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BRIEF REPORT

Seroprevalence distribution of Aichi virus among a French population in 2006–2007

Marianne Goyer · Ludwig-Serge Aho · Jean-Baptiste Bour · Katia Ambert-Balay · Pierre Pothier

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Abstract Little is known about the epidemiology of Aichi virus, which is a new member of the family *Picornaviridae*, in the genus *Kobuvirus*. We report here on seroprevalence in France. Sera were screened using an enzyme-linked immunosorbent assay (ELISA) for immunoglobulin G. Of 972 sera tested, seroprevalence ranged from 25% for the 7-month-to-9-year-old age group to about 85% for the 30-to-39-year-old age group and older age groups. Our ELISA correlated well with the microneutralization technique. This study shows that Aichi virus is quite frequent in France and that seroconversion occurs before the age of 40.

Acute gastroenteritis is one of the most common diseases worldwide. It leads to high morbidity and mortality in children. Most of these diarrheal diseases have a viral origin. The most frequent viral etiologic agents are rotaviruses, noroviruses, and adenoviruses [6]. Nevertheless, in many cases of non-bacterial gastroenteritis, no etiological agent is recovered. In this context, Aichi virus was first isolated in 1989 by Yamashita et al. [7]. It is a new member of the family *Picornaviridae* and is classified in a new genus named *Kobuvirus*. In Humans, Aichi virus seems to cause acute gastroenteritis outbreaks, especially associated with the consumption of oysters.

However, few studies report on the features of Aichi virus and its impact on human health. Most studies have been conducted in Asia: Aichi virus antigen or viral RNA was found in fecal samples collected in Japan [8]; Aichi virus was also isolated in Pakistani children, in tourists back from Southeast Asia [9] and among patients from Bangladesh, Thailand, and Vietnam [4]. It also has been encountered in Brazil, Germany, and France. The first seroprevalence study was conducted in Japan and showed that this virus is quite frequent [8]. Another study in Europe (Germany) led to similar conclusions [3].

We report here on the prevalence of Aichi virus in France, assessed using a serological study of 972 randomized samples.

Serum samples were collected between September 2006 and March 2007 from French subjects at the University Hospital of Dijon, France.

A total of 972 sera were collected. They were divided into 10 groups according to age, each group covering 10 years of age (7 months–100 years old). In each age group, 100 sera were randomly selected (except 72 sera for the 7-month-to-9-year-old group).

Sera samples were stored at −20°C.

The Aichi virus strain was kindly provided by T. Yamashita. Aichi virus was grown on Vero cells, recovered from cell lysates, and divided into aliquots at $10^{8.5}$ $TCID_{50}$/mL.

The aliquots were stored at −80°C. Viral antigen was prepared by clarification of cell lysates (2,465 g, 20 min, 4°C), then titrated at 10^7 $TCID_{50}$/mL. Mock-infected Vero cells were similarly prepared.

Plates (Maxisorp, Nunc, cat. no. 469949) were coated with viral antigen diluted at 10^5 $TCID_{50}$ in 100 μL of PBS

M. Goyer · J.-B. Bour (✉) · K. Ambert-Balay · P. Pothier
Laboratory of Virology, University Hospital of Dijon,
BP 77908, 21079 Dijon, France
e-mail: jbbour@u-bourgogne.fr

L.-S. Aho
Hygiene and Epidemiology, University Hospital of Dijon,
BP 77908, 21079 Dijon, France

(pH = 7.4) for 1 h at 37°C, then blocked (skimmed milk 3% in PBS) 30 min, 37°C. Mock plates were similarly prepared. Serum samples were applied at 1/100 dilutions in PBS and incubated at 37°C for 30 min. After five rinses with 0.1% Tween 20 in PBS, conjugate was added (anti-human IgG HRP, Southern Biotech, cat. no. 904005) at 1/20,000 in PBS for 30 min at 37°C. After another five rinses, enzymatic activity was measured 10 min later with TMB as substrate (450 vs. 620 nm). Each sample was also tested on mock wells.

In each run, positive and negative calibrators were added to antigen and mock plates respectively, allowing alignment of sample optical density (OD). Distribution of background signals (OD on mock) gave 95% of samples below OD = 0.188. Therefore, a signal >0.188 on viral antigen was considered positive. The few samples giving a noise >0.188, signal/noise ratio <1.1 were considered negative.

Thirty-two ELISA-positive sera and 31 ELISA-negative sera were randomly selected in the 10–29-year-old group. An eight-fold to 256-fold dilution of each serum was mixed with 100 µL of viral strain (100 $TCID_{50}$) and was incubated 2 h at 37°C and then overnight at 4°C. The mixtures were then inoculated on Vero cells cultivated in a 96-well plate. Neutralization titer was defined as the reciprocal of the highest dilution of serum inhibiting any cytopathic effect in Vero cells.

All analyses were performed with STATA Software (version 8). Seroprevalence levels were compared by means of 95% confidence intervals established using the binomial exact method.

Comparison of neutralization titers between the two groups (ELISA-positive and ELISA-negative) were done using Kruskall–Wallis test.

Neutralizing titers were linearly correlated with ELISA-titers by Spearman's test.

Distribution of ELISA-positive titers for each age group were analyzed by the Kruskall-Wallis test and non-parametric trend test.

Seroprevalence was calculated for each age group, based on ELISA results (Fig. 1).

Among the 972 sera collected from patients aged from 7 months to 100 years, we obtained seroprevalence increasing from 25% (age group of 7 months to 9 years old) to around 85% (age older than 30). From 30–39 to 100 years old, seroprevalence showed no significant variation.

When tested by neutralization, the titers given by ELISA-positive sera were significantly different from those given by ELISA-negative sera (Kruskall–Wallis test, $P = 0.0001$).

There was a linear correlation between neutralization titers and OD (Spearman's $\rho = 0.69$ $P = 0.0001$ for ELISA-positive; Spearman's $\rho = 0.43$ $P = 0.013$ for ELISA-negative).

Distribution of ELISA-positive titers was studied among age groups (Fig. 2).

Statistical analyses of ELISA-positive titers showed some significant differences among age groups (Kruskall-Wallis test, $P = 0.0001$). The non-parametric trend test indicated lower levels in young age groups ($P = 0.029$).

This is the first seroprevalence study on Aichi virus conducted in France. It is based on the largest sample size, and covers the broadest age spectrum, together with the most accurate confidence intervals. We used immunoenzymatic testing (direct ELISA), which proved to correlate well with the microneutralization technique.

Seroprevalence against Aichi virus increased steadily from 25% in the 7-month-to-9 year-old age group to 84% in the 30-to-39-year-old group, and then leveled off at around 85–90% in the 40-to-100-year-old group with no significant variations between age groups after age 40. This profile is quite similar to those obtained in Japan by Yamashita et al. [8], which reached a plateau (83%) at 39 years old, and in Spain [2] with 93% at 40 years old. The other seroprevalence study was conducted in Germany [3] and showed a similar level of maximum seroprevalence (86%), which was reached much earlier: all the seroconversions had occurred by the age of 19 years old. In our study, the increase in seroprevalence was regular, as was the case in Japan with the same slope for similar levels among young children. In Germany, despite the bias regarding maternal antibodies (because infants were included at birth), children were more often seropositive whatever their age. The prevalence in young Spanish children was comparable to that found in ours and to that in Japanese children. In Spain, however, seroprevalence seems to progress more quickly in those between 10 and 20 years of age.

Taken together, these serological data could indicate some differences about the epidemiology of Aichi virus in these countries. In Germany, children seem to encounter the virus very early, whereas in Spain, young teenagers tend to be infected. In France, like in Japan, the virus appears to circulate among infants as well as in young adults. In our study, there was no more progress in seroprevalence after 39 years old, indicating that most seroconversions occur before the age of 40 in France, as is the case in Japan and Spain. For similar reasons, primary infections should be rare in Germany after the age of 20.

The maximum seroprevalence we determined here was high, 92%, which is similar to levels described elsewhere. Surprisingly, this contrasts with the low incidence of Aichi virus infections, reported at least in Europe. In France, it has been found in only 6 of 110 outbreaks (9 patients among 36 investigated) [1]. Moreover, during 10 years of comprehensive surveillance in The Netherlands, and 188 outbreaks (in which no norovirus was previously found) [6], Aichi virus

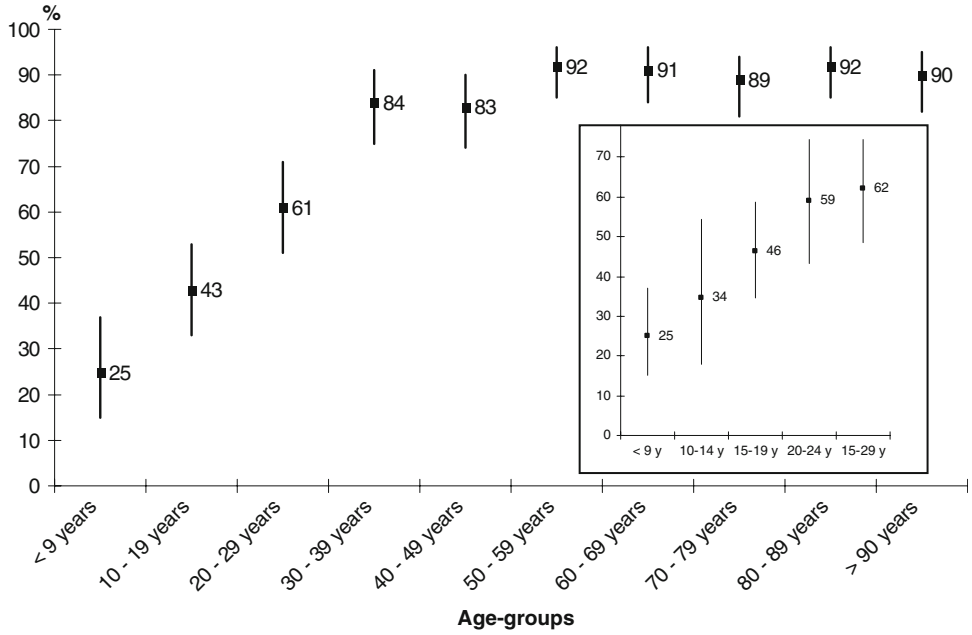

Fig. 1 Age-related seroprevalence (%) of IgG antibodies to Aichi virus in the French population. The window shows more detail below 29 years

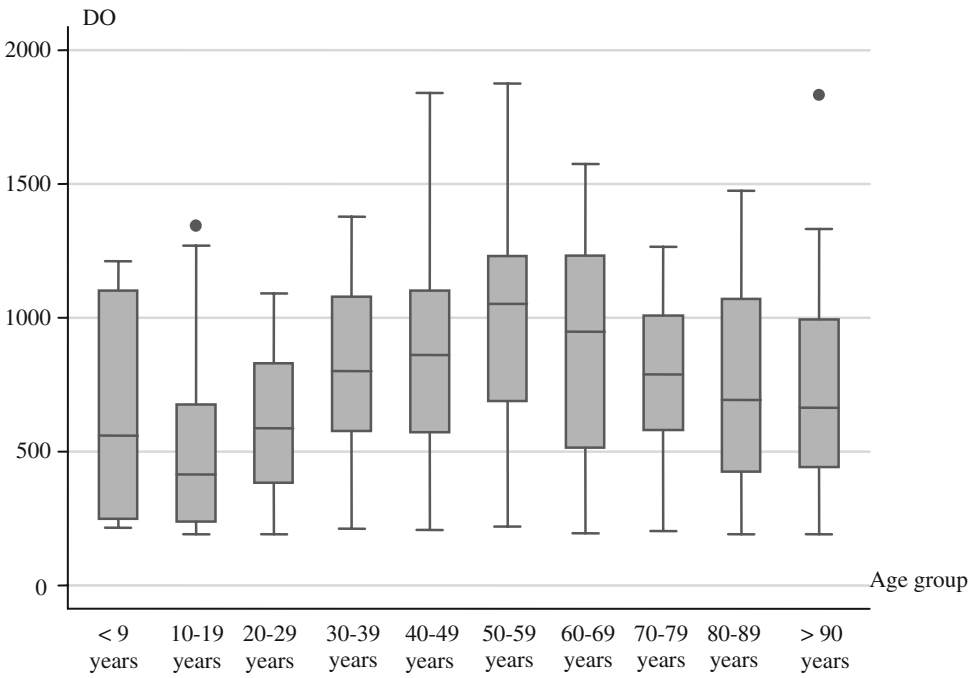

Fig. 2 Box plots detailing distribution of positive IgG titers in ELISA for each age group

was never diagnosed. Even in South East Asia, it is seldom found in patients with gastroenteritis [4, 7–9].

Analysis of the positive titers indicated that their distribution among age groups was not homogeneous: the younger the age, the lower the titer. Thus, aging seems to be associated with increase in antibody titer. This may reflect reinfections, which may be asymptomatic, leading to anamnestic responses and increased serum reactivity.

As IgM is a serological marker of recent primary infections, we tried an indirect, serological approach to determine the incidence of Aichi virus infections, looking for IgM in sera positive for IgG, randomly chosen from samples collected at ages where the prevalence progressed quickly (7 months–29 years). None of the samples gave a positive result, even at the 1/10 dilution (by IFA on infected Vero cells). This seems to corroborate what is so far known about the low incidence of Aichi virus infections, and thus it is not surprising; T. Yamashita already showed that a poor serological response can follow an authentic infection and that there may be no serological response at all: only 20 of 43 paired sera showed seroconversion or increased antibodies in their convalescent serum [8] (24 of 56 in another study [12]).

However, this also contrasts sharply with our high level of seroprevalence.

This incongruity between the rare isolation of Aichi virus and high seroprevalence suggests that the virus might circulate without causing any symptoms or that it could be responsible for some weakly symptomatic infections that do not require medical attention. Its pathogenicity has even been considered doubtful [6, 10, 11]. Nevertheless, except for only one Japanese control child, Aichi virus has never been detected in any of the published controls. Moreover, it was clearly the only diagnosed virus in the German samples [3], in 6 of 13 Aichi virus-infected French patients [1] and in two-thirds of the Tunisian children studied [5]. Furthermore, estimates of the frequency of Aichi virus could be biased in that investigations for this virus mostly occur when other viruses have not been found [4, 6], even though it could co-circulate with norovirus or other enteric viruses, as shown in Brazil [3], France [1], and Tunisia [5].

We report here serological evidence of Aichi virus circulation in France. Seroprevalence progressed from young infants to young adults, showing that most of the seroconversions occur before the age of 40. As in Japan, Spain, and Germany, the high level of maximum seroprevalence in adults indicates widespread exposure to Aichi virus during infancy. Yet Aichi virus seems to be seldom found during outbreaks; it should be screened for, even if other enteric viruses have already been found. Studies need to be conducted on the incidence and behavior of Aichi virus in order to gain better understanding of its epidemiology and pathogeny.

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References

1. Ambert-Balay K, Lorrot M, Bon F, Giraudon H, Kaplon J, Wolfer M, Lebon P, Gendrel D, Pothier P (2008) Prevalence and genetic diversity of Aichi virus community and hospitalized patients. J Clin Microbiol (In press)
2. Buesa J (2007) Personal communication, 28 Sept 2007
3. Oh DY, Silva PA, Hauroeder B, Diedrich S, Cardoso DDP, Schreier E (2006) Molecular characterization of the first Aichi viruses isolated in Europe and in South America. Arch Virol 151:1199–1206
4. Pham NT, Khamrin P, Nguyen TA, Kanti DS, Phan TG, Okitsu S, Ushijima H (2007) Isolation and molecular characterization of Aichi viruses from fecal specimens collected in Japan, Bangladesh, Thailand, and Vietnam. J Clin Microbiol 45:2287–2288
5. Sdiri K, Gharbi-Khelifi H, De Rougemont A, Chouchane S, Sakly N, Ambert-Balay K, Hassine M, Guediche MN, Aouni M, Pothier P Acute infantile gastroenteritis associated with human enteric viruses in Tunisia. J Clin Microbiol (In press)
6. Svraka S, Duizer E, Vennema H, de Bruin E, van der Veer B, Dorresteijn B, Koopmans M (2007) Etiological role of viruses in outbreaks of acute gastroenteritis in The Netherlands from 1994 through 2005. J Clin Microbiol 45:1389–1394
7. Yamashita T, Kobayashi S, Sakae K, Nakata S, Chiba S, Ishihara Y, Isomura S (1991) Isolation of cytopathic small round viruses with BS-C–1 cells from patients with gastroenteritis. J Infect Dis 164:954–957
8. Yamashita T, Sakae K, Ishihara Y, Isomura S, Utagawa E (1993) Prevalence of newly isolated, cytopathic small round virus (Aichi strain) in Japan. J Clin Microbiol 31:2938–2943
9. Yamashita T, Sakae K, Kobayashi S, Ishihara Y, Miyake T, Mubina A, Isomura S (1995) Isolation of cytopathic small round virus (Aichi virus) from Pakistani children and Japanese travelers from Southeast Asia. Microbiol Immunol 39:433–435
10. Yamashita T, Sakae K, Tsuzuki H, Suzuki Y, Ishikawa N, Takeda N, Miyamura T, Yamazaki S (1998) Complete nucleotide sequence and genetic organization of Aichi virus, a distinct member of the Picornaviridae associated with acute gastroenteritis in humans. J Virol 72:8408–8412
11. Yamashita T, Sugiyama M, Tsuzuki H, Sakae K, Suzuki Y, Miyazaki Y (2000) Application of a reverse transcription-PCR for identification and differentiation of Aichi virus, a new member of the Picornavirus family associated with gastroenteritis in humans. J Clin Microbiol 38:2955–2961
12. Yamashita T, Ito M, Tsuzuki H, Sakae K (2001) Identification of Aichi virus infection by measurement of immunoglobulin responses in an enzyme-linked immunosorbent assay. J Clin Microbiol 39:4178–4180