Clinical Commentary

Equine multinodular pulmonary fibrosis: Diagnosis and treatment

P. A. Wilkins
College of Veterinary Medicine, University of Illinois, Champaign-Urbana, Illinois, USA.
Corresponding author email: pawilkin@illinois.edu

Equine multinodular pulmonary fibrosis (EMPF) is probably not a completely new disease, appearing from nowhere in the mid-1990s, but rather a newly recognised subset of the general category of interstitial pulmonary fibrosis in horses (Wilkins 2008). It may very well be an emerging disease but recognition has increased the number of new case reports in the literature. Interstitial fibrosis with or without a granulomatous component has been described for years in the equine veterinary literature, attributed to a variety of causes, but most generally labelled ‘idiopathic interstitial fibrosis’ or ‘idiopathic granulomatous pneumonia’ (Buergelt et al. 1986; Winder et al. 1988; Berry et al. 1991; Donaldson et al. 1998; Davis et al. 2001; Pusterla et al. 2003; Pavlik et al. 2004; Schmidbauer et al. 2004; Van den Boom et al. 2008).

Equine multinodular pulmonary fibrosis is a progressive fibrosing lung disease of horses that has become associated with the presence of an equine γ-herpes virus, EHV-5 (Ainsworth 2012; Dunkel 2012). To date, the majority of cases reported in the literature have originated in the USA (Williams et al. 2007; Hart et al. 2008; Wong et al. 2008; Belgrave 2009; Kubiski et al. 2009) but the disease has a wider geographic distribution with cases reported in Sweden (Back et al. 2012), Italy (Marenzoni et al. 2011), Germany (Poth et al. 2009; Niedermaier et al. 2010; Lehmebeer et al. 2011), the UK (Soare et al. 2011), Belgium (Verryken et al. 2010) and Austria (Poth et al. 2009; Schwarz et al. 2012). First described by Williams et al. in 2007, primarily reporting the pathology and genus specific PCR findings in 27 cases gathered from multiple USA sources over 20 years, the first clinical report appeared in 2008 (Wong et al. 2008). That clinical report described 7 horses that had been diagnosed ante mortem with EMPF, 5 treated aggressively with supportive care and some potentially targeted therapeutic agents. As with many ‘new’ findings, these first 2 reports were the result of curious individuals (Kurt Williams, Pamela Wilkins and Fabio Del PIERO) being in the same place (Cornell University) at the same time (mid-1990s) and asking the same questions, continuing their conversations regarding their interests over time and changing locations, finally sharing their thoughts and discoveries with others along the way. In effect, these manuscripts resulted from overlapping interests in individuals with different specialties, areas of expertise and approaches to the problem.

In order for EHV-5 to be identified clearly as a cause of EMPF, it is desirable that Koch’s postulates be fulfilled. EHV-5 is ubiquitous and infects many horses but apparently results in EMPF in only a few (Torfason et al. 2008; Fortier et al. 2009, 2010; Marenzoni et al. 2010). No results of any such study have been reported at the time of writing. However, preliminary data have clearly demonstrated that experimental infection with EHV-5 will result in pulmonary fibrosis in horses (K. Williams, personal communication, 2013). EHV-5 is incredibly slow to grow in culture, requiring 4 passages for the cytopathic effect to be reliably observed, making infection and antiviral sensitivity testing studies particularly challenging (Fortier et al. 2010; Marenzoni et al. 2010). Additionally, there is increasing evidence that EHV-5 presents fairly large antigenic, and potentially genetic, diversity, possibly further complicating diagnosis (Ataseven et al. 2010; Fortier et al. 2010; Thorsteinsdóttir et al. 2010). The glycoprotein gD had been used as an antigenic target for EHV-1; similarly, the primary antigenic target used in identifying EHV-5 has been the gB glycoprotein (Fortier et al. 2010). Currently, while support for EHV-5 as a cause of EMPF is quite strong, the role of EHV-5 in EMPF remains somewhat controversial. In addition, γ-herpes viruses are lymphotropic viruses and have been associated with lymphoproliferative diseases and lymphoma in horses (Barton et al. 2011; Vander Werf et al. 2011; Dunkel 2012; Schwarz et al. 2012; VanderWerf and Davis 2013). Gamma-herpes viruses usually establish latency in B lymphocytes in an apparently complex relationship between virus latency and stage of B cell differentiation. Interestingly, reports of EHV-5 association with lymphoproliferative disease simultaneously with EMPF exist (Dunkel 2012; Schwarz et al. 2012).

The treatment of EMPF described in the Wong report of 2008 was based on the combined clinical experience and knowledge of the individuals involved in treating those cases. All 5 of the treated reported cases occurred quite close temporally, within 18 months, and were gathered due to the clinicians involved knowing each other and discussing the cases together. Since apparently successful treatment of EMPF with valacyclovir is reported in this issue by Schwarz et al. (2013), the remainder of this commentary will focus on recognition and treatment.

Diagnosis of EMPF

In the USA, the majority of horses diagnosed with EMPF have been older Thoroughbreds while in Europe Warmblood breeds appear to be over-represented. However, horses of many other breeds have been diagnosed with EMPF. Similar to human idiopathic pulmonary fibrosis – also thought to be associated with γ-herpes virus infection (Doran and Egan 2005; Nalysnyk et al. 2012) – EMPF appears to be a disease of the middle-aged to older individual, although the diagnosis has been made in horses as young as 2 years (Kubiski et al. 2009; Marenzoni et al. 2010; Nalysnyk et al. 2012). Abnormal physical examination findings of horses with EMPF include variable hyperthermia, weight loss, tachypnoea and tachycardia. Thoracic auscultation is usually abnormal with wheezes and crackles commonly reported both with and without rebreathing examination. Affected horses have generally been treated for fevers or infectious bronchopneumonia with little to no improvement and progression of clinical signs prior to receiving a diagnosis of EMPF. Commonly reported clinical
pathology findings in EMPF include hyperfibrinogenaemia, leucocytosis, neutrophilia, monocytosis, anaemia and hypoxaemia, particularly in cases that have had diagnosis delayed.

In evaluating these cases clinically, recurrent airway obstruction (RAO, or summer pasture associated obstructive pulmonary disease) needs to be effectively ruled out. The clinician should determine if reversible bronchospasm is contributing to the observed clinical signs of tachypnoea, dyspnoea and, in some cases, significant hypoxaemia. In horses with RAO or summer pasture associated obstructive pulmonary disease, bronchodilator administration significantly improves the horse’s breathing effort and pattern within 20–30 min of administration, while horses with EMPF show little improvement.

Transtracheal aspirates and/or bronchoalveolar lavage fluid (BALF) samples including cytological and microbiological analyses are important to rule out fungal, bacterial and silicate-induced granulomatous interstitial lung disease. The large majority of samples from EMPF cases are characterised by a predominance of nondegenerate neutrophils with fewer macrophages and lymphocytes. While this cytological pattern may also be consistent with a diagnosis of RAO or inflammatory airway disease, the bronchodilator trial should make it simple to rule these diseases out. Bacterial or fungal organisms should be rare and not identified either intra- or extracellulary in EMPF. Eosinophilic intranuclear inclusion bodies may be found in macrophages in BALF, suggesting a viral aetiology; it carefully examined. BALF samples from EMPF cases are almost uniformly polymerase chain reaction (PCR) positive for EHV-5. PCR assay for EHV-5 is first reported by Wong in 2008, originating from the observation of the eosinophilic inclusion bodies in macrophages and the knowledge that similar macrophages could be found within alveolar spaces in lung biopsy samples, suggesting that BALF would be superior to transtracheal aspirate for obtaining diagnostic samples. Testing of transtracheal aspirates and/or BALF from 661 horses with respiratory disease revealed that ~2.5% of 785 samples obtained were positive for EHV-5 by PCR (Fortier et al. 2009). BALF PCR testing appears to be commonly used and is featured in many of the newer EMPF case reports; quantitative testing is the next logical step (Kubiski et al. 2009).

Imaging studies of the thoracic cavity are also helpful in establishing a diagnosis of EMPF. Radiographic findings are fairly typical and reveal severe, diffuse, nodular interstitial pattern that is either uniformly distributed or evident primarily in the mid-ventral to crano-ventral lung lobes. Multiple radiographic views may be necessary to adequately demonstrate the presence of nodules. Repeated radiographs have been obtained prior to definitive EMPF diagnosis and treatment in some cases, such as the case reported in this issue by Schwarz et al. (2013), and may show progression of an interstitial pattern to a more nodular pattern. The typical radiographic changes rule-out recurrent airway disease but not the other causes of nodular interstitial lung disease. Ultrasoundographic examination reveals bilateral, diffuse roughening of the pleural surface and, as the disease progresses, the existence of multiple superficial discrete nodules of varying size. Both radiographic and ultrasonographic changes appear to be progressive, worsening until diagnosis is made and treatment is instituted.

Successful treatment has resulted in complete resolution of radiographic changes in many cases.

Ante mortem confirmation of EMPF is dependent upon histopathological evaluation of lung sections obtained by percutaneous, ultrasound-guided biopsy procedures. This can be challenging in tachypnoeic horses, potentially increasing the risk of lung laceration and pulmonary haemorrhage, and sedation is generally recommended. Sampling of ultrasonographically identified nodular areas superficially located in the dorsal lung feld is preferred in order to minimise complications. The author prefers to use a spring-loaded biopsy instrument for rapid retrieval of the samples, although airway haemorrhage and epistaxis are still a risk (Venner et al. 2004). Several lung biopsies are obtained unless pulmonary haemorrhage or other complications develop. Samples are submitted for histopathological evaluation of formalin-fixed tissues stained with haematoxylin and eosin; Masson trichrome and Wright-Giemsa; microbial culture/sensitivity; and PCR assay for EHV-5. Histopathology results and positive EHV-5 PCR on lung tissue are currently the ‘gold standard’ diagnostic procedures for EMPF. The horse should be observed for at least 30 min following the procedure to make certain that no secondary complications (haemothorax) have occurred. A small amount of epistaxis may be recognised once the horse lowers its head but important complications of biopsy have yet to be reported when performed for EMPF diagnosis, perhaps due to the solid mass effect of the nodular lesions being biopsied. A 5-day course of a broad spectrum antimicrobial such as oral trimethoprim sulfa (30 mg/kg bwt per os q. 12 h) is recommended unless another antimicrobial (such as beta-lactams, see below) is preferred. The use of a broad-spectrum macrolide (10–40 mg/kg bwt dissolved in 1 litre 0.9% NaCl) administered over a 20–30 min period has been recommended if significant pulmonary haemorrhage occurs (Ainsworth 2012).

**Treatment of EMPF**

Based upon our current limited experience, the diagnosis of EMPF is associated with a fair to poor prognosis. Of the treated cases reported in the literature, about 50% have responded to therapy as evidenced by an improvement in demeanour, weight gain and radiographic changes, or have self-cured. Anecdotally, the ‘cure’ rate might be a bit higher. It also appears that the response is improved in horses diagnosed early in the clinical course. Of course, there remains the possibility of self-cure! Much work remains to be done regarding both confirming the EHV-5 aetiology and defining appropriate treatment.

In the report described by Wong et al. (2008), 2 of 5 treated horses survived and resumed regular training schedules 6 and 10 months after initial presentation. Those horses were the most mildly affected, displaying mild tachypnoea (22–24 breaths/min). Initially the authors recommended treating horses with a diagnosis of EMPF for at least 6 weeks, based on clinical experience by the authors with other earlier unreported cases, to provide adequate time for the anti-inflammatory and antiviral therapy to work. The initial suggested treatment regimen included the administration of dexamethasone (0.08–0.1 mg/kg bwt i.v. q. 24–48 h); doxycycline (5–10 mg/kg bwt per os q. 12–24 h); and acyclovir (20 mg/kg bwt per os q. 6–8 h).

Corticosteroids are advocated to suppress the pulmonary inflammation by inhibiting the synthesis of inflammatory cytokines and mediators that promote ongoing fibrosis and
cellular infiltration (Montón et al. 1999; Shafiq et al. 2013; Polverino et al. 2013). The role of corticosteroids as an adjunctive therapy for EMPF is still under debate, the potential benefit based on the rationale of the anti-inflammatory effects of steroids and the potential consequent influence on disease severity and outcomes. Indeed, it has been shown that the administration of systemic corticosteroids is associated with reduced pulmonary inflammation in human patients with bacterial pneumonia (Montón et al. 1999). A recent meta-analysis of 8 randomised controlled studies (RCTs) of community acquired pneumonia (Shafiq et al. 2013), found no significant association between steroid therapy and their primary outcome of interest (hospital mortality). However, length of hospital stay was significantly shorter in the steroid group. Although adverse effects of steroid therapy were not consistently reported, most RCTs reported that hyperglycaemia was either no more common in the steroid group or did not require additional treatment. All RCTs in the most recent meta-analysis were characterised by steroid doses under 2 mg/kg b.wt/day of methylprednisolone or equivalent. This meta-analysis is also the first to demonstrate decreased length of hospital stay in hospitalised human patients with community acquired pneumonia with each of the 5 RCT studies that reported hospital stay showed the same trend. In veterinary medicine, steroid use at anti-inflammatory but not immunosuppressive doses, has been shown to importantly improve outcome in foals with acute respiratory distress syndrome/acute lung injury (Lakritz et al. 1993; Dunkel et al. 2005). However, immunosuppressant steroids may cause recrudescence of viral infections and increase the horse’s susceptibility to other infectious diseases. The dosage of any corticosteroid used in the treatment of EMPF should probably be decreased by one-third to one-half after 7–10 days of treatment or sooner if complications develop. A more current typical corticosteroid use would be to administer a dose of 0.1 mg/kg b.wt i.v. q. 36 h for 5 treatments before decreasing the dosage. Prednisolone can also be used (after a course of dexamethasone) starting at doses of 1–2 mg/kg b.wt per q. 24 h.

Doxycycline is administered as both an antimicrobial and a potential anti-inflammatory because potential benefit has been found in murine models of pulmonary disease (Rempe et al. 2007). Doxycycline interacts with bound zinc or calcium ions required for metalloproteinase activities and, as these enzymes are prevalent in pulmonary inflammatory conditions, doxycycline may reduce their activities.

The use of acyclovir or valacyclovir (an acyclovir prodrug with greater bioavailability when administered orally) is speculative, based on studies evaluating efficacy against EHV-1 in clinical studies ongoing at the time the initial reported cases of EMPF were being studied, although a targeted antiviral approach would seem to be beneficial (Wilkins et al. 2003; Wilkins 2004; Henninger et al. 2007; Wong et al. 2010). Although the pathogenic role of EHV-5 remains to be fully proven – and the susceptibility of equine γ-herpes viruses to acyclovir is unknown – the Epstein-Barr virus, a human γ-herpes virus, is reportedly susceptible (Billaud et al. 2009).

Valacyclovir is a prodrug of acyclovir – altered by linking acyclovir to L-valine by an ester – that exhibits ~60% bioavailability in the horse when administered at a dose of 30 mg/kg b.wt q. 8 h and is preferred if treatment is limited to the oral route (Garré et al. 2007, 2009; Maxwell et al. 2008). Valacyclovir is currently not available as a generic drug and is quite expensive to use, as seen in the case reported in this current issue (Schwarz et al. 2013). Acyclovir is available as a generic drug and may safely be administered intravenously at a cost effective dose. One of the earliest studies using acyclovir in horses suggests that, while acyclovir is minimally bioavailable orally, it has a very long half-life and may in fact ‘stack’ with repetitive doses (Wilkins et al. 2003, 2005; Wilkins 2004; Bentz et al. 2006; Henninger et al. 2007). One approach used by the author is to initiate treatment with i.v. acyclovir (10 mg/kg b.wt i.v. diluted in 1 litre of isotonic crystalloid fluid administered at a constant rate over 1 h b.i.d. for 1–2 days followed by oral treatment at 30 mg/kg b.wt i.d. q. 24 h). The effectiveness of either antiviral drug has not been demonstrated against EHV-5 in vitro; however, anecdotal response to treatment has convinced several clinicians to continue to pursue this treatment if financially feasible.

Additional treatment considerations include administration of i.v. fluids if the horse is dehydrated and moving the horse to a cool well-ventilated environment, particularly during hot, humid summer months. Other medications, including nonsteroidal anti-inflammatory medications, can be used for pain and fever management. Intranasal insufflation of humidified oxygen (10–15 l/min) is indicated in hypoxaemic horses.

Diagnosis of EMPF has progressed to a fairly straightforward diagnostic protocol since its first description less than 7 years ago. Treatment of the condition is evolving and, while advanced cases appear refractory to treatment, earlier recognition and treatment seems to improve outcome. We expect treatment to change as more is known about the underlying pathology and what can be done to eliminate the pathogen and ameliorate the damage.

Author’s declaration of interests
No conflicts of interest have been declared.

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