ESVCP Mystery Slide 2021

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SPECIMEN: Scanned digital slide and photomicrographs of a percutaneous transtracheal wash cytocentrifuge preparation

SIGNALMENT: Four-week-old Clydesdale colt

HISTORY AND CLINICAL FINDINGS:

A 4-week-old Clydesdale colt was presented to the Texas A&M Veterinary Medical Teaching Hospital with a chief complaint of bilateral hindlimb paralysis, which had progressed over 48 hours. He had been treated with unknown dosages of amikacin, sodium ampicillin, DMSO, flunixin meglumine, and dexamethasone prior to arrival. Prior history included failure of passive transfer of immunity at 24 hours of age, at which time he was treated with a plasma transfusion. At admission, the colt had normal vital parameters and was bright and alert but unable to stand. There was a palpable and painful soft tissue swelling near the dorsal spinous processes of T8-T10. Neurological examination revealed normal mentation, cranial nerves, forelimbs, and withdrawal reflexes of both forelimbs. Deep pain was apparent in both hindlimbs, and reflexes were increased. Complete blood count revealed an inflammatory leukogram with mild normocytic, normochromic anemia. Supportive care was provided to stabilize the patient overnight including an indwelling feeding tube, IV fluids, and anti-inflammatory drugs. Radiography and CT revealed significant osteomyelitis of thoracic vertebrae. An MRI revealed compression of the spinal cord at the level of T9. Dorsal spinous process resection with spinal stabilization of T6-T13 was performed, and aggressive antimicrobial therapy was initiated. During the next two months, the foal experienced additional complications. Bilateral inguinal hernias required a third intubation, general anesthesia, and surgical correction. The foal was intubated for general anesthesia two more times to diagnose and manage complications including additional osteomyelitic lesions, an ESBL-positive urinary tract infection, a surgical site seroma (post hernia repair), and a methicillin-resistant staphylococcus infected corneal ulcer.

Following his fifth anesthetic episode to address the corneal ulcer, he developed severe respiratory distress. His respiratory rate was persistently increased, and he had a minimal expansion of his chest wall with a severe abdominal component. Sternal recumbency and flow-by oxygen therapy did not improve his signs, so thoracic radiographs and ultrasonography were performed. A moderate, diffuse interstitial pulmonary pattern was identified on radiographs and was suspected secondary to atelectasis from prolonged recumbency. Ultrasonography was unremarkable. His respiratory signs continued to progress, but radiographic studies and thoracic auscultation remained unchanged. Roughly 2.5 weeks after the development of the respiratory signs, the colt underwent another short anesthetic episode to investigate a new osteomyelitic lesion. During the anesthesia, he became severely hypoxic. Due to the continual decline, a transtracheal wash with cytology and culture was performed. Images of the cytocentrifuge preparations made from the wash fluid are below.

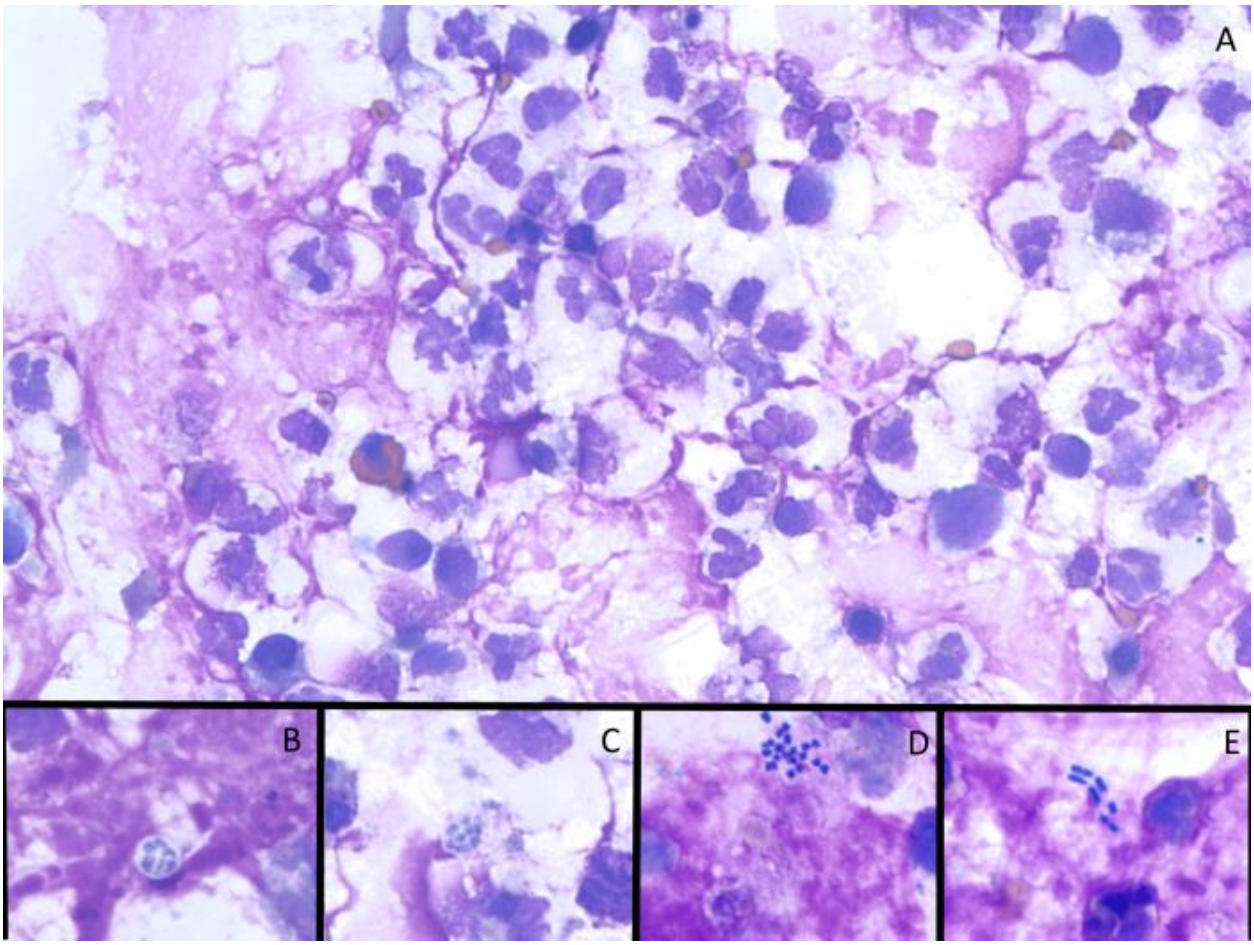

Figure 1: Cytocentrifuge preparation made from transtracheal wash fluid. Modified Wright; A. 50x objective, B-D. 100x objective.

QUESTIONS:

Question 1: What is the identity of the organisms observed in Figure 1B and C?

A. *Candida albicans*
B. *Pneumocystis carinii*
C. *Prototheca wickerhamii*
D. *Histoplasma capsulatum*

Question 2: Which diagnostic test would be least helpful in the identification of the organism from question 1?

A. GMS stain of a respiratory wash sample
B. Immunohistochemistry of a lung section
C. Fluorescent in situ hybridization of a lung section
D. Culture of a respiratory wash sample

CYTOLOGIC DESCRIPTION:

As shown in Figure 1, the smears are of high cellularity in a thick, mucinous background containing infrequent erythrocytes, extracellular bacterial cocci and rods, and rare green fungal

elements (likely barn inhalants). In addition, rare fungal cysts are observed in the background. The cysts are round, measure 5-10 µm and contain up to 8 intracystic bodies. They are consistent with *Pneumocystis carinii.* Based on a 300-cell differential count on the cytocentrifuge smear, the nucleated cells are composed of 88% degenerate neutrophils, 9% large mononuclear cells, 3% lymphocytes, and less than 1 % eosinophils. Neutrophils rarely contain phagocytized bacterial cocci. The large mononuclear cells are infrequently mildly vacuolated. In addition, occasional ciliated columnar respiratory epithelial cells are observed.

CYTOLOGIC INTERPRETATION/DIAGNOSIS:
Neutrophilic inflammation with presence of fungal cysts consistent with *Pneumocystis carinii* and intracellular and extracellular bacterial cocci and rods

ADDITIONAL DIAGNOSTIC TESTS:
GMS staining of the transtracheal wash fluid

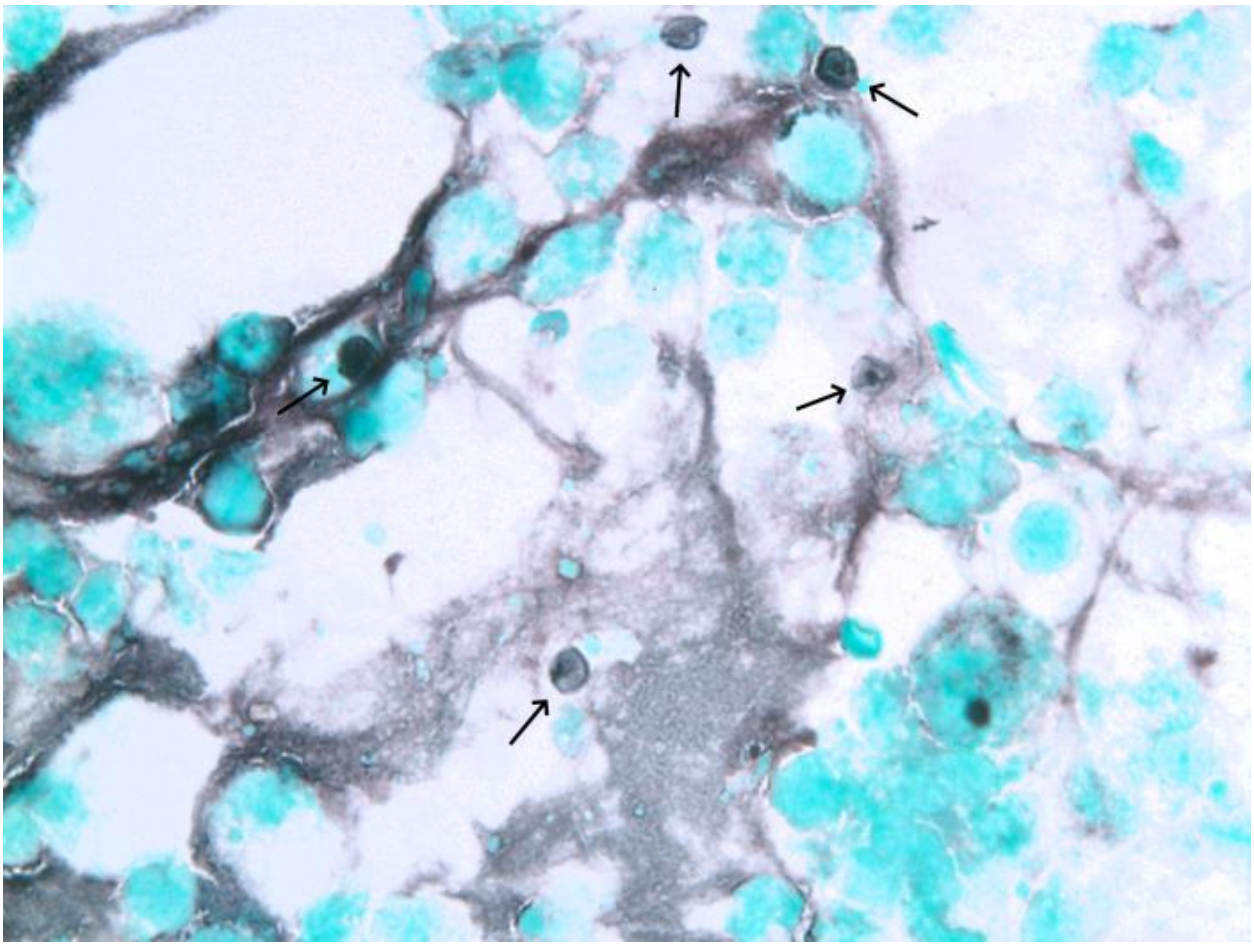

Figure 2: Several cysts of *Pneumocystis carinii* (arrows). Note the prominent intracystic dot visible in the right-most cysts which distinguishes *Pneumocystis* from other fungal organisms (sometimes referred to as a "punched-out" appearance). Cytocentrifuge preparation made from transtracheal wash fluid. GMS; 100x objective

Culture of TTW fluid: *Escherichia coli* 2+, *Klebsiella pneumoniae* 2+, *Acinetobacter baumannii* 1+, *Raoultella ornithinolytica* 1+
PCR for *Pneumocystis sp.*: Negative

DIAGNOSIS:
Mixed bacterial and pneumocystis pneumonia

CLINICAL OUTCOME/FOLLOW-UP:
In addition to the *Pneumocystis*, the colt's transtracheal wash sample cultured several multi-drug resistant pathogens. Antimicrobial therapy was continued, and he underwent a final anesthetic episode to remove the infected plates from his previous spinal stabilization. During anesthesia, he remained unstable and hypotensive. He recovered from anesthesia; however, he continued to decline and was refractory to treatment and supportive care. At this time, the owner elected to have him transferred to another facility for hyperbaric oxygen therapy. Upon arrival to that facility, the colt developed significant pericardial effusion that recurred after pericardiocentesis. Humane euthanasia was recommended due to the grave prognosis for the foal's recovery and was performed two days following his arrival at the facility.

ANSWERS TO QUESTIONS:
1) B
2) D

DISCUSSION:
Pneumocystis sp. is a unicellular, opportunistic fungal pathogen of respiratory importance in many mammals, including cats, dogs, horses, and humans.[1,3] Though it is categorized as a fungus based on high sequence homology, it is atypical in several respects. Unlike most fungi, pneumocystis has cholesterol in its cell membrane, rather than ergosterol. This difference means that classes of antifungals which target ergosterol, including amphotericin B and azoles, are ineffective.[1] Pneumocystis is also abnormal in that it has been nearly impossible to culture, which historically has complicated research into the pathobiology of the organism as well as diagnosis.[1,2] The genus *Pneumocystis* includes a diverse group of species which generally show high host specificity. Species in the pneumocystis genus include *P. jirovecii* in humans, *P. murina* in mice, *P. wakefieldiae* in rats, and *P oryctolagi* in rabbits.[2] Pneumocystis in other host species, including the horse, have not been specifically defined or named and so will be referred to in this discussion by the historical name *P. carinii*.

Infection with *Pneumocystis carinii* causes pneumocystis pneumonia (PcP).[3] Most reports of PcP in horses occur in young animals 1-6 months old, with rare reports in adults.[5,6,7,10] Transmission is most likely airborne from infected individuals or asymptomatic carriers.[2] At presentation, horses with PcP are often tachypneic and febrile and may have an alveolar to interstitial pattern on thoracic radiography.[5,6,7] Immunosuppression is considered a prerequisite to infection with *Pneumocystis* in all species and has been well documented in dogs and horses with PcP.[3,5,6,9] Therefore, a primary immunodeficiency should be considered and tested for if other predisposing factors are not identified. Alternatively, *Pneumocystis carinii* has been identified in foals suffering from a multifactorial syndrome of acute interstitial pneumonia alongside a host of other respiratory pathogens, including *E. coli*, *R. equi, K. pneumonia*, EHV-2, EHV-4, EHV-5, and equine adenoviruses.[7,10] Thus, it should also be considered that coinfection with other respiratory pathogens could weaken innate respiratory defenses predisposing to infection with *Pneumocystis*. In the current case, immune testing was not performed because of the presence of such coinfections confirmed on culture as well as the fact that there are no known heritable immunodeficiencies in Clydesdale horses. The prognosis for PcP in foals is guarded and likely depends on predisposing factors such as the presence of a primary immunodeficiency or other etiologic agents. Trimethoprim sulfa (TMS) is the treatment of choice for PcP and was used in

this case.[9] Dapsone has also been used successfully to treat a foal with PcP based on protocols from human medicine when TMS is not tolerated.[5]

In horses, as in other species, two distinct forms of the *Pneumocystis* organism may be recognized microscopically. The amoebic yeast form, sometimes referred to as a trophozoite, predominates in active infections.[2] They are 1-3 µm in length and are pyriform to elongated, light staining, and difficult to distinguish from cellular debris.[8] Though the yeast form predominates, the cyst form is much more easily recognized. The cyst form is round and measures 5-10 µm with a thick wall and up to eight distinct, dark staining intracystic bodies (Figure 1B,C).[8] Both forms may be observed extracellularly or intracellularly within macrophages. Recognition can be enhanced by the utilization of special stains such as GMS which stains cyst walls (Figure 2).[3] The organism primarily resides within alveolar spaces and so, although tracheal wash sampling may be sufficient for identification of this organism, bronchoalveolar lavage is likely the superior sampling method. The organism can also be diagnosed histologically with aid by special stains, immunohistochemistry, and fluorescent in situ hybridization.[9] Microscopic identification of the organism is critical to the diagnosis given the impossibility of culture and unavailability of molecular testing. Molecular testing may also be available in some species. A PCR test designed to detect *Pneumocystis sp.* in rodents and humans offered by IDEXX also detected the organism in a dog.[11] This same test failed to amplify DNA from *Pneumocystis sp.* in this case which emphasizes the genetic variation between different host-adapted species.

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