A Feverish Foal

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Introduction

Rhodococcus equi pneumonia or "rattles" as it is colloquially called is an important disease in growing foals. The bacteria was first isolated from a case of fatal pneumonia in 1923[5]. Foals usually infected are from 1-6 months old with signs most commonly appearing before 4 months of age[4] and rarely after 6 months of age[2]. *Rhodococcus equi* is a pleomorphic gram-positive saprophyte that lives in the soil and is ubiquitous on most breeding farms[8]. To cause disease the bacteria must contain a plasmid that encodes for proteins that accounts for its pathogenicity[10]. The most common disease is a pyogranulomatous bronchopneumonia with about half these cases involving the intestines as well. Intestinal disease alone only occurs in about 4% of cases[4]. Other extra pulmonary diseases (EPDs) include aseptic synovitis in about 25-35% of cases, abdominal pyogranulomatous lymphadenitis, uveitis, keratouveitis, panopthalmitis, osteomyelitis, and septic synovitis. Rare EPDs are endocarditis, pericarditis, cellulitis, dermatitis, subcutaneous abscesses, stomatitis, and peripheral lymphadenopathy[2].

Many infections are subclinical, and foals recover without any intervention. Infections that do not clear on their own become chronic progressive cases. These cases can less commonly present as subacute respiratory distress and sometimes these animals are found dead. More commonly they present with signs of cough, fever, lethargy and increased respiratory effort. Bilateral mucopurulent nasal discharge is an inconsistent finding. Many EPDs are subclinical and are identified at necropsy. It has been shown that foals with intestinal abscesses have a poor prognosis because of possible abdominal adhesions. Aseptic synovitis can cause non-painful effusion of multiple joints without lameness. The most common sites of effusion are the stifle, tarsocrural, carpal and fetlock joints. Septic synovitis is usually a single joint and will have pain on palpation. Patients with osteomyelitis have a poor prognosis especially if a there is vertebral

involvement. Clinical signs are stiff gait, reluctance to move, pain on palpation and soft tissue swelling. Ocular signs include epiphora, photophobia, aqueous flare, miosis and iris discoloration[2].

History and presentation

"Foal of River" was a 3-month-old Quarter Horse colt who presented to MSU-CVM equine service on Monday July 1st, 2019 for possible pneumonia. He was foaled on a breeding farm in Texas and transported to Mississippi 12 hours before presentation. No vaccination or deworming records were obtained at this time. The RDVM noticed a cough and shifting limb lameness. At the time of presentation, no therapy had been instituted.

On presentation he was quiet alert and responsive and was observed to nurse occasionally. He weighed 93.6kg, which is small for his age, and had a body condition score of 3/9. He was febrile with a temperature of 103.2 F, tachypneic with a respiratory rate of 64 and a heart rate of 88. On physical exam no lameness was observed but had bilateral tarsal and rear fetlock joint effusion which was not painful on palpation. Upon auscultation of the lungs, this colt had bilateral wheezes in all lung fields more severe cranioventrally. There was scabbing over the nose, which was most likely due to sun exposure and no nasal discharge was noted. Normal GI motility and all other physical exam parameters were within normal limits.

Diagnostics

A complete blood count was performed demonstrating a severe neutrophilia at 20,248 cells/ul (2,500-6,000 cells/ul) consistent with a left shift and infection. A mild monocytosis of 1012 cells/ul (0-800) was also present, this is consistent with a chronic granulomatous response. Usually foals with *Rhodococcus* infections present with an elevated fibrinogen[4], but that was not

present in this case. This could be due to decreased production by the liver or a nutrient deficiency.

The chemistry showed a mild hyponatremia of 129.4 mmol/L (132.0-146.0) and mild hypochloridemia of 94.4 mmol/L (98.0-106.0). When sodium and chloride are lost in proportion it is most likely due to GI loss (diarrhea or malabsorption), 3rd space loss or decreased feed intake. In this case there was no history of diarrhea, but we had not seen the foal have a normal bowel movement. There was a mild hypocapnia present 19.3 mEq/L (24.0-32.0) which is an indicator of bicarbonate, could be from due to multiple things such as GI or kidney loss. This was suggestive of a metabolic acidosis. There was a mildly increased anion gap of 19 (6-16) which supports metabolic acidosis. There was a severe hypoglycemia of 32 mg/dl (60-122), which was most likely due to decreased intake of carbohydrates. Hypoglycemia could also be due to liver damage or septicemia. There was mildly low blood urea nitrogen and creatinine, which could be due to decreased muscle mass and is clinically insignificant. There was a moderately elevated alkaline phosphatase 566U/L (61-153) most likely due to the bone isoenzyme and this elevation can be considered normal in young animals. There was a mild hypoalbuminemia 2.6 g/dl (2.8-3.9) which could be due to GI loss or normal variation. There was a mild elevation in globulin of 4.1 g/dl (2.5-4.0). There is a mild hyperphosphatemia at 6.2 mg/dl (2.4-4.0) which could be due to increased intake of phosphorus or decreased GFR. The chemistry findings were consistent with electrolyte imbalances most likely due to decreased intake or increased loss through diarrhea.

On thoracic ultrasound there were multiple (>20) hypoechoic circular nodules in the left and right lungs these vary in size from 1-3cm in diameter. They are distributed in an irregular pattern from dorsal to ventral in the lungs. Some normal lung parenchyma can be appreciated.

There is also a section of consolidated lung on the right side. These findings are consistent with multiple lung abscesses bilaterally and partial atelectasis of a caudal right lung lobe. There was moderate pulmonary edema. These findings are suggestive of *Rhodococcus equi* pneumonia due to the distribution and pattern of the lesions.

On abdominal ultrasound normal architecture of both kidneys with a distinct cortico-medullary junction and hilar vessels was noted. Normal splenic and hepatic architecture was also appreciated. There were multiple enlarged mesenteric lymph nodes measuring 1.2cmX3.2cm with multilobulated architecture. Color Doppler was used to discern these lymph nodes from mesenteric vasculature. On ventral midline there was a heterogeneous spherical structure that had a thick wall and irregular echogenicity surrounding it with a loop of small intestine adhered to it. These findings are consistent with enlarged lymph nodes are most likely from reactive lymphadenopathy/abscessation from systemic *R. equi* infection. The spherical shaped structure on ventral midline could be an abscess or granuloma adjacent to the gastrointestinal tract. The irregular structure around it could be omentum or adhesions.

Synovial fluid analysis and tarsal radiography were the next diagnostics performed to rule out septic arthritis and osteomyelitis. Left and right tarsal radiographs revealed intracapsular soft tissue swelling and periarticular new bone formation on the 3rd metatarsal bones bilaterally. Synovial fluid analysis of the right tarsocrural joint revealed a slightly hazy yellow fluid with a nucleated cell count of 2,550 cells /ul, 2,075 red cells/ul and a protein of 3.3 g/dl. The sample is mildly increased in cellularity. The cell differential consisted of 45% non-degenerate neutrophils, 9% small lymphocytes and 46% large mononuclear cells. No infectious agents or evidence of neoplasia were observed. The synovial fluid analysis of the left tarsocrural joint revealed a slightly hazy yellow and had a nucleated cell count of 6,875 cells/ul, 2,275 red cells /ul and a

protein of 3.2 g/dl. The sample is increased in cellularity. The differential consisted of 75% non-degenerate neutrophils, 1% small lymphocytes and 24% large mononuclear cells. No infectious agents or evidence of neoplasia were observed. These cytological findings were consistent with aseptic synovitis because the nucleated cell count is low and there are non-degenerate neutrophils present.

Diagnosis of *Rhodococcus equi* infection can be determined by multiple tests as well as history and clinical signs. There have been multiple research studies looking and serologic tests, but the poorly recognized antigens and decreased sensitivity of these tests make them unreliable for diagnosing infection. Presumptive diagnosis can be made on thoracic radiographs or ultrasound. A structured interstitial pattern will be seen on radiographs and multiple hypoechoic nodules will be noted on thoracic ultrasound. The gold standard diagnosis is cytology with culture and PCR. The sample can be obtained by percutaneous aspiration of the lung nodules or endoscopically. Although with endoscopy there can be contamination of the sample with non-pathogenic Rhodococcus in the upper respiratory tract. For that reason a PCR testing specifically for the virulence plasmid will determine true infection[4,10]. In the case of "Foal of River" the diagnosis made considering the history, clinical signs, ultrasound findings, complete blood count, chemistry findings and synovial cytology results.

Pathophysiology

Rhodococcus equi is a saprophyte and is ubiquitous in the on most breeding farms. There are many environmental and host immune factors that contribute to the development of clinical disease. The most common route of infection is inhalation. Dry dirt lots and increased stocking density contributes to infection. The organism can also pass through the adult GI tract without causing disease and replicate in the feces. This contributes to the increased bacterial load in

pastures[10]. Experimentally the incubation period after inhalation can range anywhere from 9 days to 4 weeks depending on the number of organisms inhaled[2]. The bacteria are taken up by local alveolar macrophages where it replicates and causes macrophage killing by necrosis rather than apoptosis. Necrosis causes the release of pro-inflammatory cytokines and leads to neutrophilic infiltration and a pyogranulomatous response.

Survival and replication in the macrophage occurs at a 80-100kb plasmid called Pvap. On this plasmid there are multiple genes encoding virulence proteins the most notable being VapA, which prevents the fusion of the phagosome with the lysosome. Experimentally in mice that VapA plasmid alone does not prevent lysosome fusion. Macrophage uptake is facilitated by complement receptor 3, this mode of entry has been shown to contribute to the survival and replication of Rhodococcus in macrophages[10]. When Rhodococcus equi is opsonized by antibodies and is then taken up by macrophages via antibody fc receptor pathways. The phagosome can fuse with the lysosome and the bacteria is eliminated[9]. This demonstrates that prior exposure and antibodies are important to prevent infection. There are still host immunity factors that have not been discovered yet. Studies show that 80-90% of foals with subclinical infections recover without antimicrobial treatment[6]. Older foals and adult horses have more developed immune systems, specifically T cell immunity that is thought to be the reason for resistance to Rhodococcus equi infection[10].

Intestinal extra pulmonary diseases (EPDs) are established from environmental ingestion of the bacteria or from swallowing of bacteria coughed up from the lungs. This infection is characterized by multifocal enterocolitis or typhlitis with granulomatous, purulent, or pyogranulomatous inflammation. This occurs around the region of the Peyer's patches and the mesenteric/colonic lymph nodes. The cause of other EPDs is due to intermittent or persistent

bacteremia seeding the bacteria throughout the body[10]. Some EPDs are not due to direct bacterial spread but are immune mediated triggered by the systemic inflammatory response elicited by Rhodococcus equi. A mechanism for aseptic synovitis is that the synovial infection begins septic and bacteria is quickly cleared leaving non-septic immune mediated inflammation[2].

Prevention and Treatment

Many foals infected with *Rhodococcus equi* eliminate the infection on their own. Because of this, it is hard to establish a protocol for breeding farms to reduce morbidity and mortality. Some farms do periodic thoracic ultrasound screenings to try and identify cases early and treat cases they think are going to become clinical[6]. To decrease exposure, producers should keep stocking densities of foals down on breeding farms and try to avoid housing foals in dry dusty lots. Foals infected with intestinal EPDs will shed the organism in their feces and increase the bacterial load in the soil. Doing this may lower the bacterial numbers in the soil but there is no feasible way to eliminate the bacteria from the soil[10].

With the discovery of the role of antibodies in opsonizing and eliminating bacteria in foals, researchers vaccinated mares to increase colostral anti-rhodococcal IgG concentrations. Although IgG concentrations did increase, it had no effect on infection rates of the foals. But a plasma transfusion with plasma of a hyperimmunized horse was proven to be partially protective[2]. However, recent research with a vaccine directed against the highly conserved microbial surface antigen poly-N-acetyl glucosamine (PNAG) in the mare has been proven to pass sufficient colostral antibodies to the foal to prevent *Rhodococcus* infection when challenged[1]. There have been immunostimulants and vaccines tested in foals using subunit and modified live vaccines. But due to the complexities of the immature immune system of the foal none have proven useful[2].

Many antibiotics show efficacy in vitro. However, the intracellular nature of the organism eliminates the effectiveness of many antibiotics. Treatment of choice is a macrolide antibiotic paired with rifampin. Macrolide antibiotics such as erythromycin, azithromycin, and clarithromycin work at the bacterial ribosomal 50s subunit to inhibit translation and are bacteriostatic. They also have a wide distribution throughout the body and intracellularly to reach *Rhodococcus* bacteria inside macrophages. Rifampin works by inhibiting DNA-dependent RNA polymerase and is considered a time dependent antibiotic in the treatment of *Rhodococcus.* It also can have the benign side effect of red tinged tears, sweat and urine. Erythromycin is used less today due to its side effect such as diarrhea, hyperthermia, and potentially fatal diarrhea in the mare if coprophagic behavior is occurring while the foal is on erythromycin. These side effects are seen with the other macrolides but are less common. A retrospective study has shown that the most effective combination is clarithromycin with rifampin [3]. Recent studies have shown that coadministration of clarithromycin or azithromycin with rifampin can cause decreased intestinal absorption and plasma levels, but the drug levels in pulmonary tissue remain within therapeutic levels[7]. This suggests that spacing oral dosing intervals out are important for rifampin and clarithromycin.

Treatment also involves symptomatic and supportive therapy. This can involve intravenous fluid therapy, gastroprotectants, non-steroidal anti-inflammatories, probiotics, additional antibiotics, and supplemental oxygen. Joint lavages and intra-articular antibiotics may also be indicated if septic arthritis is diagnosed. Ocular therapies such as topical steroids may be indicated if uveitis is present. Other specific therapies can be used depending on the extent of the patient's EPDs.

Case outcome

After initial diagnostics, Foal of River was started on Azithromycin for his Rhodococcus infection, Banamine for his fever, Ranitidine to combat stomach ulceration, Sucralfate to treat any ulcers that were already present due to his intestinal Rhodococcus infection, and Fenbendazole for because of his unknown deworming history. On day 3 in hospital, the colt broke with diarrhea, it is difficult to say if it was a side effect of the azithromycin, non-steroidal use or his concurrent GI disease because we had only noticed 2 normal bowel movements since the foal was in hospital. The foal had 4 bouts of watery diarrhea within an hour and was displaying bruxism. Measurement of the foal's urine specific gravity and the profuse diarrhea prompted us to initiate intravenous fluid therapy, a 1L bolus of lactated ringers every 2 hours was administered. The azithromycin was discontinued at this time and chloramphenicol was added along with Misoprostol to encourage enteric healing and maintain enteric blood flow, biosponge was added to absorb any clostridial toxins present from microbial imbalance, and platinum balance was given in attempt to restore normal GI flora. On day 4 he was mildly hypoglycemic, oral kayro syrup was added to his treatment due to his mild appetite and decreased nursing frequency, metronidazole was also added for possible clostridial infection.

On day 8 the diarrhea persisted, and a fecal sample was sent out for a foal diarrhea panel. The chloramphenicol was discontinued, and the azithromycin was started again. An intravenous dose of gentamycin was given to kill any extracellular Rhodococcus present. Bloodwork was repeated at this time. The complete blood count showed a severe mature neutrophilia 35,496 cells/ul (2,500-6,000) with a severe left shift with band neutrophils at 816 cells (0-100). The manual smear showed 2+ white blood cell toxicity, which is a sign of increased neutropoiesis. The chemistry showed a mild hyperchloremia 110.3 mmol/L (98.0-106.0). There was also a severe hypocapnia 12.8 mEq/L (24.0-32.0) due to GI loss of bicarbonate supporting an acidosis.

A mild hyperphosphatemia was present 6.8 mg/dl (2.4-4.0), a mild hypoalbuminemia 2.2 g/dl (2.8-3.9) most likely due to GI loss, and a mild hyperglobulinemia 4.9 g/dl (2.5-4.0). Due to his severe metabolic acidosis, sodium bicarbonate was added to his treatment plan.

On day 11 gentamycin and ranitidine were stopped and rifampin was added. At this time, the foal had become completely anorexic and the fever was consistently above 103.0 F ranging all the way up to 105.5 F at the end of the NSAID dosing interval. Ketoprofen was also tried instead of flunixin to see if the fever responded better. The foal diarrhea panel came back PCR positive for equine rotavirus. Lactaid was then added to his treatment sheet because rotavirus infection has shown to make foals temporarily lactose intolerant. On day 12, during the 6pm fluid treatment the foal started to head pressing on the foal box, became laterally recumbent, had a seizure, and died.

On necropsy there was multifocal well demarcated pyogranulomas replacing the lung parenchyma and compressing adjacent tissues. They ranged from 5mm to 3-4cm in diameter with a hyperemic rim around each. The granulomas extend onto the surface and bulge on cut surface. On histopathology they are comprised of caseous, eosinophilic debris with and abundance of degenerate neutrophils and macrophages. Inside the macrophages small coccoid basophilic organisms can be visualized. Tissue culture and sensitivity revealed Rhodococcus equi that was sensitive to Azithromycin and Rifampin.

Grossly the mesenteric lymph nodes are enlarged with the ileal and cecal lymph nodes being most severely affected. At the ileocecal junction there was a firm irregularly shaped mass measuring 24cm x 11cm x 10cm. On cut surface it is multinodular and has a friable caseous center. On histopathology the outer rim contained collagenous stroma with fibroblasts. Closer to

the center contained degenerate neutrophils with lesser numbers of macrophages. A sample of this tissue cultured E.coli, Klebsiella pneumoniae and enterococcus faecium.

The liver showed random small areas of centrolobular hepatic necrosis with an increased number of neutrophils present in the sinusoids. The joint capsules of the tarsal joints were thickened bilaterally. On histopathology the synovial membrane is hypertrophied with perivascular lymphocytes and plasma cells present. In the joint were irregular dense fibrin mats that were free floating and occasionally adhered to the joint capsule, they contained degenerate neutrophils. The heart showed pale streaks in the myocardium with some myocardiocyte atrophy.

In conclusion Rhodococcus equi is a ubiquitous disease on breeding farms. Many foals get infected and many recover without any intervention at all. The individuals that do not recover become clinical and show signs of respiratory disease. These foals can also have signs associated with EPDs. The disease is most characterized by irregularly arranged pyogranulomas in lung tissue. Treatment of choice is a macrolide with or without the use of rifampin. There is promising research being done in the field of vaccination in mares to pass colostral antibodies against PNAG that could prevent infection of the foal.

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