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Effects of Ambient Temperatures on Induction of Transmissible Gastroenteritis in Feeder Pigs

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Experiments were carried out to investigate the effects of ambient temperatures on the induction of transmissible gastroenteritis in feeder pigs 2 to 3 months old. Pigs maintained at a high temperature (30 ± 2°C) and exposed to the virulent transmissible gastroenteritis virus did not show clinical signs of the disease during their maintenance at the high temperature. On the other hand, a sudden decrease in the ambient temperature, either before or after virus inoculation, induced severe disease in feeder pigs exposed to the virus. However, continuous maintenance of pigs at the low temperature (4 ± 1°C) tended to somewhat reduce the frequency of occurrence of signs in proportion to the length of the maintenance periods at that temperature. Pigs raised at temperatures that fluctuated between 20 ± 2 and 4 ± 1°C every 24 h developed profuse diarrhea. The duration of clinical signs was longer in pigs maintained under the fluctuating temperatures than in those at the constantly low temperature. With one exception, antibody against transmissible gastroenteritis virus was demonstrated in sera collected from pigs both with and without clinical signs. Antibody titers obtained, however, were somewhat higher in sera collected from pigs that had developed clinical signs than in those from pigs that had endured the infection without showing signs.

Transmissible gastroenteritis (TGE) of pigs is a highly contagious, enteric, viral disease. The disease, characterized by severe diarrhea and vomiting, with a high mortality in newborn piglets, is caused by a coronavirus (21). The interesting epizootiological features of TGE are its seasonal appearance and the relationship between age and severity of clinical manifestations. The majority of the herd outbreaks of TGE occur during the colder months, from November to April (6). Although pigs of all ages are susceptible to TGE virus, the severity of the disease differs greatly at different ages. The mortality rate in piglets less than 2 weeks old frequently approaches 100%, whereas that in adult pigs is usually very low.

In adult pigs, clinical signs are rather mild, and inapparent infections may occur. Olson (18) and Morin et al. (16) have reported that approximately 30% of feeder pigs 4 to 6 months old did not show clinical signs of the disease after experimental exposure to TGE virus, although they sometimes had a mild focal infection of the jejunum.

Our accumulated records of the experimental infection of adult pigs with the virulent virus indicated that when the experiments were conducted in winter all pigs exposed to the virus had characteristic signs of TGE, but in summer pigs frequently resisted the infection without showing clinical signs. These facts seem to suggest that ambient temperatures may play an important role in the induction of TGE in adult pigs.

The studies presented here were carried out, therefore, to investigate the influence of ambient temperatures on the induction of TGE in feeder pigs under carefully controlled laboratory conditions.

MATERIALS AND METHODS

Viruses. The Shizuoka strain (19), a virulent TGE virus, was used for experimental inoculation of pigs. The virus had been passaged 17 times in piglets and was stored at −80°C in the form of 10% suspension of infected small intestine. Infective titers of the virus were determined by oral inoculation of piglets less than 5 days old, and the inoculum contained 10^4 pig 50% infective doses.

The TO strain of an attenuated TGE virus was used as an indicator virus in a serum neutralization (SN) test. The strain had been serially passaged in pig kidney cell cultures (8) and was used at passage 168 for the present experiments.

Experimental pigs. A total of 43 Yorkshire pigs 2 to 3 months old, weighing 20 to 25 kg, which had been serologically negative for TGE antibody, were ob-

tained from a farm where there had been no history of outbreaks of TGE.

Experimental design. Pigs were divided into 10 groups, which included 3 to 6 pigs each, and three experiments were carried out.

Ambient temperatures of animal rooms were carefully regulated at 30 ± 2 or at 4 ± 1°C by thermostats, and the rooms were designated as the high- and low-temperature room, respectively.

In the first experiment, effects on TGE infection of a sudden decrease in the ambient temperature before virus inoculation were examined (Table 1).

Pigs of groups 1 (5 pigs), 2 (6 pigs), and 3 (5 pigs) were kept in the high-temperature room for 4 days, and then transferred to the low-temperature room. They were orally exposed to 10^4 pig 50% infective doses of the virulent TGE virus immediately (group 1) and on day 4 (group 2) and day 14 (group 3) after their removal to the low-temperature room, respectively, and then continuously maintained at the low temperature. Six pigs of group 4 were kept in the high-temperature room, orally inoculated with the virulent TGE virus on day 4, and continuously maintained at the high temperature. Five pigs of group 5 served as controls. These pigs were exposed to the temperature change from 30 to 4°C on day 4, and then maintained at the low temperature without virus inoculation.

In the second experiment, groups 6 (4 pigs), 7 (3 pigs), and 8 (3 pigs) were subjected to a sudden decrease in the ambient temperature after virus inoculation (Table 2). After 4 days at the high temperature, all of the pigs were orally inoculated with the virulent TGE virus and maintained at the high temperature until, on days 4, 7, and 10 postinoculation, groups 6, 7, and 8, respectively, were transferred to the low-temperature room.

In the third experiment, groups 9 and 10, 3 pigs each, were subjected to fluctuating temperatures (Table 3). Pigs of group 9 were kept at temperatures fluctuating between 20 ± 2 and 4 ± 1°C every 24 h. On day 4 under these conditions, they were orally inoculated with the virulent TGE virus and maintained under the same conditions for the duration of the experiment. Pigs of group 10 served as controls. They were kept under the fluctuating temperatures but without virus inoculation.

All pigs were carefully examined twice daily for clinical manifestations of TGE, especially abnormalities in the consistency of the feces. Diarrhea and soft feces were regarded as positive clinical signs. Some pigs with clinical signs were sacrificed, and several segments of middle jejunum, about 1 cm in length, were collected and examined for occurrence of villous atrophy, which is a prominent lesion of TGE (11).

Serum samples were collected from remaining pigs 10, 14, and 21 days after virus inoculation and tested for SN antibody.

Pathological examination. The segments of middle jejunum were slit longitudinally, rinsed gently in phosphate-buffered saline, placed in phosphate-buffered saline, and examined under a dissecting microscope for evidence of villous atrophy.

SN test. The procedures of the SN test have been previously described (10, 20). Briefly, serial twofold

TABLE 1. *Clinical signs developed in pigs exposed to temperature change before virus inoculation*

Group	No. of pigs	Ambient temperature		Clinical signs[a]	Villous atrophy[b]
		Before inoculation	After inoculation		
1	5	30°C, 4 days	4°C	5/5[c]	2/2
2	6	30°C, 4 days; 4°C, 4 days	4°C	4/6[c]	2/2
3	5	30°C, 4 days; 4°C, 14 days	4°C	2/5[c]	1/1
4	6	30°C, 4 days	30°C	0/6	NT[d]
5 (control)[e]	5	30°C, 4 days	4°C	0/5	NT

[a] Number of pigs positive for clinical signs/number of pigs used.
[b] Number of pigs positive for villous atrophy/number of pigs tested.
[c] Total, 11/16. Statistically significant at 95% level as compared with group 4.
[d] NT, Not tested.
[e] Pigs of group 5 were not inoculated with TGE virus.

TABLE 2. *Clinical signs developed in pigs exposed to temperature change after virus inoculation*

Group	No. of pigs	Ambient temp		Clinical signs[a]		Villous atrophy[b]	
		Before inoculation	After inoculation	At 30°C	At 4°C	At 30°C	At 4°C
6	4	30°C, 4 days	30°C, 4 days; 4°C	1/4	3/3	1/1	1/1
7	3	30°C, 4 days	30°C, 7 days; 4°C	0/3	2/3	NT[c]	1/1
8	3	30°C, 4 days	30°C, 10 days; 4°C	0/3	1/3	NT	1/1
Total	10			1/10	6/9[d]		

[a] Number of pigs positive for clinical signs/number of pigs used.
[b] Number of pigs positive for villous atrophy/number of pigs tested.
[c] NT, Not tested.
[d] Statistically significant as compared with results at 30°C ($P = 0.0162$).

TABLE 3. *Effect of fluctuating temperatures on induction of TGE in pigs*

Group	No. of pigs	Clinical signs[a]	Villous atrophy[b]
9	3	3/3	1/1
10 (control)[c]	3	0/3	NT[d]

[a] Number of pigs positive for clinical signs/number of pigs used.

[b] Number of pigs positive for villous atrophy/number of pigs tested.

[c] Pigs of group 10 were not inoculated with TGE virus.

[d] NT, Not tested.

dilutions of sera were incubated at 37°C for 1 h with an equal volume of viral suspension containing 200 50% tissue culture infective doses per 0.1 ml of the TO strain. Two tubes of pig kidney cell cultures were inoculated with 0.1 ml of each serum-virus mixture.

All cultures received 0.5 ml of maintenance medium consisting of Earle balanced salt solution supplemented with 0.5% lactalbumin hydrolysate, 3% heat-inactivated bovine serum, 0.07% sodium bicarbonate, 20 μg of kanamycin per ml, and 100 μg of streptomycin per ml, and then incubated in a roller drum at 37°C for 4 days. The SN antibody titer was expressed as the reciprocal of the highest dilution of serum that completely inhibited cytopathic effect in the test.

RESULTS

Effects of decrease in ambient temperatures before virus inoculation. Frequency of clinical signs developing in pigs of groups 1, 2, 3, 4, and 5 (experiment 1) is shown in Table 1. Adverse effects of a decrease in the ambient temperature on induction of TGE in feeder pigs were evident.

All pigs inoculated with the virus immediately after their removal from the high temperature to the low temperature (group 1) showed profuse diarrhea with incubation periods of 2 to 3 days, which persisted for 2 to 4 days.

On the examination of the jejunum obtained from two pigs of group 1 sacrificed 5 days after virus inoculation, marked villous atrophy was evident.

The results obtained in groups 2 and 3 indicated that the continuous maintenance of pigs at the low temperature before virus inoculation tends to somewhat reduce the occurrence of clinical signs in proportion to the length of maintenance periods at the low temperature. Of 11 pigs in both groups, 6 pigs developed clinical signs of the disease; 5 of these had profuse diarrhea appearing after incubation periods of 3 days and persisting for 3 days thereafter. One only passed soft feces on days 4 and 5 of the infection. The other five pigs had neither diarrhea nor soft feces. Two pigs of group 2 and one pig of group 3 which had developed profuse diarrhea were sacrificed 2 days after the onset of illness and were examined for villous atrophy. Markedly shortened villi were seen throughout sections of the jejunum collected from these pigs.

In contrast, pigs of group 4, which were kept at the high temperature throughout the experiment, did not show clinical signs of TGE after exposure to the virus. There were no abnormalities in the consistency of their feces.

The differences in frequency of clinical signs developed in pigs of each experimental group were not statistically significant. This was probably due to the fact that not enough pigs were used. However, when the aggregate results obtained in groups 1, 2, and 3 were compared with those of group 4 (11/16 versus 0/6), statistical significance in frequency of clinical signs was found between the pigs exposed to temperature change and those maintained at the high temperature throughout the experiment ($P < 0.05$).

Five pigs of group 5, which were exposed without virus inoculation to the temperature change from 30 to 4°C on day 4, developed no clinical signs of the disease. Their feces were normal in appearance throughout the experiment.

Effects of decrease in ambient temperature after virus inoculation. The results obtained in groups 6, 7, and 8 are shown in Table 2. Of a total of 10 pigs exposed to the virus and maintained at 30°C, only 1 pig from group 6 developed clinical signs of TGE. The pig had severe diarrhea 4 days after virus inoculation, and marked villous atrophy was observed in the jejunum. Three pigs of group 6 that appeared normal at the high temperature developed clinical signs when the ambient temperatures were reduced to 4°C.

Two pigs showed profuse diarrhea, and the other one had soft feces 3 to 4 days after being transferred from the high- to the low-temperature room.

One of two pigs with profuse diarrhea was sacrificed 1 day after onset of the disease for pathological examination. Marked villous atrophy was seen in its jejunum.

Three pigs of group 7 remained normal at the high temperature for 7 days after exposure to the virus. Two of them, however, showed clinical signs of TGE 3 days after they were transferred to the low temperature room. One pig developed profuse diarrhea with marked villous atrophy in the jejunum, and the other had soft feces. The third pig of this group had no signs.

Similar results were obtained in pigs of group 8. No clinical signs of TGE were observed in pigs during 10 days of the maintenance at the high

temperature. However, a decrease in the ambient temperature induced the disease in one pig. This pig showed profuse diarrhea 1 day after exposure to the temperature change. The remaining two pigs of group 8 developed no clinical signs even after the ambient temperatures were reduced to 4°C. Pathological examination of the jejunum of the pig with profuse diarrhea revealed extensive villous atrophy.

The differences in the frequency of clinical signs developed in pigs of individual groups at the high and the low temperatures were not statistically significant, because the number of pigs used in each group probably was not large enough. However, statistical analysis of the aggregate results of groups 6 through 8 (1/10 versus 6/9) by the Mantel-Haenszel test (14) indicated that the frequency of clinical signs developed after transfer to the low-temperature room was significantly higher than that at the high temperature ($Z = 2.14$, $P = 0.0162$).

Effects of fluctuating temperatures. All pigs exposed to fluctuating temperatures and inoculated with the virus had profuse diarrhea (Table 3). On pathological examination of the jejunum obtained from one pig, marked villous atrophy was observed.

On the other hand, pigs of group 10 (control) remained normal throughout the observation period of 14 days. Furthermore, the duration of clinical signs was longer in pigs maintained under the fluctuating temperatures than in those of other groups; the former was 5 to 6 days, and the latter was always 2 to 4 days.

Antibody responses of pigs inoculated with TGE virus. With one exception, pigs inoculated with TGE virus developed SN antibody against the virus (Table 4). The results indicated a tendency for antibody titers to be higher in sera collected from pigs that had shown clinical signs than in those from pigs that had endured the infection without showing clinical signs.

Antibody responses in pigs of group 3, which had shown no clinical signs, were very low; one pig (no. 16) did not produce detectable SN antibody.

DISCUSSION

There are many reports on the effects of ambient temperature on the pathogenesis of viral diseases (2–5, 12, 13, 15, 23). A low ambient temperature usually intensified the severity of the diseases, whereas a high ambient temperature often resulted in an increase of resistance to the infection.

The results obtained in the present studies indicated that the induction of TGE in feeder pigs was greatly affected by the ambient temperature. With only one exception, pigs raised at the high ambient temperature did not show clinical signs of TGE after virus inoculation. This suggests that the high ambient temperature results in an increase of resistance of feeder pigs to induction of TGE. On the other hand, a sudden decrease in the ambient temperature, either before or after virus inoculation, caused a dramatic enhancement of the disease in feeder pigs. This suggests that both temperature change and the low temperature exacerbate the infection with TGE virus, and that the temperature change is an important factor in the induction of TGE in feeder pigs.

Pigs exposed to fluctuating temperatures developed severe diarrhea after virus inoculation; also the duration of clinical signs was longer in these pigs than in those maintained at the constantly low temperature.

These facts seem to indicate that fluctuating

TABLE 4. *Antibody responses of pigs inoculated with TGE virus*

Group	Pig no.	Clinical signs[a]	SN antibody titers at day postinoculation:			
			0	10	14	21
1	3	D	<1	8	16	128
	4	D	<1	NT[b]	32	NT
	5	D	<1	NT	32	NT
2	8	D	<1	8	32	128
	9	SF	<1	16	32	64
	10	N	<1	4	8	32
	11	N	<1	8	8	16
3	13	D	<1	8	32	128
	14	N	<1	1	2	1
	15	N	<1	1	1	2
	16	N	<1	<1	<1	<1
4	17	N	<1	2	4	16
	18	N	<1	1	8	32
	19	N	<1	4	8	16
	20	N	<1	2	4	16
	21	N	<1	2	16	NT
	22	N	<1	4	8	NT
6	30	D	<1	8	64	128
	31	SF	<1	4	32	64
7	33	SF	<1	4	32	16
	34	N	<1	4	8	16
8	35[c]	D	<1	2	NT	NT
	36	N	<1	2	NT	16
	37	N	<1	4	NT	32
9	39	D	<1	4	64	64
	40	D	<1	8	32	64

[a] D, SF, and N represent diarrhea, soft feces, and normal feces, respectively.

[b] NT, Not tested.

[c] Pig no. 35 had diarrhea 11 days after virus inoculation and was sacrificed on the same day.

temperatures are the most effective inducer of the disease and probably have an adverse effect on the mechanism of recovery of pigs from TGE. Findings similar to those described here have been reported by Kiorpes and Yuill (12), who found that viremia in snowshoe hares inoculated with western equine encephalomyelitis virus was more severe when the hares were exposed to low fluctuating temperatures than to a constantly low temperature.

It is of interest that two pigs of group 7 and one pig of group 8 had clinical signs after being transferred from 30 to 4°C on day 7 and 10, respectively, indicating that the virus is latently retained in the body of pigs without showing clinical signs for 7 to 10 days, and that the virus might be activated and cause the disease in pigs when they are exposed to certain stresses such as temperature change.

SN antibody could be demonstrated in pigs both with and without clinical signs, indicating that infection with TGE virus had been established even in pigs that had no signs of the disease. Antibody titers obtained, however, were somewhat higher in sera collected from pigs that had developed clinical signs than in those from pigs that had resisted the infection without showing signs. This may be due to differences in the quantity of antigen available to stimulate antibody production. Probably, a larger antigenic mass was present in the pigs that had shown clinical signs, for the virus replicated greatly in the epithelial cells of the small intestine of such pigs. Again, a similar phenomenon has been described in snowshoe hares infected with western equine encephalomyelitis virus, in which the adverse temperature induced an enhancement of antibody production as well as severe viremia (12).

It is widely known that the majority of herd outbreaks of TGE occur during the colder months (6). The findings obtained in the present studies suggest that the effects of the adverse temperature on the infection with TGE virus might explain in part the seasonal appearance of TGE outbreaks. It does appear that pigs are more susceptible to TGE virus in winter when they are exposed to the adverse temperature. Furuuchi and Shimizu (7) have stated a similar conclusion based on their observation that piglets maintained at the high temperature had been more resistant to the infection with an attenuated TGE virus than those at the low temperature.

The mechanism by which the adverse ambient temperature enhances the severity of TGE is not fully explained. TGE is frequently associated with the infection of *Escherichia coli* (17). Furthermore, TGE virus infection associated with *E. coli* causes a more severe disease than that with the virus alone (22).

Behavior of the bacterial flora in the intestinal tract might be also affected by the adverse ambient temperature. Armstrong and Cline (1) have reported that, when given an oral challenge of *E. coli*, pigs in a normal environment (28.3°C) had less diarrhea than those in a cold environment (10°C), although the environmental temperature had no significant effect on numbers of *E. coli* in fecal and intestinal samples. Because the Shizuoka strain used for challenge inoculation is cytopathogenic only after repeated passages in cell culture (9), viral multiplication in the body of pigs and viral recovery were not tested in the present studies. Therefore, the present studies could not clarify whether the severe disease induced in feeder pigs results from the effects of the adverse temperature on the behavior of TGE virus or from intestinal bacteria or both. It is possible, however, to identify the disease as TGE, because villous atrophy in the jejunum, which is a prominent lesion of TGE (11), was seen in pigs with clinical signs, and control pigs, which were exposed to the adverse temperature without virus inoculation, had no clinical signs.

The effects of ambient temperature on the physiological functions of animals might vary widely. We suspect, however, that adverse temperatures may affect the immune system, especially the initiation of immune responses in animals, and that pigs exposed to adverse temperatures probably become more susceptible to TGE virus as a result of a delay in the initiation of the immune response after inoculation with the virus. A precise explanation of the mechanism involved in the immune response should be sought.

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