HYGROMYCIN A, AN ANTITREPONEMAL SUBSTANCE

II. THERAPEUTIC EFFECT FOR SWINE DYSENTERY

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This study was conducted to evaluate hygromycin A fed to growing swine at 1, 5, 10 or 20 g/ton feed for the control of *Treponema hyodysenteriae*-caused dysentery. Pigs provided carbadox at 50 g/ton feed served as an infected treatment control group. All pigs were orally, *via* stomach intubation, administered 100 ml of a *T. hyodysenteriae* broth culture. During the *in vivo* test, rectal swabs were taken for *T. hyodysenteriae* isolation, body weights of all pigs and the feed consumption was determined. All pigs were euthanized and necropsied at study end; the large intestine was cultured for *T. hyodysenteriae* and gross intestinal lesions were noted. *T. hyodysenteriae*-caused swine dysentery was successfully controlled by feeding hygromycin A at 5 g/ton. Hygromycin A medicated pigs performed as well as or better than carbadox-medicated pigs.

Swine dysentery (SD) is a highly infectious disease of pigs. It is characterized clinically by dehydration, loss of body weight, and muco-hemorrhagic diarrhea and pathologically by marked inflammation of the colon and cecal mucosa. *Treponema hyodysenteriae* has an important role in the pathogenesis of the disease.[1~5] The interaction between *T. hyodysenteriae* and selected Gram-negative anaerobes as a cause of SD has been reported.[1,6~9] Several drugs such as carbadox,[10~12] lincomycin,[13,14] olaquindox,[11] tiamulin[15,16] (dynamutilin) and lankacidin C (sedecamycin) have been used for the treatment and control of SD. However, relapses of SD are frequently observed in swine after drug withdrawal. An apparent gradual decline in effectiveness has been noted with some drugs commonly used against SD. In previous paper,[17] we reported the *in vitro* and *in vivo* screening systems used to identify antitreponemal substances from microbial metabolites and evaluation of hygromycin A against a *T. hyodysenteriae*-caused infection in CF-1 mice. This investigation was conducted to evaluate hygromycin A, fed to growing swine at 1, 5, 10, 20 g/ton, for the control of *T. hyodysenteriae*-caused dysentery.

Materials and Methods

Pigs

Crossbred barrows and gilts (an equal number of each sex), approximately 6 weeks old, were obtained from Simpson Farms, Ault, Colorado. Pigs were fed a 17%-protein pig starter mash diet,

Table 1. Design of therapeutic experiment of hygromycin A.

Expt	Treatment designation	Drug in feed	Drug level (g/ton)	Induced dysentery	No. replicate pens	No. pigs per pen	Total No. pigs per treatment
1	A	Hygromycin A	20	Yes	2	5	10
	B	Hygromycin A	10	Yes	2	5	10
	C	Carbadox	50	Yes	2	5	10
	D	None	0	Yes	2	5	10
2	E	Hygromycin A	10	Yes	2	5	10
	F	Hygromycin A	5	Yes	2	5	10
	G	Hygromycin A	1	Yes	2	5	10
	H	Carbadox	50	Yes	2	5	10
	I	None	0	Yes	2	5	10

manufactured by Windsor Elevator, Windsor, Colorado as Lot No. 8618803, which was used both as a control ration and as a basal ration to which the hygromycin A and carbadox were incorporated to obtain the medicated test diet. Pigs were stratified by body weight and sex and were randomly assigned to pen in Table 1.

T. hyodysenteriae Culture Preparation

T. hyodysenteriae strain B204 (CARE Code T-2) obtained from Iowa State University was used in this study. This culture was grown anaerobically at 35~37°C in Trypticase soy broth (BBL) supplemented with fetal calf serum (BHI), cysteine, sodium bicarbonate and glucose. Prior to use, the prepared culture was diluted and plated to determine the number of *T. hyodysenteriae*/ml of culture.

Experimental Procedure

Experiment 1: After 4 days adjustment period, all pigs were fasted from feed for 23 hours and water for 17 hours and then were orally (*via* stomach intubation) administered 100 ml of the prepared culture. Each pig received approximately 1.2×10^9 *T. hyodysenteriae*. Treatment diets [hygromycin A: 20 g/ton, 10 g/ton and carbadox (Pfizer International Inc. Lot No. K61573060): 50 g/ton] were provided 1 hour post-challenge and continued *ad libitum* for 3 weeks followed by a two-week non-medication period. Clinical signs were noted and recorded for each pig each day post-challenge. All pigs were necropsied on day 35 post-challenge to assess gross lesions of the large intestine. Feed consumption and individual pig body weight measurements and rectal swabs for *T. hyodysenteriae* isolation were taken pre-challenge and weekly post-challenge.

Experiment 2: After 4 days adjustment period, all pigs were fasted from feed and water for 5.5 hours and then were orally (*via* stomach intubation) administered 100 ml of the prepared culture. Each pig received approximately 4×10^8 *T. hyodysenteriae*. Treatment diets [hygromycin A: 10 g/ton, 5 g/ton, 1 g/ton and carbadox (Pfizer International Inc. Lot No. K61573060): 50 g/ton] were provided 1 hour post-challenge and continued *ad libitum* for 2 weeks followed by a two-week nonmedication period. Clinical signs were noted and recorded for each pig each day post-challenge. All pigs were necropsied on day 28 post-challenge to assess gross lesions of the large intestine. Feed consumption and individual pig body weight measurements and rectal swabs for *T. hyodysenteriae* isolation were taken pre-challenge and weekly post-challenge.

Clinical Observations

Once daily during the post-challenge period, every pig in each pen was scored as follows with regard to clinical signs:

a) General appearance; 1=normal, 2=depressed, 3=moribund, 4=dead.

b) Hydration; 1=normal appearance, 2=slight gauntness of flanks and abdomen, 3=gaunt, increased thirst, muscular weakness, 4=emaciated, very thirsty, very obvious muscular weakness.

c) Fecal scores; 1=normal, 2=semi-solid, pasty, 3=watery with some solid material, 4=profuse, watery feces with little solid material, 5=blood present (any consistency).

Body Weights and Feed Consumption

Individual pig weights were determined for all groups at time of selection, approximately 6 hours prior to challenge, and weekly during the post-challenge period. Feed consumption was determined for all groups on a pen basis after 4 days of adjustment and weekly thereafter for the post-challenge period.

Detection of *T. hyodysenteriae*

Detection of *T. hyodysenteriae* was conducted by streaking rectal or colon swab specimens onto 5% citrated bovine blood agar plates containing 1,000 μg of spectinomycin/ml and incubating anaerobically at 35~37° for 7 days. Plates were examined for areas of β-hemolysis. Areas of β-hemolysis were then microscopically examined for presence of large spirochetes to confirm the sample as positive for *T. hyodysenteriae*.

Detection of Salmonellae

The rectal swab was placed in 9 ml tetrathionate broth and incubated for approximately 18~24 and 34~48 hours at 35~37°C. After each incubation period, the broth was streaked onto brilliant green agar (BGA) plates and incubated 18~24 hours at 35~37°C.

Necropsy

All surviving pigs were sacrificed at study end and necropsied for gross pathology. The large intestine was examined and lesions or conditions indicative of dysentery were noted and recorded. Any other abnormalities of the intestine and liver were also noted and recorded.

Results

Experiment 1

Clinical Observations

The nonmedicated pigs had 21.5% fewer "pig days" of normal appearance compared to the medicated pigs. There were 31.9% fewer "pig days" of normal hydration state of the nonmedicated pigs compared to medicated pigs. Fecal consistency of all the medicated pigs remained essentially normal throughout the 35 days post-challenge. The nonmedicated pigs had 49.7% fewer "pig days" of normal fecal condition. Bloody feces were present during 37.8% of the abnormal fecal status "pig days". Bloody feces were detected in 9 out of the 10 nonmedicated pigs (Table 2).

Body Weights and Gain

Average body weight on the day prior to challenge was 14.6 kg. Nonmedicated pigs overall, gained 26.5% less (624 kg) than the medicated pigs (Table 3).

Table 2. Results of Experiment 1.

Treatment	Number of pig days								Fecal score of				
	General appearance of				Hydration score of								
	1	2	3	4	1	2	3	4	1	2	3	4	5
Hygromycin A (20 g/ton)	349	1	0	0	350	0	0	0	345	5	0	0	0
Hygromycin A (10 g/ton)	349	1	0	0	346	4	0	0	345	5	0	0	0
Carbadox (50 g/ton)	349	1	0	0	347	3	0	0	343	7	0	0	0
Nonmedicated	274	76	0	0	234	65	31	17	173	64	27	29	67

Table 3. Results of Experiment 1.

Treatment	Body weight gain (kg/pig/day)	Feed conversion (kg of feed/ kg of gain)	Percent of pig positive for *Treponema hyodysenteriae* from rectal swab on post-challenge day					
			7	14	21	28	35	Mean
Hygromycin A (20 g/ton)	0.65	2.15	10	20	10	20	10	14
Hygromycin A (10 g/ton)	0.67	2.17	0	20	0	80	0	20
Carbadox (50 g/ton)	0.70	2.17	50	40	40	50	0	36
Nonmedicated	0.49	2.37	20	50	50	40	10	34

Feed Consumption and Conversions

Medicated pigs had their best feed conversion during the first 2 weeks post-challenge while nonmedicated pigs had their poorest feed conversion during this same period. Overall 35 days post-challenge, all medicated pigs had very similar feed-to-gain ratios while nonmedicated pigs had a 9.2%-poorer conversion than the medicated pigs (Table 3).

Recovery of *T. hyodysenteriae* from Rectal or Colon Swabs

During the 21-day treatment period, the fewest (7%) *T. hyodysenteriae* isolations occurred in the pigs medicated with 10 g/ton hygromycin A, followed by pigs medicated with 20 g/ton hygromycin A (13.3%) and nonmedicated pigs (40%) and pigs medicated with carbadox (43%). By 7 days post-medication, recovery of *T. hyodysenteriae* increased in all medicated pigs with the increased incidence of recovery being the greatest in the group of pigs provided 10 g/ton hygromycin A. Based on all five post-challenge samplings, the pigs medicated with hygromycin A had the fewest recoveries of *T. hyodysenteriae* and pigs medicated with carbadox and nonmedicated pigs had similar recovery incidences (Table 3).

Necropsy Results

At 35 days post-challenge very few lesions were noted in the large intestine of medicated pigs. Lesions were noted in 90% of the nonmedicated pigs, with congestion, edema, watery contents and enlarged submucosal glands as the predominant findings. Pigs medicated with hygromycin A at 20 g/ton had 50% incidence of lesions, with congestion and lymph node swelling as the predominant lesions. Very little difference was noted between lesion incidence in the pigs medicated with 10 g/ton hygromycin A and pigs medicated with carbadox (Table 4).

Experiment 2

Clinical Observations

Three pigs died on post-challenge day 10, 12, 21 in the nonmedicated group and one pig died post-challenge day 15. The nonmedicated pigs had 43% pig days of depressed or moribund appearance. There were 54% fewer pig days of normal hydration status among the nonmedicated pigs compared to medicated pigs. Hydration status of pigs throughout the post-challenge period was very similar among the hygromycin A medicated groups. Carbadox medicated pigs had 6% pig days of mild dehydration while nonmedicated pigs had 56% pig days of mild to severe dehydration. Fecal consistency of all the medicated pigs remained essentially normal throughout the 28 days post-challenge. One pig in hygromycin A (1 g/ton) medicated group had bloody feces on post-challenge days 28 and 29 (post-treatment days 13 and 14). This group also had 9% pig days of semi-solid feces as compared to 2% pig days of semi-solid feces in pigs medicated with hygromycin A at 10 g/ton. Carbadox medi-

Table 4. Necropsy results of *Treponema hyodysenteriae*-challenged pigs at 35 days post-challenge[a] in Experiment 1.

Treatment	Number of pigs with the following lesions observed in the large intestine											
	Congestion	Edema	Lymph node swelling	Fibrinous material	Bloody mucosa	Watery contents	Mucous	Enlarged submucosal glands	Thin cecal or colon wall	Necrosis	Lesion factor[b]	Number of pigs without lesions
Hygromycin A (20 g/ton)	4	1	2	0	0	1	0	0	0	0	8	4
Hygromycin A (10 g/ton)	0	0	0	0	0	1	0	0	0	0	1	9
Carbadox (50 g/ton)	2	0	1	0	0	0	0	0	0	0	3	8
Nonmedicated	4	4	1	1	0	4	1	2	0	0	17	1

[a] 10 pigs/treatment; all lesions were generally mild.

[b] Value obtained by adding together the number of pigs with lesions present for each parameter.

Pigs were challenged on day 5 and then provided respective treatment diets immediately post-challenge for a 21-day period followed by a 14-day nonmedication period.

Table 5. Results of Experiment 2.

Treatment	Number of pig days											
	General appearance of			Hydration score of				Fecal score of				
	1	2	3	1	2	3	4	1	2	3	4	5
Hygromycin A (10 g/ton)	290	0	0	287	3	0	0	283	7	0	0	0
Hygromycin A (5 g/ton)	290	0	0	289	1	0	0	270	20	0	0	0
Hygromycin A (1 g/ton)	290	0	0	287	3	0	0	261	26	1	0	2
Carbadox (50 g/ton)	273	3	0	259	16	0	0	245	27	0	1	2
Nonmedicated	141	97	10	109	57	53	29	87	30	13	30	88

Table 6. Results of Experiment 2.

Treatment	Pig died	Body weight gain (kg/pig/day)	Feed conversion (kg of feed/ kg of gain)	Percent of pig positive for *Treponema hyodysenteriae* from rectal swab on post-challenge day				
				7	14	21	28	Mean
Hygromycin A (10 g/ton)	0	0.56	2.12	10	10	10	10	10
Hygromycin A (5 g/ton)	0	0.53	2.15	10	0	0	10	5
Hygromycin A (1 g/ton)	0	0.52	2.22	0	10	40	0	13
Carbadox (50 g/ton)	1	0.53	2.17	10	10	11	0	8
Nonmedicated	3	0.12	4.88	30	50	25	43	36

cated pigs had 10% pig days of semi-solid feces and had bloody feces on 2 days. The nonmedicated pigs had 65% pig days of abnormal fecal condition. Bloody feces were detected in 9 out of the 10 nonmedicated pigs (Table 5).

Body Weight and Gain

Average body weight on the day of challenge was 11.9 kg. Nonmedicated pigs overall, gained 79% less (137.7 kg) than the medicated pigs. Pigs medicated with 10 g/ton hygromycin A had the best overall gain, 5 and 7% better than pigs medicated with hygromycin A at 5 and 1 g/ton, respectively; and 10% better than carbadox medicated pigs (Table 6).

Feed Consumption and Conversions

Overall 28 days post-challenge, nonmedicated pigs had a 55%-poorer feed conversion than the medicated pigs. Overall, pigs medicated with hygromycin A at 10 g/ton had the best feed-to-gain followed by pigs medicated with hygromycin A at 5 g/ton, pigs medicated with carbadox and pigs medicated with hygromycin A at 1 g/ton (Table 6).

Recovery of *T. hyodysenteriae* from Rectal or Colon Swabs

During the 14-day treatment period, *T. hyodysenteriae* recovery from medicated pigs ranged from 5~10% overall while recovery from nonmedicated pigs ranged from 30~50%. *T. hyodysenteriae* isolations during the 14-day post-treatment period remained the same for all previously medicated pigs, except for a 40%-recovery in pigs previously medicated with hygromycin A at 1 g/ton. Overall 28 days post-challenge, percent recovery from medicated pigs ranged from 5~13%, and 36% from nonmedicated pigs (Table 6).

Necropsy Result

At 28 days post-challenge, except for congestion and edema, very few lesions were noted in the

Table 7. Necropsy results of *Treponema hyodysenteriae*-challenged pigs at 28 days post-challenge or at death[a] in Experiment 2.

Treatment	Number of pigs with the following lesions observed in the large intestine										
	Congestion	Edema	Fibrinous material	Bloody mucosa	Watery contents	Mucous	Enlarged submucosal glands	Thin cecal or colon wall	Necrosis	Lesion factor[b]	Number of pigs without lesions
Hygromycin A (10 g/ton)	3	1	0	0	0	0	0	0	0	4	7
Hygromycin A (5 g/ton)	6	2	0	0	0	0	0	0	0	8	3
Hygromycin A (1 g/ton)	3	2	1	0	1	1	0	0	0	8	6
Carbadox (50 g/ton)	1	1	1	1	1	1	0	0	1	7	9
Nonmedicated	10	3	5	4	8	4	6	0	3	43	0

[a] 10 pigs/treatment.
[b] Value obtained by adding together the number of pigs with lesions present for each parameter.

large intestine of hygromycin A-medicated pigs. All lesions observed in the carbadox-medicated pigs were found in the single mortality that occurred on post-challenge day 15. Lesions were noted in 100% of the nonmedicated pigs, with congestion, watery contents and enlarged submucosal glands as the predominant findings. A 30, 70 and 40%-incidence of lesions was found in pigs medicated with hygromycin A at 10, 5 and 1 g/ton, respectively (Table 7).

Discussion

The study model was acceptable since dysentery was successfully produced in the nonmedicated pigs based on mortality, clinical signs, performance and recovery of *T. hyodysenteriae*. Hygromycin A fed at 5, 10 or 20 g/ton successfully controlled dysentery based on absence of clinical signs of dysentery. Additionally, the hygromycin A medicated pigs had similar or slightly better weight gains and feed conversions compared to the carbadox medicated pigs. Pigs fed hygromycin A at 10 g/ton had the lowest incidence of intestinal lesions and pigs fed hygromycin A at 5 g/ton had the lowest recovery of *T. hyodysenteriae* from rectal swabs during the 28 days post-challenge period. These data indicated that the therapeutic effect in the CF-1 mouse test parallels that of the swine test. Hygromycin A has extremely low toxicity (LD_{50} 2 g/kg in mice, iv and sc). Thus, hygromycin A seems to be an attractive drug for swine dysentery because of low toxicity, high solubility in water, and a potent therapeutic effect.

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