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# NOTES

## Antigenic Relationship Between Human and Bovine Rotaviruses as Determined by Neutralization, Immune Adherence Hemagglutination, and Complement Fixation Tests

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Guinea pig antiserum to bovine rotavirus does not neutralize human rotavirus. Bovine and human rotaviruses were, however, extensively cross-reactive when examined by complement fixation and immune adherence hemagglutination tests with antiserum to either virus. The immune adherence hemagglutination test was 16- to 32-fold more sensitive than the complement fixation test in detecting rotavirus.

The antigenic relationship among rotaviruses from different animal species has been studied by the complement fixation (CF) test (2) and by immunoelectron microscopy (5). In this communication, we report the antigenic cross-reaction between human infantile gastroenteritis virus (IGV) and neonatal calf diarrhea virus (NCDV) shown by neutralization, CF, and immune adherence hemagglutination (IAHA) tests.

Preparations of hyperimmune guinea pig antisera against NCDV and IGV were described in a previous report (3). Purified virions of NCDV and IGV were used as antigens for the CF and IAHA tests. IGV virions were prepared from fecal specimens by the method of Bishop et al. (1), and NCDV virions were prepared from infected primary monkey kidney cell cultures as described previously (3). Concentrated virions were treated with 1% (vol/vol) Nonidet P-40 for 30 min at 37°C and centrifuged through preformed gradients of 10 to 40% (wt/wt) cesium chloride at 35,000 rpm for 4 h at 4°C in a Hitachi RPS 40-T rotor. Fractions with a buoyant density of 1.36 to 1.38 g/cm$^3$ were collected. After dialysis and concentration, virions were further purified by centrifugation through 15 to 30% (wt/wt) sucrose gradients at 18,000 rpm for 3 h at 4°C in a Hitachi RPS 40-T rotor. Fractions were examined by the CF test, and the peak of antigenic activity at the middle of the gradient was collected. Electron microscopy showed that it contained nonaggregated intact virions. Table 1 shows the reaction of anti-NCDV and anti-IGV sera with NCDV in neutralization and CF tests. Anti-IGV serum reacted with NCDV to a titer equal to that of the homologous serum, but possessed a negligible neutralizing activity against NCDV. The above results indicate that most, if not all, CF antibodies do not bind with the critical antigenic site responsible for virus neutralization.

Antigenic cross-reaction was then studied by the IAHA and CF tests in checkerboard titration. Twofold serial dilutions of antigens and antisera were made in tubes and were transferred to microplates on which the IAHA and CF tests were performed. Figure 1 shows the extensive cross-reaction between NCDV and IGV. The reactions of both anti-NCDV and anti-IGV sera with a heterologous virus were nearly identical to their reaction with a homologous virus. The IAHA test was more sensitive, i.e., 16- to 32-fold more sensitive for the detection of antigens, than the CF test. The IAHA test might be useful for the detection and quantitation of rotaviruses in fecal specimens, providing a simple and sensitive method for diagnosis of infantile gastroenteritis. Preliminary ex-

TABLE 1. *Neutralizing and complement-fixing antibody titers to NCDV of anti-IGV and anti-NCDV sera*

| Serum | Antibody titer of NCDV | |
|---|---|---|
| | Neutralization | CF test |
| Anti-IGV | 180[a] | 4,096 |
| Anti-NCDV | 50,000 | 4,096 |

[a] The reciprocal of the serum dilution that caused a 50% reduction in plaque number (3).

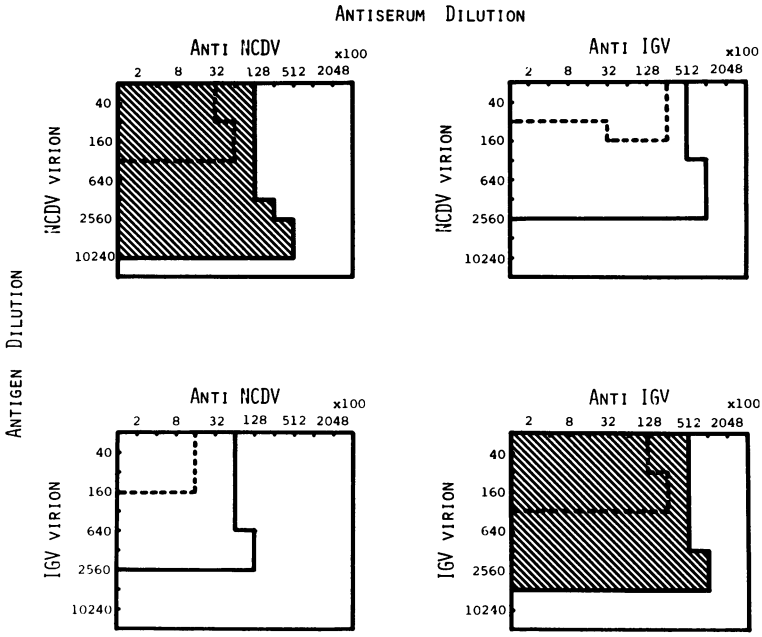


FIG. 1. *Checkerboard titration of anti-NCDV and -IGV sera against homologous and heterologous antigens. Symbols: (——) Zone of positive IAHA test; (- - -) zone of positive CF test. The IAHA test was performed by the method described by Mayumi et al. (4), and the CF test was performed by the method described in a previous report (3).*

periments have confirmed the usefulness of the test.

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