Cecal colonization and fecal shedding in rats inoculated with various Brachyspira spp.

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Introduction
Limited work has been described regarding fecal shedding in rats following inoculation with Brachyspira spp., and the reported duration of fecal shedding after infection with B. hyodysenteriae is only two days.1 Despite this short duration and low prevalence of shedding under experimental conditions, rats, along with other rodents, are commonly regarded as potential sources for Brachyspira transmission to pigs,2 and a recent investigation of wild rats revealed that 83% of the rats captured on swine and poultry farms were positive for Brachyspira spp. by culture.3 This high level of colonization in wild rats appears somewhat contradictory to the reported limited period of fecal shedding observed following experimental inoculation.

Objectives
1) To compare the duration of fecal shedding of various Brachyspira spp. in inoculated rats.
2) To compare cecal colonization in rats versus fecal shedding.

Materials and Methods
Groups
- Thirty-six Sprague-Dawley outbred rats
- 6 groups: 1 sham and 5 different Brachyspira isolates representing the following species:
  - Brachyspira intermedia
  - Brachyspira pilosicoli
  - "Brachyspira hampsonii" clade I
  - "Brachyspira hampsonii" clade II
  - Brachyspira hyodysenteriae
- There were 6 rats per group with 2 rats per box (Figure 1).
- 66% of the rats were female and 33% were male.

Inoculation
- Rats were gavage inoculated on 2 consecutive days.
- Inoculum was 1.0 ml in volume and contained approximately 1x10^8 Colony Forming Units (CFUs)

Methods (continued)
Sampling
- Sampling was performed as outlined in Figure 2.
- Fecal samples were collected two days prior to inoculation and throughout the first week post-inoculation (PI) and then again on days 10, 14, 17, 21, 24, 28, and 35 PI.
- Fecal samples were pooled by box except on days 7, 14, 21, 28, and 35 PI.
- Each box of rats (Figure 3) was considered an environmental unit as they could both potentially shed the organism and expose one another.
- On the days of pooled culture sampling, collection was initially done by physical manipulation of the fecal pellet from the animal. If this was unsuccessful, rats were placed in an individual fecal collection box for no more than 30 minutes. If the rat still had not produced fecal pellets, a determination was made of the freshest sample from the shared box and that sample was collected.
- On individual rat sampling days, if manual sampling was unsuccessful the rats were placed in the fecal collection box for no more than an hour with repeated checks for the production of feces.
- To prevent potential cross-contamination between different inoculated groups, lab coats, gloves, and collection instruments were changed.
- Gloves were sanitized between each box within a group.
- Groups were handled from least to most pathogenic Brachyspira spp. inoculated.

Culture
- Fecal samples were diluted in 0.5 ml sterile saline (Figure 4).
- All sample types were spread onto two selective agars: CVS (collistin, vancomycin, and streptomycin) and BJ.
- Inoculated media were incubated anaerobically at 42°C.
- Plates were checked for growth and hemolytic patterns at 2, 4, and 6 days.
- Results were confirmed via darkfield microscopy.

Results
- Strongly beta hemolytic spirochetes were isolated from cultures of fecal samples collected 6 PI from rats inoculated with "B. hampsonii" clade I (Figure 5).
- The identity of the isolated spirochetes was confirmed via a nose-based PCR assay.
- All other fecal culture samples were negative.
- All cecal culture samples were negative.
- Direct PCR on cecal contents collecting at necropsy were all negative.
- Necropsy results were unremarkable in all rats.

Conclusions
- These data suggest that commercially available Sprague-Dawley outbred rats, as supplied, are not an effective model for studying colonization and shedding behavior of Brachyspira spp. in rats.
- These rats may, however, prove useful in assessing potential co-infection and dietary models exploring the pathogenesis of Brachyspira infection as these rats were not readily colonized following oral inoculation alone.
- Recovery of viable spirochetes from rats at 6 DPI is significant in that this is three times longer than the previous experimental report of fecal shedding limited to just 2 DPI.
- These data reveal that rats can shed "B. hampsonii", a recently proposed novel species6 that has been recovered from pigs with clinical swine dysentery following both natural and experimental infection.8
- Pork producers and veterinarians can use this information when formulating rodent control protocols and elimination efforts for swine dysentery.

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References