MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS CULTURED FROM THE FECES OF A SOUTHERN BLACK RHINOCEROS (DICEROS BICORNIS MINOR) WITH DIARRHEA AND WEIGHT LOSS.

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Abstract: Mycobacterium avium paratuberculosis (Map) was cultured from the feces of a wild caught, female, adult southern black rhinoceros. The animal, which presented with a 4 month history of diarrhea and weight loss, was prescribed a course of anti-mycobacterial drugs. The clinical signs resolved and the feces were repeatedly culture negative thereafter. Although the rhinocerotidae are likely to be resistant to Johnes disease, this case raises the possibility that they can become transiently infected with the causative organism.

Key words: *a*nti–mycobacterial therapy, Diceros bicornis minor, Johnes disease, Mycobacterium avium paratuberculosis, southern black rhinoceros.

BRIEF COMMUNICATION

Johne's disease is a transmissible, chronic intestinal disease of domestic and non-domestic artiodactylids caused by Mycobacterium avium subspecies paratuberculosis (Map). Among animals of this Order, Johne's disease is characterized by a long pre-clinical phase and a prolonged clinical course with the variable presence of diarrhea and progressive loss of body condition leading to death. Treatment of Johne's disease is usually not attempted due to the low likelihood of eliminating infection and the high cost of anti-mycobacterial drugs. Johne's disease is rare in free ranging populations of wildlife probably because low population densities typical of such populations are inadequate to maintain infection.[14] Although primarily a disease of ruminants and camelids, naturally acquired Johne's disease has also been reported in carnivores, lagomorphs and primates [2,4,7]

Johne's disease causes significant economic loss in domestic livestock production and is the subject of formal control programs in many regions of the world. The causal organism is easily introduced and hard to eliminate from a herd due to the difficulty in detecting pre-clinical cases with the insensitive ante-mortem diagnostic tests available, and the potential for affected animals to shed and disseminate infection in the months and years prior to developing clinical signs. Similarly, Johne's disease poses a serious risk to the integrity of captive endangered species conservation breeding programs. Typically, these programs are managed cooperatively between zoos in a geographical region and comprise a small number of animals with frequent transfer between institutions. Thus, Johne's disease has the potential to disseminate widely through zoos in a region.[1] Four species of rhinoceros are the subject of multi-institution cooperative breeding programs.[3]

A female southern black rhinoceros (Diceros bicornis minor), estimated to be 4 years old, presented with progressive loss of body condition and recurrent episodes of soft, poorly formed and malodorous feces over a period of 4 months. The animal was one of a cohort of free-ranging black rhinoceros captured in Zimbabwe and transported to an Australian open range zoo 7 months previously to participate in an international conservation breeding program. [5]

Laboratory testing revealed hematological and biochemical parameters to be within normal limits. Fecal flotation was negative for endoparasite ova. Fecal culture revealed the presence of Campylobacter fecalis and appropriate antibiotic therapy was prescribed. Follow up fecal cultures were negative for this organism yet clinical signs persisted.

Additionally, fecal samples were submitted on two occasions for Johne's disease exclusion by culture using a double incubation-centrifugation decontamination procedure followed by culture on slopes of Herrold's egg yolk medium containing mycobactin J (HEYM +MJ) .[10] Culture for Map was positive after 4 months incubation from the initial fecal sampling only, while fecal smears made at the time of culture and stained by the Ziehl-Neelsen method were negative for acid-fast organisms from both fecal samples. Several years later this isolate was recovered from lyophilized cultures and subjected to restriction fragment length polymorphism (RFLP) analysis which identified it as RFLP type C5.[13] C strains of Map tend to affect cattle but not sheep and are more often incriminated in infection in other species.[13]

Based on the positive fecal culture, a diagnosis of Johne's disease was made. Despite the poor prognosis for success, a decision was made to attempt therapy due to the high conservation value of the individual. Combination anti-mycobacterial therapy was chosen comprising rifampin (Rifadin (R) Oral™, Sanofi-Aventis Australia Pty Ltd, 12-24 Talavera Rd, Macquarie Park, NSW, 2113, Australia) 15 g s.i.d. p.o. and pyrazinamide (Zinamide™, Merk, Sharp and Dohme

(Australia) Pty Ltd, 54-68 Ferndell St, South Granville, NSW, 2142, Australia) 537.5 g s.i.d. p.o. A proprietary, combination, oral anti-diarrhoea suspension which included streptomycin (Scourban™, Bomac Animal Health Pty Ltd, Unit 15/36 Leighton Place, Hornsby, NSW, 2077, Australia) was also initiated at this time and was given at a dose of 25 g of the streptomycin component sid. This was discontinued after 3 weeks coincident with the normalization of fecal consistency. Pyrazinamide therapy was continued for 10 months and rifampin therapy for 12 months. By this time, the animal's feces were normal and body condition was judged to be good. A fecal sample collected 6 months after the initiation of therapy was negative on culture for Map.

Fecal culture is regarded as the 'gold standard' for the antemortem diagnosis of Johne's disease in herds of domestic animals.[12] Despite a positive fecal culture in an animal with suggestive clinical signs, the diagnosis of Johne's disease in this case is questionable. Although domestic horses (Equus caballus) have been experimentally infected with Map resulting in a granulomatous enteritis, naturally acquired Johne's disease has not been reported in perissodactylids.[6] It is speculated, however, that naturally acquired infection with Map is implicated in some cases of idiopathic granulomatous enteritis in horses although Map has not been cultured from any of these lesions.[8]

The potential for this rhinoceros to have been exposed to Map and become infected was limited. Domestic ruminants usually acquire infection as neonates before the development of age-related resistance.[12] This animal was unlikely to have been exposed during the neonatal period in its original range, the Chete area in the west of Zimbabwe, because this is a semi–arid area with no livestock production and where Johne's disease is unknown. Although domestic calves are most susceptible, older heifers and adult cattle can become infected by consumption of contaminated

feedstuffs.[12] Exposure of the rhinoceros to Map as an adult during pre–export quarantine in Zimbabwe or post-arrival quarantine at the Australian quarantine station on the Cocos Islands is also unlikely due to hygiene standards typical of dedicated quarantine facilities. Similarly, exposure during the animal's tenure at the zoo prior to the collection of the positive fecal sample is improbable as there has been no occurrence of Johne's disease in the history of the institution. The zoo has a veterinary health program administered by staff veterinarians. Surveillance for paratuberculosis is predicated on a full necropsy examination for every susceptible animal that dies including appropriate histopathology and, where indicated, tissue culture. Additionally, pooled fecal cultures on samples collected from groups of artiodactylids for exclusion of paratuberculosis are conducted every 6 months.

If exposure to Map did occur and the rhinoceros ingested an infective dose then it can be speculated that the animal acquired a transient infection which was subsequently eliminated by chemotherapy.

While it is possible that chemotherapy was curative in this case, there are no reports of recovery from Johne's disease in the literature. Fifteen years after the original diagnosis, the rhinoceros remains bright with a good body condition and normal fecal consistency. Fecal culture had been negative for Map on at least a dozen occasions since the single original positive result. Two male offspring born over this time have no health concerns and have both returned multiple negative fecal culture results for Map.

The apparent response to anti–mycobacterial therapy in this animal may have been coincidental to the resolution of a self–limiting gastrointestinal problem or attributable to the broad spectrum antimicrobial effects of the anti-mycobacterial medication in addressing another, unidentified

microbial cause of enteropathy. Chemotherapy for Johne's disease, where it has been attempted in domestic livestock, is at best effective in leading to remission rather than a cure.[9]

Speculation that the rhinoceros did not have an active Map infection requires an explanation for the positive fecal culture results. It is possible that the positive culture result in this case was a false positive due to laboratory error. A review of laboratory submissions and results from this time suggests that laboratory error by sample mix up or cross contamination was unlikely as all other samples submitted for Map culture to the testing laboratory at that time were tissues that were culture negative. Negative culture results for each of these tissue samples were corroborated by histopathological examination.

An alternative possible explanation for a false positive result is that Map was present in the animal's feces by means of the 'pass through' phenomenon, i.e. passive excretion of Map, in the absence of infection, within 7 days of consumption of contaminated material.[11] If this was the case, and given the lack of history of occurrence of Johne's disease in the zoo, then contamination of forage or other fomite with Map would have been the most likely route of exposure.

While Johne's disease should be considered in cases with consistent clinical signs in rhinoceros, it is likely that rhinoceros, in common with their distant relative the domestic horse, share a resistance to natural infection with Map. This case raises the possibility that rhinoceros can be transiently infected with this organism.

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