STRATEGIES FOR A SUCCESSFUL CORONAVIRUS (TGE) VACCINE FOR SWINE

C. Joseph Welter

Ambico, Inc.
P.O. Box 522, Route 2
Dallas Center, IA 50063

INTRODUCTION

Transmissible gastroenteritis (TGE) is a coronaviral enteric disease in swine responsible for substantial economic loss to the swine industry. Fifteen percent of nursing piglet diarrheas in the Midwestern States can be attributed to TGE.¹ The natural route of infection is oral, with viral replication occurring in the small intestine, and leading to watery diarrhea, rapid dehydration and death of piglets under two weeks of age. Viremia and other localized infections do not appear to be prevalent during the course of the disease although infectious virus has been isolated from the lungs of clinically normal adult pigs.²

Prevention of TGE, as originally practiced, consisted of feeding infectious manure or piglet tissues to pregnant sows and gilts about three weeks pre-farrowing. This deliberate exposure of gestating animals, although effective in the induction of active immunity in the sow and passive immunity in nursing piglets, may result in perpetuation and spread of TGE and other viruses.

TGE vaccines have been commercially available since 1966.³ Until 1980, these vaccines were either inactivated or modified live virus which were used for intramuscular vaccination of pregnant sows and gilts shortly before each farrowing.⁴ An oral vaccine was developed and commercialized by our laboratory in 1980. The particular virus strain originated from the lungs of a clinically normal adult pig, although it was still virulent for piglets. This TGE virus strain was eventually modified by passage through lungs of gnotobiotic piglets, lung cell cultures and swine testicular cell cultures. During *in vitro* passages it was repeatedly exposed to low pH (simulated stomach environment) and proteolytic enzymes (simulated intestinal environment) for increased resistance to these adverse conditions. The resulting TGE vaccinal strain retained its normal morphology and did not cause disease in piglets. Safety and efficacy of this vaccine and its comparison with an intramuscularly administered vaccine has been reported previously.⁵ The principal advantage of the oral vaccine, when administered to pregnant sows, was the subsequent production of higher, more persisting TGE milk antibody levels which resulted in increased protection of TGE experimentally challenged piglets.

In this report we will review the mechanism of immunity to TGE and discuss methods for their activation under various circumstances.

IMMUNITY TO TGE

Before considering effective use of vaccination, we should first review very briefly the basic mechanisms of active and passive immunity to TGE. The virus enters the sow by the oral route and reaches the intestines where viral replication occurs in the villous enterocytes. Malfunction or destruction of these enterocytes results in diarrhea. Viral replication in the sow's intestine induces an active local immunity, sufficient to prevent subsequent viral invasion and enterocyte destruction, and sufficient to induce passive milk immunity (lactogenic) for the sow's nursing piglets. Although the exact sequential mechanism of this milk immunity remains to be proved, it appears likely that viral sensitized immunocytes in the lamina propria of the small intestine, and possibly from other locations, migrate (via lymphatic and/or blood vessels) to the supramammary lymph nodes where they remain and secrete antibodies of the IgA class into the milk. Nursing piglets are then passively protected from enteric disease so long as they continue to nurse at frequent intervals. The weaning process, therefore, terminates this lactogenic immunity for the young pig.

Colostrum and milk of sows may contain TGE antibodies principally associated with 3 immunoglobulin (Ig) classes: IgA, IgG, IgM. The pre-dominating Ig is dependent upon the method of viral sensitization. Colostrum and milk of sows which have been parenterally inoculated (intramuscular, intramammary, intraperitoneal) with either inactivated or live attenuated or virulent TGE virus, contain predominantly IgG milk antibodies. These IgG antibody levels drop sharply (ten-fold or more) within 48 hours after farrowing. On the other hand, naturally or experimentally infected (oral) sows develop predominantly IgA milk antibody which persists at higher levels throughout lactation. It was this fact which led us to concentrate our research attention to the development of an attenuated live oral TGE vaccine which would produce a similar effect.

Weaknesses in the lactogenic type of immunity for piglets are:

1. Any condition (infectious, pyrexia etc.) which interferes with lactation in the sow also interferes with passive immunity.
2. The weaning process terminates passive immunity.
3. In large litters small or weaker pigs may not consume as much milk and therefore gain less passive immunity.
4. Excessive viral contamination levels in the environment may overcome the passive immunity.

In order to overcome these difficulties, it would be desirable to actively immunize nursing piglets and this is possible only by oral inoculation.

BENEFICIAL EFFECTS OF ORAL VACCINATION OF BABY PIGS WITH TGE VACCINE

Previous studies have demonstrated that it is possible to actively immunize 7-day old baby pigs nursing immune dams.⁶ As a result of these studies active immunity in piglets was further investigated, and it was determined that piglets could be orally immunized as early as day of birth and 3-5 days were required for development of active gut immunity. Within 24 hours after oral inoculation TGE vaccinal virus was detected in the tonsils, salivary glands and oropharyngeal and mesenteric lymph nodes. Virus was never detected free in the intestinal contents. In order to determine the minimum quantity of vaccinal virus required to orally immunize piglets the following study was conducted. Twenty TGE seronegative piglets, removed from TGE seronegative sows after colostrum ingestion, were orally inoculated with different quantities of TGE vaccinal virus at one day of

Table 1
MINIMUM TGE VACCINE IMMUNIZING DOSE
IN BABY PIGS

Group	Vacc. Dose*	% Sero- Conversion	Post-Challenge	
			% Morb.	% Mort.
1	None	0	69	50
2	10^7	100	0	0
3	10^6	100	14	0
4	10^5	50	0	0
5	10^4	25	39	0

*TCID₅₀ Vaccine Virus

age and then orally challenged 10 days later with 1,000 pig infectious doses (PiD) of virulent TGE (Table 1). At least 10^6 TCID₅₀ of vaccinal virus was required to elicit 100% seroconversion to TGE virus within 10 days after vaccination although 10^5 TCID₅₀ induced only 50% seroconversion, but complete protection from morbidity (incidence and duration) and mortality due to virulent TGE challenge. Similar post-challenge morbidity and mortality results were observed in either seronegative piglets or TGE seropositive piglets originating from TGE seropositive sows. These observations have particular application under the following circumstances:

1. Oral vaccination of nonscouring baby pigs in a herd with an active outbreak of TGE.
2. Improved efficacy against TGE by vaccination of both pregnant sows and baby pigs; as a result, baby pigs receive double protection, i.e. passive protection from the sow's milk and active protection from the vaccine.
3. Orally vaccinated pigs are less sensitive to sow lactational fluctuations.
4. Orally vaccinated pigs may retain active immunity post-weaning.
5. Recommended procedure for enzootic TGE.

BENEFICIAL EFFECTS OF ORAL VACCINATION OF SOWS AND GILTS

Effective local intestinal immunity in sows and gilts is only possible by oral inoculation. Furthermore, an actively immune dam is less likely to shed virulent virus and will more effectively transfer passive milk immunity to her nursing piglets.⁵ Previous workers have demonstrated that colostrum and milk from sows infected orally with live virulent TGE virus contain IgA as the predominant class of TGE antibodies.⁷ In order to determine the Ig class of TGE antibodies in colostrum and milk of sows orally vaccinated with the Ambico TGE vaccinal strain, 11 TGE seronegative pregnant sows were orally vaccinated (10^7 TCID₅₀ per dose) at about 5 and 3 weeks preparturition and intramuscularly (IM) at about one week preparturition. Three comparable sows were retained as controls. Colostral and milk samples were collected, processed and assayed for TGE neutralizing antibodies (inhibition of cytopathology of TGE virus in cell cultures) and for IgA and IgG specific TGE antibodies (ELISA). No TGE antibodies (neutralizing, IgA or IgG) were detected in colostral or milk samples from nonvaccinated, control sows.

Figure 1: Geometric mean TGE-specific antibody titers in colostrum (Col) and milk (three and seven days postfarrowing) of orally vaccinated sows (n=11).

Figure 1 illustrates the mean TGE neutralizing, IgA and IgG antibody titers in the colostrum and milk of the 11 vaccinated sows. Both neutralizing and IgA specific TGE antibody remained high throughout the 7 day testing period, whereas IgG specific TGE antibody, which was predominant in colostrum, decreased substantially (greater than ten-fold) within 7 days post-farrowing. These data confirmed the presence of predominantly IgA antibody in orally vaccinated sows. In similar studies involving other pregnant sows it was determined that further increases (100-1,000 times) in the oral virus dose resulted in increased levels of milk IgA specific TGE antibody.

In order to determine if both the active and passive mechanisms of immunity for protection of nursing piglets from TGE would be beneficial another study was designed. In these studies both pregnant sows and subsequently their nursing piglets were orally vaccinated and later exposed to virulent TGE virus as previously described.⁸ Those nursing piglets that had both active and passive immunity were protected completely against death, and had reduced morbidity compared with those that had only passive milk immunity (Table 2). Therefore, it was apparent that induction of an active immunity to TGE in piglets receiving passive milk immunity from TGE vaccinated sows was indeed beneficial.

Table 2
Experimental Evaluation of Active and Passive Immunity Following Oral Vaccination with TGE Vaccine

Type of Immunity	No. Pigs	Morbidity Incidence-Duration* (%)	Mortality (%)	Average No. Days Sow Sick
None	42	25	43	5
Passive	10	8	20	0
Passive and Active	43	2	0	0

*Morbidity Incidence-Duration = No. days sick/total no. pig days x 100

BENEFICIAL EFFECTS OF IM VACCINATION OF PREVIOUSLY SENSITIZED SOWS AND GILTS

Since TGE is very often enzootic in nature, many sows will have been previously infected. However, their ability to transfer milk protection from TGE to their nursing piglets decreases from one gestational cycle to the next. Thus, vaccination may more often involve a boosting effect rather than a primary or initial sensitizing effect. Numerous studies conducted by our laboratory have revealed that previously infected sows respond well to only one IM inoculation of TGE vaccine shortly before farrowing. TGE neutralizing titers in serum increase 7-24 times. On the other hand, this increase in TGE antibody level is not observed in similar animals which have been fed TGE infective piglet tissues shortly before farrowing. Other investigators have reported that sows previously infected with TGE, when subsequently inoculated IM shortly before or after farrowing with Ambico TGE vaccine, developed higher TGE milk antibody titers than observed in nonvaccinated previously infected sows. Furthermore, the increased titers were associated with both IgA and IgG. These studies were recently confirmed in our laboratory (Table 3). Ten TGE naturally exposed sows were vaccinated 14 days pre-farrowing; five with a single IM dose of Ambico TGE vaccine, and five with both an oral and an IM dose of vaccine. Nine similar animals were left as unvaccinated controls. Serum and milk TGE neutralizing antibody titers (7 days post-farrowing), as well as IgG and IgA specific TGE antibody levels (ELISA assay) in the milk, were assayed. In all cases antibody levels were increased due to vaccination. Similar increases in IgA and IgG specific antibody levels were also observed for five TGE naturally exposed sows (four similar sows left as unvaccinated controls) which had been vaccinated seven days post-farrowing. These data have particular significance for improved control of enzootic TGE.

Table 3

Effect of TGE Vaccination on TGE Sensitized Sows

Vaccination Before Farrowing:

Fold Increased Geometric Mean Titer 7 Days Post-Farrowing

TGE Antibody Assay	1 x IM	Oral + IM
Serum Neutralization	10.4X	7.9X
Milk Neutralization	2.6X	3.3X
Milk IgG(ELISA)	3.0X	1.5X
Milk IgA(ELISA)	4.6X	4.0X

1 x IM Vaccination Post-Farrowing:

Fold Increased Geometric Mean Titer 21 days Post-Farrowing

Serum Neutralization	7.8X
Milk Neutralization	8.6X
Milk IgG (ELISA)	5.5X
Milk IgA (ELISA)	4.0X

EVALUATION OF SUBUNIT ANTIGENS

The development of TGE subunit antigens for immunization purposes is very appealing; however, there are few published reports on the success of such endeavors. Two approaches for the generation of such subunit antigens are apparent and both approaches have concentrated on the TGE peplomeric glycoprotein (gp 195). The complete gene for gp 195 has been identified and subsequently expressed in vaccinia and E.coli vectors, but the efficacy of resultant subunit antigens in pigs has not been reported.^{9, 10} Other investigators have prepared TGE subunit vaccines by disruption and fractionation of complete TGE virions, and the purified subunit antigens were reported to be effective immunogens against TGE.^{11, 12} Confirmation of their results in our laboratory has not been possible even though similar molecular weight subunits were evaluated. Subunit antigens have induced high TGE antibody responses in the serum and colostrum of vaccinated gilts, but when nursing piglets were exposed to virulent TGE, protection was poor. The predominant antibody in colostrum and milk was IgG associated. Further investigations relating to vaccination routes and correlation of molecular size of the immunogen to protection are in progress.

DISCUSSION

Although much has been learned about immunity to TGE and improved application of TGE vaccines has resulted, certain disadvantages with current vaccines are still apparent. Only modified live virus vaccines have so far been licensed by Veterinary Biologics, U.S.D.A. These vaccines are used only by the parenteral route in pregnant sows or as a combination oral-parenteral route (Ambico strain). In addition, the oral vaccine is also licensed for use in nursing piglets. Oral sensitization of pregnant sows is generally accepted as necessary for induction of lactogenic immunity associated predominantly with IgA type of TGE antibody. The existing TGE vaccines are more effective in animals previously sensitized to TGE, although little is understood regarding the specific nature of this response. Animal immunologic responses to primary sensitization still tends to be more variable. We have observed that a substantial increase in the oral dose of TGE antigen improves this response. Better protection of viral antigens from destruction within the gastrointestinal tract after ingestion may provide improved results. Vaccine is currently administered in a milk base. A better understanding of the mechanism of antigen uptake, processing and subsequent migration of immunocytes to the mammary gland is also needed.

Successful utilization of nonviable TGE antigens, including subunits, has not been generally confirmed. Although parenteral administration of these antigens may induce high levels (e.g. 3,000-5,000) of TGE neutralizing antibody, such antigens have failed to induce satisfactory lactogenic immunity of the IgA type.

Greater emphasis is needed on the preparation and characterization of TGE subunit immunogens from both peplomeric and nonpeplomeric proteins, and a correlation of their molecular size to immune response. Precaution must also be exercised in the selection of viral antigen since cell culture passage of TGE virus can result in considerable loss of peplomers. Application of any TGE immunogen must also take into consideration the unique immunologic mechanisms involved in not only sows, but also in nursing and weanling pigs in order to overcome the disadvantages of lactogenic immunity.

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