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toxin) and toxin B (cytotoxin), thought to act synergistically in the induction of colitis. Strains lacking exotoxin production are considered non-pathogenic. Clinical signs in horses vary in severity from asymptomatic, to mild diarrhea to fulminating colitis and death. We have identified cases of antibiotic-induced *C. difficile* colitis at the Veterinary Medical Teaching Hospital (VMTH) at the University of California at Davis. Recent antibiotic use is a consistent feature in the history. Of 187 horses (greater than 3 weeks of age) admitted between January 1995–January 1997 to isolation facilities at the VMTH for diarrhea, 116 had fecal samples submitted for culture for both *C. difficile* and *Salmonella* species. *C. difficile* alone was isolated from 41.4% of horses. *Salmonella* species were isolated from 7.8% and both *Salmonella* species and C. difficile from 6.9%. Of the *C. difficile* isolates, 85.7% were toxigenic by polymerase chain reaction for toxins A and B. Previous antimicrobial use was reported in 75% of horses with *C. difficile* compared to 56.7% (34/60) of horses that were culture negative. Survival was lower in *C. difficile* positive horses (60.7% versus 78.3%). Previous work has demonstated only 2% of normal horses have *C. difficile* in their feces. Over 40% (15/34) of *C. difficile* isolates were found to be resistant to metronidazole. Such a high rate of metronidazole resistance is not observed in human patients. No one antibiotic has been consistently associated with *C. difficile* colitis; rather several antimicrobials including trimethoprim-sulfamethoxazole, ampicillin, ceftiofur, metronidazole, penicillin and erythromycin have been associated with the disease, especially when used in combination.

Ehrlichia risticii is the etiological agent of the disease termed Potomac Horse Fever, or Equine Monocytic Ehrlichiosis. The disease is most prevalent in the Eastern United States, but it also occurs in California and other areas of the US as well as in Canada and Europe. Clinical signs include fever, anorexia, mild colic, diarrhea, dehydration, and in some cases laminitis. The disease is seasonal with cases occurring between late spring and early autumn in temperate areas. It was first observed among horses grazing in pastures along the Potomac River in Maryland. Diagnosis is hampered by the lack of specificity of the IFA test for titers to *E. risticii* and the need for cell culture systems for cultivation of the ehrlichia. Recent developments of nested PCR tests for DNA amplification have enhanced the diagnosis. The mode of transmission of *E. risticii* has been unknown. Recent DNA analysis of the 16S rRNA sequence indicated a close relationship to *Neorickettsia helminthoeca,* the causative agent of Salmon poisoning in dogs, and *Ehrlichia sennetsu,* an agent of human illness in Japan. We have recently identified Ehrlichia DNA by nested PCR in operculate snails (Pleuroceridae: Juga spp.) collected from stream water in a northern California pasture in which Potomac horse fever is enzootic. An aquarium culture system for these snails was developed and the snails exposed to temperatures above 22 C released trematode cercariae. DNA analysis of fragments of 3 genes (genes for 16S rRNA, the groESL heat shock operon, and the 51-kDa major antigen) were amplified from cercaria lysates by PCR and sequenced. Sequence analysis indicated the source organism closely resembled *E. risticii* and the sequences of all three genes were virtually identical to those of the genes of an equine E. risticii strain from a property near the snail collection site. This work suggests *E. risticii* transmission may involve exposure to infected cercariae or subsequent stages of trematodes.

Cryptosporidiosis in horses: How important is it?

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Introduction: Cryptosporidium parvum is a coccidian protozoal parasite which was first described in humans and calves in the early 1970's. It was not until 1982, however, that *C. parvum* was recognized as an enteropathogen in foals. Since then, it has been increasingly associated with foal diarrhea outbreaks. In 1988, cryptosporidiosis was considered one of the leading non-bacterial causes of foal diarrhea in Britain and Ireland.

Little is known regarding the epidemiology of equine cryptosporidiosis. The results of previously published studies have varied, depending upon the study design and the diagnostic test employed. Most have focused on mares and their foals, and sources of infection to foals have not been well defined. Consequently, a study of the prevalence and risk factors of cryptosporidiosis in horses was conducted at Texas A & M University.

Materials and methods: Three populations of horses were studied; 1) adult horses participating in a regional horse show, 2) local horses residing on farms serviced by the university ambulatory service, and 3) broodmares and their 10–21 day old foals on 4 large breeding farms. Informed consent and questionnaires were completed for each horse included in the study, and freshly-voided fecal samples were collected from each study participant. Each fecal sample was examined using immunofluorescence (IFA) and an acid-fast stain, and a subset of these were evaluated using a recently-developed flow cytometric technique. All fecal samples which were positive according to 2 of the 3 fecal tests for *C. parvum* were considered positive for the calculation of prevalences and the analysis of risk factors. Risk factors for cryptosporidial oocyst shedding were examined using logistic regression.

Results: The overall prevalence of *C. parvum* oocyst shedding was low, and varied considerably depending upon the diagnostic test used for detection. The mare-foal population had the highest prevalence of fecal oocysts. Factors which were associated with the excretion of oocysts included young age, residence on breeding farms, and a history of diarrhea. Factors which were not associated with shedding included gender, contact

with cattle, concurrent medications, and sharing a water source with other species of animals.

Discussion: Many of the findings in this study were consistent with previous reports. Foals less than 6 months of age were at highest risk of cryptosporidial cocyst shedding, and the presence of fecal oocysts was associated with diarrheal disease. Mares, however, did not seem to represent a source of infection for foals. In some cases, shedding was not associated with clinical disease, suggesting that sources of infection are not only limited to horses exhibiting clinical diarrhea.

There were strong associations between cryptosporidiosis and particular breeding farms, which confirms the role of *C. parvum* as a significant cause of foal diarrhea outbreaks. The specific sources of infection in these cases could not be identified, however; and the potential for *C. parvum* to cause outbreaks over consecutive foaling seasons on these farms could not be evaluated. The absence of *C. parvum* on other breeding farms suggests that *C. parvum* is not a ubiquitous pathogen, and is geographically localized to particular farms.

There were significant differences in the prevalence estimates depending upon the diagnostic test employed. The acid-fast stain exhibited good sensitivity, but suffered from poor specificity when compared to the IFA and flow cytometry.

Practitioners who care for foals need to consider *C. parvum* in all investigations of foal diarrhea. Although the overall mortality associated with outbreaks of cryptosporidial disease is usually low, it can result in significant financial losses associated with therapy and control. It is also zoonotic, and exposure to infected foals has resulted in human illness. Sources of infection may include water sources and other foals (even those not experiencing diarrhea), and these should be investigated. The acid-fast stain is simple to perform and is sensitive in detecting fecal oocysts. References available upon request.

Prevalence of β2-toxigenic *Clostridium perfringens* in horses with intestinal disorders

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The incidence of a new, yet unassigned toxin type of *C. perfringens* containing the α- and the recently described β2-toxin gene in horses with intestinal disorders is reported. Twenty samples of ingesta from the small and large intestine and five biopsies of the intestinal wall and 74 faeces samples were analysed bacteriologically of i) 18 horses suffering from typical typhlocolitis, 7 horses with atypical typhlocolitis and 16 horses with other intestinal disorders and ii) 58 horses without intestinal disease. *C. perfringens* isolates were typed for the presence of the α-, β1-, β2-, ε- and enterotoxin genes by PCR including a newly developed PCR for the detection of the β2-toxin gene *cpb2*. In samples from horses with typical or atypical typhlocolitis, β2-toxigenic *C. perfringens* were found in 9 of 12 (75%) ingesta specimen and biopsies and in 4 of 13 (31%) feces samples. From the 15 fatal cases of typical and atypical typhlocolitis 9 (60%) were positive for β2-toxigenic *C. perfringens* compared to only 4 (40%) of the 10 non fatal cases. Of the 9 animals with typhlocolitis which were treated with antibiotics in combination with non steroidal-antiinflammatory drug (NSAID) 8 had β2-toxigenic *C. perfringens* and died whereas of the remaining 16 cases not receiving this combination treatment only 7 died and 5 of them shed the organism ($p < 0.01$). No β2-toxigenic *C. perfringens* was found in the samples from the 58 control horses of which only one faeces sample contained *C. perfringens* type A. Surprisingly, no *C. perfringens* isolated in this study contained genes for the β1-, ε- and enterotoxin, indicating that *C. perfringens* type B, type C and type D and enterotoxigenic *C. perfringens* are not common in horses with intestinal disorders. The high incidence of β2-toxigenic *C. perfringens* in ingesta samples and biopsies of the intestinal wall of horses suffering or dying of typhlocolitis ($p < 0.001$) suggests that β2-toxigenic *C. perfringens* play an important role in the pathogenesis of typhlocolitis and may act as a lethal factor in toxic intestinal disorders in horses.

Muscle enzyme patterns and elimination of intravenous injected, homologous muscular enzymes in horses

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In myopathies of horses bloodchemistry shows often a rise in muscular enzyme activities. The purpose of this study was to get further information from bloodchemistry by determination of muscle enzyme patterns and elimination of intravenous injected, homologous muscular enzymes in horses.

Animals, material and methods: In liver, heart, diaphragm, M. masseter, M. glutaeus medius (superficial and deep part) and M. semitendinosus of 17 warm-blooded and small horses, the activities of CK, ASAT, ALD, LDH and HBDH were determined. Concentrations of haemoglobin and myoglobin were measured together. In addition, the kinetic behaviour of the cellular enzymes CK,