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LACTOGENIC IMMUNITY IN TRANSMISSIBLE GASTROENTERITIS OF SWINE:

Pregnant Sows Sensitized with Inactivated Virus and then Vaccinated Intramuscularly with Live Attenuated Virus

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The present study has shown that sows sensitized by intramuscular inoculation with inactivated virus and then revaccinated with an attenuated live virus were not able to induce a solid lactogenic immunity to nursing pigs against experimental challenge with virulent TGE virus. The pig morbidity and mortality rates were compared with pigs nursing nonvaccinated seronegative sows. The morbidlty rates for the vaceinated and nonvaccinated groups were both 100%, while the mortality rates were 48% and 91% respectively. All vaccinated sows responded with production of relatively high levels of antibodies in serum and colostrum. However, antibody levels in milk rapidly decreased after parturition. TGE antibodies in colostrum and milk were primarily or solely of the IgG class, although low levels of IgA antibodies were detected in some animais.

Transmissible gastroenteritis (TGE) is a highly contagious viral disease affecting pigs of all ages but with causing severe death losses in baby pigs under two weeks of age.[4,7] In Taiwan, the disease broke out for the first time in Ilan prefecture in January 1958, and then quickly spread to all of the prefectures.[17] In recent years, however, TGE epidemics often occurred on commercialized pig farms.

Because of a great loss from TGE in suckling piglets, considerable efforts have been devoted to develop methods and techniques for the proper immunization of pigs.[5,18,36] Immunity to TGE should be effective during the first day of a pig's life, because this is the time when the pig is most susceptible to the effects of TGE virus infection.[11] The attenuated virus administered orally is capable of stimulating circulating antibodies in newborn pigs, however, circulating antibodies provide little, if any, protection against intestinal infection with virulent TGE virus. Also several days are required to develop immunity based on active secretion of antibody, and pigs could sicken and die before it was effective.[2,11] In epizootiological studies it appeared that the disease often started in the older swine and then spread to baby pigs in the farrowing house.[6] Obviously, the involvement of sow's milk in spreading viruses to their baby pigs may play an important role.[14] It is well known that sows will,

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after a natural infection with TGE virus build up an active immunity and effectively protect their baby pigs by specific antibody in milk.[1,2,4,22] This is referred to as lactogenic immunity.[10] Lactogenic immunity is at present the only practical route of providing protection to newborn pigs. Therefore, attempts to find a suitable vaccine have been directed towards immunizing the sow as she nears farrowing.[1,2,4,5,18,22,24,26]

Some workers confirm that gut virus, the virulent virus, is the most efficient immunizing agent,[2,4,18,24] but this procedure has certain inherent dangers, especially in those herds on a frequent or continuous farrowing program, and the practice cannot be recommended.[15,16] The commercial attenuated virus vaccine is available and provides some protection when used as directed by the manufactureer.[24,26] Some workers found that colostrum antibody levels in sows given attenuated strains did not persist longer than a few days after farrowing.[2,3,26] Protection of baby pigs against TGE depends upon a continuous supply of specific viral neutralizing antibodies in the lumen of the intestine.[8,9,16,23] It is possible to sensitize the sows with killed virus and then increase the antibody activities by giving live virus a week before farrowing. This data have not been reported. The purpose of the present studies was to find out how effectively sows that had been vaccinated intramuscularly with inactivated and then with commercial attenuated virus vaccines would protect their suckling piglets against challenge with virulent TGE virus.

a. TGE-Syntex, Lot No. 8045, Modified Live Virus Porcine Tissue Culture Origin, Des Moines, Iowa, USA.
b. Miller-3 Pig Challenge Virus, Lot No. 79-8A, Virus titer 1.5×10^6 PFU/ml, Veterinary Biologics Division, Agricultural Research Service, US Department of Agriculture, Ames, Iowa, USA.
c. Pharmacia Fine Chemicals, Piscataway, NJ, USA.
d. Spectophotometer, Beckmen, DB.
e. Anti-porcine IgM serum, Commercial Product, Lot No. 12, Chemical Credentials, Miles Lab. Inc. Indiana, USA.
f. Goat anti-pig IgA and IgG Antiserum were provided by Dr. S. S. Stone, National Animal Disease Center, Ames, Iowa, USA.

MATERIALS AND METHODS

Vaccine Inactivated and live attenuated TGE viruses were used for vaccination. The inactivated vaccine was prepared from a cell culture propagated Miller-3 strain TGE virus and concentrated 100 times and then inactivated with Al $(OH)_3$. The live attenuated TGE virus vaccine is a commercial product in the USA.[a]

Vaccination of Swine A total of nine TGE seronegative pregnant sows with known breeding dates were obtained from the breeding herd maintained at the National Animal Disease Center. Of the 9 sows, five were intramuscularly injected twice at approximately 18 and 16 weeks before parturition with 2ml of inactivated virus. Approximately 2 weeks before farrowing, all of the 5 sows revaccinated with 2ml of the live attenuated virus according to directions recommended by the manufacturer. The other four sows were used as control. All of the sows farrowed in individual isolation rooms. Colostrum, milk and serum samples were collected at 0, 7th day after parturition.

Challenge Exposure of Newborn Pigs All litters were challenged orally at 3

ays of age with a dose of 5 ml of a 1:1,000 dilution of the Miller-3 strain of virulent TGE virus.[b]

Virus-Neutralization Test A continuous line of swine testis (ST) cells was used.[19] Confluent sheets of cells were obtained after 5 days of incubation at 37°C in a humidified atmosphere containing 5% CO_2. The Miller-3 isolated of TGE virus was used as the indicator virus. This virus had been serially passaged 9 times in the ST cell line and had a virus titer of 5×10^7 plaque forming units/ml. The TGE virus-neutralizing antibodies in serum, colostrum, milk and in selected gel filtrates were assayed, using the plaque reduction test as previously described.[13] Antibody titers were expressed as the reciprocal of the sample dilution resulting in a 50% reduction in plaques.

Gel Filtration Skim milk was prepared by centrifugation of whole colostrum or milk at 3,000 RPM for 30 min and by collecting the middle portion between the cream layer and the deposited debris. Whey was prepared by reaction of sample collected above with rennin (0.1mg/ml whey) and 2% $CaCl_2$ (10μl/ml whey) and then centrifugated at 20,000 RPM for 30 min to precipitate the casein. The resultant whey supernatant fraction was dialyzed against 0.1M Tris-hydrochloride and 1 M NaCl buffer solution containing 0.02% sodium azide, PH 8.6 Using Sephadex G-200, approximately 3 ml of colostrum or milk whey and 1 ml of serum were applied to a 5×50 cm column for gel filtration. The buffer system was 0.1 M Tris-hydrochloride, in 1M NaCl, adjusted to PH 8.6 with HCI. The flow rate was 50 ml/hr. Six milliliter fractions were collected and the optical density (OD) of the fractions at 280 nm were determined.[a]

Immunodiffusion Sephadex G-200 fractions of colostral and milk whey and serum were tested for the presence of IgM, IgA and IgG by the gel diffusion method against rabbit anti-porcine IgM,[e] goat anti-pig IgA and goat anti-pig IgG[f] respectively. The test used 1% agarose suspended in Tris-HCl buffer solution, adjusted to PH 8 with 5 N NaOH.

RESULTS

Antibody Response The antibody response as determined in colostrum, milk and serum of the vaccinated sows after farrowing and challenge exposure of their litters with virulent virus is listed in Table 1. Antibody titers in colostrum or serum of all vaccinated sows at the time of parturition were relatively high, but titers in milk rapidly decreased within a few days after parturition.

Morbidity and Mortality Rates After Challenge Exposure The litters nursing 5 vaccinated sows and 4 nonvaccinated control sows were challenge exposed with virulent virus at 3 days of age. All litters developed diarrhea within 1 to 3 days. Vomition and a variable degree of dehydration were also observed in all affected litters. Within 11 days after challenge exposure, mortality rate was 48% in pigs nursing 5 vaccinated sows, compared with 91% in pigs nursing 4 nonvaccinated sows (Table 1).

Immunoglobulin Classes of TGE Antibodies in Colostrum, Milk and Serum Colostrum, milk and serum samples from vaccinated sows were fractionated by gel filtration, and antibody activity was

primarily or solely associated with IgG (Table 2). Gel filtration of colostrum of vaccinated sows indicated an average antibody titer of 88 (45~162) in the IgG peak and less than 3 (<1~7) in the IgA peak of the chromatogram. The 7-day milk and serum samples from these same animals resulted in antibody activity being detected only in the IgG portion of the chromatogram.

A typical gel filtration chromatogram of colostrum, 7-day milk and serum samples from vaccinated sow 145 is illustrated (Fig 1).

Table 1. Antibody Response of Vaccinated Sows and the Immune Response of Newborn Pigs Nursing Vaccinated or Nonvaccinated Sows After Oral Challenge with Virulent TGE Virus

Sow No.	Sows TGE Neutralizing Antibody Titer*			Piglets Challenge				
	Colostrum[1]	Milk[2]	Serum[1]	No. Challenge	No. Sick	Morbidity Rate. %	No. Died	Mortality Rate. %
Vaccinated								
101	2128	100	206	6	6	100	5	83
142	4188	257	891	11	11	100	5	46
145	2023	416	1112	12	12	100	3	25
	4740	942	1778	5	5	100	2	40
138	640	316	745	10	10	100	6	60
Total (av)	2743	406	946	44	44	100	21	48
Nonvaccinated								
137	<4	NT	NT	12	12	100	12	100
141	<4	NT	NT	5	5	100	5	100
144	<4	NT	NT	14	14	100	12	86
150	NT	NT	NT	3	3	100	2	6 7
Total (av)	NT	NT	NT	34	34	100	31	91

* Expressed as reciprocal of the sample dilution resulting in a 50% reduction in plaque number in neutralization test

1 At the time of parturition

2 7 days postpartum

NT Not Tested

Table 2. TGE Antibody in Gel Filtration Fractions of Colostrum, Milk and Serum of Vaccinated Sows

Sows No.	Gel Filtration Fraction TGE Antibody Titer*					
	Colostrum[1]		Milk[2]		Serum[1]	
	IgA**	IgG***	IgA**	IgG***	IgA**	IgG***
101	2	56	< 1	2	< 1	5
142	7	162	< 1	1	< 1	21
145	< 1	45	< 1	1	< 1	25
86	< 1	48	< 1	5	< 1	45
138	3	72	< 1	1	< 1	7
Ave.	< 3	88	< 1	2	< 1	21

* Expressed as reciprocal of the sample dilution resulting in a 50% reduction in plaque number in neutralization test

** Test was conducted on a 6 ml eluate fraction where IgA was in highest concentration as determined by immunodiffusion tests

*** Test was conducted on a 6 ml eluate fraction where IgG was in highest concentration as determined by immnnodiffusion tests

1 At thetime of parturition

2 7 days postpartum

DISCUSSION

In the present study five pregnant sows were intramuscularly vaccinated with an inactivated virus and then with live attenuated virus, and all responded with production of relatively high levels of antibodies in serum and colostrum. However, antibody levels in milk against our expectation, rapidly decreased after parturition. Similar observations have been reported in swine that were parenterally vaccinated with live attenuated or killed virus.[4,5,25,26]

The lactogenic immunity resulting from challenging 3-day-old suckling pigs was only partial since there was a 100% morbidity and a 48% mortality. There was no correlation between the TGE milk antibody titer and pig mortality. This is comparable with the morbidity rate of 100% and mortality rate of 37.8%, as reported by Tamoglia[24] using only the same commercial vaccine on 9 swine. Therefore, our vaccination schedule is of limited value in preventing infection and clinical signs of TGE in suckling pigs, but just tends to reduce the high mortality associated with the occurrence of the disease in neonatal pigs.

As determined by gel filtration techniques, TGE antibodies in colostrum and in 7 day postpartum milk samples from vaccinated sows were mainly, if not solely, of the IgG class. Some researchers[23] isolated IgG antibody from colostrum and orally administered it to piglets to demonstrated its ability to prevent infection. The results suggest that not only IgA but also the IgG contained in milk may have an effect of protecting piglets from infection. But some researchers[4,21] pointed out that

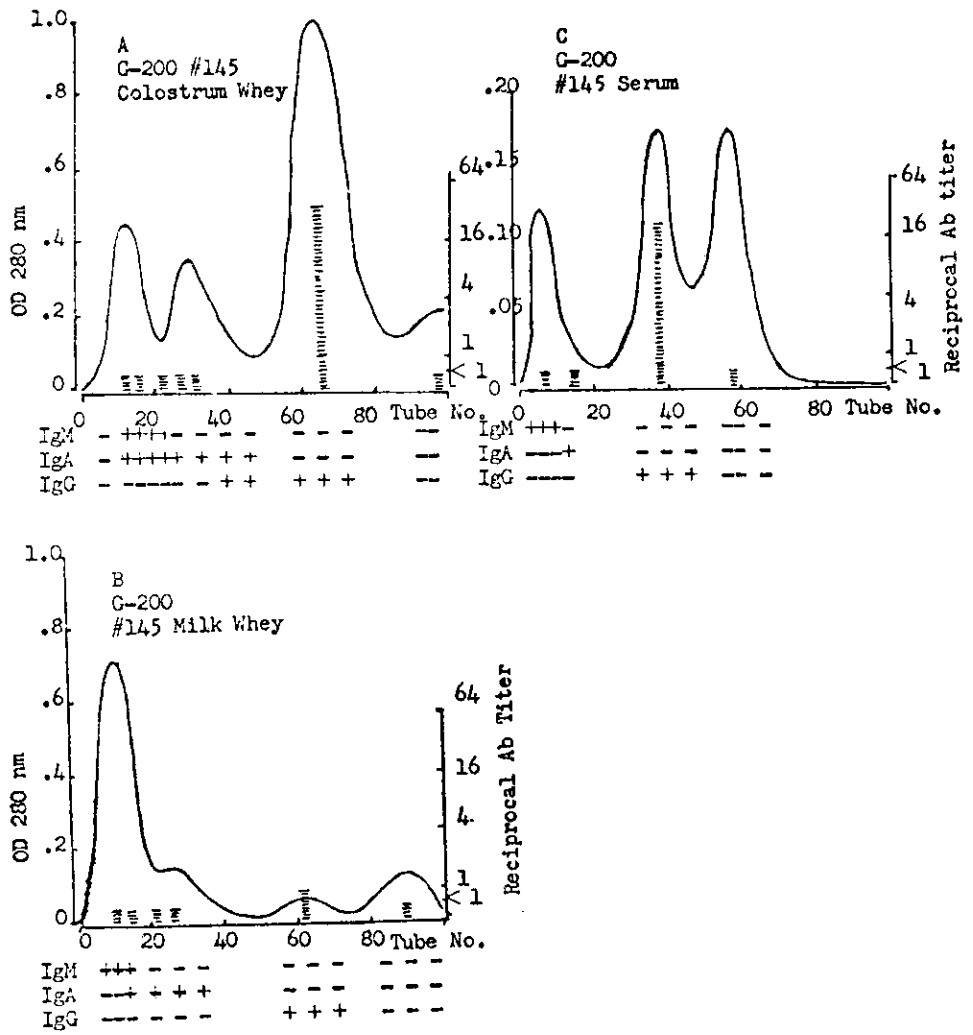

Fig 1. **A. Gel filtration of colostrum sample from vaccinated sow 145. Indicated are TGE antibody titers (represented by vertical bars) and classes of immunoglobulins in selected nonconcentrated eluate fractions**
B. Cel filtration of 7-day milk sample from the same animal.
C Cel filtration of serum sample collected at the time of parturition from the same animal.

passive immunity against intestinal infection with TGE virus was generally more complete in pigs ingesting antibodies of the IgA than the IgG class. It was estimated that IgG antibody was 500 times more efficient than was IgA for coating TGE virions, so IgA must have a greater predilection for the neutralizing site than IgG.[12] Therefore, during the lactation period of sows, the antibodies of the IgA class in milk would predominantly provide available passive immunity to their baby pigs. It has been estimated that the most effective method of stimulating production of TGE antibodies of the IgA class occurs as a result of an infection of the gastrointestinal tract.[3] Parenteral

injections or oral vaccination with a live attenuated TGE virus have resulted in the production of little, if any, IgA antibodies.[4] It appears that a live attenuated virus strain must be found which, has the antigenic characteristics of virulent virus,20 and can replicate sufficiently in the small intestine of sows after oral administration.

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猪傳染性胃腸炎之乳源免疫：懷孕母猪以不活化病毒感受，再行肌肉接種活毒弱毒疫苗

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中 文 摘 要

母猪以不活化 TGE 病毒肌肉接種先行感受，再以活毒弱毒病毒接種免疫，其仔豬以强毒攻擊後，顯示並未能獲得强固之乳源免疫。仔豬之發病率及死亡率和抗體陰性之未免疫母豬所生之仔豬比較；發病率分別爲 100 %及 100 %，而死亡率則分別爲 48 %及 91 %。所有免疫之母猪產生高力價之血清及初乳 TGE 抗體，但乳汁中之抗體於分娩後急速下降。初乳及乳汁中主要或只有 IgG 抗體，但有些母豬於初乳中也含有低力價之 IgA 抗體。

本文在美國農業部愛我華州國立動物疾病中心完成