Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Antibody against adult diarrhoea rotavirus among healthy adult population in China

Fu-Xi Qiu (Fu-Iisi Ch'iu), Ye Tian, Ji-Chang Liu (Chi-Ch'ang Liu), Xin-Sheng Zhang and Yan-Peng Hao

Institute of Epidemiology and Microbiology, Chinese Academy of Preventive Medicine, P. O. Box 5, Changping, Beijing, People's Republic of China and Medical Institute, Baimamiao, Kunming, Yunnan Province, People's Republic of China

(Accepted 29 April 1986)

This paper deals with a method of using unknown serum as the first antibody to coat wells of the microtiter plate directly in application of the sandwich ELISA method with double antibodies for the assay of the antibody against adult diarrhoea rotavirus. This method was used for assay of the antibody against adult rotavirus in 1,380 sera of healthy adults obtained from some provinces and cities of China, of which 141 showed a positive result with a total positive rate of 10.2%. The infection rate varied in different regions. In order to confirm the accuracy of these results a blocking test was carried out on 25 positive specimens, and the results revealed that the P/N ratios after blocking were all lower than those before blocking. Seventeen of these 25 positive serum specimens were obtained from rural areas of Qian'an County, Hebei Province where epidemic adult diarrhoea had occurred two years ago. The antibody against adult diarrhoea rotavirus was not detected in 50 adult sera from Xizang Autonomous Region. Only one out of 50 adult sera from Hainan Island was positive for the antibody.

Introduction

The method of enzyme-linked immunosorbent assay (ELISA) for the detection of rotavirus antigen has been widely applied (Yolken and Leister, 1981; Rodak et al., 1982; Wall et al., 1982; Inouye et al., 1984). However, a method of using unknown serum to coat the wells of the microtiter plate directly in using the sandwich ELISA method with double antibodies for the assay of the antibody against rotavirus in unknown serum has not been published. Our recent use of this method was successful in the assay of antibody against adult diarrhoea rotavirus in a total of 1,380 sera of healthy adults obtained from some provinces and cities of China. The method and our results are reported as follows.
Materials and Methods

Materials

Antigen. Fecal fluid was taken from a case of epidemic adult diarrhoea containing a large quantity of rotavirus particles, as shown by electron microscopy. A 50% suspension was made with 0.01 M phosphate buffered saline (PBS), pH 7.2, and centrifuged at 8,000 rpm for 10 min. The supernatant fluid was mixed with an equal volume of chloroform and centrifuged at 8,000 rpm for 30 min. The supernatant fluid was aspirated out and centrifuged at a superspeed of 51,000 rpm for 2 h. A small amount of PBS was used to resuspend the precipitate. Density gradient centrifugation was carried out by layering the suspension on 45% sucrose, 60% sucrose and 40% cesium chloride and centrifuging at a superspeed of 40,000 rpm for 2 h. The cesium chloride portion in the lower layer was collected and taken as the purified adult rotavirus antigen after dialysis against PBS. Electron microscopic observation showed complete rotavirus particles with typical structure together with a large number of degraded particles. Its content of virus particles was $10^6$–$10^7$/ml. RNA genome electrophoresis revealed the characteristic pattern of 11 segments of adult diarrhoea rotavirus RNA genome (Qiu et al., 1985).

Sera. (1) Rabbit anti-adult diarrhoea rotavirus immune serum. The above purified rotavirus antigen was injected subcutaneously and intramuscularly into rabbits of 2,000 g body wt three times at 2–3 week intervals. An equal volume of Freund’s complete adjuvant mixed with the virus antigen was used in the first injection, an equal volume of Freund’s incomplete adjuvant in the second injection and pure virus antigen for the third injection. A total of 4.5 ml of virus antigen was used for immunizing each rabbit. The rabbits were bled two weeks after the last injection. The titer of antibody determined by immunodiffusion test was 1:16 and the content of IgG was 17.5 mg/ml.

(2) Guinea-pig anti-adult diarrhoea rotavirus immune serum. The same method of immunization was carried out in guinea-pigs of 300 g body wt. A total of 1.5 ml of virus antigen was used for immunizing each guinea-pig. The guinea-pigs were bled two weeks after the last injection. The immunodiffusion titer was 1:8 and the content of IgG was 18.7 mg/ml.

(3) Horseradish peroxidase (HRP)-labelled goat anti-guinea-pig serum. The procedures of preparation were as follows: (a) Normal guinea-pig serum was precipitated with saturated ammonium sulfate three times and filtered through DEAE-cellulose column chromatography. The IgG obtained was confirmed to be pure by polyacrylamide electrophoresis with a content of 15 mg/ml. (b) Normal guinea-pig IgG was injected three times subcutaneously and intramuscularly into a healthy goat of 20 kg body wt. The method used was the same as above. The goat was bled 10 days after the last injection. The immunodiffusion titer was >1:64. Goat serum was precipitated with saturated ammonium sulfate twice and filtered through DEAE-cellulose column chromatography. Its IgG content was 12.6 mg/ml. (c) 10 mg of the goat anti-guinea-pig IgG was conjugated with HRP (Sigma, VI type, RZ 30) by the modified sodium periodide method. The final molar ratio of HRP-IgG conjugate was 1.29.
(4) Normal rabbit serum served as the negative control serum in the experiment.

(5) Sera of healthy adults. Sera were obtained from a total of 1,380 healthy adults with an age range of 20–40 years, of whom 362 were from Beijing and Hebei Province, 105 from Yinchuan of Ningxia Autonomous Region, 62 from Xuchang and Xinye of Henan Province, 324 from Hulunbeir Meng and Yakeshi of Nei Mongol Autonomous Region, 337 from Dexing and Gaoan of Jiangxi Province, 50 from Xizang Autonomous Region, 50 from Yanbian Autonomous Prefecture, 50 from Hainan Island and 40 from Chinghai Province (Fig. 1).
Methods
The procedures were as follows: (a) Coating of wells of the microtiter plate with the first antibody. 0.05 M carbonate buffer solution, pH 9.6, was used to dilute rabbit anti-adult diarrhoea rotavirus immune serum to 1:15, normal rabbit serum to 1:10 and 1,380 sera of healthy adults to 1:2 which were then used to coat wells of microtiter plates separately with 100 µl/well and placed at 37°C for 2 h. (b) Plates were washed repeatedly with 0.01 M PBS/Tween 20 (0.05%), pH 7.2–7.4, for 10 min. (c) The purified rotavirus antigen was diluted with 0.01 M PBS/Tween 20/EDTA, pH 7.2–7.4, to 1:20, added into wells with 100 µl/well and placed at 37°C for 2 h. (d) The plates were washed with PBS/Tween 20 repeatedly for 10 min. (e) The guinea-pig rotavirus immune serum was diluted to 1:15, added into wells with 100 µl/well and placed at 37°C for 2 h. (f) The plates were washed repeatedly as in procedure b. (g) Addition of enzyme-labelled antibody. The HRP-labelled goat anti-guinea-pig IgG was diluted with PBS/Tween 20 to 1:60, added into wells with 100 µl/well and placed at 37°C for 2 h. (h) The plates were washed repeatedly as in procedure b. (i) Addition of substrate. o-Phenylenediamine was dissolved in 0.05 M citric acid–0.1 M sodium phosphate buffer solution, pH 5.0, to 0.5 mg/ml, and added with 0.02% H₂O₂, was added to wells with 100 µl/well and placed at 37°C for 20 min. Then 2 M H₂SO₄ was added with 100 µl/well to stop the reaction. (j) Observation of results. To the naked eye, the blank control appeared colorless, the negative result appeared light yellow and the positive result appeared from deep yellow to brown. A spectrophotometer for ELISA (MB-1 type, Beijing), with a wave-length of 492 nm, was used to determine the P/N ratio (positive value/negative value). The substrate control was adjusted to 0. The negative serum control (normal rabbit serum) was 0.05. The positive serum control (rabbit anti-adult diarrhoea rotavirus immune serum) was 0.12. Those tested sera showing deep yellow to brown color and higher P/N ratios were considered

| Place                                    | No. of sera examined | No. of positives | %   |
|------------------------------------------|----------------------|------------------|-----|
| Beijing and Hebei Province               | 362                  | 25               | 6.9 |
| Yinchuan of Ningxia                      | 105                  | 16               | 15.2| Autonomic Region |
| Xuchang and Xinye of Henan Province      | 62                   | 13               | 21.0|   |
| Hulunbeir Meng and Yakeshi of Nei Mongol Autonomous Region | 324                  | 54               | 16.7|   |
| Dexing and Gaoan of Jiangxi Province     | 337                  | 22               | 6.5 |
| Xizang Autonomous Region                | 50                   | 0                | 0   |
| Yanbian Autonomous Prefecture            | 50                   | 7                | 14.0|
| Hainan Island                            | 50                   | 1                | 2.0 |
| Chinghai Province                        | 40                   | 3                | 7.5 |
| **Total**                                | **1,380**            | **141**          | **10.2**|
as positive. (k) Blocking test. Rabbit rotavirus immune serum (1:15), normal rabbit serum (1:10) and 26 sera of healthy adults (1:2), of which 25 showed positive results and 1 showed a negative result, were used in the blocking test.

Results

A total of 1,380 sera of healthy adults obtained from some provinces and cities of China were assayed for the antibody against adult diarrhoea rotavirus, of which 141 were positive, giving a total positive rate of 10.2%. The results are shown in Table 1.

In order to confirm the accuracy of the results, a blocking test was carried out on 25 positive serum specimens as well as on rabbit anti-adult diarrhoea rotavirus immune serum. Its results revealed that the P/N ratios after blocking were all lower than those before blocking. The P/N ratio of normal rabbit serum serving as the negative control showed no change after blocking.

Discussion

A method of using unknown serum as the first antibody to coat wells of microtiter plates directly in ELISA has not been described. A blocking test was carried out on 25 serum specimens positive for the antibody against adult diarrhoea rotavirus as well as on rabbit rotavirus immune serum. The results revealed that P/N ratios all declined after blocking, showing that this modified method is reliable for the assay of antibody.

Of 1,380 sera of healthy adults examined, 141 were positive for the antibody against adult diarrhoea rotavirus, showing a positive rate of 10.2%. The 25 positive specimens from Beijing and Hebei Province were traced for their sources and it was found that 17 of these were obtained from rural areas of Qian'an County, Hebei Province. According to preliminary information, epidemic adult diarrhoea had occurred in that district two years previously. Therefore, further retrospective investigations might be required.

Antibody against adult diarrhoea rotavirus was not detected in 50 sera of healthy adults from Xizang Autonomous Region. Only 1 out of 50 sera of healthy adults from Hainan Island was positive for this antibody. It is probably because their communications with the outside are not convenient, thus epidemic adult diarrhoea occurring in other provinces obviously has not spread to Xizang and Hainan Island.

References

Inouye, S., S. Matsuno and H. Yamaguchi, 1984, Efficient coating of the solid-phase with rotavirus antigens for enzyme-linked immunosorbent assay of immunoglobulin A antibody in feces. J. Clin. Microbiol. 19, 259–263.
Qiu, F.X., Y. Tian, A.Y. Xu, J.C. Liu and F.S. Li, 1985, RNA genome electrophoretic analysis of \textit{rotavirus} from feces of epidemic adult diarrhoea occurring in Shandong Province, China. \textit{J. Diar. Dis. Res.} 3, 73–77.

Rodak, L., L.A. Babiuk and S.D. Acres, 1982, Detection by radio-immunoassay and enzyme-linked immunosorbent assay of coronavirus antibodies in bovine serum and lacteal secretions. \textit{J. Clin. Microbiol.} 16, 34–40.

Wall, R.A., B.J. Mellars, P. Luton and S. Boulding, 1982, Comparison of ELISA, SPACE, and electron microscopy for the routine diagnosis of rotavirus infection. \textit{J. Clin. Pathol.} 35, 104–106.

Yolken, R.H. and F.J. Leister, 1981, Evaluation of enzyme immunoassays for the detection of human rotavirus. \textit{J. Infect. Dis.} 144, 379.