Prevalence of Antibody to Group B (Atypical) Rotavirus in Humans and Animals

D. W. G. BROWN,* G. M. BEARDS, CHEN GUANG-MU,† AND T. H. FLEWETT
Regional Virus Laboratory, East Birmingham Hospital, Birmingham B9 5ST, United Kingdom

Received 11 August 1986/Accepted 5 November 1986

Enzyme-linked immunosorbent assays were developed for the detection of group B rotavirus antigen and antibody. The specificities of both assays were evaluated for antigens and serum specific for rotavirus groups A to D. Serum collected in the United Kingdom from different animal species exhibited the following high prevalence of group B rotavirus-specific antibody: pigs, 97%; cattle, 71%; sheep, 91%; and goats, 91%. In human serum, a lower prevalence of group B-specific antibody was detected; serum from blood donors showed 10% prevalence, and serum from veterinarians showed 4% prevalence.

Until recently, it was thought that all rotaviruses shared a common group antigen; consequently, diagnostic tests based on the detection of this antigen have been adopted by many laboratories. However, when examined by electron microscopy, viruses with typical rotavirus morphology but lacking the common group antigen have now been described in several different species, including humans (3, 6, 8–11, 14–17, 19). In 1983, Pedley et al. (12) compared three rotaviruses—one rotavirus with the common group antigen and two atypical rotaviruses. They proposed that rotaviruses be classified into at least three groups, designated A, B, and C, on the basis of serological differences, determined by immunofluorescence, and nucleic acid differences, characterized by one-dimensional terminal fingerprint analysis (12). It was also noted that characteristic differences were apparent between the three groups when the electrophoretic migration patterns of the double-stranded RNA genome segments were examined. These studies have recently been extended (13), and now five groups of rotavirus have been proposed.

At present, atypical rotaviruses constitute only a minority of the rotaviruses which have been characterized; indeed, only viruses from groups B and C have been identified thus far in humans (5, 7, 9). The significance of these atypical rotavirus infections in animals is only now becoming clear. Group B rotaviruses have been shown to cause diarrhea in gnotobiotic pigs (18), and recently a high prevalence of antibody to group B rotaviruses has been described in pigs in Great Britain (4). It seems likely that they will prove to be an important cause of diarrhea in animals.

The significance of group B rotaviruses in humans is less clear. In 1982 and 1983 extensive outbreaks of diarrhea in adults were reported in two coal mining areas, Lanzhou and Jinzhou, in China (9). Widespread outbreaks of a similar nature have occurred since then. The virus isolated from this outbreak was subsequently shown to be a group B rotavirus (G. M. Chen, T. Hung, J. C. Bridger, and M. A. McCrae, Letter, Lancet ii:1123–1124, 1985). Recent limited seroepidemiological studies using countercurrent immunoelectrophoresis against this antigen have shown a low prevalence of antibody to this virus in Australia and Hong Kong and a higher prevalence in China (T. Hung, R. Fan, C. Wang, G. M. Chen, D. Chou, J. Chang, M. A. McCrae, W. Wang, W. Se, R. Dan, and M. H. Ng, Letter, Lancet ii:325–326, 1985). However, no outbreaks of diarrhea caused by this virus have so far been described outside China. We developed enzyme-linked immunosorbent assays (ELISAs) for the detection of antigen and antibody based on serum raised against the Chinese group B virus to investigate further the epidemiology of group B rotaviruses in humans and animals.

MATERIALS AND METHODS

We received group B rotavirus as a 10% fecal suspension in phosphate-buffered saline (PBS) collected from a human volunteer. This was a gift from the Institute of Virology, National Centre for Preventive Medicine, Beijing, China. We received the following representative viruses from different rotavirus groups as fecal suspensions: B/Nird-1 (a pig virus), kindly provided by Janice Bridger (12); three group B viruses (one pig virus and two lamb viruses) and one group C virus (from a calf), sent by D. Snodgrass; one group C virus (from a human), from H. G. Pereira (14); and one group C virus (from a human), provided by D. Cubitt. This latter virus has been described elsewhere (5).

Antisera. A hyperimmune guinea pig serum designated 25 was raised by repeated intramuscular injections of incomplete virus particles (1.38 g/ml at CsCl peak) which were purified by a method previously described for group A rotavirus (1) and emulsified with Freund incomplete adjuvant. Brieﬂy, the virus was precipitated from a clarified 10% fecal extract with 8% (wt/vol) polyethylene glycol 6000 followed by centrifugation through 45% (wt/wt) sucrose and isopycnic centrifugation on a self-forming cesium chloride gradient. Immunoglobulin G (IgG) was extracted from guinea pig serum 25 by ion-exchange chromatography with 20 mM phosphate buffer (pH 8.0) on a Whatman DE52 column and biotinylated for use in the ELISA by a previously described method (20).

ELISAs. Group B rotavirus antigen was detected by a simple sandwich assay using hyperimmune guinea pig serum 25 as both the capture and as a biotinylated detecting serum. The presence of virus was indicated by developing the test with a 1:200 dilution of avidin-peroxidase in PBS–0.5% Tween 20–1% bovine serum albumin at 37°C for 1 h and then with 3,5’,5’-tetramethylbenzidine substrate in citrate-acetate buffer (pH 6.2). Serum samples were tested for the presence of antibody to group B rotaviruses by a competitive

* Corresponding author.
† Present address: Institute of Virology, Beijing, China.
enzyme immunoassay; this was a modification of the standard enzyme immunoassay method (21). Briefly, the wells of irradiated polystyrene microtiter plates (Nunc 239454) were coated with guinea pig antiserum to group B rotaviruses. After incubation at 37°C for 2 h, unbound antibody was removed by washing the plates in PBS-0.5% Tween 20. The serum to be tested was then added to the plate; 20 μl of a 1:4 dilution was used as the screening dilution. An 80-μl amount of group B antigen was then added to the serum in the plate, and this serum-antigen mixture was incubated for 18 h at 4°C. The group B antigen used in the test was a dilution of the crude fecal specimen from a Chinese volunteer which was made up in PBS-0.5% Tween 20–1% bovine serum albumin. After washing, the amount of virus binding was measured by the reaction of the wells with biotinylated guinea pig antiserum to group B rotavirus, avidin-peroxidase, and TMB substrate. By this method, the presence of group B-specific antibody was demonstrated by the inhibition of binding of the biotinylated anti-group B antiserum. For the purposes of this study, serum samples were compared with both strongly reactive and nonreactive pig serum samples. A serum sample was considered to contain group B-specific antibody if it produced a >60% inhibition of binding. Serum samples generating between 40 and 60% inhibition were considered to be equivocal, and those generating <40% inhibition were considered to be negative. All results were read spectrophotometrically at an optical density at 450 nm.

**Serum samples examined.** The specificity of the ELISA of serum samples for group B-specific antibody was assessed by examining sera raised against the following four different rotavirus groups in the assay.

**Group A.** Hyperimmune rabbit serum designated 720 was raised against several different group A viruses and used in the World Health Organization ELISA for the detection of group A rotavirus antigen (2).

**Group B.** Convalescent-phase serum designated 1765, raised in a gnotobiotic pig, was kindly provided by D. Snodgrass, and hyperimmune rabbit serum raised against the Chinese group B virus was kindly sent by Hung Tao.

**Group C.** Convalescent-phase serum designated 1303 (from D. Snodgrass) was raised in a gnotobiotic piglet, and hyperimmune rabbit serum raised against the Brazilian group C human virus was kindly sent to us by H. G. Pereira.

**Group D.** A hyperimmune serum designated 132, a gift from M. Stewart McNulty, was raised in specific-pathogen-free chickens.

A total of 358 sera, categorized into six groups and derived from five different species, were tested for antibodies to group B rotavirus. The origins of these sera were as follows: 110 sera from veterinarians, veterinary students, and farm workers collected in the United Kingdom in 1983 and 1984; 100 sera collected from blood donors at the West Midlands Blood Transfusion Centre in 1986; 11 sera collected from six herds of goats, which were all less than 2 years old; 11 sera collected from 6 herds of sheep, aged between 1 and 4 years; 59 sera collected from 12 herds of dairy cattle, aged between 7 months and 12 years, collected in 1983; and 67 sera collected from 20 herds of pigs in 1983.

**RESULTS**

**Specificity of ELISA for antigen detection.** The specificity of the ELISA for antigen detection was assessed by examining fractions from a cesium chloride density gradient purification of the group B rotavirus. The results are shown in Fig. 1. The ELISA activity was concentrated in two peaks corresponding to buoyant densities in CsCl of 1.36 and 1.38 g/ml, which are those of complete and incomplete rotavirus particles, respectively. The reactivity of group A rotaviruses in this assay was assessed by testing 72 fecal specimens positive for group A rotavirus when tested in the World Health Organization ELISA (2). These specimens all gave negative results (mean optical density reading at 450 nm, 0.056; standard deviation, 0.034). Representative rotaviruses from groups B and C were also examined in the assay. The
three group C viruses tested gave negative results (optical density at 450 nm, <0.10); three of six group B viruses examined gave results >0.10 in ELISA.

**Specificity of ELISA for antibody detection.** The specificity of the ELISA for antibody detection was assessed by examining well-documented rotavirus-group-specific serum in the assay. The results are shown in Fig. 2. Sera raised against four rotavirus groups were tested; only sera raised against group B rotaviruses showed significant activity in the assay. In addition, convalescent-phase sera from gnotobiotic pigs infected with B/Nird-1, provided by Janice Bridger, also showed significant inhibition. Sera from 10 colostrum-deprived calves and from 5 guinea pigs and 5 rabbits (which had been isolated from birth) were examined; none of the sera showed significant inhibition in the assay when tested at a 1:2 dilution.

**Antibody prevalence.** Three hundred fifty-eight sera from different animal species were tested for evidence of group B-specific antibody. The results are shown in Fig. 3. A high prevalence of antibody was found in sera from pigs (97%), sheep (91%), goats (91%), and cattle (71%). However, in serum from humans, a significantly lower prevalence of antibody was detected (blood donors, 10%; veterinarians, 4%).

**DISCUSSION**

The main difficulty with developing an ELISA for a virus for which well-defined sera are not widely available is that of setting an appropriate level for positivity in the assay. In this study, we used three methods of control: using well-defined sera from animals that had only been infected with individual rotaviruses, examining the reaction of known negative sera in the assay, and looking at the distribution of inhibition in a population of known antibody prevalence (pigs) in the test. This led us to set a negative/positive cutoff at 60% inhibition for the purpose of this study. However, it may be necessary to alter this cutoff when a larger number of sera from natural infections have been investigated.

The high prevalence of antibody to group B rotavirus in serum from pigs in the United Kingdom reported here is in agreement with findings of others (4). In addition, we have shown a high prevalence of antibody to group B rotavirus in sera from cattle, which has not been previously reported. The small number of sera examined from sheep and goats suggests that group B rotaviruses are also circulating in these species. However, it remains to be established what proportion of group B rotavirus infections cause diarrhea in these animals.

There have been two previous reports of antibody studies against different group B rotaviruses in humans. In 1984, an atypical rotavirus was described in rats (19). An 88% prevalence of antibody to this rat rotavirus in humans was subsequently reported (7). The implication of this work is that group B viruses are widespread in humans but that they had previously been missed because they were difficult to detect. This is supported by the observation that the group B virus used for this study was disrupted by the phosphotungstate-negative stain routinely used for electron microscopy but not by stains based on uranium or molybdenum salts (H. Suzuki, G. M. Chen, T. Hung, G. M. Beards, and D. W. G. Brown, submitted for publication). We have found a low prevalence of antibody to group B rotavirus in serum from healthy adults in Great Britain which is consistent with the levels found in Australia and Hong Kong by immunoelectrophoresis (T. Hung et al., Letter, Lancet ii:325–326, 1985). However, this is in marked contrast to the American findings (7). This could be due to differences in sensitivity between the assays used or to the significant antigenic differences, perhaps different subgroups, within group B rotaviruses. Thus, the antibody tests described may be detecting antibodies directed against subgroup antigens in addition to the group antigen. The findings reported here suggest that group B rotavirus infections occur at a low level in humans in the United Kingdom; however, it is not yet clear whether these infections are associated with diarrhea or any other illness.

The finding of a low prevalence of antibody to this virus in
sera from humans and the Chinese report of an explosive outbreak (9) are both consistent with a zoonotic origin of infection. We have examined sera from veterinarians and farm workers as a possible "sentinel group" of a zoonotic origin of this infection. But this group had a lower prevalence of antibody than the population of blood donors examined, which suggests that, if their infections had a zoonotic origin, the reservoir was not the domestic animal population and that the virus is circulating within the human population. However, a zoonotic infection from a different source, possibly rodents, cannot be excluded.

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LITERATURE CITED