Epidemiol. Infect. (1996), **116**, 203–206

An epidemiological investigation of Norwalk virus infection in South Africa

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(*Accepted 12 October 1995*)

SUMMARY

A study was carried out to determine the incidence and seroprevalence of Norwalk virus (NV) in the Pretoria area, South Africa, using a recombinant NV (rNV) immunoassay for the detection of serum IgG antibodies. Maternal antibody was detectable in infants' sera up to approximately 6 months of age. Infection with NV was detected serologically in the second year of life and the seroprevalence of NV IgG rose from 37·1% at 7–11 months of age to 62·1% by the age of 40 years. No significant differences in seroprevalence of NV IgG antibody was evident between subjects of European or African ethnic origin, where overall seroprevalence rates were 56·4% and 53·9% respectively.

INTRODUCTION

In addition to rotavirus and adenovirus types 40 and 41, several other viruses have been shown by electron microscopy (EM) to be the cause of non-bacterial gastroenteritis [1], with the small round structured viruses (SRSVs) reportedly accounting for 2·5–4% of sporadic gastroenteritis [2]. SRSVs, including the prototype Norwalk virus (NV), are well-established causes of food and waterborne outbreaks of gastroenteritis in people of all age groups, but particularly adults and the elderly [2, 3]. However, the true incidence of NV and SRSV-associated infections is unknown as many cases of viral gastroenteritis go unreported or no definitive diagnosis is made because the detection of these viruses has, until recently, been dependent on EM [3–5] and immune electron microscopy (IEM) [6–8]. The diagnosis of these infections has been further hampered by the inability to grow SRSVs *in vitro* [9]. Limited epidemiological studies, carried out with antigens and antisera from human volunteer studies, have shown that in less-developed areas such as Bangladesh, NV is contracted early in life [10], whilst in the USA it was an uncommon childhood infection [11]. The recent cloning and sequencing of the entire NV genome [12], and the consequent production of recombinant NV antigen (rNV) [13], has facilitated the development of sensitive and specific enzyme immunoassays (EIA) for the detection of NV antigen and antibodies [14–16]. In addition, the application of the reverse transcriptase polymerase chain reaction and subsequent molecular characterization of these viruses has enabled many SRSVs, including NV, to be classified within the *Caliciviridae* [17].

NV-like viruses have been associated aetiologically with two outbreaks of gastroenteritis in adults in South Africa [18, 19], but the true incidence of NV-associated infections is unknown. This study reports the application of recombinant EIAs to determine the incidence and seroprevalence of NV among children and adults in the Pretoria area of South Africa.

MATERIALS AND METHODS

Sera

A total of 579 sera were selected from a bank of sera from children and adults up to 40 years of age, from

https://doi.org/10.1017/S0950268800052444 Published online by Cambridge University Press

both European (white Caucasian) and African ethnic origin, who had presented at the HF Verwoerd or Kalafong Hospitals, Pretoria. Blood specimens were collected during 1992 and the sera were then stored at −20 °C with limited freeze-thawing. Subjects were predominantly from an urban environment and represented all social classes. Where possible sera from immunocompromised patients were excluded from the study.

EIA for the detection of IgG antibodies to NV

The EIA system of Graham and colleagues [14], as modified by Parker and colleagues [16], was used to detect IgG antibodies to NV. In the assay system horseradish peroxidase (HRP) conjugated rabbit anti-human IgG (Dako immunoglobulins, Denmark) was used with 3,3′,5,5′-tetramethylbenzidine dihydrochloride (TMB) (Kirkegaard & Perry, Gaithersburg, MD) as substrate. An alternate substrate, *o*-phenylenediamine dihydrochloride (OPD) (Organon Teknika N.V., Boxtel, Holland) was used in some of the experiments. Samples were tested at a dilution of 1/1000 and wells giving an OD value $> 0{\cdot}1$ and at least double the negative control were considered to be positive. A titre of < 1000 in the rNV EIA has been found to be equivalent to < 50 in the radio-immunoassay (RIA) and was therefore considered to be negative [16].

Statistical analyses

Statistically significant differences between age and ethnic groups were determined by the chi-square test.

RESULTS

Prevalence of serum IgG antibodies to NV

The prevalence of IgG antibody by ethnic origin and age group, up to 40 years of age, is shown in Table 1. From these results it is evident that there is a relatively high level of NV-related infection in subjects. Infection appears to occur predominantly in children 1–2 years of age, with a progressive small increase in seroprevalence up to 40 years of age. No differences at the 0·05 level of significance could be demonstrated between the two groups investigated, with an overall seroprevalence of 56·4% (95% confidence interval [CI] = 50·8–62·0%) and 53·9% (95% CI = 48·1–59·7%) for the subjects of European and African ethnic origin respectively. There were also no significant differences between age groups within the group of African ethnic origin. A statistically significant ($P < 0{\cdot}05$) rise in seroprevalence was however noted in the 19–29 year old group within the group of European ethnic origin. The pattern of seropositivity in each ethnic group was however similar, i.e. maternal antibodies declined at approxiamtely 6 months of age, with little evidence of infection up to 1 year of age. In the group of African ethnic origin, the seroprevalence in the neonates (54·0%; 95% CI = 41·7–66·3%) was similar to that found in women of childbearing age (55·6%; 95% CI = 32·7–78·5%). After 1–2 years of age the seroprevalence appears to remain static, followed by a small increase in seroprevalence in subjects 30–40 years of age (63·6%; 95% CI = 43·5–83·7%). Although not as clearly defined, a similar pattern of seroprevalence is discernable amongst subjects of European ethnic origin.

DISCUSSION

In this investigation the observed seroprevalence in adults aged 30–40 years (62·1%; 95% CI = 46·6–77·8%) was of the same order as that reported for nursing and medical staff in this age group in London (63%; 95% CI = 53·4–73·1%) [20], and lower than those reported for adults in other areas in England (80·8–90·0%) [21], Ecuador (89·7%) [22], Belgium (76·7%) [22], Indonesia (100%) [23], and for Australian aborigines (94%; 95% CI = 80·0–99·3%) and Saudi Arabian Bedouins (72%; 95% CI = 58·1–85·4%) [20]. A number of seroepidemiological studies have been performed in various countries to determine age prevalence of NV antibody. In these investigations the age of acquisition of NV antibodies varied between countries, i.e. 14–19 months of age in Bangladesh [10], 2–10 years of age in the United Kingdom [20], and $\geqslant 12$ years of age in Japan [20, 23] and the USA [20]. Regional differences in the acquisition of NV antibody within one country have also been recorded [21]. In the present study, the age of acquisition of NV antibody in urban South Africans occurs very early, i.e. within the second year of life. This is similar to the pattern demonstrated in the less developed or more rural regions of the world, e.g. Bangladesh [10] and in Australian aborigines [20]. Whether this is due to geographical location, population densities, or water quality has yet to be established. There is also no obvious window between the decay of maternal antibody and primary infection.

Table 1. *Seroprevalence by age of Norwalk virus IgG in groups of different ethnic origin in South Africa*

	European			African			Total		
Age range	No.*	% Pos†	95% CI‡	No.	% Pos	95% CI	No.	% Pos	95% CI
0–6 mo	50	56·0	(42·2–69·8%)	63	54·0	(41·7–66·3%)	113	54·9	(45·7–64·1%)
7–11 mo	14	35·7	(10·6–60·8%)	21	38·1	(17·3–58·9%)	35	37·1	(21·1–53·1%)
12–23 mo	23	60·9	(40·9–80·9%)	40	55·0	(39·6–70·3%)	63	57·1	(44·9–69·3%)
2–4 y	66	62·1	(50·4–74·1%)	40	52·5	(37·0–68·0%)	106	58·5	(49·1–67·9%)
5–10 y	59	55·9	(43·2–68·6%)	50	56·0	(42·2–69·8%)	109	56·0	(46·7–65·3%)
11–18 y	45	42·2	(27·8–56·6%)	27	55·6	(36·9–74·3%)	72	47·2	(35·7–58·7%)
19–29 y	26	73·1	(56·1–90·1%)	18	55·6	(32·7–78·5%)	44	65·9	(51·9–79·9%)
30–40 y	15	60·0	(35·2–84·8%)	22	63·6	(43·5–83·7%)	37	62·1	(46·6–77·8%)
Total	298	56·4	(50·8–62·0%)	281	54·1	(48·1–59·7%)	579	55·5	(51·4–59·5%)

* Number of serum samples tested.
† Percentage seropositive.
‡ 95% confidence interval.

A similar pattern was obtained when sera from London children were screened for the presence of antibodies to another SRSV, Mexico virus [24]. This lack of a distinct trough was ascribed to the early age of acquisition of the infection and the high sensitivity of recombinant protein EIAs [24].

Although the overall seroprevalence rates in both ethnic groups were similar, there are no data with regard to the epidemic vulnerability of either group. In an investigation of a NV-associated outbreak of gastroenteritis in the same area the presence of pre-existing NV IgG antibody in white symptomatic patients was not protective, while a subject of African ethnic origin, with pre-existing NV antibody, was asymptomatic [18]. Blacklow and colleagues [25] suggest that either a genetic factor is involved in protective immunity or repeated exposures to the virus is necessary to elicit a protective immune response. The irregular pattern observed in the seroprevalence rates between age groups in the subjects of European origin may reflect sporadic contact with NV, while the more regular pattern shown for subjects of African origin may be due to repeated exposures to the virus.

An additional confounder which has to be considered in the interpretation of serological results is antigenic relatedness among the Norwalk-like agents [26]. The seroprevalence of NV IgG in the different ethnic groups may reflect exposure to NV or an antigenically related virus. This is possible as varying levels of seroresponse to NV and SRSVs from other genogroups have been shown in adult volunteer studies [27]. This is also supported by the fact that two different, but closely related, viruses from the Norwalk-like genogroup have been shown to circulate in different geographic regions of South Africa at different time periods [19]. The NV seroprevalence rates in urban and rural communities in different geographic regions of South Africa needs to be ascertained before the full impact of NV infection in South Africa can be assessed, and the source of virus identified.

The results of this investigation support the premise that NV infection is ubiquitous [22], and although antibodies are acquired early in life the role of NV as a paediatric pathogen has yet to be established. In addition the mechanisms responsible for resistance or susceptibility to NV infection are still unclear. However, data presented provides valuable information toward the elucidation of NV epidemiology.

ACKNOWLEDGEMENTS

We would like to thank Professor M. K. Estes and Dr Xi Jiang from the Division of Molecular Virology, Baylor College of Medicine, Texas Medical Center, Houston, Texas for the reagents for the rNV EIAs; Dr M. B. Taylor acknowledges travel grants from the Poliomyelitis Research Foundation, and the Faculty of Medicine, University of Pretoria, South Africa to visit the Institute of Child Health, London. Thanks are also due to Ms L. Engelbrecht for technical assistance.

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