

Antibody Responses in Serum, Colostrum, and Milk of Swine After Infection or Vaccination with Transmissible Gastroenteritis Virus

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The antibody response of pregnant swine to transmissible gastroenteritis (TGE) virus was studied, with special reference to the titers and the immunoglobulin (Ig) class of TGE neutralizing antibodies in colostrum and milk. Animals vaccinated twice intramuscularly or intramammarily with live attenuated TGE virus developed high levels of antibodies in serum and colostrum, but the levels in milk declined markedly within a few days post-farrowing. In contrast, animals naturally or experimentally infected with virulent virus generally developed lower levels of antibodies in serum and colostrum but maintained higher levels in milk, as compared to the vaccinated animals. Gel filtration studies indicated that antibodies in milk from vaccinated animals were primarily of the IgG class, whereas those from the naturally or experimentally infected animals were primarily of the IgA class. The ability of sows to transmit a high degree of passive immunity to their suckling progeny was more closely associated with TGE antibodies of the IgA than the IgG class. Present evidence suggests that high levels of TGE antibodies of the IgA class occur in milk as a result of an infection of the intestinal tract. Probable reasons for this are discussed.

Transmissible gastroenteritis (TGE) of swine is a highly contagious, enteric, viral disease characterized by severe diarrhea and high mortality in pigs under 2 weeks of age. The TGE virus, a member of the coronavirus group (4, 25), primarily infects the epithelial cells of the small intestines, which results in an atrophy of the villi and a malabsorption syndrome (15).

Of special interest is information on the mechanism of passive immunity in this disease and how such can be provided to newborn pigs. Sows which have recovered from TGE are capable of transmitting immunity to their suckling pigs (1), apparently by providing a continual source of milk antibody which will protect the luminal surface of the intestinal epithelial cells (14). However, attempts to duplicate this effect by parenteral injections of pregnant swine with inactivated or attenuated TGE viral preparations have generally provided a lesser or even questionable degree of immunity (2, 4, 26).

Recently, we have briefly reported some of the

characteristics of the antibody response of pregnant swine following infection or vaccination with TGE viral preparations, with emphasis on the immunoglobulin (Ig) classes of antibodies in colostrum and milk (5). This report will further elaborate on such studies and will indicate the probable importance of TGE antibodies of the IgA class in milk for providing passive immunity.

MATERIALS AND METHODS

Cell cultures and media. Primary cultures of porcine kidney (PK) cells were prepared in 4-oz (ca. 120 ml) glass prescription bottles similar to a previously described method (16). Cells were grown in Hanks lactalbumin hydrolysate (HLH) medium containing 0.5% lactalbumin hydrolysate, 5% bovine serum, and 0.088% NaHCO₃.

The agar overlay medium was similar to the HLH medium except it contained 0.132% NaHCO₃, 1% Noble agar, and 0.0007% neutral red. The viral, serum, and milk diluent used for antibody titrations was HLH medium without serum.

Viruses. (i) A virulent Miller strain of TGE virus was prepared in such a way as to attempt to insure its microbial purity and virulence (3). The Miller strain was isolated in PK cell cultures from the small intestine of a young pig with typical clinical signs of TGE, passed 13 times in PK cell cultures, and plaque-

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purified twice. To increase its virulence for swine, it was then passed three times in gnotobiotic pigs. A large volume of stock virus was prepared and is referred to as Miller no. 3 (M-3). It represented a 5% bacterial-free suspension of the intestines of five infected 7-day-old gnotobiotic pigs, of the third pig passage, and had a titer of approximately 10^6 pig infective doses per ml.

(ii) A virulent Purdue strain, as used in these studies, was prepared as a 5% bacterial-free suspension of the intestines of two infected 7-day-old gnotobiotic pigs and is referred to as Purdue no. 1 (P-1). This strain was initially obtained from E. O. Haelterman, Purdue University, as a 10% suspension of infected pig gut. It was subsequently passed several times in newborn pigs in our laboratory prior to preparing the P-1 stock virus.

(iii) A high passaged Purdue (HPP) strain of TGE virus, which had been serially passed 111 to 114 times in PK cell cultures, was used as a vaccine and as the test virus in determining neutralizing antibody titers. The virus used for vaccination purposes contained approximately 2×10^6 plaque-forming units (PFU) per ml and was propagated in a medium containing porcine rather than bovine serum.

This strain was initially obtained from E. O. Haelterman in the form of infected gut tissue, as referred to above. A cytopathic and plaque-producing virus was isolated from the small intestines of a newborn pig experimentally infected with this gut virus, but only after several blind passages on PK cells. By pathogenic and serologic studies, this virus was shown to be etiologically associated with TGE. As it was progressively passed on PK cells, its cytopathic effect and plaque-producing ability became more pronounced, and its virulence for young pigs became reduced (6).

Plaque technique. Bottles were emptied of growth medium, and 0.2 ml of the appropriate viral preparation was added to each bottle. Inoculated bottles were incubated at 37 C for 90 min to allow adsorption of virus. The monolayers were then overlaid with the agar medium. The bottles were inverted and incubated at 37 C for at least 6 days. Plaques were counted beginning on the third day.

Antibody titrations. Neutralizing antibody for TGE virus was assayed by the plaque-reduction test. Serum, colostrum or milk specimens were inactivated (56 C, 30 min) and fourfold dilutions were made. The HPP strain was diluted to an estimated 60 PFU/0.1 ml, and was added to an equal volume of the diluted specimen. Virus-specimen mixtures were incubated at 37 C for 60 min, and 0.2 ml of each was inoculated into each of two bottle cultures. The remainder of the procedure was as described for the plaque technique. Antibody titer was expressed as the reciprocal of the specimen dilution which resulted in an 80% reduction in the number of plaques.

Source of pregnant swine. All pregnant swine originated from three herds which were under close clinical and serological surveillance for TGE. All animals used for experimental infection or vaccination were from herds which had not recently experienced clinical signs of TGE and which were serologically negative

for TGE antibodies by the plaque-reduction test utilizing undiluted serum.

Experimental infection with virulent virus. Pregnant swine were exposed to either the M-3 or P-1 virus stocks, of the Miller or Purdue strains, respectively. Approximately 5 ml of the virus was given orally, 1 ml was given in each nostril with the aid of a long blunt needle and syringe, and 5 ml was placed on the feed of each exposed animal.

Vaccination procedures. The live attenuated HPP strain was used for vaccination by the following two routes: (i) Intramuscular injections were given deep in the ham or behind the ear, using a 5-ml dose which was repeated in 2 to 4 weeks. (ii) Intramammary injections were given into three glands on the left side of the udder using a 21- or 22-gauge, 1-inch needle. The inoculum was injected either in the milk cistern or in the adjacent mammary tissue at the base of the teats. The dosage was 5 ml, divided between the 3 glands, and was repeated in 2 to 4 weeks.

Challenge procedures. The procedure used for challenging the passive immunity of suckling pigs of a litter was as follows. A 1-ml amount of a 1:1000 dilution of the M-3 viral preparation was given orally to all but one of the suckling pigs at approximately 72 hr of age. The one unexposed pig helped serve as a control since it did not usually become sick until 24 hr after the other pigs. As a further control, especially to insure the infectivity of the diluted viral inoculum, one pig was removed from the sow to an isolation cage about 24 hr before the litter was to be challenged. It was fed a canned milk preparation (SPF-lac, The Borden Co., New York) and was challenged with the same inoculum as used for the other pigs of the litter. Pigs weaned and exposed in this fashion are susceptible to TGE, regardless of the immune status of the dam (15).

The M-3 viral preparation had been prepared in a large volume, and samples were placed in many small vials and stored at -70 C. Just prior to use, a vial of virus was thawed and diluted with HLH without serum. The diluted virus was kept cool and protected from light as much as possible. Using this procedure, the infective titer of the diluted virus prepared from samples over a period of several months remained fairly constant, so that 1 ml of a 1:1000 dilution represents about 100 pig-infective doses.

Collection and preparation of samples. Blood samples were obtained from the anterior vena cava. Milk samples were obtained manually following an intravenous injection of 20 to 40 USP units of oxytocin, which stimulated a let-down of milk. However, colostrum samples were often collected at time of farrowing or shortly thereafter without resorting to an injection of oxytocin. An effort was made to be as precise as possible when indicating the time of colostrum collection since the levels of total protein and Ig of various classes decrease significantly within a few hours after farrowing (10, 20). Blood and milk were not usually obtained from the sow at the time her pigs were challenged to avoid any possible interference with normal lactation. A single milk sample represented the pooled milkings from at least three glands to minimize possible fluctuations as might

occur from individual glands. When the term "colostrum" is used in this report it refers to mammary secretions of the first 24 hr after farrowing.

Skim milk was prepared by centrifugation of whole milk or colostrum at $2,000 \times g$ for 30 min and by collecting the middle portion between the cream layer and the deposited debris. Whey was prepared by centrifugation at $90,000 \times g$ for 60 min at 4 C. The clarified whey was carefully collected and filtered through 0.45- μ m membrane filters (Millipore Corp., Bedford, Mass.). Either skim milk or whey was used for conducting neutralization tests, but only whey was used for gel filtration chromatography. Samples were stored at -20 C.

Gel filtration chromatography. Colostral and milk whey were fractionated by passing through 2 columns (2.5 by 45 cm) containing Sephadex G-200 (Pharmacia, Uppsala, Sweden), so that the flow was descending in the first column and ascending in the second. Specimens were usually applied to the columns in volumes of 2 to 4 ml. The buffer system was 0.1 M tris (hydroxymethyl)aminomethane, in 0.2 M NaCl, adjusted to pH 8 with HCl. The flow rate was adjusted to about 15 ml/hr, accomplished either by gravity or by a peristaltic pump. The eluate was collected in fractions of 3 ml and their optical densities (OD) were determined by reading at 280 nm in a Gilford 240 spectrophotometer. Selected fractions or pooled fractions were tested also for TGE neutralizing antibody titer and for the presence of IgM, IgA, and IgG.

As used in this report, the IgA position on the Sephadex G-200 chromatogram will refer to that portion where the fractions give maximal reactions in the immunodiffusion (ID) tests with monospecific anti-porcine IgA. With colostrum, the IgA position was about one-half down the descending limb of the first peak, which was often indicated by a shoulder. As reported by others, this is the elution area of the porcine IgA 11S molecule (22). As lactation advanced, the predominant IgA position in milk gradually occurred higher on the descending limb of the first peak, often demonstrated by a distinct shoulder or a small peak, so that late in lactation (42 to 70 days post-farrowing) it occurred at the top or on the ascending limb of the first peak. The position of the highest concentration of IgG, as judged by ID tests, was the top of the second peak in either colostrum or milk. This represents the elution area of the 7S molecule (19). IgM was detected primarily in the ascending limb of the first peak. The demonstration of a distinct shoulder or peak in the 11S portion of the chromatogram varied from one column to another. Reasons for this are not known, but may be related to slight differences in how the columns were packed.

Immunodiffusion. Sephadex G-200 fractions of colostral and milk whey were tested for the presence of IgM, IgA, and IgG by the double micro-immunodiffusion technique utilizing 1% Noble agar (Difco), 1% NaCl, and 1:10,000 Merthiolate. For these determinations, monospecific rabbit antisera against porcine IgM, IgA, or IgG sera, as prepared in our laboratory, were utilized (L. Saif and E. H. Bohl, *in preparation*). Monospecific anti-IgA serum was obtained from P. Porter (Unilever Laboratories, Eng-

land) and A. Richardson (University of Vermont), and monospecific anti-IgM serum from P. Porter. Similar results were obtained on the ID tests with these reagents as with those produced in our own laboratory.

Examination of small intestines. The small intestines of euthanized pigs were examined for evidence of villous atrophy, mucosal lactase, and chyle in the mesenteric lymphatics by procedures previously described (9).

RESULTS

Natural infection with virulent virus. Studies were conducted on swine from two herds which had been naturally infected with TGE. In both herds, serum samples collected from several animals in the acute phase of the disease had TGE antibody titers less than 4, whereas serum from the same animals in the convalescent phase had titers greater than 16.

Serum and milk samples were periodically obtained from seven sows and were tested for TGE antibodies (Table 1). Antibody titers in colostrum were considerably higher than in serum and, usually, two- to fivefold higher than in the 4- to 6-day milk sample. In six of the seven sows, titers were higher in milk even after 4 days of lactation than in serum of the same animal.

The litters of four of the seven sows were challenged at 2 to 4 days of age with TGE virus, resulting in a rise of antibody in both serum and milk of three sows (Table 1). None of the sows became sick. Mortality of the pigs in the four challenged litters was 5% (Table 2). Diarrhea occurred in 52% of the pigs but was much less severe than that observed in pigs nursing non-immune control sows. The incubation period prior to diarrhea was 3 to 8 days, in contrast to the 1 to 2 days observed in susceptible pigs. Only one litter remained entirely free of diarrhea, and this was from sow no. 81-3, an older animal which had farrowed previously. In contrast, the other three sows of this group had not farrowed previously.

Gel filtration of colostrum of sow no. 81-3 indicated that antibody activity was associated with both IgA and IgG, but primarily with IgA as judged by ID tests (Fig. 1a). With a 4-day milk sample from the same animal, antibody activity was detected in the IgA but not the IgG portion of the chromatogram (Fig. 1b). A comparison of chromatograms from colostrum and 4-day milk indicated a marked decrease in IgG and a lesser decrease in IgA, and this held true for all animals in this study. Colostrum samples from four other sows were similarly fractionated by gel filtration, and antibody activity was primarily associated with IgA in three of the four animals (Table 3).

TABLE 1. TGE antibody titers in serum and milk of sows after natural infection and following challenge of their suckling pigs

Sow no.	Approx. day pre-farrowing when exposed ^a	Challenge of pigs (age) ^b	Specimen	Sequential antibody titers expressed as: titer (post-farrowing day) ^c			
146	135	NC	Serum	56 (.2)	58 (5)	55 (28)	
			Milk	540 (.2)	290 (5)	105 (28)	
14-9	120	NC	Serum		115 (12)	100 (22)	
			Milk	560 (0)	165 (12)	110 (22)	
7-7	130	NC	Serum		170 (2)		150 (79)
			Milk	380 (0)	220 (2)	140 (13)	
32-8	125	3	Serum		32 (4)	140 (32)	
			Milk	150 (0)	36 (4)	490 (32)	
100	150	2, 4	Serum	20 (.2)	32 (4)	350 (30)	
			Milk	225 (.2)	72 (4)	410 (30)	
81-3	80	3	Serum		93 (4)		95 (45)
			Milk	800 (0)	72 (4)	78 (9)	
126	240	2	Serum	34 (.3)	28 (6)	120 (34)	64 (45)
			Milk	130 (.3)	45 (6)	100 (34)	

^a Indicates when the herd outbreak occurred, prior to time the sows farrowed.

^b Pigs in certain litters were challenged with TGE virus at the age (day) indicated. NC = not challenged.

^c Number in parenthesis indicates the day or portion of day after farrowing (0) when sample was collected.

TABLE 2. Transmission of passive immunity to suckling pigs challenged with TGE virus

Groups and history of sows	Sow no.	Age of pigs at challenge (days)	Mortality		Euthanized ^a
			Percent (no./total)	Mean	
Naturally infected	32-8	3	0 (0/4)	5 (1/20)	1
	100	3	20 (1/5)		
	126	2	0 (0/6)		
	81-3	3	0 (0/5)		
Experimentally infected	63	2	17 (1/6)	7 (1/15)	1
	76-10	3	0 (0/4)		
	185	3	0 (0/5)		
Intramuscularly vaccinated	224	3	60 (3/5)	60 (6/10)	3
	74-1	4	60 (3/5)		
Intramammarily vaccinated	5-1	3	0 (0/4)	14 (2/14)	1
	179	3	20 (1/5)		
	6-5	2	20 (1/5)		
Controls, susceptible	243	3	100 (7/7)	92 (34/37)	1
	78-1	3	100 (6/6)		
	17-9	3	80 (4/5)		
	19-8	3	60 (3/5)		
	26-9	3	100 (7/7)		
	245	3	100 (7/7)		

^a Some pigs with diarrhea were euthanized so that the small intestine could be examined for villous atrophy and lactase activity.

Experimental infection with virulent virus. Table 4 provides information on the experimental infection of seven pregnant swine and on the resulting TGE antibody titers in serum and milk. All but one animal (no. 185) were observed sick during the 2- to 7-day post-exposure period, exhibiting inappetence, depression, and diarrhea. Antibodies

were detected in the serum of five animals as early as 9 to 15 days post-exposure. Antibody titers in serum, colostrum, and milk were very similar to those observed with the naturally infected animals. In both groups of infected animals, antibody titers were invariably higher in milk than in serum.

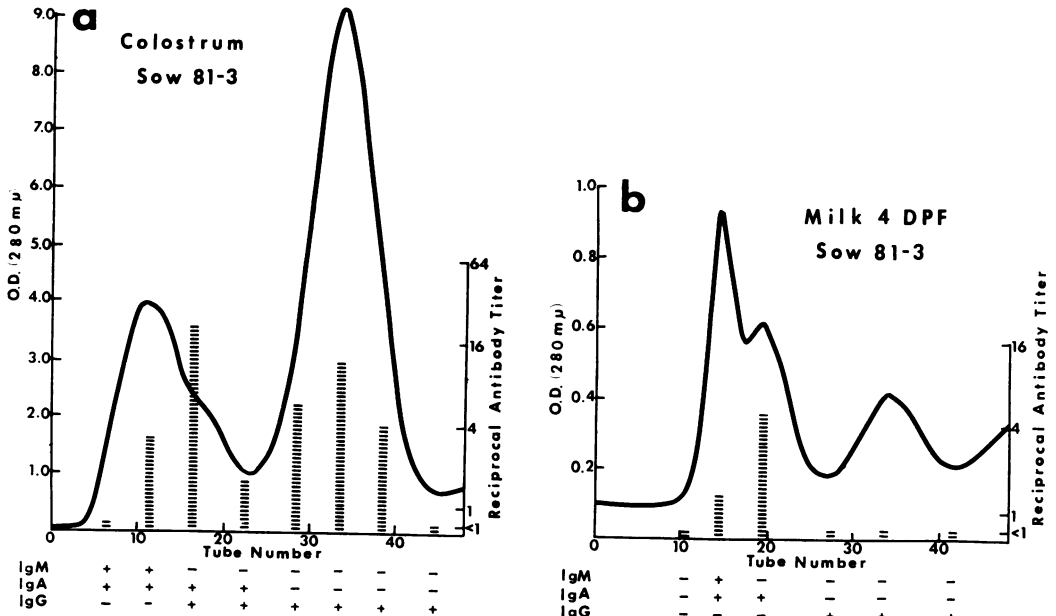


FIG. 1. Gel filtration on Sephadex G-200 of (a) colostrum and (b) 4-day milk from sow no. 81-3 which had been infected naturally with TGE virus. Indicated are TGE antibody titers, represented by vertical bars, and classes of immunoglobulins in selected unconcentrated eluate fractions.

The litters of three sows were challenged with TGE virus, but no distinct rise in antibody titer, either in serum or milk, could be detected in the sows (Table 4) nor did they become sick. The mortality of the pigs in the three challenged litters was 7% (Table 2). Although all pigs developed diarrhea, the incubation period was extended, being from 3 to 9 days. Diarrhea was generally mild as was dehydration when it occurred.

Gel filtration of colostrum of sow no. 76-10 indicated that antibody activity was associated with both IgA and the IgG, but primarily with the former (Fig. 2a). A 4-day milk sample from the same animal, similarly tested, resulted in antibody activity being detected only in the IgA portion of the chromatogram (Fig. 2b). Colostrum or milk samples from three other sows were similarly fractionated by gel filtration, and antibody activity was primarily associated with IgA (Table 3). This was especially evident in the 4- and 5-day milk samples.

Intramuscular vaccination. Table 5 provides information on the intramuscular vaccination of five pregnant swine and on the resulting TGE antibody titers in serum and milk. Following two intramuscular injections of live attenuated TGE virus, serum antibody titers were usually much higher than in the naturally or experimentally infected animals. Antibody titers in colostrum were generally high; for example, 9,600 in sow no. 74-1. However, antibody levels in milk de-

clined markedly after farrowing, so that after 4 to 7 days they were many fold less than in colostrum and several fold less than in serum. The latter observation is in marked contrast to the findings on the naturally or experimentally infected animals.

The litters of two sows were challenged with TGE virus, resulting in a distinct rise of antibodies in the serum and milk of both animals (Table 5). Sow no. 224 was not observed sick whereas the other sow exhibited anorexia and agalactiae for 2 days which was probably due to a mild form of TGE. All the challenged pigs developed diarrhea and the mortality was 60% (Table 2).

Gel filtration of colostrum of sow no. 74-1 indicated that the antibody titer was 345 in the IgG peak and 2 in the IgA portion of the chromatogram (Fig. 3a). A 7-day milk sample from the same animal, similarly tested, resulted in antibody activity being detected only in the IgG portion of the chromatogram (Fig. 3b). Colostrum or milk samples from two other sows were similarly fractionated by gel filtration, and antibody activity was primarily or solely associated with IgG (Table 3).

Intramammary vaccination. Table 6 provides information on the intramammary vaccination of five pregnant swine and on the resulting TGE antibody titers in serum and in milk of both injected and noninjected glands. Milk from the

TABLE 3. *TGE antibody titers in colostrum or milk whey and in the IgA and IgG fractions following gel filtration on Sephadex G-200*

Groups and history of sows	Sow no.	Day post-farrowing sample collected	TGE antibody titers		
			Whole milk whey	Gel filtration fractions	
				IgA ^a	IgG ^b
Naturally infected	51	0	410	9	2
	146	.2	540	37	6
	101	0	2,000	74	6
	7-7	0	380	12	13
Experimentally infected	185	0	400	12	6
	63	4	110	17	1
	107	5	340	19	1
Intramuscularly vaccinated	224	0	2,000	8	80
	224	4	60	1	9
	449	.2	903	<1	36
Intramammarily vaccinated ^c	5-1	.2	20,000	27	380
	5-1	4	1,024	1	25
	179	0	20,000	17	1,400
	179	4	1,500	6	64
	6-5	4	400	1	12

^a Conducted on a 3-ml eluate fraction from the top of the shoulder, or small peak, on the descending limb of the first peak, where IgA was in highest concentration as judged by immunodiffusion tests. In colostrum this fraction also always contained small amounts of IgG.

^b Conducted on a 3-ml eluate fraction from the top of the second peak, where IgG was in highest concentration as judged by immunodiffusion tests.

^c All samples were from injected glands.

TABLE 4. *TGE antibody titers in serum and milk after experimental oral infection of pregnant swine with virulent TGE virus and following challenge of their suckling pigs*

Sow no.	Day pre-farrowing when infected	Infected with virus strain	Challenge of pigs (age) ^a	Specimen	Sequential antibody titers expressed as: titer (pre- or post-farrowing day) ^b			
13-5	52	P-1	NC	Serum	14 (-43)	28 (.3)	42 (7)	41 (14)
				Milk		2,100 (.3)	152 (7)	80 (14)
12-10	46	P-1	NC	Serum	21 (.5)	27 (7)	25 (15)	
				Milk	450 (.5)	140 (7)	135 (15)	
107	32	M-3	NC	Serum	13 (-18)	57 (1.7)	44 (5)	22 (59)
				Milk		1,100 (1.7)	340 (5)	120 (59)
304	31	P-1	NC	Serum	84 (1.3)	74 (3)	80 (8)	67 (15)
				Milk	120 (1.3)	84 (3)	84 (8)	47 (15)
185	32	M-3	4	Serum	18 (-17)		68 (4)	86 (29)
				Milk		400 (0)	110 (4)	115 (29)
76-10	33	M-3	3	Serum	28 (-19)	100 (0)	82 (4)	82 (18)
				Milk		740 (0)	81 (4)	84 (18)
63	33	M-3	3	Serum	7 (-19)	110 (1.2)	90 (4)	64 (44)
				Milk		263 (1.2)	110 (4)	100 (44)

^a See footnote b, Table 1.

^b Number in parenthesis indicates the day or portion of day before (-) or after farrowing (0) when samples were collected.

noninjected glands was obtained only from the right side of the udder, the side which did not receive any injected virus. Following two intramammary injections of live attenuated TGE virus, serum antibody titers ranged from 300 to 1,700

as the highest levels in the individual animals. Antibody levels in colostrum were as high as 20,000 but declined rather markedly in milk within a few days after farrowing. Antibody levels were usually higher, sometimes two- or

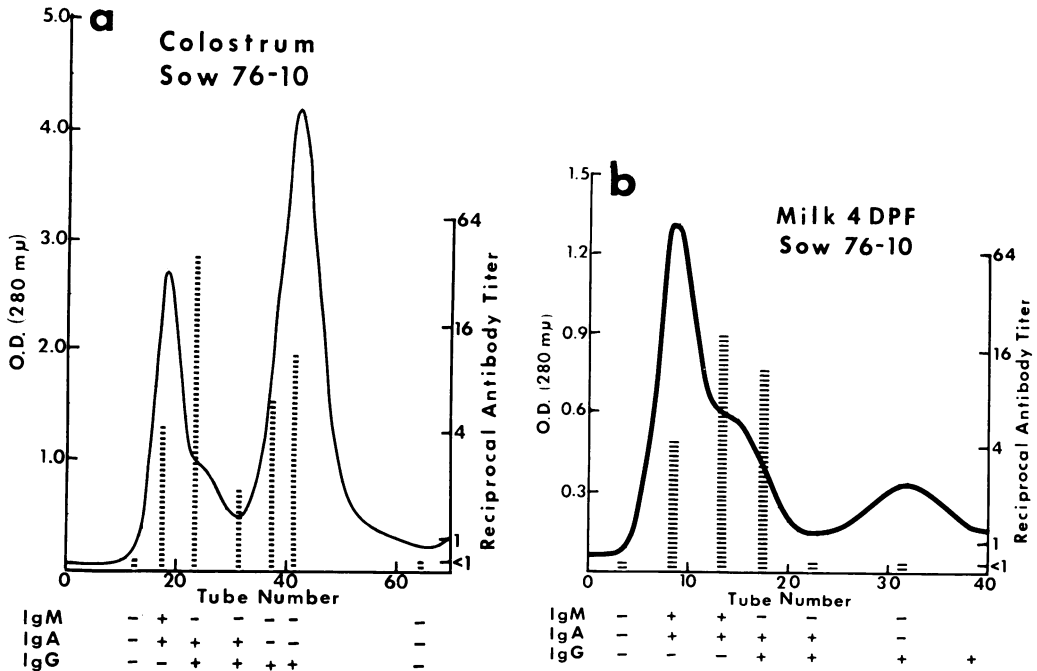


FIG. 2. Gel filtration on Sephadex G-200 of (a) colostrum and (b) 4-day milk from sow no. 76-10 which had been infected experimentally with virulent TGE virus, 33 days pre-farrowing. Indicated are TGE antibody titers, represented by vertical bars, and classes of immunoglobulins in selected unconcentrated eluate fractions.

TABLE 5. TGE antibody titers in serum and milk after intramuscular vaccination of pregnant swine with live attenuated TGE virus and following challenge of their suckling pigs

Sow no.	Days pre-farrowing vaccinated	Challenge of pigs (age) ^a	Specimen	Sequential antibody titers expressed as: titer (pre- or post-farrowing day) ^b					
				1	2	3	4	5	6
25-10	33,17	NC	Serum	144 (-11)		78 (.5)	76 (2)	68 (4)	71 (6)
			Milk		>1,024 (0)	350 (.5)	32 (2)	10 (4)	2 (6)
21-5	51,36	NC	Serum	10 (-36)	320 (-30)	130 (-8)	72 (1)	54 (4)	82 (8)
			Milk			27 (1)	4 (4)	5 (8)	
190	32,18	NC	Serum	31 (-18)	330 (1.5)	350 (5)	310 (15)	225 (29)	
			Milk		300 (1.5)	14 (5)	8 (15)	3 (29)	
74-1	41,13	4	Serum	100 (-13)	4,800 (-7)	1,500 (0)		1,200 (7)	7,000 (21)
			Milk			9,600 (0)	340 (3)	150 (7)	1,200 (21)
224	30,14	3	Serum	12 (-14)		160 (4)	5,200 (11)	5,200 (18)	
			Milk		2,000 (0)	60 (4)	440 (11)	1,400 (18)	

^a See footnote b, Table 1.

^b See footnote b, Table 4.

threefold, in colostrum or milk from injected than from noninjected glands.

The litters of four sows were challenged with TGE virus, and a distinct rise of antibodies in both serum and milk occurred in the three sows which were specifically tested for this purpose (Table 6). Two of the sows (no. 5-1, no. 179) became sick 4 to 7 days after the litters were challenged, having clinical signs compatible with

those of TGE. All challenged pigs developed diarrhea and the mortality was 14% (Table 2). The incubation period in many of these pigs was longer than normal, being 2 to 9 days.

Colostrum or milk from the injected glands of four sows was fractionated by gel filtration and the results indicated in Fig. 4 and Table 3. Antibody activity was highest in the IgG portion of the chromatogram. There was little if any indica-

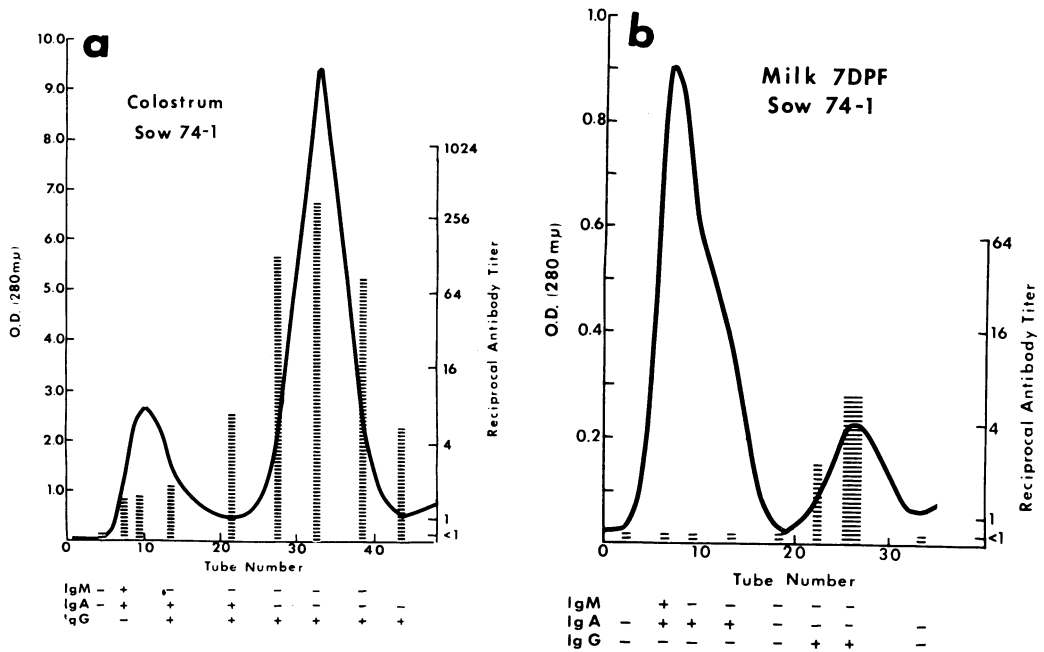


FIG. 3. Gel filtration on Sephadex G-200 of (a) colostrum and (b) 7-day milk from sow no. 74-1 which had been vaccinated intramuscularly with live, attenuated TGE virus, 41 and 13 days pre-farrowing. Indicated are TGE antibody titers, represented by vertical bars, and classes of immunoglobulins in selected unconcentrated eluate fractions.

TABLE 6. TGE antibody titers in serum and milk after intramammary vaccination^a of pregnant swine with live attenuated TGE virus and following challenge of their suckling pigs

Sow no.	Days pre-farrowing vaccinated	Challenge of pigs (age) ^b	Specimen	Sequential antibody titers expressed as: titer (pre- or post-farrowing day) ^c					
74	42, 28	NC	Serum	37 (-28)	210 (1.3)	300 (7)	256 (14)	133 (27)	
			Milk						
			I. gl. ^d		190 (1.3)	19 (7)	6 (14)	8 (27)	
5-1	43, 14	3	N.I. gl. ^e			19 (7)	7 (14)	8 (27)	
			Serum	160 (-14)	2,000 (.2)	1,500 (4)	1,650 (8)	6,000 (34)	5,200 (52)
			Milk						
179	34, 12	3	I. gl.		20,000 (.2)	1,024 (4)	64 (8)	270 (34)	120 (52)
			N.I. gl.		8,200 (.2)	430 (4)	48 (8)	500 (34)	130 (52)
			Serum	320 (-12)		1,400 (4)	1,300 (10)	5,500 (29)	
19	40, 18	38	Milk						
			I. gl.		20,000 (0)	1,500 (4)	220 (10)	1,050 (29)	
			N.I. gl.		18,000 (0)	350 (4)	210 (10)	1,020 (29)	
6-5	34, 14	2	Serum	390 (-18)	1,300 (.3)	1,700 (4)	800 (29)	950 (38)	9,900 (70)
			Milk						
			I. gl.		6,100 (.3)	225 (4)	10 (29)	18 (38)	
6-5	34, 14	2	N.I. gl.		5,200 (.3)	120 (4)	9 (29)	9 (38)	256 (70)
			Serum	150 (-14)	1,350 (0)	1,300 (4)	960 (8)		
			Milk						
6-5	34, 14	2	I. gl.		21,500 (0)	400 (4)	34 (8)		
			N.I. gl.		6,500 (0)	380 (4)	24 (8)		

^a Three mammary glands on the same side were injected.

^b See footnote b, Table 1.

^c See footnote b, Table 4.

^d I. gl. = milk from injected glands.

^e N.I. gl. = milk from non-injected glands.

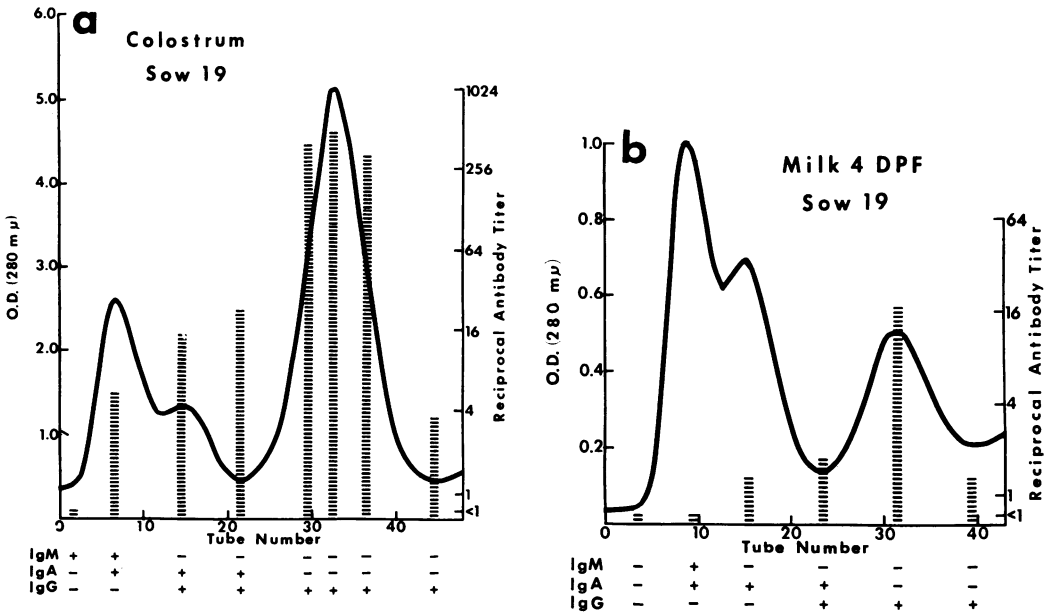


FIG. 4. Gel filtration on Sephadex G-200 of (a) colostrum and (b) 4-day milk from sow no. 19 which had been vaccinated intramammarily with live, attenuated TGE virus, 40 and 18 days pre-farrowing. Indicated are TGE antibody titers, represented by vertical bars, and classes of immunoglobulins in selected unconcentrated eluate fractions.

tion of antibody activity being associated with IgA. The location of antibody activity in the chromatograms of colostrum or milk from non-injected glands was similar to that from injected glands. Although antibody activity was detected in the descending limb of the first peak from colostrum specimens, it was much less than in the IgG peak, and may have been due to IgG which is always present in small amounts in this portion of the chromatogram.

Susceptible sows. Litters from six serologically negative sows were challenged with TGE virus (Table 2). The incubation period prior to diarrhea was usually 21 to 36 hr. The morbidity was 100% and was characterized by transient vomiting in many of the pigs, followed by severe diarrhea and dehydration in all pigs. Mortality was 92%—34 of the 37 challenged pigs died. Death occurred 2 to 11 days post-challenge. Four of the six sows were sick, 3 to 8 days following challenge of pigs, characterized by depression, anorexia, and agalactiae, and two had diarrhea. All sows became serologically positive for TGE.

Small intestines of challenged pigs. As indicated in Table 2, several pigs from the various groups of sows were euthanized, following onset of diarrhea, and their small intestines were examined for villous atrophy and in some cases for lactase activity. The purpose of these examinations was to determine if passively acquired antibodies might be altering the course of the intestinal infection.

Typical findings for TGE were observed in three pigs of the control, susceptible group and included extensive villous atrophy for the entire length of the small intestine except for the first few centimeters, absence of lactase in mucosal sections of the jejunum and ileum, and an absence of chyle in the mesenteric lymphatics (9).

Three pigs from the naturally and experimentally infected groups had slightly shortened villi mainly in the ileum. Slight lactase activity was detected in mucosal sections of the jejunum and ileum from a pig of sow no. 76-10.

Three pigs from the intramuscularly vaccinated group were examined. Two pigs from sow no. 224 had normal-length villi for about the first 1.8 m but had shortened villi caudally. In one of these pigs, examined about 24 hr after onset of diarrhea, lactase activity was detected in mucosal segments from the duodenum and jejunum but not from the ileum. Chyle was present in the mesenteric lymphatics adjacent to that portion of the intestine with normal-length villi. No lactase activity or chyle was evident in the other pig, which was examined about 48 hr after onset of diarrhea. A third pig of this litter, examined about 28 hr after onset of diarrhea, had shortened villi throughout the small intestine.

Four pigs from the intramammarily vaccinated group were examined. Three pigs, one from sow no. 5-1 and two from sow no. 6-5, had normal-length villi in the first 1.3 to 2.7 m of the small intestine, but had shortened villi caudally. Chyle

was evident in the mesenteric lymphatics adjacent to that portion of the intestine with normal-length villi. Lactase activity was detected in the intestinal segments taken about 1 m from the pylorus but not in those taken 2 m from the ileocecal valve. Diarrhea had been present in these three pigs for 12 to 52 hr prior to euthanasia. One pig from sow no. 179, examined 42 hr after onset of diarrhea, had moderately shortened and fused villi and no chyle was evident.

DISCUSSION

Some of the characteristics of the antibody response of swine following infection or vaccination with TGE virus will be discussed. Emphasis will be given to the titer and Ig class of TGE antibodies in colostrum and milk and to the ability of the infected or vaccinated sows to transmit passive immunity.

Antibody titers in sera were usually considerably higher in the intramuscularly or intramammarily vaccinated animals (range of 144 to 4,800) than in the experimentally infected animals (range of 27 to 110). This comparison is based on the highest serum titer detected in individual animals during a period of 21 to 50 days after vaccination or infection, prior to challenge of their litters. These results are probably due to more viral antigen being available to antibody-producing cells in parenteral tissues following intramuscular or intramammary vaccination than following experimental infection, which in adult animals is recognized as a localized intestinal infection.

Antibody titers in colostrum were generally highest in the intramammarily vaccinated animals, with titers reaching 20,000 in three animals. High titers were also detected in the other groups, especially the intramuscularly vaccinated animals. In all of the infected or vaccinated animals, antibody titers were several times higher in colostrum than in serum. These findings are undoubtedly due to the higher levels of Ig in colostrum than in serum.

Antibody titers in milk of all infected or vaccinated animals declined during the first few days post-farrowing, but was particularly marked in the vaccinated animals. Titers remained consistently higher during the lactation period in infected than in vaccinated animals. A comparison of titers in milk versus serum, collected concurrently from the same animal, revealed a marked difference between the infected and the vaccinated animals. This was especially true if samples were collected at least 7 days post-farrowing. In infected animals, milk titers were invariably higher than those in serum whereas in vaccinated animals, milk titers were several times lower than in

serum. A probable explanation is that TGE antibodies in milk of infected animals are primarily of the IgA class, whereas in vaccinated animals they are primarily of the IgG class. These findings and the interpretation agree fairly well with reports on the values of IgA and IgG in porcine serum and milk collected at least 7 days post-farrowing. For example, IgA was higher in milk than in serum (10, 30), whereas Ig was about 17 times less in milk than in serum (10). Also, IgA constitutes the predominant class of Ig in milk (10, 20, 30).

Of considerable interest is information on the Ig class identity of TGE antibodies in serum, colostrum, and milk. Fractionation of colostrum and milk specimens by gel filtration and the ratio of antibodies in serum to those in milk were used in attempting to determine the probable Ig identity of TGE antibodies. In the naturally or experimentally infected animals, TGE antibodies in colostrum were associated with both IgA and IgG whereas those in milk were primarily, if not solely, IgA. In the vaccinated animals, TGE antibodies in colostrum or milk appeared to be primarily, if not solely, of the IgG class. Especially in milk of the vaccinated animals, there was little if any evidence for the existence of TGE antibodies of the IgA class. However, other procedures must be utilized to determine more precisely the Ig class identity of these antibodies. In another report (L. Saif, R. K. P. Gupta, and E. H. Bohl, *in preparation*), absorption techniques, utilizing monospecific sera against IgM, IgA, and IgG, are described which will further clarify the Ig class identity of the TGE antibodies.

The ability of sows to transmit passive immunity for TGE to their suckling progeny was determined in a small number of litters (Table 2). The resulting mortality in the different groups of sows was as follows: 5% of the pigs in the naturally infected, 7% in the experimentally infected, 14% in the intramammarily vaccinated, 60% in the intramuscularly vaccinated, and 92% in the susceptible, control group. The severity of diarrhea and dehydration in pigs of the various groups were also comparable to the mortality figures.

Certain problems are encountered when attempting to evaluate the passive immunity of suckling pigs for this disease. Passive immunity occurs when the epithelial cells of the small intestines are protected either to avoid viral infection or to reduce the severity of the infection. Available information indicates that this can be accomplished only if protective antibodies are almost continually present in the lumen of the intestinal tract (14), as occurs when pigs nurse immune sows at frequent intervals, which nor-

mally is every 1 to 2 hr. Thus, if an immune sow is not providing sufficient milk or if the pigs do not nurse at frequent intervals, there may be an impairment in the transfer of passive immunity. The viral inoculum used for challenge purpose must be of suitable virulence, purity, and at an appropriate dosage so as not to overwhelm the immune system. The viral inoculum used in the present study was especially prepared and used to meet these requirements. A further problem occurs when one or more pigs in a challenged litter is inadequately protected and becomes infected. The resulting diarrhea will disseminate large amounts of virus in the environment and will provide a secondary severe challenge to the remaining pigs. It is probably for these reasons that challenged pigs, even though nursing immune sows, will often develop diarrhea but the severity of the disease and mortality will be reduced.

The antibody response of swine to TGE virus has also been studied by Harada et al. (17). They reported that antibody titers rose significantly following two intramuscular injections of attenuated virus but that the inoculated swine became ill after oral challenge, indicating that circulating antibodies do not provide protection against this intestinal infection.

The results of this study give additional data indicating that IgA TGE antibodies are capable of providing more adequate passive immunity than are IgG TGE antibodies, as was previously suggested (5). However, high levels of IgG TGE antibodies in colostrum and milk can apparently provide a variable degree of protection as indicated by the decreased mortality and severity of the disease in pigs nursing vaccinated sows when compared to pigs nursing susceptible sows (Table 2). This was especially noticeable with the intramammarily vaccinated sows which produced very high levels of IgG antibodies.

A rather striking feature was the presence of lactase activity and normal-appearing villi in only the first 1 or 2 m of the small intestines of several of the post-challenged pigs of the vaccinated sows, indicating an immunity of only the first portion of the intestinal tract. This might be associated with the lability of IgG to proteolytic enzymes, in contrast to the stability of secretory IgA, as has been reported (24, 29). That is, IgG antibodies might be enzymatically degraded in the stomach or intestines, thus restricting their effectiveness so that only the first portion of the intestinal tract would be protected. It is also possible that the mode of action of IgA and IgG antibodies on mucous membranes is entirely different, with the former being able to adhere to and protect epithelial cells better than the latter.

Current information suggests that secretory

IgA is produced locally (29). Porter and colleagues have provided evidence, somewhat indirect, suggesting that IgA occurs in porcine mammary secretions as a result of local synthesis rather than by a process of selective transfer from blood (20, 21). If this is true, what provides the stimulation for the elaboration of IgA TGE antibodies in mammary glands with its subsequent secretion in milk? Two possible mechanisms are suggested. One involves the stimulation of IgA-producing cells in the mammary glands by TGE virus, with the latter arriving at the site either via blood or teat meatus. However, our failure to demonstrate any appreciable level of IgA TGE antibody in milk following intramammary injections of live attenuated TGE virus would tend to cast doubt on this mechanism. The second possible mechanism, as we previously suggested (5), might involve a relocation of TGE viral sensitized immunocytes from the lamina propria of the intestinal tract to the mammary gland, possibly via lymphatic and blood vessels. Crabbe et al. (8) have expressed the view that IgA antibody-producing cells, after having received their antigenic stimulation in the intestinal mucosa, might emigrate to extraintestinal sites where IgA antibody would be produced. The second mechanism seems to be more compatible with the findings in this report. For example, appreciable levels of IgA TGE antibodies in milk have been detected only in those animals which have recovered from a natural or experimental infection with virulent virus which normally results, primarily, in an intestinal tract infection. A close immunologic relationship between the intestinal tract and the mammary gland, which would provide high levels of IgA antibodies in colostrum and milk, would be highly advantageous for the protection of the newborn against those enteric infections that are endemic in the parent population.

Vaccination of pregnant swine so as to provide passive immunity to suckling pigs against TGE is a goal of considerable economic importance. A high level of immunity can be accomplished by oral exposure of pregnant swine to virulent TGE virus at least 3 weeks prior to farrowing (1), but this has the disadvantage of perpetuating the disease and, possibly, of producing new foci of infection. A commercial vaccine (TGE-Vac, Diamond Laboratories, Des Moines, Iowa), composed of live attenuated TGE virus, which is to be administered intramuscularly at approximately 6 weeks and again at 2 weeks prior to farrowing, is currently available (12). Following challenge of 3-day-old pigs, Tamoglia (26) reported a mortality of 38% in pigs nursing sows which had been vaccinated with this commercial

vaccine, 71% in pigs nursing nonimmune control sows, and 0% in pigs nursing sows which had been experimentally infected with virulent virus during gestation. The limited effectiveness of live, attenuated TGE vaccine, especially when administered by the intramuscular route, is probably due to the production of IgG rather than IgA antibodies in mammary secretions.

Oral vaccination with a live, attenuated TGE vaccine results in the production of neutralizing antibodies, but immunity to suckling pigs has been poor (2). The predominant class of antibody found in milk was IgG, rather than IgA (E. H. Bohl, *in preparation*). The failure of the oral vaccine to produce adequate immunity may be due (i) to the inability of the attenuated virus to infect the intestinal tract, consequently not stimulating the appropriate immunocytes, or (ii) to an antigenic alteration in the attenuated virus. However, we believe the former to be the more probable explanation.

Thorsen and Djurikovic have reported that intramammary injections of live, virulent (11), live, cell-cultured (27), or inactivated (28) TGE viral preparations provided good protection against TGE to the suckling progeny. The Ig class identity of the antibodies in milk was not indicated. Ristic and Abou-Youssef (23) have also reported similar favorable results following intramammary injections of live, virulent TGE virus, with elaboration of IgA antibodies in colostrum and milk. However, it is possible that parenterally injected virulent virus can infect the intestinal tract, resulting in the stimulation of IgA antibodies. For example, Bay et al. (1) reported that one of four pregnant swine developed diarrhea following intramuscular injection of virulent TGE virus.

By the procedures used in this study, higher levels of TGE antibodies were produced in colostrum and milk following injections of live, attenuated virus by the intramammary route than by the intramuscular route. By either route, the antibody appeared to be primarily, if not solely, of the IgG class. In the limited number of litters which were challenged, the intramammary route also provided better protection to the suckling pigs. However, the feasibility of vaccinating swine by this route under field conditions can be questioned.

Intramammary injections of rabbits with bovine serum albumin have resulted in the production of both IgG and IgA antibodies in milk (13, 24). However, adjuvants were used with the antigen which differed from our procedure and which may have influenced the type of antibody response.

The important role of IgA antibodies in the

alimentary tract for providing protection against enteric infections has been suggested by many authors (7, 8, 21, 29). Our results, as described herein and in a previous report (5), support this concept and indicate that TGE in swine provides a good model for investigating the immune mechanisms of the intestinal tract. Information on immunizing procedures which will result in appreciable levels of IgA antibodies in mammary secretions is needed so as to provide passive immunity to suckling animals.

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