

## Prevalence of Antibody to the Norwalk Virus in Various Countries

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Serum samples from children and adults from several countries were tested by radioimmunoassay for antibody to the Norwalk virus. Antibody was commonly found in adults from all the countries tested. Antibody appears to be acquired more rapidly in children from underdeveloped countries than in children from the United States.

The Norwalk virus is the most thoroughly studied member of a family of small, 27-nm viral particles which are an important cause of acute nonbacterial gastroenteritis in humans (7). These viruses have not been successfully cultivated in tissue culture, and their host range appears to be restricted to humans and chimpanzees. The Norwalk strain, the prototype virus of this group, has been associated most frequently with epidemics and family outbreaks of gastroenteritis in older children and adults (1a). In a recent serological survey, this virus appeared to be the cause of more than 25% of nonbacterial gastroenteritis outbreaks in families and communities (1a).

Very little information has been available concerning the prevalence of Norwalk infection throughout the world, due in large part to the lack of a sensitive specific serological assay for Norwalk antibody. Recently, an immune adherence hemagglutination assay and a solid-phase blocking radioimmunoassay for antibody to Norwalk virus were developed (1, 3). Both tests were shown to be sensitive and specific, the solid-phase blocking radioimmunoassay being somewhat more sensitive (1). In a preliminary study using the less sensitive immune adherence hemagglutination assay, we showed that Norwalk antibody, as opposed to rotavirus antibody, was acquired gradually during the first 5 decades of life in an urban and suburban U.S. population. In this paper we have extended our studies on the acquisition of Norwalk antibody to several different populations and have used the more

sensitive radioimmunoassay for antibody detection. Our findings indicate that Norwalk infection appears to be relatively ubiquitous and that in some populations, especially in underdeveloped countries, antibody acquisition occurs quite early in life.

### MATERIALS AND METHODS

**Radioimmunoassay blocking test.** Basically, the radioimmunoassay blocking test was done as previously reported (1). Polyvinyl microtiter plates pre-coated with Norwalk antibody were inoculated with 25  $\mu$ l of a single standard human volunteer crude stool suspension containing Norwalk antigen. This antigen preparation was shown to be as sensitive and specific as a partially purified virus preparation for use in antibody detection (1a). The antigen preparation contained approximately 4 "binding" units and gave a positive-over-negative ratio of greater than 4 (1). After an overnight incubation at room temperature, the plates were washed and inoculated with 50  $\mu$ l of a 1:100 dilution of the serum sample to be tested. Samples were diluted in phosphate-buffered saline with 0.1% sodium azide and 1% bovine serum albumin. All specimens were tested in duplicate. The plates were again incubated overnight at room temperature, and then 25  $\mu$ l of <sup>125</sup>I anti-Norwalk immuno-globulin G was added to each well and incubation was continued for 4 h at 37°C. Plates were then washed and cut, and individual wells were counted in a gamma counter. A 50% or greater reduction in residual radioactivity produced by a serum sample as compared with a buffer control was taken as evidence of the presence of Norwalk antibody.

**Serum specimens.** Serum samples from adults living in the United States, Belgium, Switzerland, and Yugoslavia were obtained primarily from randomly

selected urban volunteer blood donors. These same specimens have previously been studied for the prevalence of hepatitis A antibody (8). The serum samples from several villages in rural Bangladesh were gathered as part of a longitudinal family study of infection with various gastroenteritis agents.

The specimens from Ecuador were gathered from a rural Indian group, the Waorani Indians of eastern Ecuador. Various clinical and epidemiological characteristics of this population have already been described (4). Indians from three villages (Tiwaeno, Tzapino, and Bai's) were analyzed together (Tables 1 and 2) and described simply as Ecuador Indians. Individuals from these villages had moderate contact with other tribal groups and Western visitors over the past 20 years. Another group of Indians, described as Ecuador Indians (Gabaro) lived in an extremely isolated village and had little if any contact with neighboring tribes or Westerners before 1972 and very infrequent contact since then. The inhabitants of the Gabaro village have had some hostile contact with other Indians and outsiders, but this has not been of a sustained nature (4).

The specimens from Nepal were obtained from healthy adults living in the upper Karnali valley in northwest Nepal in the villages of Yakba and Thymkyn. This is a rural area of Nepal with limited contact with more urban populations in Kathmandu. Sera from the various adult populations studied were all gathered between 1974 and 1977.

Specimens from healthy children from Yugoslavia were obtained in 1977 as part of a hepatitis serological survey. These children came primarily from urban areas of Yugoslavia. The serum samples of U.S. children were obtained at Children's Hospital, Washington, D.C., over the past 20 years. For the most part, they represent admission blood specimens from children with respiratory tract disease and other nongastrointestinal illnesses.

An additional series of 86 sera from adult homosexuals (61 males and 25 females) was tested. These sera had previously been tested for antibody to hepatitis A and B (9). All sera had been stored at  $-20$  or  $-70^{\circ}\text{C}$  until tested. All sera were tested without knowledge of the age or sex of the subjects.

## RESULTS

Table 1 shows the age and sex distribution of adults included in the survey. Antibody to the

Norwalk virus was found in serum from individuals living in each of the areas studied. 71% of 861 individuals tested had antibody (Table 2). There did not seem to be a striking difference in antibody prevalence in adults between Western countries (United States, Belgium, and Switzerland) and less-developed countries such as Nepal, Bangladesh, or Ecuador. During the fourth decade (Fig. 1), antibody acquisition approached 70% in all the countries studied. Of interest was the Norwalk antibody prevalence data obtained from the highly isolated Ecuador Indian tribe in Gabaro. None of the 16 adults studied had evidence of prior Norwalk infection (Table 2). This is in marked contrast with the three other Waorani villages studied, where almost 90% of the adults had antibody. The prevalence of antibody in adult male and female homosexuals was approximately the same (57.4 and 65, respectively) and not appreciatively different from the other adult U.S. blood donors studied (the homosexual prevalence data is not included in the figures or tables).

The Norwalk antibody prevalence data for children is seen in Fig. 2. Children from the United States and, to a lesser extent, Yugoslavia acquired antibody more slowly than did children from less well developed countries like Ecuador

TABLE 2. Prevalence of serum antibody to Norwalk virus in healthy adults from various parts of the world

Country	No. tested	No. with antibody	% positive for Norwalk antibody
USA	295	195	66.1
Ecuador	107	96	89.7
Ecuador (Gabaro)	16	0	0
Belgium	133	102	76.7
Switzerland	91	63	69.2
Yugoslavia	76	41	53.9
Bangladesh	104	72	69.2
Nepal	55	41	75

TABLE 1. Age and sex distribution of healthy adults from various parts of the world tested for antibody to Norwalk virus

Country	Age (yr)					Sex <sup>a</sup>		Total
	18-19	20-29	30-39	40-49	≥50	M	F	
United States	40	72	68	63	52	149	146	295
Ecuador	14	44	25	12	12	55	52	107
Ecuador (Gabaro)	5	6	2	3	—	8	8	16
Belgium	—	25	29	44	35	80	53	133
Switzerland	20	20	18	16	17	91	—	91
Yugoslavia	22	19	15	14	6	38	38	76
Bangladesh	12	40	31	10	11	—	—	104
Nepal	2	13	15	10	15	25	30	55

<sup>a</sup> M, Male; F, female.

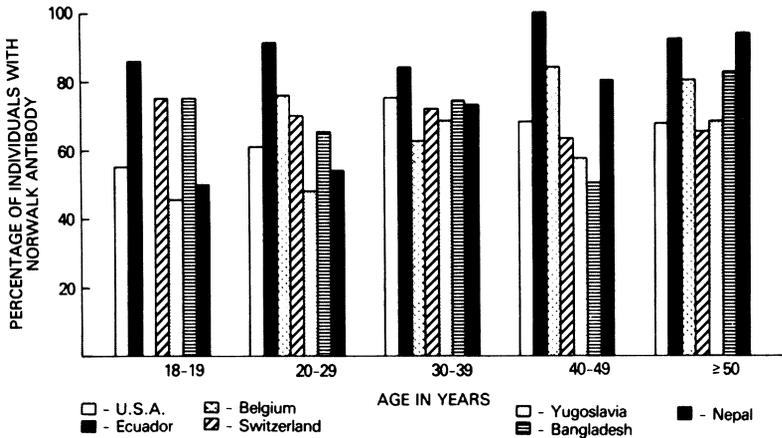


FIG. 1. Prevalence by age of serum antibody to Norwalk virus in healthy adults from various parts of the world. Note that no specimens were tested in the 18- to 19-year age group of Belgian donors.

or Bangladesh. Norwalk infection appeared to be relatively uncommon in early childhood in the United States and Yugoslavia. On the other hand, in the underdeveloped countries studied, Norwalk infection occurred rather early in life. However (Fig. 2), none of the Ecuadorian Indian children in the isolated Gabaro village had antibody to Norwalk.

DISCUSSION

The Norwalk virus was originally isolated from a community outbreak of acute gastroenteritis in Norwalk, Ohio. Subsequent studies have linked this virus, or serologically related viruses, to about 25% of investigated epidemics of acute nonbacterial gastroenteritis (2). However, little information was available concerning the prevalence of Norwalk infection on a worldwide basis. The preliminary studies presented here suggest that Norwalk virus infection is ubiquitous. All the adult populations studied (excluding Gabaro) had similar prevalence rates. The high prevalence rates probably mean that the Norwalk virus is a common gastrointestinal pathogen over much of the world. At present, data are not available concerning the persistence of serum antibody after infection. It is likely that in some persons it may decline to an undetectable level with time. The 70% prevalence rate found in most adult populations probably reflects a steady-state condition in which reinfection with an antibody response balances disappearance of antibody. In a recent volunteer study we observed that some adults who lack serum antibody are not susceptible to Norwalk infection and do not develop serum antibody after challenge. Parrino et al. have previously

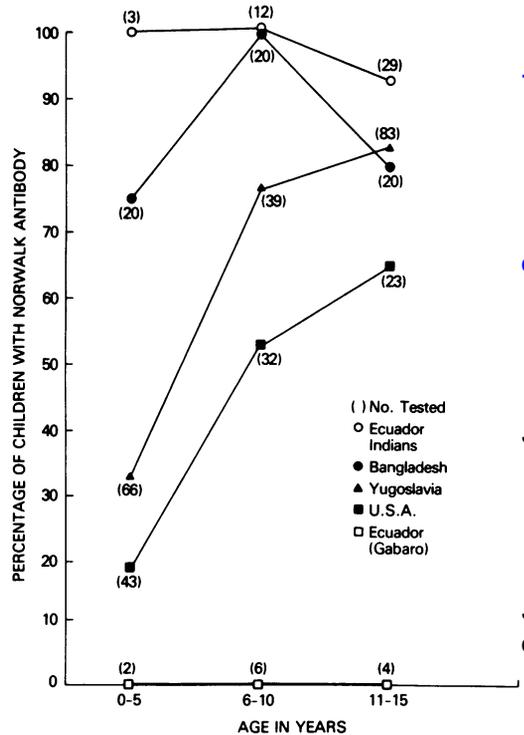


FIG. 2. Age-related prevalence of serum antibody to Norwalk virus in children from various countries.

reported similar findings (5).

The gradual pattern of antibody acquisition seen in the U.S. children is similar to data published previously (3). It is in agreement with our inability to demonstrate Norwalk infection as a common cause of early childhood gastroenteritis

in U.S. children. The high pediatric antibody prevalence rates in Bangladesh and Ecuador were unexpected. This finding may indicate that in underdeveloped countries infection with Norwalk virus occurs very early in life. What morbidity, if any, is associated with this early infection remains to be determined. Rotavirus infection has recently been shown to be a major cause of childhood gastroenteritis in the 6-month to 3-year age group (2). This has been true both in the United States and in underdeveloped countries (2, 6). However, a substantial portion of diarrhea in the 6-month to 3-year age group remains undiagnosed. This is also true in the less-developed countries. Further study is needed to determine whether Norwalk virus plays an important role as a pediatric pathogen.

Finally, it is of some interest that one highly isolated population was identified in which Norwalk virus appeared to be absent. The residents of the Gabaro village are felt to be closely related to the surrounding Indians (4) so that their lack of antibody is unlikely to be due to a genetic influence. Apparently, Norwalk had not circulated in this isolated group in the past 40 years. Perhaps a small population cannot perpetuate this virus. There is no evidence for chronic infection with Norwalk virus, and the absence of antibody in the Gabaro population is in keeping with the lack of a chronic carrier state. Similarly, the virgin antibody status of this population is evidence against a common animal vector for Norwalk virus. It is interesting to note that this population does have close to a 100% prevalence of antibody to rotavirus, respiratory syncytial virus, and hepatitis A virus, but has no evidence of exposure to the hepatitis B virus (anti-HBc negative) (unpublished data).

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