Influence of Coronavirus (Transmissible Gastroenteritis) Infection on Jejunal Myoelectrical Activity of the Neonatal Pig

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Four silver–silver chloride electrodes were surgically implanted at 5-cm intervals on the jejunal serosa of 7 neonatal pigs. Daily recordings, 7 h in duration, were made from each piglet beginning 3 days after surgery. Characteristic migrating motility complexes and short, distinct (2.5–5.0 s), rapidly aboral migrating bursts of intense spike activity ("migrating action potential complexes") were seen in all preinfection recordings. Piglets were inoculated with a 1-ml oral dose of a 0.1% gut suspension from coronavirus (transmissible gastroenteritis) infected pigs. This resulted in inappetence, vomiting, and diarrhea, most marked on the second day postinfection, but which had abated by the third day. When compared to recordings from both fed and fasted noninfected (control) animals, infection significantly altered jejunal myoelectrical activity by (a) shortening the duration of the migrating motility complex on day 1 postinfection and prolonging it on day 2, (b) increasing the number of abnormal activity fronts, and (c) decreasing the number of migrating action potential complexes. Slow wave frequency and the duration of phase 3 of the migrating motility complex were unaffected. When compared to fed control animals, infected piglets also showed a slight shortening of phase 1 of the migrating motility complex on day 1 postinfection and a prolongation on days 2 and 3, as well as a shortening of phase 2 on the second and third days postinfection. Changes in myoelectrical activity were not solely due to decreases in food intake, as abnormalities persisted when food intake returned to normal on postinfection day 3, and disruption of the activity front and migrating motility complex duration were purely transmissible-gastroenteritis-virus-induced phenomena. These findings suggest that infection with transmissible gastroenteritis virus disrupts organized propulsive activity in the jejunum of the neonatal pig.

There is increasing interest in the effects of various enteropathogens on intestinal motility, as well as in the interrelationships between motility, absorption, and secretion in the etiopathogenesis of diarrhea (1). Most studies of the effect of enteric enteropathogens on small intestinal motility have been conducted by Mathias and coworkers. Using an anesthetized rabbit model, these investigators have shown that bacterial enteropathogens or their toxins induce two distinct abnormal myoelectrical complexes: (a) the migrating action potential complex (MAPC), and (b) repetitive bursts of action potentials, the incidence of which depends on the nature of the inciting agent (2–4).

Whereas the anesthetized rabbit has been very useful for initial studies in this field, it may not entirely reflect changes in the conscious animal. This is so because, with the exception of the specific myoelectrical response to the enteropathogenic insult, action potential activity is virtually absent under general anesthesia. This model also fails to allow for examination of any change in activity during the course of the disease process. Therefore, in order to fully understand the small intestinal myoelectrical response to an enteropathogen, it seems

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Abbreviations used in this paper: MAPC, migrating action potential complex; MMC, migrating motility complex; TGE, transmissible gastroenteritis.

essential to use conscious animals with chronically implanted electrodes. The neonatal piglet has great potential for use in this type of study because it is susceptible to a variety of viral and bacterial enteropathogens, tolerates chronic electrode implantation well, and readily adapts to the laboratory environment.

The effect of viral enteropathogens on gastrointestinal myoelectrical activity has not been investigated. Thus, it was our objective to examine the activity of the jejunum of the conscious neonatal pig before and after oral infection with the coronavirus that causes transmissible gastroenteritis (TGE), an acute self-limiting disease of the small intestine (5).

Methods

The infection study was conducted on 7 healthy parasite-free piglets obtained from a local herd at 3 days of age. Piglets were housed in individual stainless-steel cages and fed a mixture of pasteurized cow's milk and enriched white bread at 8 AM, 12 noon, and 5 PM daily.

At one wk of age, the piglets were anesthetized with a mixture of halothane and nitrous oxide, and four bipolar silver–silver chloride electrodes were surgically implanted on the jejunum at 5-cm intervals, beginning 20 cm below the ligament of Treitz. Wires from the electrodes were tunneled subcutaneously to a microconnector (Cannon 9PL1, ITT Cannon Electric Company, Santa Ana, Calif.) situated behind the scapula just to the left of the dorsal midline.

Three days were allowed for surgical recovery, during which time the piglets were acclimatized to a recording box. The box was dark, allowed limited movement, and contained a heating pad to ensure the animals' comfort and relative quiescence during each study.

Measurement of jejunal myoelectrical activity commenced on the fourth postoperative day. Animals were placed in the recording box immediately after the morning meal and were connected via a matching microconnector (Cannon 9SL1) through a type 9853A coupler to a Beckman R711 dynograph recorder (Beckman Instruments, Inc., Schiller Park, Ill.). Recordings were made with the low-frequency cutoff at 5.3 Hz and the high-frequency cutoff at 100 Hz. Recordings commenced at 9 AM daily, 1 h after the morning meal and lasted for 7 h; piglets were fed in the recording box at 12 noon. Four to six preinfection (control) recordings were made from each piglet. At 8 PM on the evening of the fifth or sixth postoperative day, the piglets were infected with a 1-ml oral dose (12×10^5 plaque-forming units) of a 0.1% bacteria-free gut suspension obtained from TGE-infected piglets (OARDC pool [M-6], S-2, AB, 10/17/79). Postinfection recordings were continued in a similar manner to the preinfection studies, beginning at 8 AM the next morning and continuing for the next 3 days. Records were kept of the response to infection, specifically noting attitude, appetite, and any vomiting or diarrhea. At the end of the third day the piglets were killed by an intravenous overdose of sodium pentobarbital. The small intestine was immediately removed and sections of the duodenum, jejunum, and ileum were fixed for histologic examination.

In addition to the control recordings in fed piglets, five or six daily recordings were made from each of 4 separate but similarly prepared piglets after an overnight fast of 17 h. In a separate study, each of these animals was also given an identical oral dose of ether inactivated viral preparation.

Each recording was analyzed visually to assess (a) slow wave frequency; (b) frequency (periodicity), duration, and conduction velocity of phase 3 (the activity front) of the migrating motility complex (MMC), (c) the duration of phase 1 and phase 2 of the MMC, (d) the percentage of abnormal (nonconducted) phase 3 activity, and (e) the frequency and propagation velocity of short, rapidly aborally propagated migrating bursts of intense spike activity, henceforth referred to as MAPCs.

To facilitate analysis, patterns of spike activity were coded for each study using a modification of the technique described by Code and Marlett (6). For this, the number of slow waves with associated spike activity was converted to a percentage and coded to give a histogram of spike activity for each electrode and minute of recording. Phase 1 of the MMC was defined as a period 2 min or longer in which slow waves had no associated spike activity; phase 2 was defined as any period in which slow waves were associated with irregular or random spike activity. Phase 3 (the activity front) was defined as a distinct period 2 min or longer when all slow waves were associated with intense spike activity that appeared sequentially over adjacent electrodes. The duration (periodicity) of each cycle of the MMC was determined as the time from the end of one activity front to the end of the next.

Data from each piglet and electrode were pooled, and relationships between fed control, fasted control, and infected periods were evaluated by Duncan's multiple range test using the SAS user's guide for 1982 (7). All results were expressed as the mean ± SEM and were considered significantly different if $p < 0.05$.

Results

Clinical Disease

Infection with TGE virus produced typical signs of the disease. Piglets became depressed and slightly inappetent on the first day (12–24 h) postinfection. Inappetence became more severe on the second day (36–48 h) with food intake decreasing to 60%–75% of normal. All piglets developed watery fetid diarrhea; retching and vomiting were observed in 4 animals. One piglet died at the end of the second day, but signs of recovery were apparent in the others by the third day postinfection, because appetite, attitude, and fecal consistency returned to normal or near normal. Histologic examination of the small intestine of pigs killed at the end of the third day postinfection showed villus atrophy consistent

Table 1. Influence of Transmissible Gastroenteritis (Coronavirus) Infection on Jejunal Myoelectrical Activity in the Neonatal Pig

	Myoelectrical parameters						
		Migrating motility complex					
	Slow wave frequency (*cycles/min*)	Overall duration (periodicity, *min*)	Duration of phase 1 (quiescent period, *min*)	Duration of phase 2 (irregular spike activity, *min*)	Duration of phase 3 (activity front, *min*)	Percent of abnormal activity fronts[c]	Migrating action potential complexes (*frequency/h*)
Fed	17.2 ± 0.2[a]	45.5 ± 1.1[a]	6.8 ± 0.3[a]	22.4 ± 0.9[a]	4.8 ± 0.1[a]	5.1 ± 2.7[a]	10.1 ± 1.7[a]
Day post infection							
1	16.8 ± 0.3[a]	38.3 ± 1.8[b]	5.7 ± 0.4[b]	18.2 ± 1.4[b,c]	5.1 ± 0.2[a]	43.1 ± 12.6[b]	3.4 ± 1.4[b]
2	15.2 ± 0.8[a]	62.6 ± 4.0[c]	9.0 ± 0.9[c]	16.2 ± 1.7[c,d]	4.8 ± 0.1[a]	48.1 ± 16.9[b]	1.6 ± 1.0[a,c]
3	16.0 ± 0.8[a]	49.7 ± 3.4[a]	9.6 ± 0.7[c]	21.3 ± 2.1[a,b]	4.5 ± 0.1[a]	39.4 ± 14.4[b]	1.0 ± 0.7[b,c]
Fasted controls	16.9 ± 0.2[a]	50.9 ± 2.3[a]	17.9 ± 0.9[d]	15.4 ± 0.9[d]	4.4 ± 0.1[a]	2.0 ± 0.9[a]	0.9 ± 0.2[c]

Least squares of mean ± SEM values. Within each column, means with different superscripts (a,b,c,d) are different at $p < 0.05$. [c] An abnormal or disrupted activity front (phase 3) was defined as one or more of the following: (1) a failure of phase 3 of the migrating motility complex to propagate sequentially over all four electrodes, (2) splitting into two shorter components, or (3) retrograde propagation over two or more adjacent electrodes.

with published descriptions of the histology of the disease (5).

Myoelectrical Activity in Normal Piglets

Slow wave and spike activity were recorded in all animals; preinfection and postinfection myoelectrical data are summarized in Table 1.

Spike activity occurred in characteristic MMCs analogous to those reported in the dog (6,8) (Figure 1). Each activity front (phase 3 of the MMC) was readily identified as a region of intense spike activity. This was propagated aborally at a velocity ranging from 5 to 20 cm/min, and recurred with a range of 4–156 min in fed and 8–102 min in fasted piglets. However, unlike the dog (6,8), periods of phase 1 activity (quiescence) that lasted from 2 to 12 min were interspersed between periods of phase 2 activity, and periods of quiescence immediately after an activity front were not a regular occurrence. In addition, all piglets exhibited a number of bursts of intense spike activity each lasting 2.5–5.0 s, which were conducted aborally at a velocity of ~2 cm/s. These MAPCs (Figures 1 and 2) predominated at the end of phase 2 of the MMC immediately before an activity front, but could be identified during all phases of the cycle (Figure 2). They were more prominent in the fed state (Table 1).

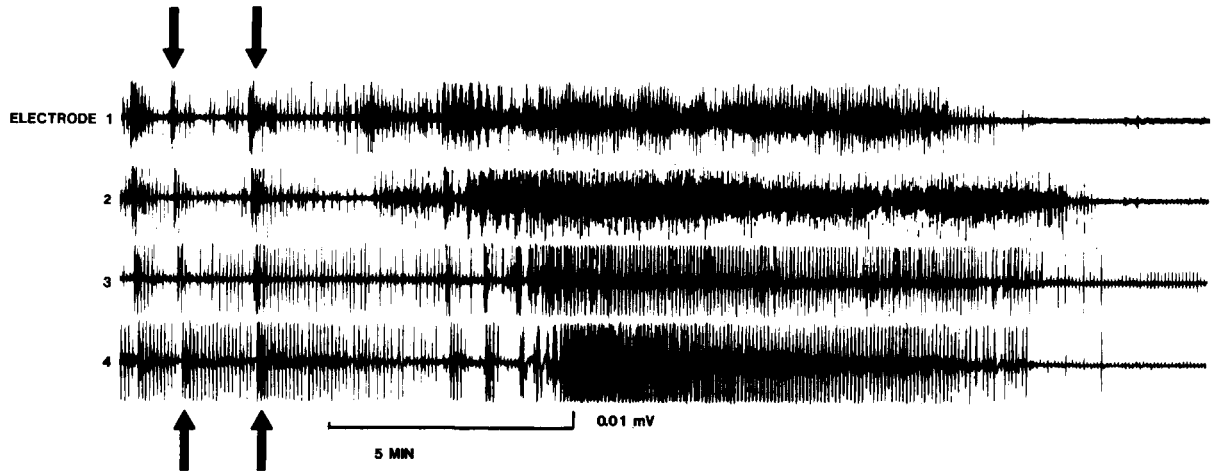

Figure 1. A representative segment of a recording of myoelectrical activity from the jejunum of a normal 9-day-old piglet. Electrodes are located 20, 25, 30, and 35 cm below the ligament of Treitz. A burst of intense spike activity (phase 3) can be seen to be propagated aborally over all four recording sites. A period of less regular and intense activity (phase 2) precedes phase 3, which is followed by a short period devoid of all spike activity (phase 1). In addition, two short bursts of intense spike activity that are rapidly conducted in an aboral direction (migrating action potential complexes) can be seen to precede the activity front (*arrows*).

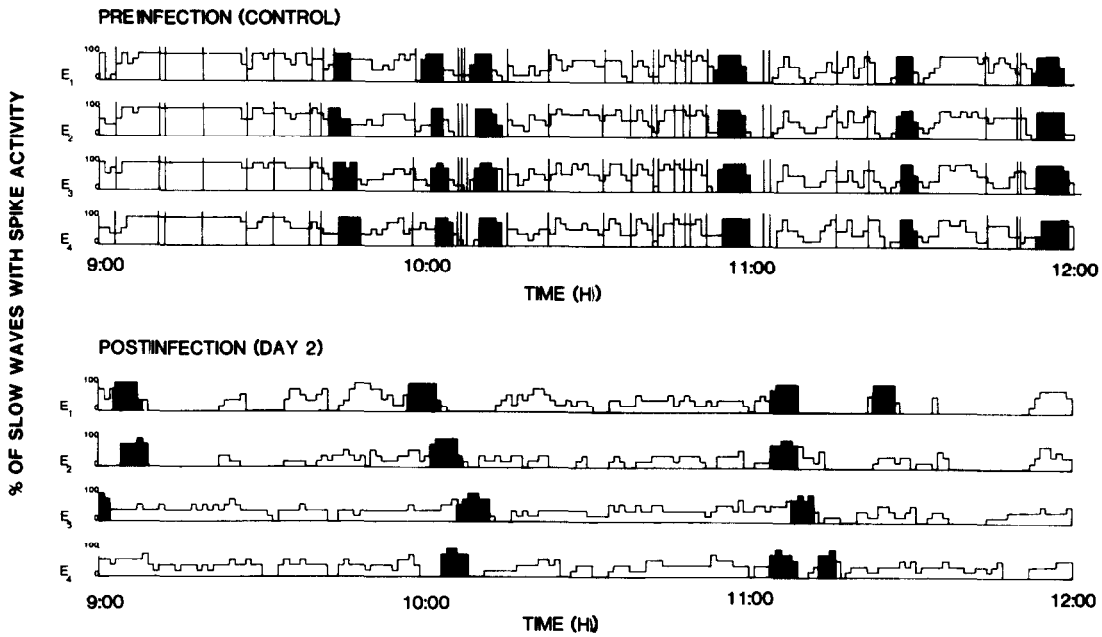

Figure 2. Histograms of spike activity for a 3-h recording period from a preinfection (fed control) piglet and the same piglet 2 days after infection with transmissible gastroenteritis virus. Phase 1 activity is denoted by a *horizontal line*, phase 2 activity by *open blocks*, and phase 3 activity by *solid blocks*. Migrating action potential complexes are denoted by *vertical lines*. Infection resulted in an increase in the duration of phase 1, a decrease in the duration of phase 2, and a disruption in the organized aboral propagation of phase 3; it also abolished the migrating action potential complexes. When compared to the preinfection recording, the percentage of activity in phase 2 was also decreased after infection.

Differences between the overall duration (periodicity) of the MMC and the sum of its component parts (phases 1, 2, and 3 in Table 1) are due to the fact that more than one period of phase 1 and phase 2 activity usually occurred between successive activity fronts.

Myoelectrical Activity in Infected Piglets

Infection with TGE virus decreased overall spike activity and disrupted the duration (periodicity) of the MMC and the regular aboral propagation of phase 3 of the complex. Infection also decreased overall spike activity when compared with fed controls; this resulted from an increase in the duration of phase 1 and a corresponding decrease in the duration of phase 2 (Table 1). The decrease was most marked on the second day postinfection when signs of disease were most severe. Infection was also associated with a marked reduction in the overall percentage of spike activity (Figure 2).

The MAPC frequency was significantly reduced on the first day postinfection and the complex was virtually abolished on the second and third days (Table 1 and Figure 2). This progressive decrease contrasted with the return toward normal of other clinical and myoelectrical parameters by the third day (Table 1).

Whereas TGE infection did not affect the duration of the individual activity fronts (phase 3) of the MMC, it did first enhance and then delay their periodicity. The MMC periodicity decreased from a mean of 45.5 ± 1.1 min in normal fed piglets to 38.3 ± 1.8 min on the first day postinfection, and then increased to 62.6 ± 3.9 min on the second day. Infection also disrupted the regular aboral propagation of phase 3; this was indicated by (a) a failure to propagate sequentially over all four electrodes, (b) splitting into two shorter components, or (c) retrograde propagation over two or more adjacent electrodes (Figure 3). Disruption occurred in 5% of activity fronts before infection but in nearly 50% of activity fronts by the second day postinfection (Table 1). Disruption was much more frequent and severe in piglets in which vomiting was observed. Infection had no effect on slow wave frequency.

Piglets infected with inactivated TGE virus showed no disruption of myoelectrical activity. Their appetites remained normal and they had no vomiting or diarrhea.

Discussion

The pathogenesis of diarrhea is complex; water and electrolyte transport abnormalities together with the loss of epithelial integrity are generally agreed to be major factors in most diarrheal diseases, whereas the role of abnormal intestinal motility is

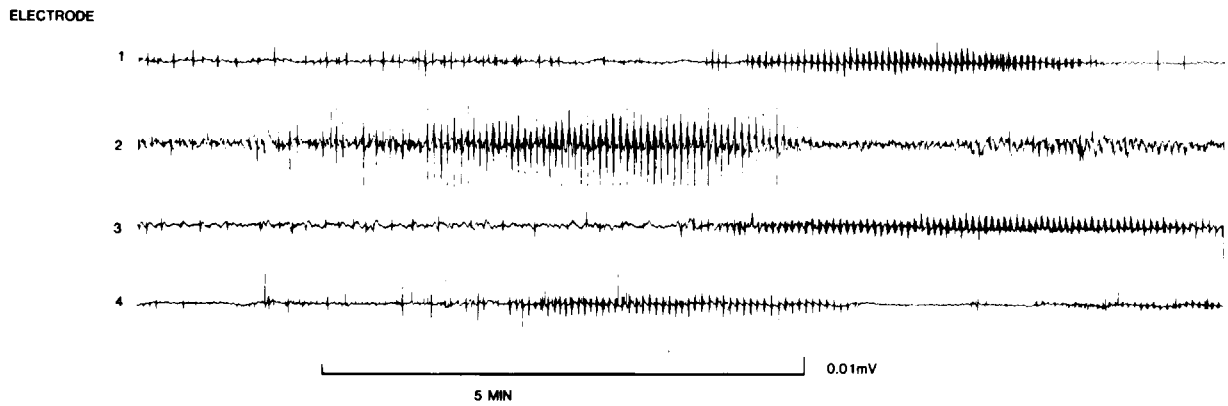

Figure 3. Disruption of phase 3 of the migrating motility complex in a piglet 2 days after infection with transmissible gastroenteritis virus. A burst of intense spike activity characteristic of phase 3 begins on electrode number 2, then appears sequentially on electrodes 4, 3, and 1, giving the impression of retrograde propagation.

less well understood (1). It is now recognized that disturbed motility and transport may coexist, and that both might contribute to diarrhea caused by bacterial enteropathogens (2–4,9). However, the effect of an enteropathogenic virus on intestinal motility has not been previously studied, and a decrease in small intestinal myoelectrical activity, and therefore presumably in motility, has not been reported after infection with any enteropathogen.

The virus that causes TGE, a member of the coronavirus family, selectively multiplies in and destroys villus absorptive cells. This results in villus atrophy, with subsequent loss of surface digestive properties and impaired absorptive capacity (5,10). Diarrhea caused by TGE is considered to be osmotic due to a decrease in absorptive area and a defect in glucose-stimulated Na^+ transport (11,12). There is also evidence for a secretory component secondary to increased proliferation and migration of immature crypt epithelial cells (5,13). Disrupted motility has not been previously demonstrated in this disease.

It is unclear how the decrease in myoelectrical spike activity in TGE is initiated and maintained. The virus affects only the intestinal mucosa and immunofluorescent studies have not shown viral particles below the level of the villi (14). It is possible that changes in the duration of phases 1 and 2 of the MMC are related to decreases in food intake, because the duration of phase 1 was between that of the fed and fasted control animals on the second and third days postinfection, and phase 2 duration approximated that of the fasted controls on day 2 of the study when food intake was lowest (Table 1). This is supported by observations in dogs, which show that the degree of MMC disruption by feeding has a linear relationship to caloric intake (15). It must, however, be emphasized that infected piglets were only transiently inappetent, and that prolongation of phase 1 activity could not be related to changes in food intake because this was near normal on day 3 postinfection. Changes in food intake were also unrelated to disruption of MMC periodicity, to disruption of the normal propagation of the activity front, and to the progressive decrease in MAPCs that occurred postinfection.

The most notable effects of infection were disruption of MMC periodicity, an increase in the number of abnormal activity fronts, and a decrease in MAPC frequency. It is possible that damage to mucosal nerve endings might have an effect on the MMC through an effect on the myenteric plexus. The plexus appears to control MMC initiation and propagation (16,17) and disruption of normal phase 3 conduction and periodicity suggests that the plexus may require an intact small intestinal mucosa in order to generate normal MMCs. An effect on intestinal myoelectrical activity by a substance that causes strictly mucosal damage is not without precedent. Histologic changes caused by ricinoleic acid, for example, are restricted to the tips of the villi (18,19), yet the compound has been shown to induce MAPCs in the anesthetized rabbit (20), and in the conscious dog it induces MAPC-like activity while reducing overall spike activity (21).

Inhibition and disruption of spike activity by TGE virus may not be a primary event; these effects could, for example, be secondary to mucosal damage by an accumulation of fluid in the intestinal lumen, to the presence of unabsorbed nutrients, or to the products of the bacterial degradation of these nutrients. In the rat, MMC disruption has been shown to predispose to enteric microbial proliferation (22), an event that might be enhanced in the presence of undigested and unabsorbed nutrients such as occur in TGE (5,11,13). It is therefore possible that bacterial overgrowth, either directly or indirectly, might be associated with the deranged motility seen in TGE.

The MAPC was first described and named as such

by Mathias and coworkers as a short, rapidly propagated spike burst induced in isolated ileal loops of anesthetized rabbits by cholera toxin (2). Apparently identical complexes have been recorded from the small intestine of conscious healthy adult pigs (23), sheep (24), rabbits (25), calves (26), dogs (27), and now from piglets. The relationship of MAPC frequency to the fed and fasted state has not been previously examined. Whereas the complexes are present in published recordings of intestinal myoelectrical activity from the species mentioned above, little note was taken of them in the text of many publications. Exceptions are those of Bueno and Ruckebusch (23) and Summers and Dusdieker (28), who found an association between MAPC-like activity and the aboral movement of luminal contents in pigs and dogs, respectively.

In the present study, infection markedly reduced MAPC frequency to a point that was comparable to the effect of an 18-h fast (Table 1). Piglets, however, were never totally anorexic postinfection, and food intake had returned to normal or near normal on the third day when MAPC frequency was lowest. This implies, as was the case with the disrupted phase 3 complexes, that destruction of the small intestinal mucosa may be responsible for the decreased frequency of MAPCs. However, this interpretation probably does not apply to all situations, in that the presence of ricinoleic acid in the intestinal lumen induces an increase in the frequency of MAPC-like activity in the conscious dog (21). The net effect, whether an increase or a decrease in activity, might well depend on species differences or perhaps on the degree of mucosal damage.

The results of this study show that infection of piglets with TGE virus, a strict mucosal pathogen, reduces organized jejunal propulsive activity. Specific relationships between disrupted myoelectrical activity and the diarrhea and vomiting seen in this disease remain to be elucidated. This infection model emphasizes the importance of an intact mucosa in the maintenance of normal small intestinal motility.

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