Case Report and Short Communication: Rectal prolapse associated with an unusual combination of pinworms and citrobacter species infection in FVB mice colony

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Summary
Spontaneous cases of rectal prolapse in a breeding colony of FVB mice were found to be due to infection with *Syphacia obvelata* and *Citrobacter freundii*. Microbiology, biochemical and parasitological examination revealed *Citrobacter freundii* and eggs of *Syphacia obvelata*. After treatment with antibiotics, antihelminthic drugs and manual reduction prevented further occurrence.

Introduction
Colitis in mice is associated with a high incidence of rectal prolapse due to heavy infestation by Citrobacter freundii *(Ediger et al., 1974)*. Rectal prolapse due to a natural outbreak of Syphacia infection *(Hoag, 1961; Harwell & Boyd, 1968)* and due to Citrobacter *freundii* were reported by Barhold (1980). Other causes for rectal prolapse involved pelvic floor disorder in male transgenic mouse strain deficient in urokinase – plasminogen activator (UPA -/-) *(Yiou et al., 2001)* and in large bowel disease caused by *Helicobacter hepaticus* in immunodeficient mice (Ncr-nu/nu, Balb/ c an Ncr nu/ nu, C57 BL / 6 Ncr – nu / nu) *(Ward et al, 1996)*. Rectal prolapse with colonic mucosal hyperplasia was recorded due to citrobacter infection by Barhold et al.. (1977). In this paper we present a case report describing the rectal prolapse associated with both pinworm and *Citrobacter freundii* infection in a FVB mice colony and its curative measures.

Case presentation
In the breeding colony of our small-animal facility, spontaneous rectal prolapses were observed in a group of the FVB strain. The animals remained housed in individual cages in accordance with the guideline for care and use of animals in scientific research (Indian National Science Academy, New Delhi, India) in a CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals) registered animal facility. The animals were maintained under the standard environmental condition (Temp. 22° C) relative humidity 45-55%) with 12: 12 hrs dark / light photopheriod and fed commercial autoclaved pellet feed and water *ad libitum*. For parasitological examination we used the cellophane tape method in which cellophane adhesive impressions of the anus were applied over the glass slides and examined under a microscope. In addition, a swab from a rectal prolapse was taken, dissolved in a 0.1% NaCl solution, centrifuged at 2500 rpm for five minutes, the supernatant solution discarded, and a drop taken for light microscopic examination.
Aseptically collected faecal swabs from the prolapsed animals were subjected to culture in blood agar plates at 37° C for 48 hours under anaerobic conditions as described *(Ediger et al., 1974; Hansen, 2000)*. The pure colonies were subjected to colony morphology; gram staining reaction (Gram staining kit, Hi Media, India) and biochemical tests were carried out by a commercial kit for Enterobacteriaceae (HIMVIC Biochemical test Kit KB 003, Hi Media, India). After confirmation the animals were treated with Tetracycline VET water soluble (Intervet, India) at a dose rate of 1g / liter of water for three days and four consecutive doses of Ivermectin and the prolapsed mass was manually reduced.

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Results

On physical examination, the animals had ruffled fur and pasty diarrheic faeces around the prolapsed mass, base of the tail and perineum. The mass appeared edematous, enlarged with hemorrhagic rectal mucosa. (Fig 1)

Figure 1: FVB Male mice with Rectal prolapse

Microscopic examination of faecal samples showed numerous eggs of Syphacia species. The eggs appeared asymmetrical, thin shelled, transparent, and slightly flattened on one side and resembled a banana shape.

Culture examination revealed typical large round colonies with a smooth edge surface. Gram staining reaction of pure colonies showed multiple numbers of gram-negative bacteria that were pale in color with pleomorphic rod-shaped organisms. Biochemical examination of pure colonies using a commercial kit showed positive for sucrose, H_2S and negative for lysine, ornithine decarboxylase and the VP test. Based on these results we decided to treat the animal with tetracycline hydrochloride 1 gm / liter of water for 3 days and Ivermectin 4 mg/ kg body weight *(Klement et al., 1996)* for four consecutive days. After treatment, all clinical signs disappeared except the prolapsed mass. The prolapsed mass was cleaned with normal saline and reduced manually; following reduction there was no recurrence.

Discussion

The case describes rectal prolapse associated with both pinworm and Citrobacter species infection and their treatment. Heavy infestation of pinworms has previously been reported to cause rectal prolapse, constipation, intersucception or faecal impaction but usually without diagnostic procedures being used to exclude other diseases as primary or contributing causes *(Wescott et al., 1982)*. Clinical signs associated with pinworms are poor condition, rough hair coats, reduced growth rate and rectal prolapse *(Hoag 1961; Harwell & Boyd, 1968)*. Based on the microscopic examination of faecal samples, we identified Syphacia species but the clinical signs persisted even after treating with antihelminthic drugs. Cultural examination followed by a biochemical test for the characterization of the bacteria in the fecal sample revealed the typical colony morphology and biochemical character of Citrobacter species *(Hansen et al., 2000)*. Hence we concluded that the colony was infected with Citrobacter bacteria. Based on that, we decided to treat with an antibiotic and continued with antihelminthics. While antihelminthics are effective in eliminating a high percentage of adult pinworm, these are inefficient in clearing immature worms *(Wescot et al., 1982)*. The affected animals returned to normal after five days of treatment.

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