Lincomycin-induced Severe Colitis in Ponies: Association with *Clostridium cadaveris*

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ABSTRACT

Four groups of two ponies, free of fecal *Salmonella* and *Clostridium cadaveris*, were treated as follows: Group A, control group; B, single nasogastrically administered dose of lincomycin (25 mg/kg) followed 48 h later by 3 L of *C. cadaveris* (10^9 organisms/mL); C, the same dose of lincomycin as group B; D, the same dose of *C. cadaveris* as group B on each of three occasions at 12 h intervals. Groups A and D remained healthy, but groups B and C developed severe colitis 48–56 h (B) or 72 h (C) after administration of lincomycin. Three ponies were euthanized and one in group B died. *Clostridium cadaveris* was isolated at about 10^6/mL of colonic contents from these ponies, but one pony in group B also yielded *Salmonella typhimurium* from the colon. Subsequent challenge of group A ponies (3 L of *C. cadaveris* 10^9/mL, three times at 12 h intervals) did not produce colitis. Nasogastric administration of lincomycin (25 mg/kg) to group A and D ponies, 20 days after administration of *C. cadaveris*, resulted in severe colitis in all ponies within 48–72 h. *Salmonella agona* was isolated from the colonic contents of one pony and *C. cadaveris* (10^6/mL) from all four ponies. *Clostridium cadaveris* was not isolated from the colonic content of 45 healthy horses examined immediately after death. These studies confirm the potential for lincomycin to induce severe enterocolitis in ponies and implicate *C. cadaveris* further as a cause of "idiopathic colitis" in ponies.

RÉSUMÉ

Quatre groupes de 2 poneys exempts de *Salmonella* et de *Clostridium cadaveris* fécaux ont reçu les traitements suivants: Groupe A: témoin; groupe B: administration d'une dose de lincomycine (25 mg/kg) par intubation nasogastrique suivie, 48 heures plus tard, de 3 litres de *C. cadaveris* (10^9 organismes/mL); groupe C: la même dose de lincomycine que le groupe B; groupe D: trois administrations à 12 heures d'intervalle de la dose de *C. cadaveris* administrée au groupe B. Les poneys des groupes A et D sont demeurés en santé, mais les animaux des groupes B et C ont développé une colite sévère débutant entre 48–56 heures (B) ou 72 heures (C) après l'administration de lincomycine. Trois poneys ont été euthanasiés et un poney du groupe B est mort. Le contenu du côlon de ces poneys contenait approximativement 10^6 colonies par mL de *Clostridium cadaveris* et, chez un poney du groupe B, *Salmonella typhimurium* fut également isolé. Une dose additionnelle (3 L de *C. cadaveris* 10^9/mL, 3 fois à 12 heures d'intervalle) aux poneys du groupe A ne fut pas associée à l'établissement de colite. L'administration de lincomycine (25 mg/kg) par intubation nasogastrique aux poneys des groupes A et D fut suivie, entre 48–72 h, d'une colite sévère chez tous les poneys. *Salmonella agona* fut isolé du contenu digestif d'un poney et *C. cadaveris* (10^6/mL) fut isolé des 4 autres poneys. *Clostridium cadaveris* ne fut pas isolé du contenu du côlon de 45 chevaux normaux immédiatement suivant leur mort. Ces études confirment que la lincomycine peut induire des entérocolites sévères chez les poneys et impliqueraient principalement *C. cadaveris* comme agent causal des colites idiopathiques des poneys. (*Traduit par Dr Jean-Pierre Lavoie*)

Fatal idiopathic colitis (colitis X) is a well recognized sporadic, acute, severe, and often fatal disease of horses but the causes are unclear (1–4). A method for inducing fatal idiopathic colitis in the horse had been previously reported, involving oral administration of lincomycin to horses to upset the anaerobic colonic microflora, followed by a small quantity of colonic content from horses dead with fatal idiopathic colitis (5). In those cases of induced colitis, *Clostridium cadaveris* was the predominant clostridium isolated from colonic ingesta (5). This report describes a model for producing severe colitis with oral lincomycin, with or without administration of *C. cadaveris*, and further implicates *C. cadaveris* as a potential pathogen in undifferentiated acute enterocolitis in the horse.

Eight healthy adult ponies were obtained from random sources through a local stockyard. Repeated fecal culture (eight times) showed them to be free of *Salmonella* species, *Yersinia enterocolitica* and *C. cadaveris* (5). They were housed in pairs (A,B,C,D) in four isolation rooms. In experiment one, group A served as untreated controls, group B received 25 mg/kg lincomycin (Lincocin®, Upjohn, Tuco Products, Orangeville, Ontario) by stomach tube once, followed 48 h later by 3 L of *C. cadaveris* strain J1 (10^9 organisms/mL) cultured at 37°C for 36 h in thioglycollate broth (Difco, Detroit, Michigan). Group C received lincomycin by stomach tube (25 mg/kg) once and group D received orally 3 L of *C. cadaveris* (10^9/mL) on each of three occasions at 12 h intervals. All animals were monitored at regular frequent intervals for development of diarrhea and clinical signs of colitis. Once clinical signs of colitis developed (heart rate > 60, with signs of colic) affected animals were immediately euthanized and were necropsied without delay. Tissues were fixed in 10% formalin, embedded in paraffin blocks, sectioned and stained with hematoxylin-eosin according to standard methods. Colonic contents from healthy horses and from ponies with

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induced colitis were examined for clostridia by the methods described (5,6). All experiments followed the guidelines of the "Guide to the Care and Use of Experimental Animals" issued by the Canadian Council on Animal Care.

Group A remained normal. One pony of group B developed severe watery diarrhea after 48 h. It showed elevated heart rate, clinical signs of abdominal discomfort, dehydration and toxic shock, and was therefore euthanized. The second pony of group B did not show clinical signs of colic or diarrhea but it was found dead 6 h later. On postmortem, the first pony showed gross and histopathological lesions of severe acute erosive colitis and typhlitis. *Salmonella typhimurium* but no *C. cadaveris* were cultured from the colon. The other pony had a gross and histopathological appearance of acute regional colitis and signs of acute toxemic shock. Large numbers (10^6/g colonic contents) of *C. cadaveris* were cultured from cecal and colonic contents. Both ponies of group C were euthanized at 72 h because of clinical colitis. On postmortem, both had gross and histopathological evidence of acute severe fibrinous typhlocolitis. *Clostridium cadaveris* was cultured in large numbers (10^6/g) from colonic ingesta. Ponies of group D showed mild signs of colic between 72 to 96 h after initial *C. cadaveris* treatment but had normally formed feces and recovered uneventfully.

In a subsequent second experiment, group A ponies were intubated with 3 L of *C. cadaveris* (10^9/mL) on each of three occasions at 12 h intervals. The animals were monitored as described. Two other healthy adult ponies with cecal fistulae (group E) were inoculated intracecally with 3 L of *C. cadaveris* (10^9/mL) broth on each of three occasions at 12 h intervals. During the following 20 days group A and E ponies remained normal.

In a third experiment, ponies of groups A and D received, 20 days after the *C. cadaveris* administration described above, 25 mg/kg oral lincomycin on a single occasion. All four ponies had to be euthanized 48–72 h later because of severe, acute colitis. *Salmonella agona* and *C. cadaveris* were isolated from the colon of one pony. The other three ponies yielded *C. cadaveris* in high numbers (10^6/g) from colonic contents.

Colonic ingesta were obtained immediately after death from 45 apparently healthy horses at a local slaughterhouse. Samples were taken to the laboratory on ice and cultured for *C. cadaveris* within 6 h but no *C. cadaveris* were recovered.

In this study, eight ponies developed severe enterocolitis after treatment with a single dose of lincomycin. Of these ponies six had been given *C. cadaveris* before (20 days, four ponies) or after (48 h, two ponies) the lincomycin, and two ponies received lincomycin alone. In two cases salmonella may have been the cause of the enterocolitis. *Clostridium cadaveris* was isolated in large numbers from seven ponies. Neither salmonella nor *C. cadaveris* were detected before the experiments. The gross and microscopic changes of typhlocolitis in this series of ponies were uniformly consistent with those previously reported for colitis X in horses (3,5).

The administration of *C. cadaveris* alone was not associated with the development of enterocolitis in four ponies, even when administered in large numbers intracecally in two animals.

Animal models of antibiotic-induced clostridial colitis involve at least three essential factors to produce disease (7). First, the animal used has to be susceptible to the organism overgrowing in the large colon, second, the clostridium (usually *C. difficile*) has to be toxigenic, and third, a broad spectrum antibiotic has to disturb the local residential protective flora to allow overgrowth of the pathogen (7). Normal hamsters are highly resistant to colonization and infection with *C. difficile*, even with massive oral challenge, unless the colonic flora is previously disturbed with antibiotic (7). Therefore, failure to induce acute fatal colitis with *C. cadaveris* administered orally without lincomycin is not conclusive evidence that this organism is not involved in colitis in ponies. However, in contrast to *C. difficile*, *C. cadaveris* has not been shown to produce any toxin associated with pathogenicity (8). Supporting evidence for a causative role of *C. cadaveris* in equine enterocolitis, however, was the isolation of large numbers from seven of eight horses with lincomycin-induced enterocolitis and the apparent absence of the organism from healthy horses. These studies nevertheless show that *C. cadaveris* is likely present in the colonic contents of healthy horses although at levels undetected by the culture methods used. Our studies do not however rule out other pathogens which may be uncovered by oral administration of lincomycin, as shown in two cases of salmonellosis. While further work is needed to define the role of *C. cadaveris* in severe enterocolitis of ponies and horses, administration of lincomycin and *C. cadaveris* to ponies appears reliably to produce severe enterocolitis and may allow objective assessment of preventive treatment measures for idiopathic colitis in equine species.

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