

# Shifting Seroepidemiology of Hepatitis A in Japan, 1973–2003

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**Abstract: Background.** Hepatitis A infection is caused by hepatitis A virus (HAV) contracted through fecal-oral transmission. Life-long immunity is conferred after infection. Improved sanitary conditions have generally resulted in a significant decline in the incidence of hepatitis A. However, a low incidence of infection results in increased HAV susceptibility. The present study investigates the prevalence of anti-HAV antibody and clarifies the current HAV status and HAV susceptibility in Japan at 2003. **Methods.** A total of 2,430 serum specimens collected during 2003 from Japanese individuals ranging in age from 0–92 years, were tested for anti-HAV antibody using an inhibition enzyme linked immunosorbent assay. All specimens were obtained from the WHO and the National Serum Reference Bank/National Institute of Infectious Diseases, Tokyo, Japan. **Results.** The overall seroprevalence was 12.2%. Anti-HAV antibodies were rarely detected in individuals between 0–44 years of age. Starting from the age of 45–49 years, seropositivity gradually increased through age 65 years and above. Seroprevalence was not affected by gender, and geographic distribution did not affect age-specific seroprevalence until the age of 60 years. **Conclusions.** HAV susceptibility in Japan is increasing annually. Particularly, the prevalence of anti-HAV antibody in individuals older than 50 years in 2003 was 50.3%, which is significantly lower than that of corresponding studies in 1994 (74.3%), 1984 (96.9%) and 1973 (96.9%). The growing susceptible population of advanced age results in more frequent HAV infection among them. The surveillance of anti-HAV antibody prevalence is useful for implementing preventive measures and for controlling the spread of HAV.

**Key words:** Hepatitis A, Seroepidemiology, Anti-HAV antibody

Hepatitis A virus (HAV) is a pathogen of human acute hepatitis and is considered to be one of epidemiologically important viruses. It is a positive-strand RNA virus belonging to the Family *Picornaviridae*, genus *hepatovirus* (17).

Most HAV infections occur through fecal-oral transmission, either by direct contact with an infected person or by ingestion of food or water contaminated with HAV (5). Improved sanitary conditions and hygiene practices have reduced the incidence of hepatitis A infection. Children who become infected are usually asymptomatic or develop mild symptoms, whereas adults infected with hepatitis A develop fever, fatigue,

malaise and jaundice that lasts between 4 and 10 weeks. Regardless of symptoms, seroconversion occurs after infection and convalescent individuals develop permanent immunity (13).

Hepatitis A virus has only one known serotype and anti-HAV antibody (anti-HAV) induced by infection or vaccination protects individuals against infection with any HAV strains (14). Both immunoglobulin M (IgM) and immunoglobulin G (IgG) types of anti-HAV antibodies are detectable during the early stages of illness. Levels of IgM antibody usually diminish within 2 or 3 months after infection, but IgG antibody confers life-long immunity. Individuals without anti-HAV antibodies are susceptible to HAV infection.

Improved socioeconomic status, urbanization, ethnic origin, and access to clean water and sanitation have

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**Abbreviations:** anti-HAV, anti-hepatitis A virus antibody; ELISA, enzyme linked immunosorbent assay; GMT, geometric mean titer; HAV, hepatitis A virus; IgG, immunoglobulin G; IgM, immunoglobulin M; OD, optical density.

changed hepatitis A epidemiology. The distribution of anti-HAV seroprevalence by age group might reflect current hepatitis A status in various countries and regions (7, 11).

In highly endemic areas, people become infected with HAV during early childhood and acquire anti-HAV antibodies before 10 years of age. As infections in early childhood are either asymptomatic or mild, hepatitis A is not a clinical problem and the virus is recognizable only by serological studies or the occurrence of infection among visitors.

In intermediate or transition areas, HAV circulation has been reduced by improving hygiene conditions and socioeconomic status. Those who become infected are older in such areas than in highly endemic areas. The incidence of symptomatic hepatitis A is higher in adolescents and young adults. Frequent outbreaks of hepatitis A are recognized as serious public health problems (21).

In low endemic areas, rare incidence results in the accumulation of non-immune populations. Susceptible adults who are exposed to HAV infection and symptomatic hepatitis A are mostly high risk groups such as child care providers, hospital workers or family members with direct patient contact, travelers to endemic areas, drug abusers and men who have sex with men.

The incidence of hepatitis A has decreased in Japan mainly due to improved sanitary conditions and hygiene practices (Fig. 1) (15) and thus an HAV-susceptible population has developed.

This study examines the current seroprevalence of hepatitis A in Japan, and describes the seroepidemiological shift over the past three decades. The results

should provide useful epidemiological information upon which to base decisions that will prevent and control the spread of hepatitis A.

## Materials and Methods

*Serum specimens.* A total of 2,430 serum specimens (1,242 and 1,188 from males and females, respectively, aged between 0 and 92 years) were collected from 12 Japanese prefectures during 2003 and tested for anti-HAV. All specimens were obtained through the WHO and National Serum Reference Bank/National Institute of Infectious Diseases, Tokyo, Japan and stored at  $-20^{\circ}\text{C}$ . The specimens were separated into groups based on age, gender and geographic location for further analysis.

*Anti-HAV screening.* Total anti-HAV antibody was determined for all specimens using an inhibition enzyme linked immunosorbent assay (ELISA) as described (12). Briefly, purified inactivated HAV (KRM003 strain, genotype IIIB) was bound to microtiter plates (Nunc, F96 CERT.MAXISOAP, Denmark) coated with anti-HAV rabbit serum (HAV-plates). Non HAV-binding wells prepared on each HAV-plate comprised positive controls (100% inhibition). Negative controls comprised several HAV-binding wells. Diluted test specimens (1:32) were added to appropriate wells. Equal volumes of diluents (phosphate-buffered saline (pH 7.2) containing 0.5% skim milk and 0.05% Tween 20) were added to both positive and negative control wells. Plates were incubated overnight at  $4^{\circ}\text{C}$  and then the wells were emptied. Diluted horseradish peroxidase (HRPO)-conjugated anti-HAV rabbit IgG was added to the wells and incubated at  $37^{\circ}\text{C}$  for 2 hr in

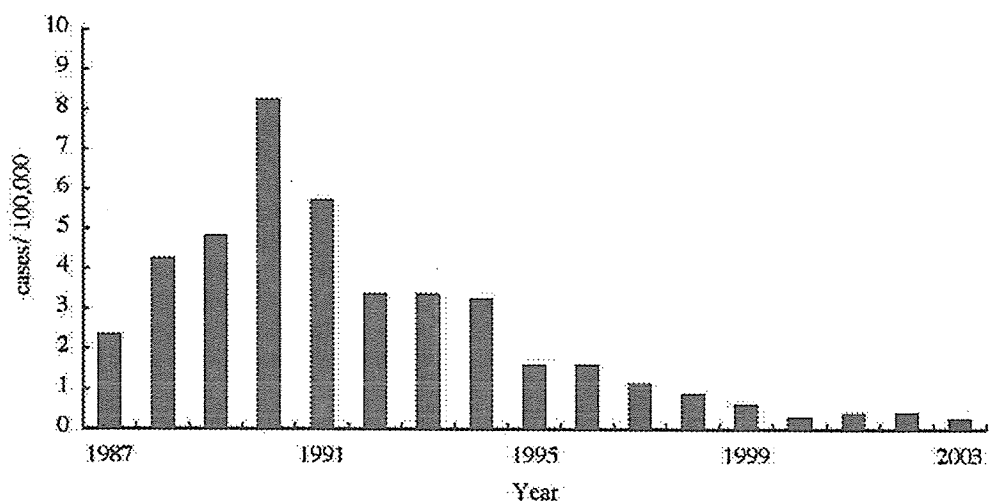


Fig. 1. Reported incidence of hepatitis A, Japan, 1987–2003. Data are cited from Ref. 15.

a humidified atmosphere. Plates were washed and 100  $\mu$ l of substrate *o*-phenylenediamine (Tokyo Chemical Industry Co., Ltd., Japan) was applied to each well. Thirty minutes later, reactions were stopped by adding 100  $\mu$ l of 2 N H<sub>2</sub>SO<sub>4</sub> and the optimal density (OD) was measured at 492 nm in an ELISA plate reader (Corona, Japan). The inhibition rate was measured using the following formula:

Inhibition rate (%) =  $(N-S)/(N-P) \times 100$  where  $N$  = mean OD<sub>492</sub> value of negative controls,  $P$  = mean OD<sub>492</sub> value of positive controls and  $S$  = mean OD<sub>492</sub> value of specimens. Specimens with non-specific responses detected in the same manner using the non HAV-binding plates were excluded from this study.

**Titration of anti-HAV positive specimens.** Titers of anti-HAV were determined in all positive specimens and are expressed as mIU/ml using the parallel line assay (19) with a national human anti-HAV reference calibrated with a WHO standardized preparation. All positive sera were separated into five age categories as follows: 0–49 years ( $n=32$ ), 50–59 years ( $n=103$ ), 60–69 years ( $n=71$ ), 70–79 years ( $n=60$ ) and 80–92 years ( $n=31$ ). The geometric mean titer (GMT) of anti-HAV in each age group was determined.

**Data analysis.** All data were statistically assessed by the  $\chi^2$ -test or one-factor ANOVA using Statcel software (version 1; OMS, Japan, 1998).  $P < 0.05$  was accepted as the minimal level of significance.

## Results

### Prevalence of Anti-HAV

Figure 2 shows that all specimens plotted by inhibition rate were divided into two groups. Specimens with inhibition rates above 80% were regarded as anti-HAV positive. The cutoff inhibition rate of 80% was equivalent to 352.3 mIU/ml. Of the 2,340 serum specimens, 297 were anti-HAV positive (Table 1). Anti-HAV prevalence remained very low until the age of 45–49 years. Most individuals below or equal to 49 years of age were HAV susceptible. Seroprevalence gradually increased among persons above 50 years of age. The highest seroprevalence was 86.5% in the oldest age group ( $\geq 65$  years). Seroprevalence between males (158/1,242) and females (139/1,188) did not significantly differ ( $P=0.59$ ).

Geographic differences in anti-HAV seroprevalence in the Tohoku (north area), Kanto (central east area), Kansai (central west area) and Kyushu-Yamaguchi (south area) regions of Japan were analyzed over the age groups 0–9, 10–19, 20–29, 30–39, 40–49, 50–59 and over 60 years old (Fig. 3). Age-specific seroprevalence was very similar in populations under 59 years of age in each area. Statistical differences were evident in individuals over 60 years of age between Tohoku and Kansai ( $P=0.0066$ ), and between Kansai and Kyushu-

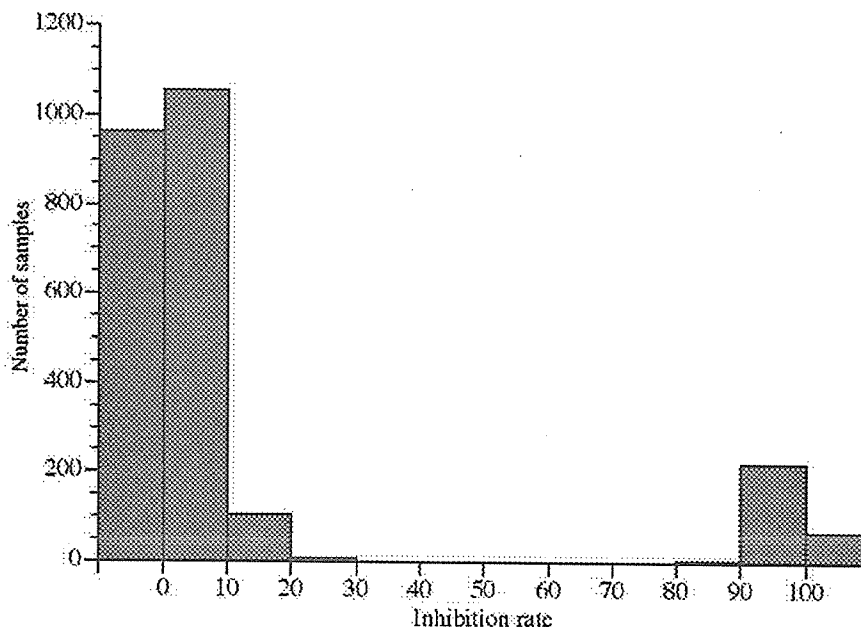


Fig. 2. Distribution of inhibition rates. Sera were divided into two groups by inhibition rate. Sera over 80% inhibition corresponding to 352.3 mIU/ml of anti-HAV antibody were identified as anti-HAV positive.

Yamaguchi ( $P=0.012$ ).

#### Titration of Anti-HAV Positive Specimens

The GMT values of anti-HAV were 7,486 (95% confidence interval, 4,437–12,629), 7,397 (6,061–9,027), 6,210 (4,742–8,132), 7,944 (5,917–10,666), and 5,007 (3,351–7,481) mIU/ml for the age groups 0–49, 50–59, 60–69, 70–79 and 80–92 years old, respectively (no significant difference,  $P=0.34$ ).

#### Discussion

In 2003, the overall anti-HAV prevalence in Japan was 12.2% (Table 1) and gender did not affect this value. The GMT of anti-HAV in five age groups

remained high and did not change with age ( $P=0.34$ ). This confirmed that individuals who acquired anti-HAV immunity would retain high titers of anti-HAV and life-long immunity. Fifty percent of individuals over 50 years of age had anti-HAV whereas only 1.68% of people younger than 50 years of age had immunity. Because of the low incidence of hepatitis A, fewer younger people would acquire HAV-immunity, whereas anti-HAV positive individuals who mostly belong to older age groups have retained anti-HAV from past exposure. Seroprevalence also remained very low in individuals under the age of 40–44 years who were born around 1960. This finding implies that the incidence of hepatitis A in Japan has been rare since 1960. The seroprevalence of the oldest age group (over 65

Table 1. Age-specific prevalence of anti-HAV in Japan, 2003

Area Prefecture Age (years)	Tohoku						Kanto							
	Akita		Yamagata		Miyagi		Fukushima		Niigata		Ibaraki		Nagano	
	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
0–4	13	0	NA	NA			16	0	NA		19	0	9	0
5–9	23	0	NA	NA			11	0	NA		21	0	23	0
10–14	8	0	NA		2	0	14	0	NA		15	0	NA	
15–19	17	0	NA		27	0	17	0	NA		7	0	4	0
20–24	4	0	1	0	14	0	2	0	NA		9	0	43	1
25–29	14	0	NA		11	0	23	0	1	0	14	0	12	0
30–34	9	0	NA		7	0	17	0	32	1	14	0	21	0
35–39	11	2	NA		1	0	8	0	29	3	11	0	14	1
40–44	15	0	NA		9	1	21	0	26	1	6	0	24	0
45–49	9	2	19	0	8	0	4	0	19	3	10	2	27	0
50–54	14	5	13	2	14	2	11	3	26	6	9	1	19	3
55–59	11	6	12	7	6	1	14	5	20	11	9	2	14	8
60–64	4	4	6	3	1	1	5	4	2	1	5	3	8	7
65–	23	23	19	19		NA	18	14		NA		NA	14	13
Total	175	42	70	31	100	5	181	26	155	26	149	8	232	33

Area Prefecture Age (years)	Kansai				Kyushu-Yamaguchi						Total		
	Fukui		Kyoto		Yamaguchi		Fukuoka		Saga		Tested	Positive	%
	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	%
0–4	NA		25	0	34	2	16	0	24	0	156	2	1.3
5–9	NA		34	0	35	0	46	0	26	0	219	0	0
10–14	NA		24	0	71	0	35	0	18	0	187	0	0
15–19	NA		19	0	44	0	45	0	18	0	198	0	0
20–24	1	0	24	0	42	1	43	0	12	0	195	2	1
25–29	2	0	27	0	46	3	38	1	15	1	203	5	2.5
30–34	NA		19	0	41	0	37	0	14	0	211	1	0.5
35–39	NA		24	0	42	0	34	1	12	0	186	7	3.8
40–44	13	0	32	0	17	1	12	0	13	0	188	3	1.6
45–49	5	1	23	3	11	0	13	1	12	0	160	12	7.5
50–54	15	5	32	4	17	5	8	0	15	6	193	42	21.8
55–59	4	2	26	12	11	3	3	2	7	2	137	61	44.5
60–64	NA		9	5	2	2	1	1	6	3	49	34	69.4
65–	NA		26	19	26	23		NA	22	17	148	128	86.5
Total	40	8	344	43	439	40	331	6	214	29	2,430	297	12.2

NA: not available.

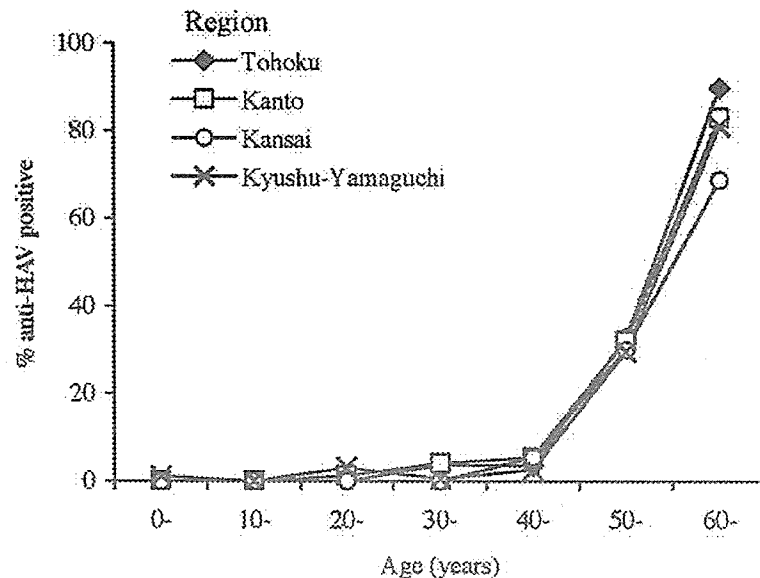


Fig. 3. Geographic distribution of anti-HAV antibody. Geographic distribution does not significantly differ among 4 areas of Japan until the population reaches 60 years of age. Seroprevalence in individuals over 60 years old significantly differs between Kansai and Tohoku ( $P=0.0066$ ), and between Kansai and Kyushu-Yamaguchi ( $P=0.0121$ ).

years old) is the highest at 86.5%. Individuals within this population, born before 1940, presumably acquired immunity during an era when hepatitis A was endemic in Japan. Between 1940 and 1960, risk of HAV-exposure was decreased by rapid infrastructural improvement, including hygiene conditions particularly after World War II.

Interestingly, regional differences in seroprevalence were evident only in individuals over 60 years of age (Fig. 3). We surmise that when HAV was endemic over 60 years ago, the frequency of hepatitis A endemicity varied across locations. However, regional features might have no impact on current hepatitis A status.

Figure 4 shows the shift in anti-HAV seroprevalence in Japan during the past three decades. The shapes of seroprevalence curves derived from studies reported in 1973, 1984, 1994 (12) and 2003 have remained similarly sigmoidal, and shift towards older groups in 10-year increments. Numbers of HAV-susceptible individuals have accumulated not only among younger generations, but also among older age groups. The seroprevalence of anti-HAV in individuals older than 50 years in 2003 was 50.3%, which is significantly lower than that of corresponding studies in 1994 (74.3%), 1984 (96.9%) and 1973 (96.9%). This has led to a shift in hepatitis A patients towards older age groups, in which clinical illness is more frequent and severe (Fig. 5) (8, 13).

Hepatitis A is one of the major vaccine preventable

diseases. Hepatitis A vaccine is an inactivated preparation of cell-culture adapted HAV, and has been used in many parts of the world (20). Several hepatitis A mass vaccination programs have been started since 1997 in regions of intermediate endemicity. A universal vaccination program in the United States of America resulted in a 76% decline in the overall incidence of hepatitis A to 2.6 per 100,000 reported in 2003, compared with 10.7 per 100,000 during the baseline period of 1990–1997 (18). A study in Israel found that a toddler-only universal vaccination program effectively reduced the incidence of hepatitis A by 95% (4). In that study, the annual pre-vaccination incidence of 50.4 per 100,000 declined post-vaccination to 2.2–2.5 per 100,000. The authors of these reports suggested that this decline was not solely a result of the universal vaccination program but was also associated with improved environmental and hygiene conditions. However, both studies suggest that the implementation of a universal hepatitis A vaccination program for children provides a significant degree of immunity for individuals who live in intermediate epidemic regions. Determining the impact of hepatitis A on current health care resources and defining the benefit and cost of hepatitis A vaccination will greatly influence the decision as to whether to implement such programs in particular countries and population groups (1, 3). The recent incidence of hepatitis A of 0.34 per 100,000 in Japan is lower than the post-vac-

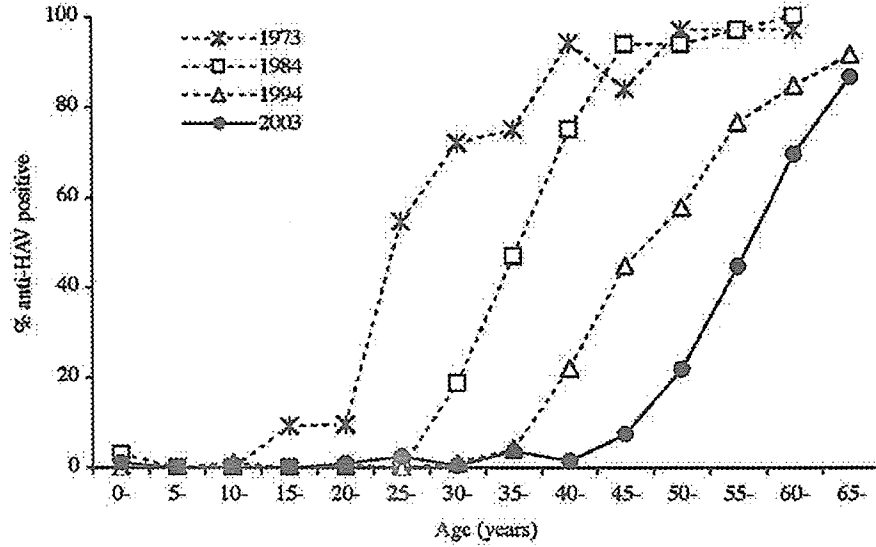


Fig. 4. Age-specific seroprevalence of anti-HAV from 1973 to 2003 in Japan. Data for 1973, 1984 and 1994 are cited from Ref. 12.

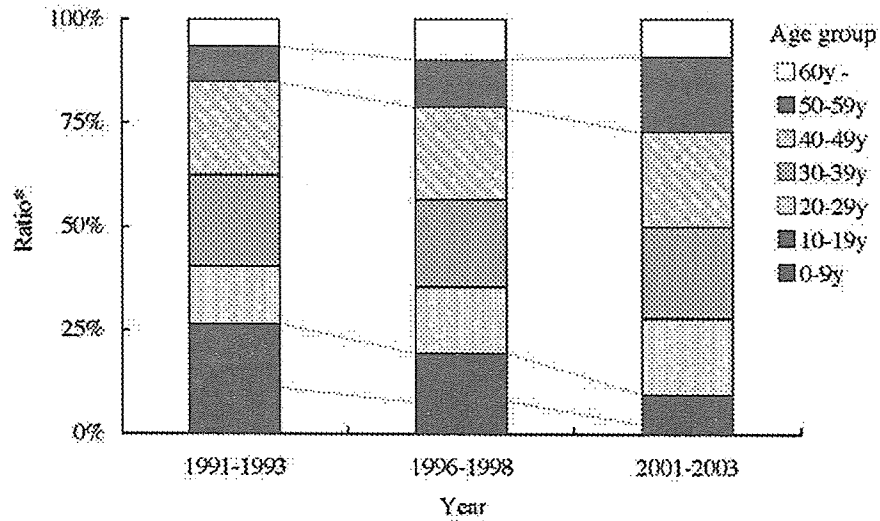


Fig. 5. Age-distribution of hepatitis A reported cases in Japan from 1991 to 2003. Data are cited from Ref. 15. Ratio of reported cases aged 50–59 increased during the period 1989 to 2003. Simultaneously, ratio of the 0–9 and 10–19 age groups decreased. \* Ratio=Number of reported cases per age group in each study period/all reported cases in each study period (%). Study period was separated into 3-year intervals.

ination incidence in studies reported from the United States and Israel, namely 2.6 and 2.2–2.5 per 100,000, respectively. The current incidence of hepatitis A in Japan might be too low to warrant the introduction of a universal vaccination policy.

Hepatitis A infection might not be considered as a disease of great importance in Japan due to its low frequency and low mortality rate. However, our study

suggests that susceptibility to hepatitis A among Japanese is increasing. As in other low HAV-endemic areas of the world, the major risk factors for hepatitis A in Japan have been travel to endemic areas (16), consumption of contaminated food including that imported from HAV endemic areas (6), patients medicated with clotting factors (9) and men who have sex with men (2). In addition, immunization of food handlers would seem

worthwhile, to prevent the possibility of food-borne, common-source outbreaks of hepatitis A. Furthermore, people with chronic liver disease are not more likely to be infected with hepatitis A virus, but they are more likely to develop fulminant hepatitis and die if infected (10).

In conclusion, the timely release of information regarding the current status of hepatitis A together with a vaccination program for high-risk groups, food handlers and persons with chronic liver disease in Japan would be suitable for the control of this virus at present.

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