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Detection of Antirotavirus Immunoglobulins A, G, and M in Swine Colostrum, Milk, and Feces by Enzyme-Linked Immunosorbent Assay

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An enzyme-linked immunosorbent assay was developed to allow direct detection of class-specific antirotavirus antibodies. In colostrum and in milk, antirotavirus antibodies were found in the three immunoglobulin classes. Antirotavirus immunoglobulins G and M were predominant in colostrum, whereas antirotavirus immunoglobulin A was predominant in milk and feces.

Rotavirus and transmissible gastroenteritis virus were shown to induce diarrhea in pigs (for a review, see reference 4). The immune response against transmissible gastroenteritis virus was extensively described (6, 7, 12, 19), and the importance of immunoglobulin classes sharing function against this virus was demonstrated (5, 7, 21, 22). Concerning rotavirus infections, the relative role of immunoglobulin classes in protection of piglets has not been published yet. It has been shown that the enzyme-linked immunosorbent assay (ELISA) has the ability to determine class-specific antibodies (15-18, 23).

In the present work we report the development of an ELISA which allows the direct detection of class-specific antirotavirus antibodies. This assay was used for quantitative assay of antibodies in pig colostrum, milk, and feces.

Pools of sow colostrum and milk samples were lyophilized and reconstituted with distilled water before use. Feces from healthy pigs were collected during the winter of 1979-1980 in a conventional pig farm where no clinical signs of diarrhea were observed in pigs at any age. Nevertheless, 90% of these animals had serum antibodies against rotavirus. Fecal samples from 20 pigs with diarrhea were collected in 20 different farms in Brittany. All fecal samples were diluted 1:10 in phosphate-buffered saline containing 0.05% Tween 20 and 0.02% sodium azide (PTSA) and clarified. Calf rotavirus (single-shelled particles) was extracted from feces of diarrheic calves and purified as previously described (11).

Pure pig immunoglobulin and specific antisera were prepared according to previous reports (8, 14; J. Franz and G. Corthier, Clin. Exp. Immunol., in press).

The anti-rabbit immunoglobulin antibodies were prepared by hyperimmunization of a pig, using pure rabbit immunoglobulin G (IgG) in Freund complete adjuvant. The antisera were adsorbed onto rabbit immunoglobulin aggregated with glutaraldehyde (3). Anti-rabbit antibodies were eluted with 0.1 M glycine hydrochloride buffer (pH 2.8), and then 1 M tris(hydroxymethyl)aminomethane was added to adjust the pH to 8.0.

Coupling of the antibodies or immunoglobulin with alkaline phosphatase was carried out as described by Avrameas (2). These conjugates were used at concentrations of 5 μg/ml. Immunoglobulin quantitation was performed by competitive ELISA (16).

Detection of class-specific rotaviral antibodies was performed as follows: microplate wells (Linbro polystyrene plates) were treated with 1% glutaraldehyde (1 h at 4°C), washed thoroughly with water, and coated with 100 μl of purified calf rotavirus suspension (2 μg/ml) in 0.05 M carbonate buffer (pH 9.6). The plates were incubated for 3 h at 37°C and then rinsed three times with PTSA. Samples to be analyzed were diluted threefold serially in PTSA, and 100 μl of each dilution was added to each well. After an overnight incubation at room temperature, the plates were washed three times, and 100 μl of rabbit anti-pig IgA, IgG, or IgM heavy chains was added. A further incubation period of 3 h at 37°C was followed by a threefold wash and addition of the pig anti-rabbit conjugate. The plates were incubated for 3 h at 37°C, rinsed four times, and then reacted with the substrate solution (*p*-nitrophenyl phosphate [1 mg/ml] in 0.1 M diethanolamine buffer [pH 9.8] for 1 h at 37°C). Each plate contained serial dilutions of a standard positive serum (pig hyperimmunized with pig rotavirus, OSU strain) and a phosphate-buffered saline control.

Absorbance of the content of the wells was measured at 405 nm in a Titertek Multiskan. The results of ELISA were expressed as an

endpoint titration: the highest sample dilution that yielded an optimal density of 0.2. The specific activity was expressed by the logarithm of the reciprocal of the dilution divided by the concentration of immunoglobulin (milligrams per milliliter) in the test sample.

The specificity of the rabbit anti-IgA, IgG, and IgM heavy chains was determined using antirotavirus IgA, IgG, and IgM extracted from serum or milk collected from naturally infected adult pigs. When antirotavirus IgG was used in the test, no reaction could be detected with anti-IgA or anti-IgM heavy chains, whereas absorbance with anti-IgG heavy chain antiserum was very high. Similar results were observed with antirotavirus IgA or IgM. Every anti-immunoglobulin serum was specific for the relevant immunoglobulin (data not shown).

In colostrum, the antirotavirus IgG (aRIgG) titer was higher than the aRIgM titer ($P < 0.01$) or aRIgA titer ($P < 0.05$) (Fig. 1). However, it must be taken into account that IgG was the predominant class in colostrum. Thus the specific activity of aRIgG, aRIgM, or aRIgA appeared not significantly different (Fig. 1).

In milk, the most important antirotavirus activity was found in the IgA class, significantly different from the IgM and IgG classes ($P < 0.01$; Fig. 2). In lacteal secretions IgA was predominant, but the specific activity of aRIgA was higher than that of aRIgG ($P < 0.01$). When milk and colostrum aRIgA were compared, titer and specific activity were not significantly different.

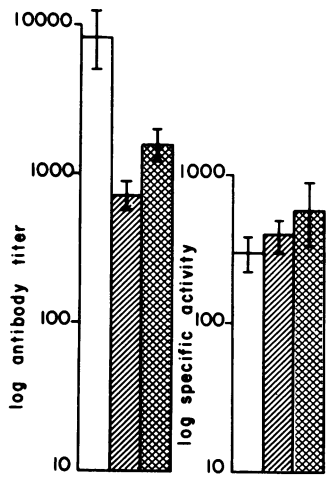

FIG. 1. *Class-specific antirotavirus activity in pooled colostrum, expressed as antibody titer (left) or specific activity (titer divided by immunoglobulin concentration; right). Bars indicate standard error of the mean. Antisera used: □, anti-IgG heavy chain; ▨, anti-IgM heavy chain; ▩, anti-IgA heavy chain.*

A total of 53 fecal samples were also analyzed. Of these, only 17 specimens had antirotavirus antibodies: in the healthy pig group, 6 of 15 suckling piglets, 3 of 16 weaned pigs, and 1 of 9 adults; in the diarrheic pig group, 4 of 9 piglets and 3 of 14 adults.

As shown in Fig. 3, in the healthy animal

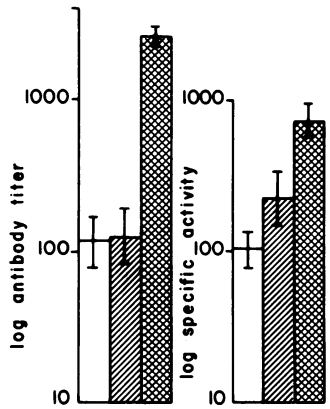

FIG. 2. *Class-specific antirotavirus activity in pooled milk, expressed as antibody titer (left) or specific activity (right). Bars indicate standard error of the mean. Antisera used: □, anti-IgG heavy chain; ▨, anti-IgM heavy chain; ▩, anti-IgA heavy chain.*

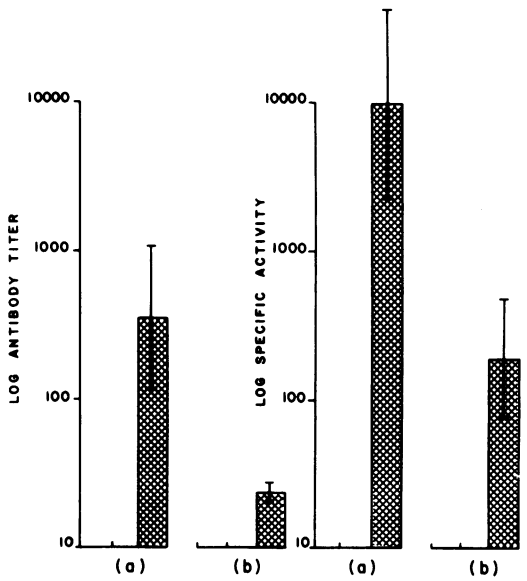

FIG. 3. *Class-specific antirotavirus activity in feces of healthy pigs, expressed as antibody titer (left) or specific activity (right). (a) Feces from suckling piglets; (b) feces from weaned and adult pigs. Bars indicate standard error of the mean. Anti IgA heavy-chain antiserum was used in the ELISA. Reactions with anti-IgG and IgM heavy-chain antisera were negative (little lines next to the bars).*

group, the aRIgA titer or specific activity was higher in suckling piglets than in weaned or adult pigs ($P < 0.02$). The absence of aRIgG may be explained by the fact that small amounts of IgG were found in feces (Franz and Corthier, in press). The same argument was not valid in the case of aRIgM, since IgM was found to be predominant in the feces of suckling piglets (65%; Franz and Corthier, in press).

In feces collected from pigs with diarrhea (Fig. 4), aRIgA, aRIgG, and aRIgM were collected. The concentration of immunoglobulin in these diarrheic feces was significantly higher ($P < 0.02$) than in normal feces. Antirotavirus antibodies from the IgA class remained predominant, aRIgA titer being higher than aRIgG titer ($P < 0.05$). The specific activities of aRIgA were higher than those of aRIgM or aRIgG ($P < 0.05$).

Our class-specific antirotavirus ELISA procedure appeared to be an efficient technique for detecting antirotavirus antibodies in colostrum, milk, or feces. However, antibodies having high and low affinity may not be detected equally by ELISA (1, 10).

We have to mention that the present results are related to group-specific antigens of rotavirus, since purified single-shelled calf rotavirus particles were used in the assay.

All of the pooled colostrum and milk samples that were analyzed in this work contained antibodies against rotavirus; this is consistent with the fact that in France 90% of adult pigs were shown to be seropositive for rotavirus (13).

With respect to the pig intestinal immune response against transmissible gastroenteritis virus, it was previously shown that IgA is the predominant class sharing antibody functions as a consequence of immunity following natural infection (for review, see reference 20). In suckling piglets, antibody function was also shared by IgA. In this case, however, the antibodies may originate either from milk or intestinal mucosa or both. Nevertheless, the results we obtained in weaned or adult pigs strongly suggest that antirotavirus activity is essentially due to IgA.

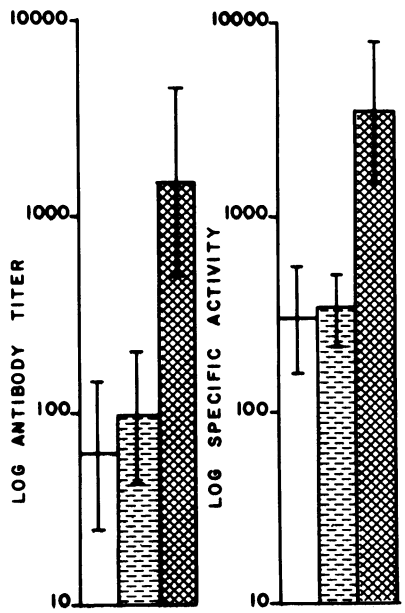

FIG. 4. *Class-specific antirotavirus activity in feces of pigs with diarrhea, expressed as antibody titer (left) or specific activity (right). Bars indicate standard error of the mean. Antisera used: □, anti-IgG heavy chain; ▩, anti-IgM heavy chain; ■, anti-IgA heavy chain.*

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LITERATURE CITED

1. **Ahlstedt, S., J. Holmgren, and L. A. Hanson.** 1974. Protective capacity of antibodies against *E. coli* O antigen with special reference to the avidity. Int. Arch. Allergy **46**:470–480.
2. **Avrameas, S.** 1969. Coupling of enzymes to proteins with glutaraldehyde: use of the conjugates for detection of antibodies. Immunochemistry **6**:43–52.
3. **Avrameas, S., and T. Ternynck.** 1969. The cross-linking of protein with glutaraldehyde and its use for preparation of immunoadsorbents. Immunochemistry **6**:53–66.
4. **Bohl, E. H.** 1975. Viral disease, p. 168–188. *In* H. W. Dunne (ed.), Diseases of swine, 4th ed. The Iowa State University Press, Ames, Iowa.
5. **Bohl, E. H., R. K. P. Gupta, L. W. Closkey, and L. Saif.** 1972. Immunology of transmissible gastroenteritis. J. Am. Vet. Med. Assoc. **160**:543–549.
6. **Bohl, E. H., R. K. P. Gupta, M. U. F. Olquin, and L. J. Saif.** 1972. Antibody response in serum, colostrum, and milk of swine after infection or vaccination with transmissible gastroenteritis virus. Infect. Immun. **6**:289–301.
7. **Bohl, E. H., and L. J. Saif.** 1975. Passive immunity in transmissible gastroenteritis of swine: immunoglobulin characteristics of antibodies in milk after inoculating virus by different routes. Infect. Immun. **11**:23–32.
8. **Bourne, F. J.** 1969. IgA immunoglobulin from porcine milk. Biochim. Biophys. Acta **181**:485–487.
9. **Bourne, F. J.** 1973. The immunoglobulin system of the suckling pig. Proc. Nutr. Soc. **32**:205–215.
10. **Butler, J. E., T. L. Feldbush, P. L. Mc Givern, and N. Stewart.** 1978. The enzyme-linked immunosorbent assay (ELISA): a measure of antibody concentration or affinity. Immunochemistry **15**:131–136.
11. **Cohen, J.** 1977. Ribonucleic acid polymerase activity associated with purified calf rotavirus. J. Gen. Virol. **36**: 395–402.
12. **Corbel, M. J., M. Lucas, and S. F. Cartwright.** 1972. Immunoglobulin class in relation to neutralization of transmissible gastro-enteritis virus. Vet. Rec. **90**:658–659.
13. **Corthier, G., J. F. Vautherot, and P. Vannier.** 1979. Mise en évidence sérologique d'infections à rotavirus au sein de l'élevage porcin français (région de Bretagne).

Ann. Rech. Vet. **10**:65–69.

14. **Curtis, J., and F. J. Bourne.** 1971. Immunoglobulin quantitation in sow serum, colostrum and milk and in the serum of young pigs. Biochim. Biophys. Acta **236**: 319–332.
15. **Dittmar, D., T. J. Cleary, and A. Castro.** 1979. Immunoglobulin G- and M-specific enzyme-linked immunosorbent assay for detection of dengue antibodies. J. Clin. Microbiol. **9**:498–502.
16. **Holmgren, J., and A. M. Svennerholm.** 1973. Enzyme-linked immunosorbent assay for cholera serology. Infect. Immun. **7**:759–763.
17. **Keren, D. F.** 1979. Enzyme-linked immunosorbent assay for immunoglobulin G and immunoglobulin A antibodies to *Shigella flexneri* antigens. Infect. Immun. **24**: 441–448.
18. **Locarnini, S. A., A. G. Coulepis, A. M. Stratton, J. Kaldor, and I. D. Gust.** 1979. Solid-phase enzyme-linked immunosorbent assay for detection of hepatitis A-specific immunoglobulin M. J. Clin. Microbiol. **9**:459–465.
19. **Ristig, M., and M. H. Abou-Youssef.** 1972. Comments on the immunology of transmissible gastroenteritis. J. Am. Vet. Med. Assoc. **160**:549–553.
20. **Saif, L. J., and E. H. Bohl.** 1979. Role of secretory IgA in passive immunity of swine to enteric viral infections, p. 237–255. *In* P. L. Ogra (ed.), Immunology of breast milk.
21. **Sprino, P. J., A. Morilla, and M. Ristic.** 1976. Intestinal immune response of feeder pigs to infection with transmissible gastroenteritis virus. Am. J. Vet. Res. **37**:171–175.
22. **Stone, S. S., L. S. Kemeny, R. D. Woods, and M. T. Jensen.** 1977. Efficacy of isolated colostral IgA, IgG or IgM to protect neonatal pigs against the coronavirus of transmissible gastroenteritis. Am. J. Vet. Res. **38**:1285–1288.
23. **Yolken, R. H., R. G. Wyatt, H. W. Kim, A. Z. Kapikian, and R. M. Chanock.** 1978. Immunological response to infection with human reovirus-like agent: measurement of anti-human reovirus-like agent immunoglobulin G and M levels by the method of enzyme-linked immunosorbent assay. Infect. Immun. **19**:540–546.