Pathological Studies on a mini outbreak of Pleuritis/Pleuropneumonia in a group of Horses associated with infection by *Mycoplasma felis*.

By

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**SUMMARY**

Three horses in a group of thirteen horses from one farm had acute onset of respiratory distress, fever, and anorexia. All treated with antibiotics, intravenous fluids, and oxygen with no significant improvement. Due to poor prognosis, all horses were euthanized six days after the appearance of the clinical signs and were submitted for necropsy. The characteristic lesions in all horses were marked bilateral diffuse fibrinous pleuritis. Multifocal areas of lung necrosis with mild to moderate fibronsuppurative bronchopneumonia were present in 2 horses. *Mycoplasma felis* was the sole organism isolated and identified from the lungs of these horses. The importance of *Mycoplasma felis* as an equine respiratory pathogen was discussed.

**INTRODUCTION**

*Mycoplasma felis* (*M. felis*) is considered to be a normal flora of feline respiratory tract, however, several natural cases of conjunctivitis and arthritis in cats were caused by the infection with *M. felis* (*Haesebrouck et al. 1991, Randolphi et al. 1993, Chalker et al. 2004, Leihmann et al. 2006, Caswell and Williams 2007*). In Human, *M. felis* induced arthritis in an immunodeficient woman after been exposed to a cat (*Bonilla et al. 1997*).

The role of *Mycoplasma spp.* in respiratory infections, in species other than bovine and porcine, has been a source of debate. In Equine, *M. felis* is considered as an uncommon etiology for equine pleuritis and lower respiratory disease, however, it has been isolated from the upper respiratory tract of clinically normal horses (*Ogilvie et al. 1983, Rosendal et al. 1986, Hoffman et al. 1992, Wood et al. 1997, Caswell and Williams 2007*). The role of *M. felis* in inducing pleuritis was confirmed when acute fibrinous pleuritis was reproduced experimentally in a pony (*Ogilvie et al. 1983*). Pleuritis is an important and relatively common pathologic condition of the lower respiratory system of horses particularly after transportation of young adult horses (*Sprayberry and Byras 1999, Caswell and Williams 2007*).
Equine pleuritis was described secondary to bronchopneumonia (pleuropneumonia), in association with bacterial septicemia (e.g. *Actinobacillus equuli*, and *Streptococcus zooepidemicus*), secondary to ruptured chest or mediastinal abscess, in association with aspiration pneumonia and following a perforated rib fracture (Byras and Becht, 1991, Chaffin and Carter 1993, Chaffin et al. 1994, Kollias-baker and Johnson 1999, Sprayberry and Byras 1999, Caswell and Williams 2007).

Described here, the clinical findings and the pathologic changes present in 3 horses from one farm that had *M. felis*-associated pleuritis.

**MATERIALS AND METHODS**

**Clinical History and necropsy:**
In December 2005, three horses (Table 1) in a group of 13 horses at one farm had acute onset of respiratory distress, fever, and anorexia. The referring veterinarian made an initial diagnosis of pleuropneumonia and all affected horses were treated with antibiotics, intravenous fluids, and oxygen. The exact information about the type and doses of these treatments were not available. The horses responded poorly to the treatment, their clinical condition got worse and progressed to recumbence. Euthanasia was elected six days after the appearance of the first clinical signs and euthanized horses were sent for Postmortem examination.

Table (1): Clinical history of the studied horses.

<table>
<thead>
<tr>
<th>Horse/Breed</th>
<th>Age</th>
<th>Sex</th>
<th>General health status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse 1/Standbred</td>
<td>34 months</td>
<td>Female</td>
<td>Healthy and vaccinations are up to date.</td>
</tr>
<tr>
<td>Horse 2/Standbred</td>
<td>33 months</td>
<td>Female</td>
<td>Healthy and vaccinations are up to date.</td>
</tr>
<tr>
<td>Horse 3/Standbred</td>
<td>21 months</td>
<td>Male</td>
<td>Generally healthy but had intermitted rhinitis especially in the fall season.</td>
</tr>
</tbody>
</table>

**Pathology:**
Signalment, history and gross lesions were recorded. Selected tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 3–5 µm, and stained with hematoxylin and eosin (H & E). Few lung sections were
stained with Gram stain to demonstrate any possible bacteria.

**Bacteriology:**

At necropsy, specimens from lung and/or pleural exudates were collected aseptically and sent for routine bacterial cultures. Routine aerobic and anaerobic cultures were done on all collected samples based on standard operation protocols (SOP) used by the Microbiology laboratory in the Animal Health laboratory, University of Guelph, Guelph, Ontario, Canada.

**Virology:**

Samples from lung and pleura were sent to the virology laboratory in the Animal Health laboratory, University of Guelph, Guelph, Ontario, Canada. These samples were investigated for the presence of viruses by inoculation into tissue culture (Rabbit kidney cells (RK13) and Madin-Darby canine kidney (MDK)) and eggs (9- to 11-day-old embryonated chicken egg) (Peek et al., 2004, Hornyak et al., 2006). Cytopathic effects were monitored. Using the manufacturers protocols, standard immunofluorescence (IF) test was performed using a rabbit polyclonal antiserum against Equine herpes virus 1 and 4 (EHV-1 and EHV-4), Equine arteritis virus, and Equine influenza virus. (Chemical and kits purchased from Kirk-ergaard & Perry Laboratories Inc., Maryland, MD, USA, and KIO-chemicals, Guelph, ONT, Canada).

**Mycoplasmolology:**

For *M. felis* isolation, lung samples were immersed in a tube containing 2 ml modified SP4 medium (SP4-II) and incubated with agitation at 37 C for 1 day and then followed by subculture onto solid tryptic soy medium with 5% sheep blood (Ramirez et al., 1997). Presumptive *M. felis* colonies appeared in 3-7 days post-inoculation. Confirmation of *M. felis* to the species level is then achieved by Qualitative real time PCR (Chalker et al., 2004, Larry et al., 2005).

**RESULTS**

**Microbiology:**

Other than 2 colonies of *non-hemolytic Escherichia coli* grown from the lung of horse 2, no significant bacteria or viruses were isolated or identified from the lungs of all examined horses. High numbers of *Mycoplasma* colonies were detected from the lung specimens of all the three horses 3-7 days post inoculation and were identified as *M. felis* using Qualitative real time PCR.

**Gross Pathology:**

The most characteristic lesions in all horses were present in the lungs and the pleura. These lesions are described below in details. Unless otherwise described, no signifi-
ciant lesions were present on sections examined from liver, bronchial lymph nodes, kidneys, spleen, gastrointestinal tract, and heart.

**Horse 1**: There was marked subcutaneous edema of the ventral body wall. There was diffuse bilateral pleural thickening. The affected pleura appeared opaque, edematous, had few acute fibrin tags, and was mildly to moderately thicker (Fig. 1). There was mature adhesion between the pleura and pericardium. The lesions in the lung were bilateral, predominantly the cranio-ventral parts, with the left side two third affected and the right side one third affected. The affected lung areas were firm and congested, and there were multiple areas of consolidation that can be palpated. On cut section there were alternating areas of necrosis, edema and hemorrhage. Necrotic areas had mild firmer consistency but they were almost in the same surface level of the lung (Fig. 2). Two sequestra (2-2.5 cm) were present in the right caudal lung lobe and appeared as dry inspissiated necrotic material within a cystic fibrotic wall.

**Horse 2**: The pleura on the right and left lung was yellow, diffusely thickened, edematous, and had a gelatinous consistency. On cut section, the pleura was five times (1.5-2 cm thick), its normal size and composed of yellow gelatinous fibrinous material. The pleura was strongly adherent to the underlying lung. Affecting predominantly the cranio-ventral parts of the right and left lungs, there were random multifocal areas of necrosis (0.1 to 0.8 cm) that appeared paler and firmer than adjacent normal parenchyma and each necrotic area surrounded by a 0.1 cm fibrous capsule. The bronchial lymph nodes were markedly edematous.

**Horse 3**: Approximately half liter of serosanginous fluid was present in the thoracic cavity. Pleura in both sides were diffusely thicker, appeared glistening, and covered with yellow gelatinous fluid and large amount of fibrin mats. The pleura adhered to the chest wall with multiple fibrin tags. On cut section, the pleura was pale yellow, 1 cm in thickness, and was filled with yellow gelatinous exudates. The lung itself had no significant lesions. The bronchial lymph nodes were markedly enlarged and edematous and on cut sections they oozed a clear serous fluid.

**Histopathology:**

Significant histologic lesions were present only on the lungs of the examined horses. The histologic lesions present on horses 1 and 2 were almost similar and they will be described together. No bacteria were present when some selected
lung sections from all horses stained with Gram stain.

**Horses 1 and 2:** The pleura was markedly thickened (1-2 cm) with edema, fibrous tissue and mild infiltration of macrophages, lymphocytes, and plasma cells (Figs. 3 and 4). At some areas of the pleura, there was acute fibrin deposition associated with few degenerate neutrophils. The interlobular septa were very prominent and thickened with deposition of fibrin, edema, lymphatic emboli, and mild to moderate infiltration of mononuclear cells and fewer neutrophils. The lung had numerous multifocal random necrotic areas composed of basophilic and eosinophilic necrotic debris in the center and surrounded by a layer of inflammatory cells composed mainly of macrophages that had streaming nuclei (oat cells), neutrophils and fewer lymphocytes (Fig. 5). The alveoli were mildly to moderately filled with degenerate neutrophils, macrophages, and fibrin. The interalveolar tissue was mildly thickened by edema, few fibrin and neutrophils. There was mild pneumocytes type II proliferation with mobilization of alveolar macrophages. There was mild perivascular edema and cuffing with few lymphocytes and plasma cells. The small bronchioles had moderately hyperplastic epithelium with multifocal areas of attenuation and erosions and mild peribronchiolar lympho-plasmacytic cuffing. The subcapsular and medullary sinuses of bronchial lymph nodes were markedly edematous and were mildly filled with small lymphocytes, plasma cells, and few macrophages.
Fig. 1: Lung: Pleura is thickened (arrow), inflamed (A), and is covered by thick fibrin mats (B). Lung has multifocal areas of necrosis (arrow head).

Fig. 2: Lung has multifocal necrotic areas that are lined by a white zone (arrow heads).
Fig. 3: The lung is covered by very thick pleura (arrow). Bar = 0.2 cm.

Fig. 4: Higher magnification of Fig. 3. The pleura is markedly thickened by fibrous tissue and pleocellular exudates (A). The alveoli have mildly thickened wall but their lumens are almost devoid of inflammatory cells (B). Bar= 500 microns.
DISCUSSION

In this study, *M. felis* was the only pathogen identified and isolated from the lungs of three horses suffered from pleuritis and acute respiratory distress. The gross and histologic lesions were those of severe pulmonary fibrosis to fibrinopurpurative pleuropneumonia occasionally with presence of multifocal areas of lung necrosis. Previous reports identified *M. felis* as the cause of pleuritis and pneumothorax in many horses, however, the gross and histologic lesions of the lung in these cases were not described (Ogilvie et al., 1983; Hoffman, et al. 1992; Wood et al., 1997). To the best of the author knowledge, this is the first paper describe the gross and histologic lesions associated with natural *M. felis* infection in horses. On the other hand, the histopathology of experimental *M. felis* infection was described in one pony, wherein, the intrapleural inoculation of *M. felis* led to severe acute pleuritis (Ogilvie et al., 1983).

Pleuritis with or without pneumonia (pleuropneumonia) is a common respiratory disease in young adult horses particularly after transportation, viral infection, general anesthesia, and stress (Raphel and Beech 1982, Sweeney 2002, Caswell and Williams 2007). Transportation and general anesthesia are
thought to cause aspiration pneumonia followed by formation of an abscess which might ruptures and lead to pleuritis and pyothorax (Raphel and Beech, 1982; Sweeney, 2002). Stress factors such as cold, intensive exercise and transportation affect the immunity of the host making the lung more vulnerable for infection (Horohov et al., 1999; Ishizaki and Kariya, 1999; Folsom et al., 2001). Respiratory viruses destroy the mucosal barriers and predispose the lung for opportunistic infection (Caswell and Williams, 2007). In this outbreak, the current horses exposed to two days of extreme cold weather four days before the clinical signs appear. Although pleuritis was reproduced clinically by the inoculation of *M. felis* into a pony’s lung, the exact pathogenesis of *M. felis* pleuritis is not completely understood. The isolation of *M. felis* from clinically normal horses and the occurrence of most natural cases after natural stress time (e.g. cold or transportation), suggests that *M. felis* is an opportunistic pathogen that occasionally become pathogenic at times of immunosuppression (e.g. after viral infection or at time of stress).

Pathogenic microorganisms reach the pleura mainly either through blood (hematogenous route), direct extension from the lung, or due penetration wound to the chest wall (Caswell and Williams, 2007). How *M. felis* reach the pleura or become more pathogenic is not clear. Other Mycoplasma species such as *Mycoplasma hyopneumoniae* or *hyorhinis* in swine and *Mycoplasma mycoides subspecies mycoides small colony type* in bovine are considered to be a highly pathogenic primary organisms that solely can induce severe lower respiratory disease and pleuritis (McGavin et al., 2001; Caswell and Williams 2007). The virulence factors and pathogenesis of these species were extensively studied and presumptively *M. felis* might have similar virulence factors. Examples for the virulence factors of pathogenic Mycoplasmas include induction of ciliostasis and loss of cilia (DeBey and Ross, 1994; Caswell and Williams, 2007), stimulation of lymphocytes apoptosis (Caswell and Williams, 2007) and modulation of proinflammatory cytokines (Thanawongnuwech and Thacker, 2003; Muneta et al., 2006). Whether *M. felis* has all of these factors or some of them, is yet to be determined.

It is not clear if *M. felis* is the primary pathogen in natural equine pleuritis/pleuropneumonia cases or it must be preceded or associated with bacterial or viral infection. Several bacteria were isolated from natural cases of equine pleuritis and pleuroneumonia (Raphel and Beech, 1982; Sweeney, 2002; Ca-
swell and Williams, 2007). Failure to grow bacteria from some cases including the current outbreak may be due to massive treatment with antibiotics. In the current outbreak, no significant bacteria were isolated on culture or identified on special stains, however, the current horses were heavily treated with antibiotics which might precluded the possible bacterial growth. Culture for common equine viruses either on cell culture or on eggs also were negative.

In conclusion, the lesions associated with natural infection with *M. felis* in horses were described for the first time. *M. felis* should be included in the differential diagnosis of any lower respiratory infection particularly if this infection proceeded by stress such as transportation or cold.

REFERENCES


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