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Short Communication

SERUM ANTIBODY RESPONSES OF NEONATAL AND YOUNG ADULT PIGS TO TRANSMISSIBLE GASTROENTERITIS CORONAVIRUS

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ABSTRACT

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Serum titers of virus-neutralizing (VN) antibody were 10 to 16 times higher in neonatal pigs than in young adult pigs, after single oral doses of virulent transmissible gastroenteritis virus (TGEV). To determine the reason for this higher response, sera from neonatal and young adult pigs, 18 to 21 days after exposure to TGEV, were collected and assayed for VN antibody by plaque reduction. In addition, sera of VN-positive and VN-negative neonatal pigs were analyzed for immunoglobulin classes by radial immunodiffusion technique.

The competence of neonatal pigs to produce VN antibody with increased IgG levels was demonstrated. The higher antibody response seen in neonatal pigs, when compared to sera of young adult pigs, may be attributed to the increased replication of TGEV in the intestinal tracts of neonatal pigs or to the lack of other immunogens that may interfere or compete with the production of specific antibody.

INTRODUCTION

In a previous study (Stone et al., 1977), we observed that transmissible gastroenteritis virus (TGEV) neutralization serum titers of neonatal pigs that survived the viral infection greatly exceeded those of adults given a comparable oral dose of virus.

There is some conflict as to the immunological competence of pigs at birth (Bourne et al., 1974; Kim et al., 1967; Sterzl et al., 1965). It is generally accepted: (a) that maternal antibodies are not transferred across the placenta in swine, (b) neonatal pigs are aglobulinemic at birth, and (c) after colostrum feeding, colostral globulins will transfer to the serum.

In this study, we investigated antibody responses of neonatal and young adult pigs to TGE viral antigens to determine if the higher VN titer is due to a specific antibody present in the sera of colostrum-fed pigs.

MATERIALS AND METHODS

Pigs

Eight hysterectomy derived, colostrum deprived pigs; 66 colostrum-fed pigs; and 51 young adult pigs were used in this study. All pigs were from herds known to be free of TGE. The colostrum deprived pigs were fed a commercial bovine milk formula and kept in individual isolators equipped with air filters. The neonatal pigs were infected with TGEV at 3 to 4 days of age. The young adults were infected at 6 to 8 months of age. All pigs were bled for serum 18 to 21 days after inoculation and the serum samples were kept at -20 C and heated to 56 C for 30 minutes before use.

Virus

The virulent Miller-3 strain of TGEV was given orally to infect all pigs (Stone et al., 1977).

Virus neutralization

The 8th serial passaged cell culture TGEV was diluted to contain about 100 plaque-forming units per 0.1 ml in virus neutralization (VN) tests (Kemeny, 1976). Cultures of a continuous line of swine testicular cells grown in plastic plates were inoculated with 0.2 ml of the serum-virus mixtures and allowed to adsorb for 60 minutes at 37 C. The inoculated cell cultures were overlaid with agar and examined for plaques after incubation for 48 hours at 37 C in an atmosphere of 5% CO₂. Neutralization endpoints were calculated as the highest dilution of serum needed for a 50% reduction in the number of plaques.

Protein and immunoglobulin determinations

Total protein was determined refractometrically. Concentrations of individual immunoglobulins were estimated by the radial immunodiffusion technique (Mancini et al., 1965) using heavy chain specific rabbit antiporcine IgA, IgG, and IgM.

RESULTS

Antibody response

Neonatal pigs and young adult pigs developed measurable VN antibody 18 to 21 days after exposure to virulent TGEV. The geometric mean VN titer for the neonatal pigs was 10 to 16 times higher than that of young adults (TABLE I).

TABLE I

Antibody response of neonatal and young adult swine after oral inoculation with transmissible gastroenteritis virus

No. in	Pigs	Virus neutralization serum titer ^a		
group		Geometric mean	Range	
8	Colostrum-deprived	1:1216	1:300-1:2000	
63	Colostrum-fed	1:800	1:100-1:1400	
51	Young adults	1:74	1:16-1:512	

^aHighest dilution of serum needed for a 50% reduction in the number of plaques.

Protein and immunoglobulin levels

The results of total protein and immunoglobulin determinations in sera from 6 colostrum-fed pigs are presented in TABLE II.

TABLE II

Virus neutralization (VN) titers, total serum protein values and immunoglobulin concentrations in sera of six colostrum-fed neonatal pigs^a

Pig no.	vn ^b	Total protein (g/100 ml)	Ig mg/ml		
			IgA	IgG	IgM
1	Negative	6.0	1.0	13.4	3.0
2	Negative	5.8	1.2	13.4	3.0
3	Negative	5.8	1.2	10.4	3.8
4	1:256	6.0	1.5	23.2	1.9
5	1:512	5.7	1.4	24.8	0.7
6	1:256	5.8	1.7	26.4	2.4
Average of	6 neonates	5.85	1.3	18.6	2.5
Average of	12 adults	7.15	NT ^C	NT	NT

^aAll pigs received colostrum on day 1, and were removed from the sow on day 2. Pigs numbered 4, 5, and 6 were orally administered transmissible gastroenteritis virus on day 3.

 $^{\mathrm{b}}$ Highest dilution of serum needed for a 50% reduction in number of plaques. $^{\mathrm{c}}$ Not tested.

The mean value for total protein in the 6 neonatal sera was lower than for the mean value of 12 young adult pigs. An examination of the total protein or IgA contents in sera from 3 VN-negative and 3 VN-positive colostrum-fed pigs revealed little difference. However, there was a marked difference in the concentrations of IgG and IgM in these sera. The IgG values in the VN-positive sera were about twice the concentration of the VN-negative sera. In contrast, the IgM values were higher in the VN-negative sera.

DISCUSSION

The data clearly show the competence of the neonatal pig to produce high levels of VN antibody. Others have reported the immunological competency of the porcine fetus to <u>Salmonella</u> paratyphi B (Sterzl et al., 1965), to parvovirus (Mengeling and Cutlip, 1976), and to sheep red blood cells (Schultz et al., 1971). The differences seen between the IgG and IgM levels of the VN-negative and VN-positive pigs (TABLE II) may represent a recent immune response of the VN-negative pigs to organisms in the environment, or the IgG in these pigs may be residual from the ingested colostrum. The colostral contribution of IgM would be low as the calculated mean half-life of IgM is 4.8 days, whereas the half-life of IgG is 13.8 days (Curtis and Bourne, 1973).

Moon et al., 1975, demonstrated that 3-day-old pigs are more susceptible to TGEV infection and produce more virus in their intestines than older pigs. The increased replication of the virus in the intestinal tracts of neonatal pigs may provide a greater antigenic mass and may have caused a greater antibody response in neonatal pigs. Alternatively, the neonatal pigs may have had fewer other (extraneous) antigens to interfere with the formation of specific antibody.

REFERENCES

- Bourne, F. J., Curtis, J., Johnson, R. H. and Collings, D. F., 1974. Antibody formation in porcine fetuses. Res. Vet. Sci., 16:223-227.
- Curtis, J. and Bourne, F. J., 1973. Half-lives of immunoglobulins IgG, IgA, and IgM in the serum of newborn pigs. Immunology, 24:147-155.
- Kemeny, L. J., 1976. Antibody response in pigs inoculated with transmissible gastroenteritis virus and cross-reactions among ten isolates. Can. J. Comp. Med., 40:209-214.
- Kim, Y. B., Bradley, S. G. and Watson, D., 1967. Ontogeny of the immune response. III. Characterization of components in germ-free colostrum-deprived piglets. J. Immunol., 98:868-873.
- Mancini, G., Carbonara, A. O. and Heremans, J. F., 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry, 2:235-254.
- Mengeling, W. L. and Cutlip, R. C., 1976. Reproductive disease experimentally induced by exposing pregnant gilts to porcine parvovirus. Am. J. Vet. Res., 37:1393-1400.

Moon, W. H., Kemeny, L. J., Lambert, G., Stark, S. L. and Booth, G. D., 1975. Age-dependent resistance to transmissible gastroenteritis of swine. III. Effects of epithelial cell kinetics on coronavirus production and on atrophy of intestinal villi. Vet. Pathol., 12:434-445.

Schultz, R. D., Wang, J. T. and Dunne, H. W., 1971. Development of humoral immune response of the pig. Am. J. Vet. Res. 32:1331-1336.

- Sterzl, J., Mandel, L., Miler, I. and Riha, I., 1965. Development of immune reactions in the absence or presence of an antigenic stimulus. In: J. Sterzl, P. Hahn and J. Rudinger (Editors), Molecular and Cellular Basis of Antibody Formation. Academic Press, New York, pp. 351-370.
- Stone, S. S., Kemeny, L. J., Woods, R. D. and Jensen, M. T., 1977. Efficacy of isolated colostral IgA, IgG, and IgM(A) to protect neonatal pigs against the coronavirus of transmissible gastroenteritis. Am. J. Vet. Res., 38:1285-1288.