

Efficacy of Antiserum Produced in Goats and Pigs to Passively Protect Piglets against Virulent Transmissible Gastroenteritis Virus

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ABSTRACT

The protective effect of sera produced in swine and goats exposed to virulent transmissible gastroenteritis virus (TGEV) or modified-live TGEV was tested in hysterectomy-derived, colostrum-deprived three-day-old pigs. Pigs were given serum with their daily ration of milk, and their immunity to virulent TGEV was determined. The pigs were observed for ten days for clinical signs of TGEV infection. One of nine pigs receiving goat serum was protected whereas all three pigs receiving three doses of swine serum per day were protected. Because virus was not isolated from the goats after oral/intranasal vaccination, it is suggested the virus did not replicate in either the respiratory or digestive tract of the goat.

RÉSUMÉ

Des sérums immuns provenant de porc et de chèvres exposés à une souche virulente du virus de la gastro-entérite transmissible (VGET) ou à une souche vivante modifiée de ce même virus ont servi à vérifier leur effet protecteur chez des porcelets de trois jours obtenus par césarienne et n'ayant pas reçu de colostrum. Les sérums immuns ont été mélangés au lait et par la suite la résistance des porcelets à l'infection a été évaluée avec la souche virulente du VGET pendant une période de dix jours. Un seul des porcelets ayant reçu du sérum immun de chèvre a survécu à l'infection tandis que tous les porcelets ayant reçu du sérum immun porcien

ont survécu, et ce, indépendamment de la dose de sérum reçu. Le virus de la CET n'ayant pas été isolé des chèvres suite à la vaccination orale ou intranasale, les auteurs suggèrent qu'il n'y aurait pas eu de réplication virale dans leur tractus digestif ou respiratoire. (*Traduit par Dr Pascal Dubreuil*)

A goal of veterinary virologists has been to develop a vaccine that could be given to newborn pigs without causing disease and yet when given to pregnant sows, it would infect the gut resulting in a stimulation of the gut-mammary immunological system (1). Vaccines (2,3) tested for the control of transmissible gastroenteritis virus (TGEV) infection include virulent, heterologous, modified-live, killed, and subunit products administered by various routes [intranasal (IN), orally (O), intramuscular (IM), intramammary (IMm), or enteric coated]. After identification of the causative agent as a virus, it was found that feeding intestinal homogenates from baby pigs dying of TGEV infection to pregnant sows 3 wk before they farrowed would elicit an immune response, and the sows would protect their nursing pigs against TGEV (4). This is the only consistently reproducible method able to induce a protective antibody response in exposed animals (3). The use of virulent virus as a vaccine has several disadvantages: TGEV may spread to adjacent susceptible herds, the vaccine could contain other swine pathogens, an enzootic or chronic type of TGEV infection would be established, the vaccine cannot be given to baby pigs,

and exposed animals could not be sold for export.

Serum from swine (2), colostrum from a cow (5), and TGEV neutralizing monoclonal antibodies (6) have been tested as passive methods to control the disease. Reports have varied on the efficacy of these methods, but swine serum has been the most effective and monoclonal antibodies have been the least effective (1,6).

The purpose of this report is to present information on the use of serum produced in goats and swine against virulent or modified-live TGEV for providing passive protection for baby pigs against TGEV. The study followed the guidelines of the Guide to the Care and Use of Experimental Animals of the Canadian Council on Animal Care.

The McClurkin swine testicular (ST) cell line was cultured as reported (7). Virus plaque (PFU) and 50% plaque reduction serum neutralization (SN) titers were determined on 12×75 mm² petri dishes of confluent ST cells as previously described (6).

The history of the virulent pig-passaged TGEV has been reported (5). The virus had a titer of 3.6×10^6 PFU/mL on ST cells. The commercial modified-live TGEV vaccine (Ambico Pro System 1, Ambico Inc., Dallas Center, Iowa) had a titer of 6.3×10^6 PFU/mL on ST cells.

Three goats, serologically negative for TGEV SN-antibodies, were obtained from a herd maintained at the National Animal Disease Center. The goats were housed in individual isolation rooms and fed a commercial protein diet, alfalfa cubes, and water

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ad libitum. A pool of normal control sera was collected from the goats before they were exposed to TGEV. Goat 1 was vaccinated with seven 2 mL doses of virulent TGEV over a two-month period; the first and last doses were given IM (2 mL) and five doses were given O (1 mL per os)/IN (0.5 mL per naris). Goat 2 was vaccinated with four 2 mL doses of virulent TGEV over a two-month period; the first and last doses were given IM and the second and third doses were given O/IN. Goat 3 received seven 2 mL doses (2 IM and 5 O/IN) of a modified-live TGEV over a two-month period. For IM inoculations, the virus was added to an equal volume of Freund's incomplete adjuvant and mixed until a water in oil emulsion was achieved, and injected at four sites (0.5 mL/site). Serum samples were collected from the goats at the time of each vaccination, and a large pool was collected from each goat 2 wk after the last IM injection. Serum was separated and stored frozen at -20°C . The goats did not develop clinical signs of infection. Transmissible gastroenteritis virus was not isolated from nasal or rectal swabs collected 48 to 120 h from any goat after O/IN exposure. Serum neutralization antibodies were detected in the goats 2 wk after the first IM vaccination, and the SN titer remained constant or declined through the O/IN exposures. An anamnestic response was detected after the last IM vaccination. The final 50% TGEV SN-antibody titers were: goat 1, 1:128; goat 2, 1:128; and goat 3, 1:256.

The history of the swine serum used in this study has been previously reported (6). This serum had a SN titer of 1:1280 against virulent TGEV, and when given in three 4 mL doses per day to susceptible pigs, it protected them against virulent TGEV.

Sixteen hysterectomy-derived, colostrum-deprived pigs were obtained from sows that were negative for TGEV SN-antibodies. Baby pigs were housed in individual isolators in a room maintained at 35°C . The isolators were equipped with HEPA air filters and kept under slightly negative air pressure to minimize aerosol contamination. The pigs were fed a daily ration of a sows' milk replacer (SPF-lac, Borden Inc., Norfolk, Virginia) administered in three feedings (7:00 a.m.,

TABLE I. Response to challenge with virulent TGEV of three-day-old pigs which had ingested swine or goat serum

Experimental group	Morbidity No./total	Mortality No./total
1 Swine serum (1 dose)	1/1 ^a	1/1 ^b
2 Swine serum (3 \times /day)	0/3	0/3
3 Goat 1 (7 doses virulent virus)	3/3	3/3
4 Goat 2 (4 doses virulent virus)	3/3	2/3
5 Goat 3 (modified-live virus)	3/3	3/3
6 Normal goat serum	3/3	3/3

^aNumber of pigs sick/number of pigs per group

^bNumber of pigs that died over number of pigs challenged

12:00 noon and 4:00 p.m.). Initially the pigs received 60 mL per feeding that was gradually increased to 120 mL by ten days postchallenge (PC).

At three days of age, the pigs were randomly divided into six groups, each containing three pigs except group 1 that contained one pig. One pig (group 1) received a single 1 mL dose of swine serum, group 2 pigs were given 1 mL of swine serum per feeding, group 3 pigs were given 20 mL of serum from goat 1 per feeding, group 4 pigs were given 20 mL of serum from goat 2 per feeding, group 5 pigs were given 20 mL of serum from goat 3 per feeding and group 6 pigs were given 20 mL of control goat serum per feeding. Each pig, except the single dose pig in group 1, was given an initial dose of serum in the milk at the 7:00 a.m. feeding, and at noon all 16 pigs were given, via stomach tube, a mixture of swine or goat serum and 1000 pig lethal doses of TGEV that had been incubated at 37°C for 1 h. Serum was added to each subsequent feeding of milk for the next ten days, except the pig in group 1. The pigs were observed three times per day for clinical signs of TGEV infection.

The pig that received a single dose of swine serum developed clinical signs of TGEV (vomiting, diarrhea, rough hair coat, loss of weight, drinking water, listless) within 48 h after challenge and died four days later (Table I).

Pigs in group 2 (3 mL swine serum per day) were protected against TGEV as they remained clinically normal. Serum samples collected from these pigs 28 days PC were negative for TGEV SN-antibodies.

Pigs in groups 3 and 4 (60 mL of serum per day from virulent TGEV exposed goats) developed clinical signs of TGEV infection within 48 h and five of the six pigs were dead by eight

days PC. One pig in group 4 survived challenge, although it was very ill during the observation period and developed SN-antibodies against TGEV suggesting that the pig's immune system was exposed to virus. Pigs in group 5 (60 mL of serum per day from modified-live virus exposed goat) developed clinical signs of TGEV infection within 48 h and were dead by six days PC. Thus, although the goat serum could neutralize TGEV *in vitro*, it was unable to protect pigs against challenge.

Pigs in group 6 (60 mL per day of normal goat serum) developed clinical signs of a TGEV infection within 30 h and were dead by six days PC.

This study confirms previous reports that serum from swine exposed to virulent TGEV will passively protect pigs against challenge (6,9,10). In a previous study, three 4 mL doses of swine serum per day protected pigs against virulent TGEV, and in this study, three 1 mL doses provided the same level of protection. While the minimum quantity of swine serum necessary to protect a pig against virulent challenge was not determined, this study proved that multiple doses are necessary. Serum samples from group 2 pigs 28 days PC were negative for TGEV SN-antibodies suggesting that the pigs' immune systems were not exposed to TGEV antigen. Because the group 1 pig died of TGEV infection, we believe that the virus given to group 2 pigs was infectious but was neutralized *in vivo* by the swine serum.

Colostrum from a cow vaccinated IMm with virulent TGEV passively protected baby pigs against a virulent challenge (5). Because the cow is not considered a host animal for TGEV, the presence of protective antibodies in the colostrum does not support the theory that replicating virus is neces-

sary to induce protective immune responses (1). If protective antibodies can be produced in the cow, then it may be possible to produce them in another ruminant. Goat serum was selected for use because it can be regularly collected, and goats are routinely used to produce high quality diagnostic reagents. While SN-antibodies for TGEV were produced in goat serum, the serum did not passively protect pigs against a virulent challenge. Factors other than host-specificity could account for the differences in protection observed between colostrum from the cow and serum derived from goats. The goat's pharyngonasal area may lack receptors for the O/IN administered virus or the virus was destroyed in the rumen before it reached the intestinal tract. The route of vaccination also may have played a role since the cow was vaccinated IM and the goats were vaccinated IM and O/IN. An IM inoculation is more likely to elicit an IgA response than an IM inoculation (8,13). In piglets, the IgA type of antibody has been reported to be more protective against enteric infections than the IgG type of antibody (14).

Serum antibodies from swine exposed to virulent TGEV protected pigs against challenge, while serum from swine vaccinated with inactivated virulent virus did not protect pigs (3). The protective antigen may be related to a virulence

factor. The failure of inactivated virulent and modified-live virus vaccines to elicit protective antibodies can be explained as follows. While the inactivated virus contains the appropriate antigen, it does not replicate, and while the modified-live virus replicates, it does not contain the protective antigen or virulence factor. Thus, finding a safe and effective vaccine for the control of TGEV remains a very difficult task.

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