

Case Report

Rhodococcus equi pneumonia in an adult horse

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Summary

A 20-year-old, Thoroughbred mare in the fifth month of gestation was examined for weight loss, pyrexia and lethargy. Physical examination, ultrasonography and radiography revealed a severe abscessing pneumonia and a dead fetus. The mare did not respond to symptomatic treatment and died suddenly. Necropsy revealed multifocal pulmonary abscessation.

***Rhodococcus equi* was isolated from the lungs, liver and kidneys. Specific immune function of the mare and presence of the virulence associated protein A (VapA) of the *R. equi* isolated was not determined. It is likely that immunosuppression is required for systemic *R. equi* infections in adult horses; however, it is unknown if VapA is necessary to produce disease in adult horses.**

Introduction

Rhodococcus equi is a Gram-positive pleomorphic rod that is a free-living soil saprophyte with widespread distribution. Horses may come into contact with the organism through ingestion and inhalation. The bacterium can also become a facultative intracellular pathogen within macrophages (Woolcock *et al.* 1980; Zink *et al.* 1986). Phagolysosomes containing virulent *R. equi* are not as acidic as those containing avirulent bacteria. Virulence factors may somehow suppress acidification of phagolysosomes, enabling *R. equi* to survive and replicate in phagocytic cells (Toyooka *et al.* 2005).

Rhodococcus equi is most commonly considered as a cause of pneumonia and occasional enterocolitis in foals at or under weaning age (5–6 months), with most foals showing clinical signs before 4 months of age. Most frequently, clinical disease in foals is caused by chronic suppurative bronchopneumonia with abscessation and lymphadenitis; however, approximately 50% of foals have mesenteric

lymphadenitis and ulcerative mucosal enterocolitis at necropsy (Zink *et al.* 1986). Immune-mediated polysynovitis, most often affecting the tibiotarsal and femorotibial joints is seen in approximately one-third of affected foals (Sweeney *et al.* 1987). *Rhodococcus equi* bacteraemia, usually with concurrent pneumonic or intestinal disease, can be a sporadic cause of septic arthritis or osteomyelitis in foals.

Rhodococcus equi is a rare cause of infection in adult horses, even on farms where clinical disease in foals is endemic (Hines *et al.* 2001). There are few detailed reports of respiratory *R. equi* infections in adult horses (Roberts *et al.* 1980; Genetzky *et al.* 1982; Zink *et al.* 1986; Freestone *et al.* 1987; Vengust *et al.* 2002). Pulmonary or intrathoracic *R. equi* infection led to spontaneous death or euthanasia in all these cases except for one (Zink *et al.* 1986). Most reported cases had at least some clinical signs of respiratory disease such as dyspnoea and pyrexia (Roberts *et al.* 1980; Zink *et al.* 1986; Vengust *et al.* 2002). Other cases had nonspecific clinical signs without overt respiratory disease that included chronic weight loss, lethargy, anorexia and depression (Genetzky *et al.* 1982; Freestone *et al.* 1987). The diagnosis of intrathoracic *R. equi* infection was not made until *post mortem* examination in several cases (Roberts *et al.* 1980; Genetzky *et al.* 1982; Zink *et al.* 1986).

Equine abortion and fetal pneumonia caused by *R. equi* has been reported (Fitzgerald and Yamini 1995; Patterson-Kane *et al.* 2002; Szeredi *et al.* 2006). Foals were aborted from 7–8 months of gestation (Fitzgerald and Yamini 1995; Szeredi *et al.* 2006) to late term (Patterson-Kane *et al.* 2002). Pulmonary pathology in aborted fetuses was similar to that seen in foals affected with *R. equi* pneumonia. The route of fetal infection is speculative because the placenta could not be examined in all cases. However, *R. equi* could be cultured from the lungs and stomach contents of most fetuses, which suggests infection through swallowing of amniotic fluid or inhalation during fetal breathing movements (Fitzgerald and Yamini 1995; Patterson-Kane *et al.* 2002; Szeredi *et al.* 2006). The placenta was available for examination in only one of these cases (Patterson-Kane *et al.* 2002) and histopathological lesions indicated possible haematogenous rather than

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ascending placental infection because of diffuse, suballantoic and largely perivascular distribution of inflammatory cells. The chorioallantoic stroma was also infiltrated by macrophages containing numerous Gram-positive coccobacilli, consistent with *R. equi*.

Case details

History and clinical findings

A 20-year-old Thoroughbred mare in the fifth month of gestation was examined for weight loss, pyrexia and lethargy. The mare had a normal rectal temperature (37.0°C), but heart (88 beats/min) and respiratory (48 breaths/min) rates were elevated. Physical examination revealed pale mucous membranes, increased respiratory effort and bronchovesicular sounds over both sides of the thorax. The mare did not appear to be clinically dehydrated, based on skin turgor and the mucous membranes were moist. Petechial and ecchymotic haemorrhages were not observed on any of the mucous membranes.

Results of a complete blood count revealed anaemia (PCV 0.10 l/l; reference range [rr] 0.30–0.42 l/l), mild leucopenia (5.8×10^9 cells/l; rr 6.0–12.0/ $\times 10^9$ cells/l) and thrombocytopenia (52×10^9 /l; rr 100–500 $\times 10^9$ /l). Differential cell count was characterised as a mild neutrophilia (74% segmented neutrophils; rr 50–70%) with lymphopenia (16% lymphocytes; rr 20–40%) and a slight left shift (4% band neutrophils; rr 0–2%). Platelet count was repeated using a blood sample collected into sodium citrate with similar results. Fibrinogen was slightly elevated at 5.0 g/l (rr 1.0–4.0 g/l). Serum chemistry abnormalities included elevated activities of aspartate aminotransferase (AST) (674 iu/l; rr 80–240 iu/l), alkaline phosphatase (SAP) (497 iu/l; rr 50–150 iu/l), lactate dehydrogenase (LDH) (558 iu/l; rr 52–240 iu/l), sorbitol dehydrogenase (SDH) (900 iu/l; rr 50–250 iu/l) and γ -glutamyltranspeptidase (GGT) (298 iu/l; rr 6–24 iu/l). Hyperbilirubinaemia (total bilirubin 53.0 μ mol/l;



Fig 1: Transthoracic ultrasound image showing pleural roughening and abscessation.



Fig 2: Lateral thoracic radiograph showing extensive abscessation of the lung fields.

rr 13.7–34.2 μ mol/l), hypoproteinaemia (55 g/l; rr 60–76 g/l) and hypoalbuminaemia (20 g/l; rr 24–50 g/l) were also present. Electrolyte abnormalities included hyponatraemia (sodium 121 mmol/l; rr 136–144 mmol/l) and decreased serum bicarbonate concentration (17 mmol/l; rr 21–28 mmol/l).

Ultrasound examination revealed extensive pleural roughening and several large (5–10 cm) hypoechoic areas within both sides of the thorax, consistent with abscessation (**Fig 1**). There was mild hepatomegaly and slightly increased amounts of peritoneal fluid. The fetus could be identified, but no fetal movements or heartbeats were observed. Thoracic radiographs showed several large abscesses within the lungs (**Fig 2**).

Although the echogenicity of peritoneal fluid did not appear to be consistent with blood, abdominocentesis was performed to determine if the severe anaemia was due to haemoperitoneum. Results of peritoneal fluid analysis were normal. A Coombs test was performed to determine if anaemia and thrombocytopenia may have been caused by immune mediated disease and results were negative. Cytological examination of bone marrow obtained from the sternum had a normal myeloid:erythroid ratio with normal numbers of megakaryocytes. A transtracheal wash was also performed and submitted for bacterial culture and antibiotic sensitivity. Transtracheal wash cytology revealed many degenerate and nondegenerate neutrophils and numerous rod-shaped bacteria.

Treatment and clinical management

Initial therapy consisted of potassium penicillin (22,000 u/kg bwt, i.v., q. 6 h), gentamicin (6.6 mg/kg bwt, i.v., q. 24 h) and flunixin meglumine (1.1 mg/kg bwt, i.v., q. 12 h), and i.v. isotonic saline was administered at 60 ml/kg bwt/h. The mare was transfused with 8 l of freshly collected, cross-matched whole blood because of the severity of anaemia and the chronicity of blood loss was unknown. After transfusion, PCV

and total protein concentration raised to 0.17 l/l and 64 g/l, respectively.

Complete blood count and serum chemistries were repeated on the third day of hospitalisation. Anaemia had improved (PCV 0.18 l/l; rr 0.30–0.42 l/l) and white blood cell count was normal (9.5×10^9 cells/l). Differential cell count was characterised as a mature neutrophilia (80% segmented neutrophils; normal 50–70%) with a left shift (5% band neutrophils; normal 0–2%) and lymphopenia (15% lymphocytes; normal 20–40%). Abnormalities of the serum chemistries included persistently elevated activities of AST (616 iu/l; rr 80–240 iu/l), SAP (519 iu/l; rr 50–150 iu/l), LDH (780 iu/l; rr 52–240 iu/l), SDH (1692 iu/l; rr 50–250 iu/l) and GGT (257 iu/l; rr 6–24 iu/l). Bile acid concentration was elevated at 32 $\mu\text{mol/l}$ (rr <15 $\mu\text{mol/l}$). Hyperbilirubinaemia (total bilirubin 71.8 $\mu\text{mol/l}$; rr 13.7–34.2 $\mu\text{mol/l}$), hypoproteinaemia (56 g/l; rr 60–76 g/l) and hypoalbuminaemia (21 g/l; rr 24–50 g/l) also continued. Electrolyte abnormalities included hyponatraemia (sodium 128 mmol/l; rr 136–144 mmol/l) and decreased serum bicarbonate concentration (18 mmol/l; rr 21–28 mmol/l).

Klebsiella pneumoniae, sensitive to fluoroquinolones and resistant to penicillin and aminoglycosides, was isolated from the transtracheal wash. Enrofloxacin (5.5 mg/kg bwt, i.v., q. 24 h) was then added to the treatment plan. Penicillin and gentamicin were continued to provide broad spectrum antibiotic coverage. Cloprostenol (0.25 $\mu\text{g/kg}$ bwt, i.m.) was administered beginning on the third day of hospitalisation to promote passage of the dead fetus, after multiple transabdominal ultrasound examinations failed to identify any fetal movements or heartbeats and gas echoes could be seen around the fetus.

Outcome

The mare became progressively more depressed, tachycardic (heart rate 112 beats/min) and tachypnoeic (respiratory rate 52 breaths/min) on Day 3 of hospitalisation and died suddenly. *Ante mortem* PCV was 0.17 l/l and total protein concentration was 63 g/l.

Necropsy findings

Gross necropsy examination revealed mild generalised icterus and multifocal 2–8 cm pulmonary abscesses (Fig 3). Petechial haemorrhages were present on the splenic capsule and tricuspid valve. The liver had slightly rounded edges and a reticulated pattern on cut surface. Histologically, there was marked necrosis of the lungs. The alveoli contained large numbers of intact and degenerate neutrophils and macrophages. There were low numbers of multinucleated giant cells, macrophages containing bacteria, and moderate amounts of acidophilic proteinaceous material, cellular debris and fibrin. Interlobular septae and pleura were mildly oedematous and infiltrated by low numbers of neutrophils and macrophages. The liver contained multifocal areas of mid-zonal to periacinar hepatocellular degeneration and necrosis.

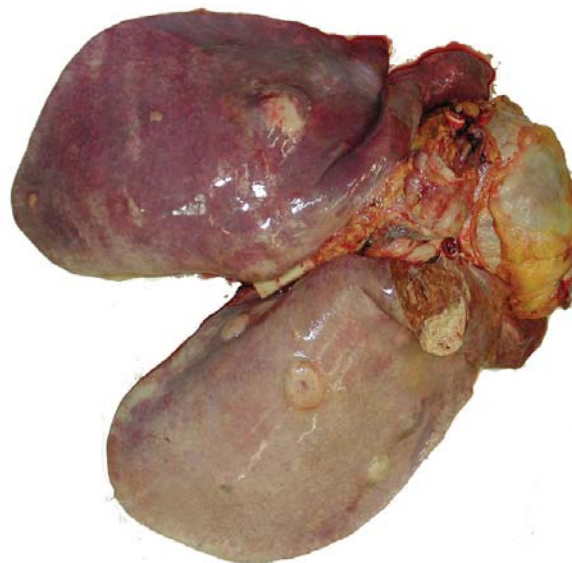


Fig 3: Gross necropsy specimen of the lower respiratory tract with multifocal abscessation.

Intermixed with the areas of necrosis were low numbers of neutrophils and macrophages. There was hepatocellular individualisation and disruption of limiting plates. Portal regions contained low to moderate numbers of lymphocytes and mild amounts of fibrosis. Hepatic sinusoids and portal areas contained haemosiderin-laden macrophages. There was moderate cholestasis present. The renal interstitium was infiltrated by low to moderate numbers of lymphocytes, plasma cells, macrophages, neutrophils and occasional multinucleated giant cells. Occasional renal tubules had degenerative and necrotic epithelium and contained moderate amounts of neutrophils and macrophages, acidophilic proteinaceous material and cellular debris. There was severe congestion of the red pulp and moderate depletion of lymphoid nodules of the spleen. *Rhodococcus equi* was isolated from the lungs, liver and kidneys at necropsy. Additional testing was not performed to determine if this *R. equi* isolate contained virulence associated protein A (VapA). The fetus and placenta appeared grossly normal and were not further examined.

Discussion

Although all horse farms are likely to be infected with *R. equi*, clinical disease ranges from enzootic and devastating on some farms, to sporadic or undiagnosed on others. This may reflect differences in environment (temperature, irritant dust, soil pH) and management conditions, as well as differences in the virulence of isolates (Takai *et al.* 1991). *Rhodococcus equi* may be found in areas never inhabited by horses, although greater concentrations occur where horses are present. It is surmised that volatile fatty acids present in equine manure enhance the growth of *R. equi* (Hughes and Sulaiman 1987). Adult horses passively carry the organism in their intestine, probably the result of inoculation from contaminated soil. However, replication can occur in the intestine of foals up to 3 months of

age (Takai *et al.* 1986). Faecal shedding of both virulent and avirulent *R. equi* is common in mares during the periparturient period (Cohen *et al.* 2006). In one study, all mares were positive at least once when sampled at 1 and 2 weeks *prepartum* and 1 week *post partum*. Thirty-three percent of mares were positive for virulent *R. equi* in their faeces at all sampling times.

Immunodeficiency is probably necessary for adult horses to develop rhodococcal infections because *R. equi* pneumonia is so common in foals and respiratory or reproductive infections are very rare in adults. Freestone *et al.* (1987) reported *R. equi* pneumonia and bacteraemia in a 7-year-old Appaloosa gelding that was found to have low or undetectable immunoglobulin concentrations, lymphoid depletion and abnormal lymphocyte stimulation activity. Immunosuppression of pregnancy may predispose some mares to developing reproductive *R. equi* infections (Patterson-Kane *et al.* 2002). In a review of *R. equi* infections in man (Kedlaya *et al.* 2001), mortality rates range from around 11% in immunocompetent patients to 20–55% in immunocompromised patients. Pulmonary infections accounted for 42% of cases in immunocompetent human patients, compared to 84% in immunocompromised patients. Specific immune function testing was not performed in the case reported here, but the mare had normal globulin concentrations and a persistent fairly mild lymphopenia. The mare did not have any prior clinical signs or history suggestive of decreased immune function. Because of the ubiquity of *R. equi* in the faeces of horses, detection of the VapA protein confirms infection with virulent bacteria. VapA was found in isolates taken from lung abscesses in a 10-year-old Warmblood gelding (Vengust *et al.* 2002) and 2 aborted foals (Szeredi *et al.* 2006). Although virulent *R. equi* isolates have been previously confirmed as pathogens in adult horses (Vengust *et al.* 2002; Szeredi *et al.* 2006), it remains unknown if expression of the VapA protein is necessary for *R. equi* to cause disease in adult horses. Detection of the VapA protein in this case would have been useful to determine if pathogenic *R. equi* was responsible for pneumonia and septicaemia.

Adult horses challenged intrabronchially with virulent *R. equi* developed minimal clinical signs of respiratory disease, except for transient fever in 1 of 12 horses (Hines *et al.* 2001). Clinicopathological changes were also minor; all horses had normal white blood cell counts and only 4 of 12 horses developed hyperfibrinogenaemia. The immunological memory response of adult horses experimentally challenged with virulent *R. equi* involves an influx of CD4⁺ and CD8⁺ T lymphocytes into the lung, an increase in the relative proportion of lymphocytes to macrophages in bronchoalveolar fluid (BALF) and secretion of interferon gamma (IFN- γ) (Hines *et al.* 2001, 2003). Experimental intrabronchial challenge of adult horses with avirulent *R. equi* was cleared without a significant increase in IFN- γ -producing T lymphocytes in BALF (Hines *et al.* 2003). Immunity against *R. equi* in immunocompetent adult horses appears to involve localised pulmonary antigen-specific memory immune responses resulting in increased CD4⁺ and CD8⁺ T lymphocytes in BALF, IFN- γ production and activation of macrophages (Hines *et al.*

2003). Age-related differences between the immune response of adult horses and foals related to antibody specificity and Th1 (cell-mediated) versus Th2 (humoral) immune responses may predispose foals to *R. equi* infection (Hooper-McGrevy *et al.* 2003). Foals that contract *R. equi* pneumonia may have an ineffective Th2-biased immune response, in contrast to immunocompetent adults and foals that do not become ill and mount a Th1-biased immune response. Expression of virulence associated plasmids or the exposed dose of virulent *R. equi* may determine if an effective Th1-biased immune response occurs.

In the case reported in this paper, *ante mortem* transtracheal wash yielded only *Klebsiella pneumoniae* and *R. equi* was isolated at necropsy from lung abscesses, liver and kidneys. *R. equi* may be easily overgrown in mixed cultures because of its slow growth (Kedlaya *et al.* 2001). If *R. equi* had been isolated *ante mortem*, antibiotic therapy with i.v. potassium penicillin and enrofloxacin would have been continued with the addition of rifampin (5 mg/kg bwt, *per os*, q. 12 h). Enrofloxacin achieves high intracellular concentrations and has excellent *in vitro* efficacy against *R. equi* isolated from cases examined at Rood and Riddle Equine Hospital.

Both the cases reported by Freestone *et al.* (1987) and in this report had thrombocytopenia, which may be the result of bacteraemia or sepsis induced coagulopathy, increased consumption of platelets, or bone marrow depression.

Rhodococcus equi was isolated from multiple blood cultures from a horse with acquired immunodeficiency (Freestone *et al.* 1987). At necropsy, the horse reported by Freestone *et al.* (1987) had multiple miliary pulmonary abscesses and bacteria were observed within Kupffer cells of the liver. Isolation of *R. equi* from multiple organs from the horse of this report suggests that bacteraemia had been present at some time during illness. In human patients, *R. equi* was cultured from blood in 30% of immunocompetent patients, compared to 83% of HIV patients and 100% of HIV patients with pulmonary infections (Kedlaya *et al.* 2001).

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