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

Kate Sawyer, Bailey Wilberts, Joann Kinyon, Eric Burrough
Iowa State University, College of Veterinary Medicine, Ames, IA

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-sex 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.
  - Rats of the same gender were housed together.
- Rats were provided a standard pelleted rodent diet as well as free-choice access to commercial pig feed consistent with what might be encountered in commercial swine finishing operations.
  - Both feed types as well as water were given *ad libitum*.

Inoculation

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

Figure 1. Housing structure for rats. There were 2 rats per box. There were a total of 18 boxes with 6 treatment groups.

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 produced no fecal pellet, 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 (colistin, vancomycin, and streptomycin) and BJ[4].
- 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.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
	Day -2 Fecal samples		**Day 1** • Inoculation #1	**Day 2** • Inoculation #2 • Fecal samples	**Day 3** Fecal samples	**Day 4** Fecal samples
Day 5 Fecal samples	**Day 6** Fecal samples	**Day 7** Fecal samples			**Day 10** Fecal samples	
		Day 14 Fecal samples			**Day 17** Fecal samples	
		Day 21 • Fecal samples • Cull 2 rats/group (#12) • Cecal samples			**Day 24** Fecal samples	
		Day 28 • Fecal samples • Cull 2 rats/group (#12) • Cecal samples			**Day 31** Fecal samples	
		Day 35 • Fecal samples • Cull last 2 rats/group • (#12) • Cecal samples				

Figure 2. Schedule of sampling for culture. Days listed in black are pooled samples between 2 rats within a box. Days listed in red were individual rat samples.

Figure 3. Close-up view of a single rat box. Boxes were taken to a separate table to handle the animals and for fecal pellet collection.

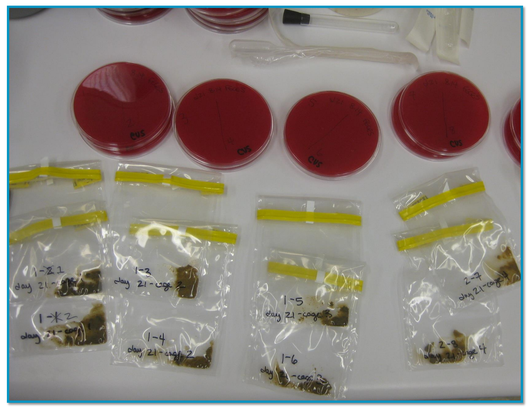

Figure 4. Dilution of fecal samples in saline. Samples were then plated on both CVS and BJ selective agar plates.

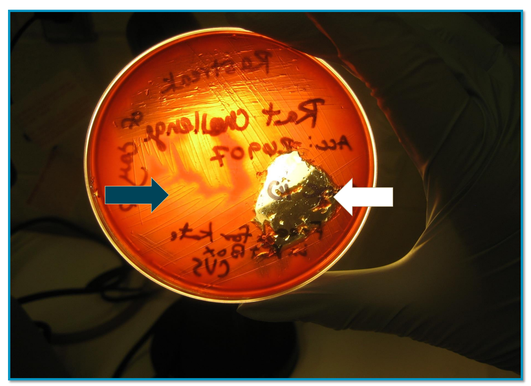

Figure 5: Positive plate for "*B. hampsonii*" clade I. Hemolytic pattern of the organism can be viewed against a back light (blue arrow). Note : An area of agar was removed for save back and PCR (white arrow).

Results

- Strongly beta-hemolytic spirochetes were isolated from cultures of fecal samples collected **day 6** PI from rats inoculated with **"*B. hampsonii*" clade I (Figure 5).**
  - The identity of the isolated spirochetes was confirmed via a *nox*-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 species[5] that has been recovered from pigs with clinical swine dysentery following both natural and experimental infection.[6]
- Pork producers and veterinarians can use this information when formulating rodent control protocols and elimination efforts for swine dysentery.

Acknowledgement

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