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# Comparison of Human Fecal and Serum Parvo-Like Viruses

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Parvovirus-like particles found in the sera of two blood donors had the size and appearance on electron microscopy of a virus (B19) found in the serum of a blood donor by Cossart et al. (1975), and those of a virus found in the feces of a normal subject. Antibody to these viruses was detected by immune electron microscopy and immunoelectro-osmophoresis in the sera of 50 children aged 10 to 15 years. Of these, 36% had antibody to the fecal virus, 36% had antibody to B19, and 54% had antibody to the two other serum viruses. The results of these tests suggest that serologically the three serum viruses were similar to one another, but that the fecal virus was distinct. The two blood donors had nonspecific symptoms at the time of viremia. Both donors had developed immunoglobulin M antibody to the virus when tested 3.5 and 4.5 weeks later, but no viruses were detected in the feces or urine.

In 1973, Paver et al. (8) described the presence of parovirus-like particles in the feces of some people with gastroenteritis and also in others without symptoms. Subsequently it was shown (9) that these particles had the appearance, size, and density of known animal parvoviruses. Similar particles have been seen in human feces by Ferris et al. (5) in Australia, by Flewett et al. (6) and Almeida et al. (1) in the United Kingdom, and by Dienstag et al. (4) in the United States. In 1975, Cossart et al. (3) described a parvo-like virus in the serum of nine blood donors and two patients.

The present paper reports the finding of a similar virus in the serum of two donors, as well as the results of comparing this virus with one of the previously described serum viruses (B19) (3) and with one of the parvo-like human fecal viruses (10).

## MATERIALS AND METHODS

**Subjects.** The two blood donors in whose serum the virus was found were detected during routine immunoelectro-osmophoresis (IEOP) for hepatitis B (HB) antigen. The human serum used in the tests contained antibody to both the HB antigen and the parvo-like virus. Neither of the donors had HB antigen in the serum, as shown by immune electron microscopy (IEM). B19 serum containing parvovirus-like particles was kindly supplied by Y. Cossart. The fecal virus was obtained from subject A (8, 9), who excreted parvovirus-like particles in relatively large numbers in his feces for several weeks.

The sera for antibody studies were obtained from 50 patients aged 10 to 15 years with a variety of illnesses and were sent to the laboratory for antistreptolysin O or virus antibody tests.

**Examination of blood donors for parvo-like viruses.** Serum was examined by IEM with a human serum known to contain antibody to the parvovirus-like particles. Feces were emulsified (10 to 25%) in distilled water and clarified by centrifugation at 10,000 × *g* for 30 min. They were then recentrifuged at 111,000 × *g* for 4 h or 320,000 × *g* for 2 h. The deposit was examined by IEM with a human serum found to clump the subject's serum virus. Urine was clarified and then spun at 48,000 × *g* for 2 h. The deposit was examined by IEM in the same way.

**Purification of virus from feces of subject A for IEOP and IEM.** A 10% emulsion of feces in distilled water was clarified by centrifugation at 10,000 × *g* for 1 h. Polyethylene glycol was added to the supernatant fluid to a final concentration of 8%, and the mixture was left overnight at 4°C and then centrifuged at 10,000 × *g* for 1 h. The resuspended deposit was mixed with CsCl to give a 38.12% solution of CsCl and centrifuged for 21 h at 314,000 × g. Fractions with a density of 1.37 to 1.41 g/ml were pooled and the density was adjusted to 1.39. This preparation was again centrifuged at 314,000 × g for 21 h, and the fraction with a density of approximately 1.39 g/ml was dialyzed against distilled water and used in IEM tests.

**IEOP.** This was performed in square petri dishes with agarose buffered to pH 8.2. Reagents were placed in 2-mm wells 5 mm apart and subjected to a current of 30 mA for 75 min. Antisera were used undiluted, and viruses were used undiluted or diluted $^1/_5$ or $^1/_{10}$.

**IEM.** Serum viruses were diluted $^1/_{50}$ and the purified fecal virus was diluted $^1/_4$. Antisera were diluted $^1/_{20}$ to $^1/_{80}$. Antibody-antigen mixtures were incubated at room temperature for 1 h and then centrifuged at 35,000 × g for 2 h. The deposit was examined by electron microscopy after staining with 1.5% phosphotungstic acid at pH 6.5. Grids were examined under code. Clumps with more than three particles and with visible antibody were considered as evidence of the presence of antibody.

**Examination of sera for the presence of specific IgM.** For examination of sera for specific immunoglobulin M (IgM), sera were fractionated in sucrose density gradients (2). Fractions were examined for antibody by IEM either untreated or after incubation for 1 h at room temperature with 0.10 volume of 0.5 M 2-mercaptoethanol.

## RESULTS

**Subjects with virus particles in serum.** Parvovirus-like particles were found in large numbers by IEM in the sera of two donors.

**(i) Subject 1.** This man gave a pint of blood in January 1975. At about this time he felt unwell and vomited several times during the night, but none of his companions had similar symptoms. His serum contained numerous parvovirus-like particles that were not aggregated. Parvovirus-like particles were not seen in serum taken 3.5 weeks later, but antibody was found, both in the IgG and IgM fractions of the serum. The first serum sample could not be tested for antibody because it contained large numbers of particles. Similar particles were not found in feces or urine collected at this time.

**(ii) Subject 2.** A 36-year-old woman, who frequently had minor symptoms, donated a pint of blood in February 1975. The following day she complained of aching in the calves and forearms, which spread to the shoulder, and she developed a stiff neck. The symptoms lasted a few days. Her blood contained numerous parvovirus-like particles that were not aggregated. A second specimen taken 4.5 weeks later showed only a few parvovirus-like particles, but antibody was detected in the IgG and IgM fractions of the serum. The first serum specimen could not be tested for antibody because it contained large numbers of particles. No parvovirus-like particles were found in her feces at this time.

Three of this woman's four children had itchy rashes that appeared 3½, 4½, and 5 weeks after she gave blood. Her husband had myalgia, headaches, and diarrhea 3 weeks after the blood donation. His serum, taken 10 days later, showed no virus particles, but a small amount of antibody was present in the IgG, but not the IgM, fraction. It was therefore unlikely that he had been recently infected with the parvo-like virus.

**Properties of the serum virus.** The serum and fecal viruses were identical in appearance (Fig. 1). The clumped virus particles were measured from electron micrographs; between 30 and 70 measurements were made of each virus. Mean diameters of the various viruses were as follows: the fecal virus from subject A, 24.3 ± 1.0 nm; the serum virus from subject 1, 23.5 ± 1.5 nm; the serum virus from subject 2, 24.0 ± 1.0 nm; and the serum virus from B19, 23.4 ± 1.6 nm. It was found by the same technique that the mean diameters of the two animal parvoviruses, mink enteritis virus and porcine parvovirus, were 23.3 ± 1.0 nm and 23.5 ± 1.1 nm, respectively, and the mean diameter of coxsackievirus B5, an enterovirus, was 28.9 ± 1.8 nm.

**Serological properties.** It was not possible to obtain enough of the fecal virus to immunize animals, and no human paired sera showing an increase in antibody were available. Therefore, to compare the viruses serologically, sera from 50 children aged 10 to 15 years were tested by IEM for antibody to the fecal virus from subject A and the serum viruses from subjects 1 and 2 and B19 (Table 1). None of the four viruses was aggregated by incubation in phosphate-buffered saline.

The three serum viruses were also tested by IEOP (Table 2), but it was not possible to concentrate the fecal virus sufficiently to produce precipitation in this test. All the sera showing antibody by IEOP also showed antibody by IEM. By IEM, 36% of the children had antibody to the fecal virus and to B19, and 54% had antibody to the serum viruses from subjects 1 and 2. The prevalence data for antibody to the three serum viruses showed a strong positive association, with concordances of 84, 90, and 94% between pairs by IEOP and 76, 82, and 82% between pairs by IEM ($\chi^2$ = 12.18, 20.64, and 20.64; $P < 0.001$). Thus, the three serum viruses seemed serologically related; however, the fecal virus seemed to differ from the three serum viruses (concordances were 34, 38, and 40% between pairs by IEM).

## DISCUSSION

In both size and appearance, the fecal virus and the three serum viruses resembled each other and some known animal parvoviruses. With the current laboratory techniques the sizes were slightly larger than previously reported (9), 24 instead of 22 nm, but all the viruses were of similar size. Cossart et al. (3) found that the buoyant density in CsCl of the serum virus B19 was between 1.36 and 1.40 g/ml, and the fecal virus has been shown previously to have a buoyant density in CsCl of 1.38 g/ml, which was similar to that obtained for two animal parvoviruses tested at the same time (9). Final classification of these viruses must await further study. It is to be expected that a human parvovirus might be found in the feces, since animal parvoviruses are probably spread by the fecal-oral route (7).

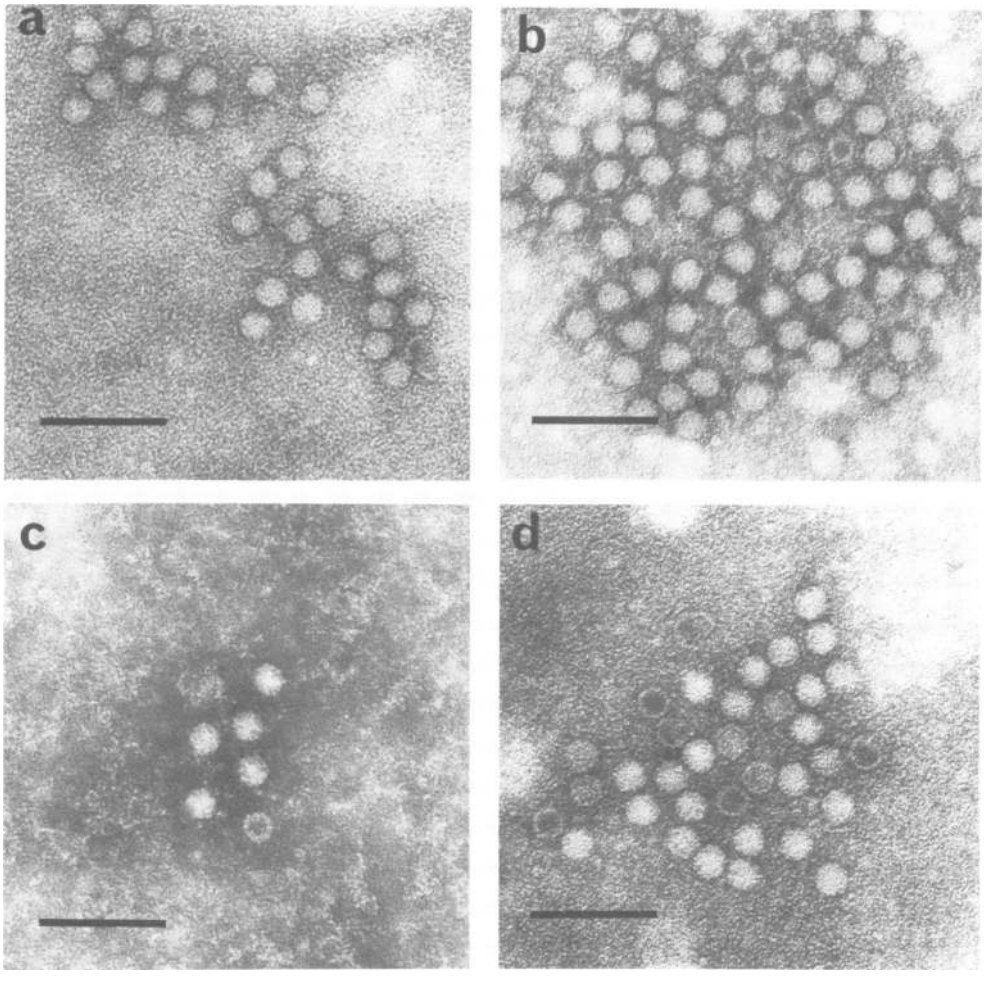


FIG. 1. *Electron micrograph of negatively stained particles (×165,000): (a) from serum of subject 1; (b) from serum of subject 2; (c) from serum B19; and (d) from feces of subject A. Serum B19 and serum from subjects 1 and 2 were diluted* 1/25 *and mixed with a second sample of serum from subject 2 at a dilution of* 1/20. *The fecal virus from subject A was precipitated with polyethylene glycol and purified by cesium chloride density gradient centrifugation. Purified virus was diluted* 1/4 *and mixed with antiserum from subject A at a dilution of* 1/20. *Bar represents 100 nm.*

IEM is a more sensitive test than IEOP, but it is necessary to use optimum proportions of antigen and antibody. When there is an excess of antigen over antibody, there is no clumping. On the other hand, if there is an excess of antibody, the virus particles are surrounded by a thick "fuzz" of antibody, and, although there may be some clumping, the clumps are small and usually difficult to identify because of poor staining. These two extremes are easily distinguished. In the present study, sera that had been shown to be strongly antibody positive by IEOP were tested by IEM at higher dilutions than weakly positive and negative sera. By IEM no antibody to serum virus B19 was detected in 15 sera that had antibody to one or both of the other two serum viruses. Conversely, all the sera with antibody to B19 had antibody to the other two. By IEOP, the results varied little irrespective of which antigen was used. More variation was found by IEM. It is possible that B19 was a higher-titered antigen than the other two serum viruses, and therefore those sera with little antibody did not clump the virus because of antigen excess. The degree of concordance between the presence of antibodies to these three viruses was higher than would be expected by chance if they were totally antigenically distinct, and bearing in mind the above arguments, it was concluded

TABLE 1. *Detection of antibody in the serum of 50 children by IEM against 22-nm viruses*

| No. of children with antibody | Fecal virus–subject A | Serum viruses | | |
|---|---|---|---|---|
| | | B19 | Subject 1 | Subject 2 |
| 3 | + | + | + | + |
| 15 | 0 | + | + | + |
| 1 | + | 0 | + | + |
| 2 | 0 | 0 | + | + |
| 3 | + | 0 | 0 | + |
| 3 | 0 | 0 | 0 | + |
| 2 | + | 0 | + | 0 |
| 4 | 0 | 0 | + | 0 |
| 9 | + | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 |
| % of children with antibody | 36 | 36 | 54 | 54 |

TABLE 2. *Detection of antibody in the serum of 50 children by IEOP against 22-nm viruses in sera*

| No. of children with antibody | Serum viruses | | |
|---|---|---|---|
| | B19 | Subject 1 | Subject 2 |
| 10 | + | + | + |
| 5 | + | + | 0 |
| 3 | 0 | + | 0 |
| 32 | 0 | 0 | 0 |
| % of children with antibody | 30 | 36 | 20 |

that these three viruses were serologically similar. A higher degree of concordance might be expected where the social conditions would encourage infection of a child with one of these viruses and increase the chance of infection with one of the other viruses. However, such a high degree of concordance as was found would be unlikely to be due to this cause. When the fecal virus was compared with the three serum viruses, the degree of concordance was no more than would be expected by chance if the fecal virus was antigenically completely different from the serum viruses. It was not possible to explain this difference in the same way as the difference between B19 and the other two serum viruses. Unfortunately, no other fecal virus could be included in the comparison for lack of feces containing sufficient virus particles.

Cossart et al. (3) found that the serum parvo-like viruses were serologically distinct from the adeno-associated viruses. The fecal virus from subject A has not yet been tested for any relationship to these viruses. It is unlikely that these parvovirus-like viruses in human feces are undescribed bacteriophages (11).

It is uncertain whether the symptoms described by the two subjects reported here were related to the presence of virus in the blood. B. Cant and T. Widdows (Abstr. Conf. Int. Soc. Blood Transfusion, 1975, p. 73) examined very carefully four of the donors found by Cossart to have parvovirus-like particles in the serum. One had no symptoms; one had a rubelliform rash 4 days after the viremia; one had an attack of fatigue and aching limbs lasting several days and starting the day after the viremia. The fourth had had fatigue for 3 months and was found to have a mild lymphadenopathy and a severe leukopenia. These workers estimated that this viremia was found in 1 in 40,000 donations. This frequency is of the same order as would be expected if the virus caused a short viremia during a primary infection. In virus infections in general, viremia commonly occurs in the incubation period; therefore, it is not surprising that these people felt well enough to donate blood at that time.

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