Isolation of *Treponema hyodysenteriae* from Wild Rodents

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Rodents from swine-producing farms were examined for the presence of *Treponema hyodysenteriae*. Wild mice ($n = 257$) and rats ($n = 41$) were trapped on eight farms. Ceca were removed aseptically, and the contents and mucosal scrapings were cultured on selective medium (blood agar containing 400 μg of spectinomycin per ml). *T. hyodysenteriae* was detected in the cecal scrapings of four mice from three different farms where swine dysentery had occurred. Gross lesions were detected in the ceca in two of the four mice. In addition, *Treponema innocens* was detected in the cecal scrapings of 12 mice and 13 rats. Three of the four *T. hyodysenteriae* isolates were pathogenic when inoculated intragastrically into swine. The results of this investigation suggest that wild rodents may be carriers of *T. hyodysenteriae*.

Treponema hyodysenteriae is an anaerobic spirochete that produces enteric lesions in orally inoculated pigs and laboratory mice (4, 5, 10, 14, 20). Pigs are considered the primary hosts of the organism (1, 3, 18). Asymptomatic carriers of swine dysentery (SD) are able to transmit the disease to susceptible sentinel pigs as long as 70 days after initial infection (18). Recently, our laboratory has shown that laboratory mice infected with *T. hyodysenteriae* shed the organism in their feces for 180 days and are able to transmit infection to susceptible swine after only 5 to 7 days (9).

With the exception of one report of a transitory infection in a dog (17), the organism has been found only in swine. This report describes the examination of wild rodents on swine-producing farms for the presence of *T. hyodysenteriae*.

MATERIALS AND METHODS

Farms. Eight swine-producing farms in central Iowa were visited. SD had occurred on six farms but had not occurred on two farms.

Trapping rodents. Mice and rats were trapped in conventional (Animal Trap Co., Lititz, Pa.) or live traps (J. J. Dill Co., Kalamazoo, Mich.). The traps were examined every other day and reset and baited if needed. Rodents were placed in bags or plastic containers and transported back to the laboratory for necropsy.

Necropsy. The cecum was aseptically removed from each rodent, opened longitudinally, and sampled at four different mucosal sites. Mucosal scrapings were placed onto selective agar and cultured for isolation of *T. hyodysenteriae* (19). The plates were placed in an anaerobic GasPak (BBL Microbiology Systems, Cockeysville, Md.) container and incubated under an atmosphere of 50:50 $H_2:CO_2$ at 42°C for 2 to 6 days. A beta-hemolytic zone without the presence of surface growth indicated the presence of *T. hyodysenteriae* in the cecal scrapings. A weakly beta-hemolytic zone indicated the presence of *Treponema innocens* (12).

Bacterial isolation. Agar plugs of hemolytic zones were picked and subcultured into Trypticase (BBL) soy broth (TSB) supplemented with fetal calf serum (10% vol/vol) and incubated under an atmosphere of 50:50 $H_2:CO_2$ at 38°C (11). Broth cultures of either *T. hyodysenteriae* or *T. innocens* were examined for purity and then frozen at −70°C in 1-ml samples. All further testing except the API-ZYM and drug sensitivity tests was performed from broth cultures.

Control cultures. *T. hyodysenteriae* strains B204 and B78 were obtained from pigs affected with SD and were shown to be pathogenic for pigs (14). *T. innocens* strain B256 was obtained from a pig with signs of postweaning diarrhea, but it was nonpathogenic for pigs (14). The swine isolates were used as controls in the in vitro and in vivo testing of rodent isolates.

Hemolysin production. Augmentation of hemolytic activity was determined by the method of Picard et al. (16) with the following modifications: (i) the basal broth used was TSB with fetal calf serum incubated under an atmosphere of 50:50 $H_2:CO_2$ (11); (ii) the cultures were tested for hemolytic activity at 24 h; and (iii) equine erythrocytes were used as the indicator system.

Susceptibility testing. In vitro susceptibility tests were conducted similar to those described previously (13). Isolates were subcultured on Trypticase (BBL) soy agar with 5% bovine blood. Antimicrobial agents were prepared at several concentrations in sterile distilled water, mixed with molten Trypticase soy agar plus bovine blood, and then poured into quadrant petri dishes. Control media were also prepared without antimicrobial agents. Inoculum was prepared from each isolate by the mixing of agar cultures with TSB and glass beads. The resulting suspension was spot-inoculated onto the susceptibility test media in a replicate pattern. The tests were incubated as described and the results were recorded after 3 days. The

minimum inhibitory concentration of an antimicrobial agent from a given isolate was the lowest concentration which inhibited its growth and hemolysis. If growth was present at or above the accepted minimum inhibitory concentration level (13), the isolate was termed resistant.

Enzyme tests. The methods used for testing the reactions of treponemes in the API ZYM kit (Analytab Products, Plainview, N.Y.) were as previously described (8) with the following exceptions: (i) cultures were grown on 5% bovine blood-Trypticase (BBL) soy agar plates, and (ii) the plates were incubated under an atmosphere of 80:20 $H_2:CO_2$ at 42°C.

Serotyping of isolates. The beta-hemolytic isolates were cultured in 200 ml of TSB supplemented with fetal calf serum and extracted with hot (68°C) phenol-water by a modification of the method of Baum and Joens (2). A 48-h culture was harvested, and the cells were washed once in phosphate-buffered saline (pH 7.2, 0.01 M). The cells were pelleted and extracted as previously described (2). The aqueous phase was dialyzed against 100 volumes of distilled H_2O overnight and precipitated with 6 volumes of 90% ethanol and 10 mg of sodium acetate at −20°C overnight. The precipitate was resuspended in 2 ml of distilled H_2O and tested against reference sera to the four serotypes in a gel-diffusion test (2).

Enteropathogenicity testing. Beta-hemolytic isolates were tested for pathogenicity in swine colonic loops, mice (strain CF1), and pigs (10, 14). The beta-hemolytic mouse isolates were cultured in TSB-fetal calf serum and inoculated at the time of log-phase growth into ligated colonic loops (single 10-ml dose) or into mice and pigs intragastrically on 2 consecutive days. The weakly beta-hemolytic isolates were tested for pathogenicity in mice (strain CF1) and pigs. Observations were recorded and lesions were noted at necropsy.

RESULTS

Cecal culture results. A total of 157 mice (*Peromyscus* spp., $n = 5$; *Microtus* spp., $n = 8$; and *Mus musculus*, $n = 144$) and 21 rats (*Rattus norvegicus*) were trapped on farms where SD had occurred. Four mice (*Peromyscus* spp., $n = 1$; *M. musculus*, $n = 3$) from three different farms were positive by culture for *T. hyodysenteriae*, and 12 mice (*Peromyscus* spp., $n = 5$; *M. musculus*, $n = 7$) from five different farms were positive by culture for *T. innocens*. Seven rats from three farms were positive for *T. innocens*. *T. hyodysenteriae* was not isolated from rats.

A total of 100 mice (*M. musculus*) and 20 rats (*R. norvegicus*) were trapped on farms without a prior occurrence of SD. *T. hyodysenteriae* was not detected in the ceca of mice or rats trapped on these farms. *T. innocens* was isolated from 13 of 20 rats.

Gross observations. Lesions consisting of edema and catarrhal inflammation were detected in the ceca of two mice infected with *T. hyodysenteriae* and trapped on the same farm. Mice infected with *T. hyodysenteriae* from the other two farms did not have detectable lesions. Lesions were not detected in the ceca of mice and rats that harbored *T. innocens* isolates.

Hemolysis titration. The addition of sodium ribonucleate to the medium increased the hemolytic activity of the *T. hyodysenteriae* mouse isolates 8- to 32-fold. The *T. hyodysenteriae* isolates of porcine source had a 32- to 64-fold change in hemolytic activity when sodium ribonucleate was added to the medium. The change

TABLE 1. In vitro testing of *T. hyodysenteriae* and *T. innocens* isolates for hemolysin production, sensitivity to antimicrobial agents, and enzyme production

Isolate source	Hemolytic activity[a]		Minimum inhibitory concentration of antimicrobial agent (μg/ml)[b]							API-ZYM tests[c]			
	Without NaR[d]	With NaR	L	V	I	D	A	T	C	13	14	16	17
T. hyodysenteriae													
T22 mouse	1:4	1:28	12.5	1	1	0.25	50	50	0.005	0	5+	±	1+
T38 mouse	1:8	1:64	12.5	1	0.05	0.05	50	100	0.0005	0	5+	0	3+
T39 mouse	1:4	1:128	25	5	1	0.25	>100	>100	0.015	0	5+	±	3+
T40 mouse	1:4	1:128	25	2	1	0.25	100	>100	0.005	0	5+	±	3+
B204 pig	1:8	1:256	25	5	0.25	0.1	100	>100	0.005	0	5+	±	3+
B78 pig	1:2	1:128	12.5	5	2	>1	>100	50	0.015	0	5+	±	4+
T. innocens													
T25 mouse	<1:2	1:16	ND	ND	ND	ND	ND	ND	ND	0	5+	±	0
T35 rat	<1:2	1:16	ND	ND	ND	ND	ND	ND	ND	1+	5+	±	4+
B256 pig	1:2	1:8	50	2	0.5	0.25	100	>100	0.005	4	5	0	4+

[a] Expressed as reciprocal of last dilution to hemolyze 50% of erythrocytes.

[b] L, Lincomycin; V, virginamycin; I, ipronidazole; D, dimetridazole; A, arsanilic acid; T, tylosin; C, carbadox; ND, not done.

[c] Enzyme 13, alpha-galactosidase; enzyme 14, beta-galactosidase; enzyme 16, alpha-glucosidase; enzyme 17, beta-glucosidase. Values rate the level of enzyme on a scale of 0 to 5+.

[d] NaR, Sodium ribonucleate.

TABLE 2. Enteropathogenicity of *T. hyodysenteriae* and *T. innocens* isolates in mice and pigs

Isolates	Source	Ligated loops of pig colon[a]	Oral inoculation[b]	
			Mice	Pigs
T. hyodysenteriae				
T22	Field mouse	3/6	3/5	0/6
T38	House mouse	ND	3/5	3/4
T39	House mouse	ND	4/5	2/2
T40	House mouse	ND	4/5	2/2
B204	Pig	6/8	5/5	3/5
T. innocens				
T25	House mouse	ND	0/5	0/2
T35	Rat	ND	0/5	0/2
B256	Pig	0/3	0/5	0/2

[a] Values expressed as number of ligated loops of pig colon infected with *T. hyodysenteriae* or *T. innocens* that was passaged once in CF1 mice per number examined. ND, Not done.
[b] Values expressed as number of animals positive by culture per number of animals examined.

in the hemolytic activity of *T. innocens* isolates was 4- to 16-fold (Table 1).

Enzyme tests. Alpha-galactosidase was present in two of the three *T. innocens* isolates tested, but absent in the *T. hyodysenteriae* isolates. Beta-glucosidase was detected in large amounts in all isolates examined except isolate T22 *T. hyodysenteriae* and isolate T25 *T. innocens*. No differences were detected between isolates examined for beta-galactosidase or alpha-glucosidase activity.

Drug sensitivities. Isolate T38 was susceptible to ipronidazole and dimetridazole at very low concentrations. There was no resistance demonstrated by the mouse isolates to any of the antimicrobial agents (Table 1).

Serotype results. The aqueous phase antigen of mouse isolates T22 and T38 reacted against antisera to serotype 1, and the antigen extracted from the mouse isolates T39 and T40 reacted against antisera to serotype 2. The extracts of the *T. innocens* isolates did not react to the *T. hyodysenteriae* antisera.

Enteropathogenicity testing. The four *T. hyodysenteriae* isolates induced cecal lesions similar to those of SD in orally inoculated mice (Table 2). Three of the four isolates of *T. hyodysenteriae* were also pathogenic in orally inoculated pigs and in ligated colonic loops of pigs and produced gross and microscopic lesions of SD. Isolate T22 was nonpathogenic in orally inoculated swine and in ligated colonic loops. However, after one passage through a mouse (strain CF1), it was pathogenic in ligated colonic loops, but not in orally inoculated swine. The mouse and rat *T. innocens* isolates were nonpathogenic in orally inoculated mice and pigs.

DISCUSSION

This is the first report of the isolation of *T. hyodysenteriae* from wild rodents. These data

and a previous study (9) indicate that rodents may be involved in the spread and transmission of SD. In addition, the detection of *T. innocens* in a large number of mice and rats from both infected and noninfected premises indicates that this organism is probably endemic in the rodent population.

The amount of hemolysin produced by the mouse *T. hyodysenteriae* isolates was similar to that of the control isolates B78 and B204. After the addition of ribonucleate, the *T. hyodysenteriae* isolates could be differentiated from the *T. innocens* isolates by the increase in hemolytic activity of the *T. hyodysenteriae* isolates. These results were similar to those reported by Picard et al. (16).

The sensitivities of the mouse isolates to the antimicrobial compounds were similar to those of the control pig isolates. Isolate T38 seems slightly more sensitive to ipronidazole and dimetridazole than the other isolates. Mice inhabiting pig feeders probably feed on the drug-treated rations. Thus, the high degree of sensitivity shown by these *T. hyodysenteriae* isolates may be the reason for the low incidence of this organism in the wild rodent population.

All of the *Treponema* isolates produced beta-galactosidase, but only the *T. innocens* isolates produced alpha-galactosidase. Hunter and Wood (8) have suggested that the presence of alpha-galactosidase distinguishes the nonpathogenic from the pathogenic treponemes, and our results partially concur with this idea. However, we cannot separate the pathogens from the nonpathogens by the presence of beta-glucosidase, as suggested by Hunter and Wood (8), because this enzyme was present in all but one of the isolates tested.

With the exception of isolate T22, enteropathogenicity testing of the mouse isolates produced results similar to those from studies with

porcine isolates (6, 7, 14). Isolate T22, which was obtained from the cecum of a field mouse (*Peromyscus* spp.), was avirulent in orally inoculated swine. This is one of two reports (15) of an isolate that produces the beta-hemolysin but is nonpathogenic in pigs. This isolate may be just one of many such organisms in the wild that is lacking one or more of the virulence factors but, upon being cultured, resembles pathogenic types.

With the presence of *T. hyodysenteriae* in the rodent population, it may be necessary to establish a rodent eradication program to completely disinfect a facility in which an SD outbreak has occurred.

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