wood mouse (*Apodemus sylvaticus*), is associated with lower viral titres than in laboratory mice (Hughes and others 2010). Therefore, the outcome of MHV-68 infection in laboratory mice should be interpreted with caution, when inferring parallels with other natural gammaherpesvirus infections. In short, further investigation into the pathogenesis of pulmonary fibrosis and its potential association with gammaherpesviruses is required.

This is the first report of EMPF in the UK, a disease that is being increasingly recognised and further diagnoses of which are likely to be made in the future. EMPF should be considered in equine cases of respiratory disease with a history of decreased appetite, weight loss, cough, tachypnoea, respiratory distress and persistent pyrexia. The striking radiographic (Williams and Wilkins 2009) and ultrasonographic appearance (Fig 1a, b) are the most prominent diagnostic features.

Acknowledgements

The authors wish to thank their colleagues in the Histology Laboratory and the Electron Microscopy Unit, Veterinary Laboratory Services, University of Liverpool, for excellent technical support and Dr. Andrew Davison, MRC-University of Glasgow Centre for Virus Research, who kindly provided EHV-5 DNA. TS was financially supported by a scholarship from the Agency for Credits and Studies, Romanian Ministry of Education, Research and Innovation. RM's Senior Clinical Training Scholarship in Equine Internal Medicine is supported by The Horse Trust.

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Veterinary Record published online August 31, 2011
doi: 10.1136/vr.d4348