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Epidemiological and serological study of hepatitis A virus outbreaks in France: a comparison between immunoadherence and radioimmunoassay

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SUMMARY

An epidemiological study and the simultaneous evaluation of anti-HAV antibody using radio immunoassay (RIA) and immunoadherence hemagglutination assay (IAHA) was performed during three hepatitis A epidemics in the Tours area (France). Fifty-seven sera from 35 subjects with viral hepatitis type A and 16 sera from nine children who did not develop any clinical signs of hepatitis were studied. The more explosive epidemic occurred in an institution for mentally retarded children (attack rate 68%). The two major outbreaks observed were due to the introduction in the institutions of individuals infected with hepatitis A virus. Two out of three of the index cases had a seafood dinner three to four weeks before onset of jaundice. Sera taken one week after jaundice were always found to be anti-HAV positive by both RIA and IAHA, and sera taken more than three days before the appearance of jaundice were negative by both methods. Sera taken at the peak of the transaminase elevation were anti-HAV positive by RIA but only one out of two were positive by IAHA. The anti-HAV titre by RIA increased from the time of the appearance of jaundice and the highest titres, over 1/20000 were seen only after several months. Observations of subjects in close contact with patients who seroconverted without any manifestation of hepatitis, confirmed the existence of clinically mute infections.

INTRODUCTION

Hepatitis A virus (HAV) was first demonstrated in the faeces of volunteers infected experimentally with the MS1 strain, using immune electron microscopy (IEM) (Feinstone, Kapikian & Purcell, 1973). This technique was also used to detect antibody to HAV. This historical technique was time consuming and limited

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by interpretation errors (Almeida, Gay & Wreghitt, 1974; Gravelle *et al.* 1975; Dienstag *et al.* 1976). Since then, other serological methods have been developed such as complement fixation test (Provost *et al.* 1975) and immunoadherence hemagglutination assay (IAHA) (Miller *et al.* 1975). The latter method was able to detect anti-HAV in the early convalescence period of HAV infection. More recently, radioimmunoassay (RIA) and enzyme-linked immunoabsorbent assay (ELISA) have been developed for the detection of both HAV antigen and antibody (Purcell *et al.* 1976; Hall *et al.* 1977; Mathiesen *et al.* 1978; Drucker *et al.* 1979*b*). These more sensitive methods allow the detection of anti-HAV during the acute phase of the illness. Therefore, since anti-HAV in serum is evidenced by IAHA only during convalescence, a serum anti-HAV positive by RIA and negative by IAHA during the acute phase of the disease was assumed to indicate acute hepatitis A infection (Rakela *et al.* 1978). The detection of specific IgM antibody by RIA (Bradley *et al.* 1977) has also been shown to be suitable to differentiating between acute and convalescent phases. Hepatitis A epidemics from 1976 to 1978 in the Tours area (France) provided an opportunity to investigate the epidemiology of HAV infection. Simultaneous use of RIA and IAHA for the detection of anti-HAV was evaluated as the diagnostic tool. The kinetics of anti-HAV antibody after hepatitis A virus infection were studied.

POPULATION AND METHODS

Case definition

A case of hepatitis A infection was defined as an illness consisting of at least one of the following symptoms: excessive fatigue, nausea, vomiting, dark urine, abdominal discomfort and accompanied by elevation of liver enzyme (ALT). The index case in each outbreak was interviewed to establish his source of exposure to hepatitis A virus.

Study population

Two epidemics occurred in and around Tours in 1976 (Coursaget *et al.* 1977): 19 cases in a group of 60 children (epidemic I) and 3 cases in a group of 50 military recruits stationed in a supply depot (epidemic II). At the end of 1977 and the beginning of 1978, 24 cases of hepatitis A were diagnosed in a group of mentally retarded children and 5 other cases among their families and staff members (epidemic III). Fifty-seven serum samples were taken from 35 subjects who developed clinical hepatitis and 16 serum samples from 9 children who did not develop any clinical sign of hepatitis.

Investigation of the etiologic agent

Twenty-six stools collected from 23 hepatitis A cases involved in the three outbreaks were tested for the presence of HAV by immune electron microscopy (Coursaget *et al.* 1977).

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Anti-HAV detection

Immunoadherence hemagglutination assay

The technique used is similar to the one described by Moritsugu *et al.* (1976). The HAV used in this test was extracted from the livers of marmosets infected experimentally (Drucker *et al.* 1979). The antigen was purified by ultracentrifugation and CsCl banding. The immune adherence titre of the final preparation used as reactive antigen was 1/8.

Radioimmunoassay

HAVAB test (Abbott Laboratories). In this test sera were diluted to 1/20, 1/200, 1/2000 and 1/20000 in PBS.

Transaminases

The level of alanine amino transferase (ALT) was measured by automated kinetic method (system TR, Beckman Instruments). Results were expressed in international units (Normal value < 25 IU).

RESULTS

Epidemic curve (Fig. 1)

The outbreaks of hepatitis A occurred between four weeks and eight months after the onset of symptoms in the index cases. The time of onset of symptoms in the 19 hepatitis cases from epidemic I and in the 29 hepatitis cases from epidemic III are shown in Fig. 1. The two major outbreaks were started in the two institutions by a single case of hepatitis followed four to five weeks later by other cases affecting a significant number of residents (attack rate 32 and 68 % respectively). Epidemic I lasted eight months with secondary waves of cases. Epidemic III in an institution for mentally retarded children was more explosive and occurred in three waves of cases. No other departments in the hospital were infected.

In epidemics I and III, the time lapse between two cases did not exceed five to six weeks, and was sometimes as short as two weeks (a resident and his grandmother). However, in epidemic I, 11 weeks elapsed between the sixth and seventh hepatitis cases.

In the second institution (epidemic III) the area was disinfected by extensive washing with sodium hypochlorite. This did not appear to slow down the spread of the epidemic.

Index cases

In the military supply depot outbreak the first two cases diagnosed had eaten a seafood dinner four weeks before the onset of clinical signs. The index case of epidemic I also had a seafood dinner between three and four weeks before onset. In epidemic III the index case, a 5-year-old boy, had returned from holiday one month before.

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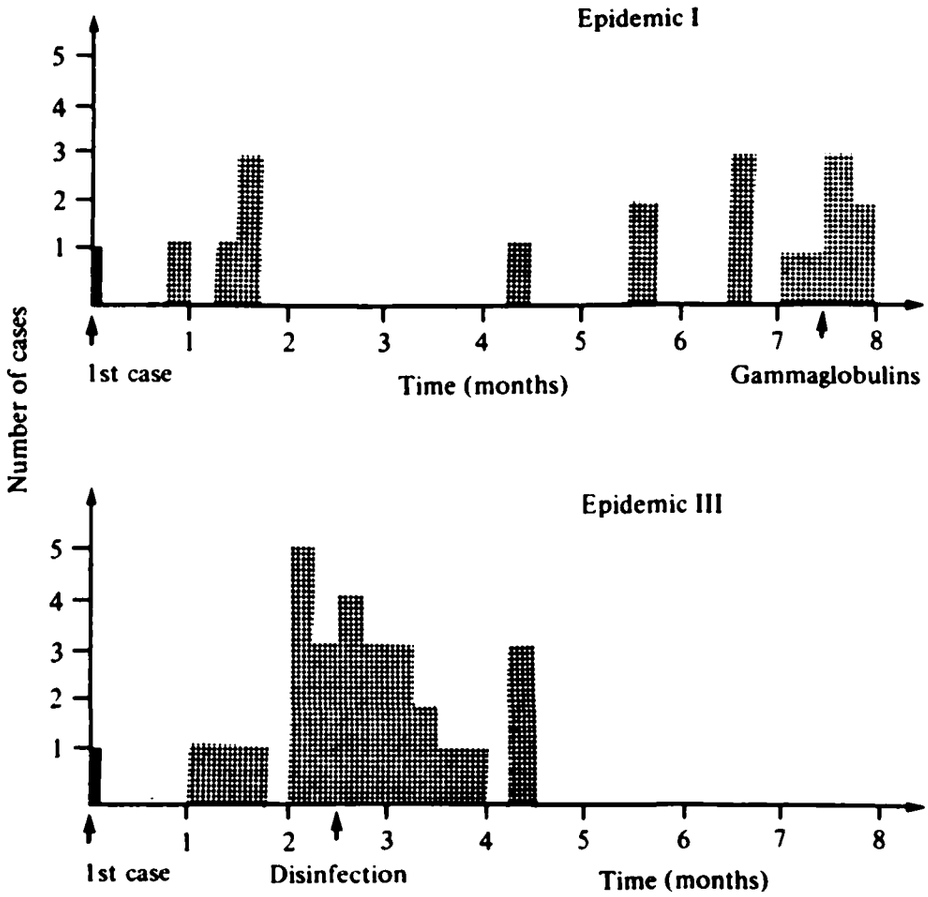

Fig. 1. Curves of epidemics of hepatitis A in two children's institutions.

Detection of the etiologic agent

HAV was evidenced in the stools of 11 patients. The strains isolated were morphologically and antigenically identical to the MS_1 reference strain (Coursaget *et al.* 1977).

Anti-HAV antibodies

Thirteen serum samples taken more than three days before the appearance of jaundice did not contain anti-HAV (RIA and IAHA tests). Only one of these sera showed evidence of a high level of transaminase (Table 1). Anti-HAV was detected by RIA in 14 sera taken at the time that a transaminase increase was evidenced (three days before to five days after onset of jaundice). Only six of these sera had detectable anti-HAV (by IAHA), while three were anticomplementary. All sera taken later in the course of the disease were found to be positive for anti-HAV by both RIA and IAHA. A seroconversion was observed in 12 cases when testing for anti-HAV by IAHA, but only eight of them seroconverted when testing with RIA.

The anti-HAV titre by RIA reached 1/200 at the time of appearance of jaundice, then increased more slowly to reach 1/2000 on the fifteenth day. The highest titres, up to 1/20000, were seen only after four months following the acute phase (Table 2).

Only one of nine children in contact with the infected subjects but without clinical and biochemical sign of hepatitis was found anti-HAV positive by both RIA and IAHA at the time of the outbreak. Blood samplings were drawn later from 6 of 8 of the remaining children. Testings revealed that four had seroconverted without symptoms in the intervening six to nine months (Table 3).

Table 1. *Anti-HAV antibody in children who developed hepatitis A*

Patient code no.	Age (years)	Time*	SGPT	Anti-HAV RIA	Anti-HAV IA	Patient code no.	Age (years)	Time	SGPT	Anti-HAV RIA	Anti-HAV IA
1	8	−60 d	25	−	−	27	4	7 d	430	+	+
2	11	−40 d	25	−	−	28	23	10 d	NT	+	+
3	6	−28 d	25	−	−	21	24	10 d	NT	+	+
4	10	−26 d	25	−	−	26	21	15 d	200	+	+
5	7	−26 d	25	−	−	23	20	19 d	NT	+	+
6	7	−26 d	25	−	−	29	4	23 d	50	+	+
7	11	−24 d	25	−	−	18	5	26 d	NT	+	+
8	7	−16 d	25	−	−	24	6	27 d	NT	+	+
9	5	−11 d	25	−	−	19	5	27 d	NT	+	+
10	5	−10 d	25	−	−	20	5	27 d	NT	+	+
11	6	−5 d	25	−	−	22	21	28 d	180	+	+
12	6	−4 d	780	−	−	23	20	33 d	NT	+	+
13	4	−4 d	25	−	−	17	4	2 m	25	+	+
14	7	−3 d	100	+	+	30	7	3 m	25	+	+
15	7	−2 d	200	+	AC	31	24	3 m	NT	+	+
16	9	−1 d	53	+	+	26	21	4 m	25	+	+
17	4	0	1200	+	−	1	8	4 m	25	+	+
13	4	0	NT	+	AC	2	11	4 m	25	+	+
18	5	1 d	2100	+	−	7	11	5 m	25	+	+
19	5	1 d	740	+	−	4	10	5 m	NT	+	+
20	5	2 d	700	+	+	6	7	5 m	NT	+	+
21	24	2 d	2000	+	AC	8	7	6 m	25	+	+
22	20	3 d	1620	+	−	17	4	6 m	NT	+	+
23	20	4 d	480	+	+	32	6	6 m	25	+	+
24	6	4 d	440	+	+	3	6	6 m	NT	+	+
25	7	4 d	NT	+	+	14	7	6 m	NT	+	+
26	21	5 d	440	+	−	33	9	6 m	25	+	+
						34	5	6 m	25	+	+
						35	25	7 m	25	+	+
						16	9	7 m	25	+	+

* Time between the onset of jaundice or clinical signs and the collection of serum sample.
d = day, m = month, AC = anti complementary, NT = not tested.

Table 2. *Anti-HAV titre by RIA at different times of hepatitis A infection*

Time*	Anti-HAV titres (RIA) Negative	1/20	1/200	1/2000	1/20000
> − 4 d	13	—	—	—	—
−3 d to +5 d	—	1	8	5	—
+7d to +33 d	—	—	3	9	—
2 to 7 m	—	—	1	9	5

* Time between the onset of jaundice or clinical signs and serum samples taken.
d = day, m = month.

Table 3. *Anti-HAV antibody in children who did not develop any clinical signs of hepatitis during hepatitis A epidemic no. III*

Patient code no.	age (years)	1st sample ALT	1st sample anti-HAV RIA	1st sample anti-HAV IAHA	2nd sample ALT	2nd sample anti-HAV RIA	2nd sample anti-HAV IAHA
A	9	< 25	−	−	< 25	+	+
B	2	< 25				NA	
C	6	< 25	−	−	< 25	+	+
D	3	< 25	−	−	< 25	+	+
E	4	< 25	−	−	< 25	−	−
F	9	< 25	+	+	< 25	+	+
G	7	< 25	−	−	< 25	−	−
H	5	< 25	−	−	< 25	−	−
I	6	< 25	−	−		NA	

NA = not available.

DISCUSSION

Data from the epidemics in the Tours area showed that the two major outbreaks of hepatitis A studied were due to the introduction in the institutions of an individual infected with HAV and not by contaminated water or food as generally described (Viswanathan, 1957; Denes *et al.* 1977; Hooper *et al.* 1977). However, it is likely that two out of three index cases observed had been contaminated by ingestion of seafood as in other outbreaks (Dienstag *et al.* 1976). These data suggest the hypothesis that HAV maintains itself in human populations through serial propagation rather than by a carrier reservoir (Mosley, 1975). The delay between two cases was generally of four to five weeks. This is similar to that described in experimental infection (Ward *et al.* 1958). However, in the present study, a delay as short as two weeks was noted in a few cases. This finding is in agreement with the observation that the faecal shedding of HAV is at a maximum during the week preceding clinical signs of the illness. Contact transmission during the incubation period from infected to uninfected children seemed to be prevalent. In one epidemic we observed a delay of 11 weeks before a new case appeared. From this it was impossible to know whether two different epidemics occurred in the institution or whether there were asymptomatic infected individuals spreading the disease. However, seroconversion was observed in four subjects without

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evidence of clinical hepatitis. This confirmed that, in children, hepatitis A infection is frequently asymptomatic (Drucker *et al.* 1979*a*). Anti-HAV was detected by RIA as soon as the first clinical signs of illness appeared. On the contrary, anti-HAV was detectable later (one week) by IAHA (Krugman, Friedman & Lattimer, 1975; Miller *et al.* 1975; Moritsugu *et al.* 1976). Thus, RIA could be used to make a very early diagnosis of the disease providing it is improved by separation of the IgM fraction (Bradley *et al.* 1977). In this study an increase in the anti-HAV titre was difficult to demonstrate by RIA when using a ten-fold dilution. IAHA was found to be very convenient for assessing seroconversion. Rakela *et al.* (1978) have proposed a serological diagnosis of hepatitis A based on simultaneous detection of anti-HAV by RIA and IAHA in the same serum sample. Using this method, a positive RIA and negative IAHA would show an acute hepatitis A infection. We obtained this result in only 6 out of 14 cases tested at the beginning of the jaundice. Thus it appeared rather unreliable to ascertain a diagnosis of acute hepatitis A infection in individuals using this discrepancy between RIA and IAHA. Furthermore, it has been shown that such results are observed in 20% of children without any signs of hepatitis (Drucker *et al.* 1979).

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