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Survival of Pathogenic Intestinal Spirochetes Kept in Pure Cultures and in Pig Feces Held at Four Different Temperatures

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Summary and Implications

Porcine colonic spirochetosis (PCS) caused by Serpulina pilosicoli has been identified as a contributing cause of diarrhea and reduced performance of growing pigs in all major swine producing countries. The current view that transmission of PCS occurs through contamination of the environment by acutely or persistently infected pigs is based on the assumption that the spirochetes remain viable in the environment. The purpose of this study was to compare the viability of Serpulina pilosicoli kept in pure culture or mixed with feces at four different temperatures over time with that of Serpulina hyodysenteriae. The results of the present study indicated Serpulina pilosicoli survived considerably longer than Serpulina hyodysenteriae in pure cultures held at 75°F and 99°F, and at all temperatures in spiked fecal materials. Pure cultures of Serpulina pilosicoli survived for at least 63 days at -158°F, seven to 14 days at 39°F 14 to 28 days at 75°F and seven to 28 days at 99°F. The survival of each spirochete mixed with feces was similar as pure cultures for samples kept at -158°F and 39°F but was reduced to one to seven days at 75°F and one to three days at 99°F for Serpulina pilosicoli and <five days at 75°F and < one day at 99°F for Serpulina hyodysenteriae. Information on the survival of Serpulina pilosicoli outside the pig's body provides a basis to improve strategies for PCS control.

Introduction

Recent advances in genetic-based identification have led to the recognition of *Serpulina pilosicoli,* a new pathogenic intestinal spirochete difference from *Serpulina hyodysenteriae,* the cause of swine dysentery. First identified in 1980 in the United Kingdom, *Serpulina pilosicoli* was recognized as the etiologic agent of porcine colonic spirochetosis (PCS) in 1996. Since then, PCS has been identified as a contributing cause of diarrhea and reduced performance of growing pigs in North America, Europe and Australia.

The clinical signs of PCS consist of transient diarrhea, which is gray to green in color and the consistency of wet cement. Persistent infections cause a lack of uniformity in weight gain, increased days to market and increased feed costs. The disruption of pig flow and increased number of pigs with lighter weights at the end of the feeding period are major problems in all-in/all-out management systems.

Although the significance of PCS as a disease of growing-finishing pigs is well documented, the mode of transmission of *Serpulina pilosicoli* between pigs is poorly understood. A major risk factor for PCS is a history of moving and mixing of weaner or grower pigs to new accommodations and a change of diet. The current view that *Serpulina pilosicoli* is transmitted by oral exposure to contaminated fecal materials is based on the assumption

Table 1. Survival of *Serpulina hyodysenteriae* and *Serpulina pilosicoli* in spiked fecal pools held at four different temperatures over 63 days.

Temperature	Strain/replicates		Positive (+) or negative (-) culture results from day 0 to day 63 of incubation												
			0	1	3	5	7	14	21	28	35	42	49	56	63
-158°F	*S. hyodysenteriae*	1	++	++	++	--	--	--	--						
		2	++	-+	--	+-	--	--	--						
	S. pilosicoli	1	++	++	++	++	++	++	--	--	-+	+-	-+	--	--
		2	++	++	+-	--	--	--	--	--	--	+-	--	--	--
39°F	*S. hyodysenteriae*	1	++	++	+-	+-	+-	--	--	--					
		2	++	++	--	--	--	--	--	--					
	S. pilosicoli	2	++	++	++	++	++	++	--	--	--				
		2	++	++	++	++	++	+-	--	--	--				
75°F	*S. hyodysenteriae*	1	++	++	--	+-	--	--	--						
		2	++	--	--	--	--	--	--						
	S. pilosicoli	1	++	++	++	++	-+	--	--	--					
		2	++	++	--	--	--	--	--						
99°F	*S. hyodysenteriae*	1	++	-+	--	--	--	--							
		2	++	--	--	--	--	--							
	S. pilosicoli	2	++	++	-+	--	--	--							
		2	++	-+	--	--	--	--							

Replicates: 1 = duplicates of spiked finisher pig fecal pool; 2 = duplicates of spiked grower pig fecal pool.

that the spirochetes remain viable after shedding in the environment. However, with the exception of *Serpulina hyodysenteriae*, little is known about the survival of spirochetes outside of the pig's body. The purpose of this study was to compare the viability of *Serpulina pilosicoli* in pure culture or mixed with feces at four different temperatures over time with that of *Serpulina hyodysenteriae*.

Materials and Methods

Stock cultures of *Serpulina pilosicoli* and *Serpulina hyodysenteriae* were propagated using a standard protocol for anaerobic culture of spirochetes in a liquid medium. The optical density, total bacterial count, viability count and purity of each stock culture were determined by standard laboratory methods.

Fecal materials were collected from four healthy pigs: two finisher (230 pound) and two grower (110 pound) pigs, fed a corn/soybean meal-based diet without a antimicrobial. Pigs were selected on the basis of prior spirochetes-negative fecal cultures. The fecal specimens were refrigerated until the next day when two pools, a finisher and a grower pig fecal pool, were prepared. Spiked fecal pools were prepared by mixing ten times concentrated spirochete cultures with ten times the volume of fecal pool using a mechanical mixer. Four replicates of each pure culture and duplicates of each spiked fecal pool were aliquoted into sterile tubes and held at either -158°F, 39°F, 75°F or 99°F until processing for determination of viability on day zero (sample preparation), one, three, five, seven and at weekly intervals until day 63. The viability of the spirochetes was determined by anaerobic incubation of aliquots of either pure culture or spiked fecal pool onto selective agar medium. Each culture was evaluated blindly after a two-, four- and seven-day incubation period and recorded as positive or negative for the presence of spirochetes.

Results

The pure cultures of *Serpulina pilosicoli* and *Serpulina hyodysenteriae* contained approximately 3 X 10^{11} and 4 X 10^{10} spirochetes per ml, respectively. Pure cultures of both spirochetes survived for the entire observation period of 63 days at -158°C, whereas survival at 39°F was reduced to seven to 14 days for both spirochetes. At 75°F and 99°F, *Serpulina pilosicoli* survived for 14 to 28 days and seven to 28 days, whereas *Serpulina hyodysenteriae* survived for less than one day at each temperature.

The viability of *Serpulina hyodysenteriae* and *Serpulina pilosicoli* in each spiked fecal pool held at each temperature over 63 days is presented in Table 1. Overall, *Serpulina pilosicoli* kept under identical conditions survived longer than *Serpulina hyodysenteriae*. However, the viability of each spirochete held at 75°F and 99°F over time was markedly reduced when inoculated into the grower pig fecal pool when compared with the finisher pig fecal pool (Table 1). Both spirochetes were recovered from all of the spiked fecal pools on the day of inoculation (day zero), but *Serpulina hyodysenteriae* survived less than five days at -158°F, 39°F and 75°F, and less

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than one day at 99°F. Although *Serpulina pilosicoli* showed a gradual loss of viability with increasing temperatures over time, it survived up to 49 days at -158°F, 14 days at 39°F, one to seven days at 75°F and less than three days at 99°F.

Discussion

The results of this study indicate *Serpulina pilosicoli* survives longer than *Serpulina hyodysenteriae* in pure cultures held at 75°F and 99°F, and at all temperatures in spiked fecal materials. Reduced viability of both spirochetes was found in spiked feces over time, an effect was more marked at 75°F and 99°F, and possibly attributable to a direct effect of temperature on the viability of spirochetes exposed to ambient air. However, in a biological model such as spiked feces, the interaction of the spirochetes with the normal fecal bacteria may require rapid induction of adaptative survival mechanisms. For example, the number of bacteria in normal human feces is estimated at 10^{11} per gram, therefore competition with the resident bacteria for limited nutrients may be involved. Additionally, the source of the fecal pools appeared to have an effect on the viability of the spirochetes; the viability of each spirochete was less over time when held at 75°F and 99°F in the grower pig fecal pool compared with the finisher pig fecal pool. The reason for this variation is unknown.

Determination of the duration of potential infectivity of *Serpulina pilosicoli* is critical to management practices such as all-in/all-out and optimal timing for reintroduction of pigs after cleaning. Although the viability of *Serpulina pilosicoli* in fecal materials obtained from naturally infected pigs would have to be examined before definitive conclusions can be made, the data suggested at least seven days may be required for elimination of *Serpulina pilosicoli* from the environment without decontamination.

Serpulina pilosicoli can be isolated from the large intestine of challenge-inoculated pigs for up to six weeks post-inoculation, even though diarrhea may have ceased. This suggests transmission of PCS is from shedding of *Serpulina pilosicoli* in the feces of persistently infected pigs. Carrier-shedder pigs are an important reservoir of *Serpulina pilosicoli* on infected farms, and movement of infected pigs is the most likely means of transmission of *Serpulina pilosicoli* between farms. However, considering *Serpulina pilosicoli* is viable for up to 14 days at less than 39°F, transmission by contaminated fecal material also is likely to occur between groups of pigs or between pens, particularly during winter. This is consistent with high prevalence of clinical signs of PCS in management systems that favor fecal-oral recycling, such as open-flush gutters and recycled lagoon water.

In all-in/all-out multi-site production systems, transmission most likely results from commingling susceptible and shedder pigs. In continuous flow production systems, spirochetes are most likely transmitted by feces from older pigs coming in contact with younger *Serpulina pilosicoli*-naive pigs or from the contaminated environment. Indirect transmission arising from contaminated vehicles or movement of personnel with contaminated clothes or boots also is possible. The possibility also exists that hosts other than pigs may act as potential sources of *Serpulina pilosicoli*, emphasizing the need for biosecurity. Access of dogs, mice and wildlife, including birds, to the pigs and feedstuffs should be restricted.

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