SHORT REPORT
Hepatitis A virus in urban sewage from two Mediterranean countries

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SUMMARY

Molecular methods for the detection and typing of hepatitis A virus (HAV) strains in sewage were applied to determine its distribution in Cairo and Barcelona. The study revealed the occurrence of different patterns of hepatitis A endemicity in each city. The circulating strains characterized, whether in Cairo or Barcelona, were genotype IB. The effects of a child vaccination programme and the increase in the immigrant population on the overall hepatitis A occurrence in Barcelona were evaluated. While vaccination contributed to a significant decrease in the number of clinical cases, the huge recent immigration flow has probably been responsible for the re-emergence of the disease in the last year of study, in the form of small outbreaks among the non-vaccinated population.

The distribution patterns of hepatitis A in different geographical areas of the world are closely related to their socioeconomic development [1, 2]. The endemicity is low in developed regions and high in under-developed countries. An epidemiological shift, from high to low prevalence, has been noticed in recent decades in the countries of Southern Europe, including Spain, Italy and Greece [3–5]. Consequently, the Mediterranean basin as a whole should no longer be considered as an endemic area. Most northern shore countries are at present of low endemicity [3–5], although some of them, such as Albania, are still areas of high endemicity [6], while most southern/eastern shore countries are highly endemic, with exceptions such as Israel, where a clear epidemiological shifting is presently occurring [7]. However, due to the great immigration flows from North Africa to Spain, Italy and France a new epidemiological pattern may emerge in the near future.

The epidemiological pattern has important implications on the average age of exposure and hence on the severity of the clinical disease. Since hepatitis A infection induces a life-long immunity [2], severe infections among adults are rare in highly endemic regions where most children are infected early in life, usually without clinical symptoms. In contrast, in low endemic areas the disease occurs mostly in adulthood, mainly as a consequence of travelling to endemic regions or as food- or water-borne outbreaks, and hence the likelihood of developing severe symptomatic illness is high.

Molecular epidemiology studies of hepatitis A virus (HAV) strains isolated from different countries, including low, intermediate and high endemic areas, reveal the circulation of dominant or even unique strains in highly endemic areas, in contrast with the

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occurrence of mixed imported strains in conjunction with endemic strains in certain high-risk or ethnic groups in low endemic areas [2, 6, 8–12]. Optimal clinical specimens for the isolation and characterization of HAV strains include stool samples taken at the onset of jaundice [2] and serum samples obtained up to several weeks after the onset of symptoms [8, 11, 13, 14]. Since these types of samples are quite difficult to collect in high endemic areas, where most infections develop asymptomatically, an attractive alternative that will detect the most abundant viral strains circulating in a given population is the analysis of raw sewage samples [15].

Raw sewage samples from three sewage treatment plants from Cairo (Balaks, Zenin and El Berka), were collected monthly from November 1998 to October 1999 ($n = 35$). Raw sewage from Sant Adrià del Besòs sewage treatment plant in Barcelona, was sampled twice a month during a period of ∼4 years, from June 1998 to December 2002 ($n = 249$) with a gap between July and October 2000. Sewage samples were concentrated following previously described methods [15, 16], and RNA was purified from $50\,\mu l$ of the concentrated samples by guanidine thiocyanate extraction [17]. Generic HAV detection was performed as described elsewhere [14], based on the amplification by reverse transcription–polymerase chain reaction (RT–PCR) of a 172-nt fragment of 5′-NCR. Primers HAV240 (5′-GGAGAGCCCTGGAAGAA-AGA-3′, reverse primer) and HAV68 (5′-TCACCGC-CGTTTGCCTAG-3′, forward primer) were employed. PCR amplification products were confirmed by Southern blot hybridization with an internal digoxygenin-labelled probe (5′-TTAATTCCTGCA-GGTTCAGG-3′). All positive samples were further characterized in order to establish their genotype. The previously published primers [10], -3285 (5′-AGTCACACCTCTCCAGGAAAACTT-3′, reverse primer) and $+2949$ (5′-TATTTGTCTGTCACAGA-ACAATCAG-3′, forward primer) were used for the amplification of a fragment of the VP1X2A region containing an internal sequence (positions 3024–3191) of 168 bp that has been extensively used for genotyping [9, 10, 12, 14]. Sequencing of RT–PCR products was performed and multiple sequence alignments were carried out with the ClustalW program (European Bioinformatics Institute).

HAV was detected in 25 out of 35 sewage samples from Cairo (71%). A total lack of seasonal distribution was observed. Twenty-one out of 25 positive samples (84%) could be genotyped. All the strains characterized were identical in the genotyping region and belonged to genotype IB (Genbank accession no. AY867867). However, if a longer positions 2984–3284) sequence is employed in the comparative analysis a certain degree of nucleotide variability up to 1·7% is observed. In Barcelona only 28 out of 249 sewage samples (11%) were HAV positive and only three out of these 28 could be genotyped and were of genotype IB. Two of these strains, isolated within a month's interval (Genbank accession no. AY867862), were identical while the third strain (Genbank accession no. AY867864), isolated 1½ years later, was 98% homologous with the former two. The homology between the isolated Cairo and Barcelona IB strains in the genotyping region was 97%. It is of note that genotype I was divided in subgenotypes IA and IB differing from each other at this particular region at ∼7·5% [10]. The percentage of positive HAV detection in sewage samples from Cairo (71%) and Barcelona (11%) reflects the different infection patterns occurring in both cities. The actual situation in Cairo is the occurrence of high levels of asymptomatic excreters leading to the discharge of high numbers of viruses.

In contrast, the picture observed in Barcelona is the occurrence of low numbers of asymptomatic infections in conjunction with few acute cases and consequently a low level of virus spread. When sewage contamination is low, the detection of positive samples is a difficult task, because of technical limitations. For this reason, samples, positive only after Southern blot hybridization, are even more arduous to genotype. Positive sewage samples from Barcelona were more frequently of this type. In fact, the HAV genotyping threshold has been estimated to be 10^5 genomes/ml, a level quite difficult to achieve from raw sewage from a low endemic area, even after sewage concentration. Although the study in Barcelona exceeded 4 years, the positive sewage samples were not uniformly distributed during the overall period. The percentage of positivity was of 14·7, 11·7, 15·4, 5·9 and 6·7 for years 1998, 1999, 2000, 2001 and 2002 respectively. A significant decrease in sewage detection was observed after November 2000, which correlated with a progressive decline of the number of hepatitis A cases observed in Barcelona since the introduction of a vaccination programme [18] in October 1998 (Fig.). This campaign, which is still currently being implemented, is dedicated to the pre-adolescent population through the administration of vaccine to all children aged 12 years. A total of 137
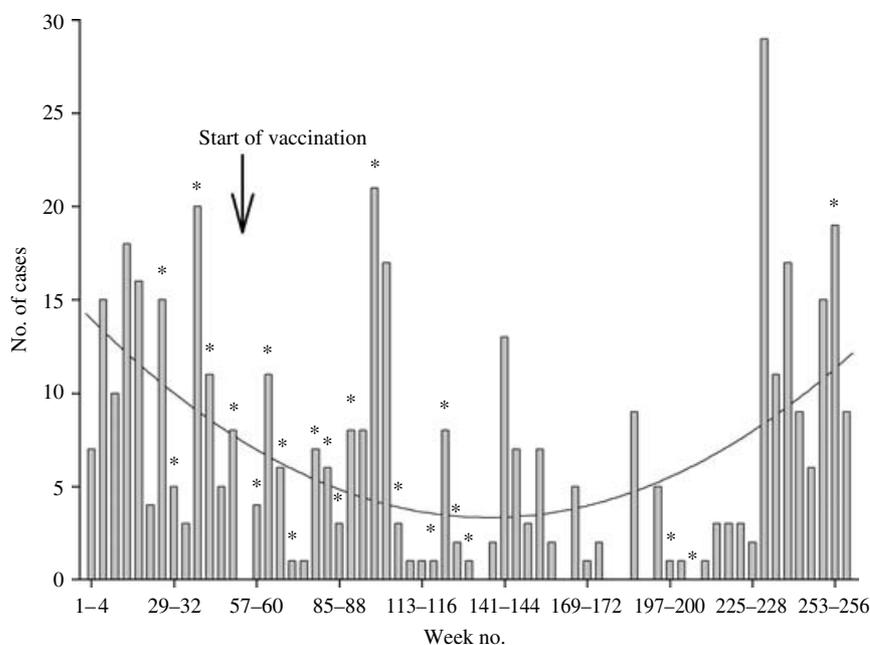
Fig. Hepatitis A cases for the period 1998–2002 in Barcelona. Hepatitis A is a notifiable disease. Weeks 1–52 (1998); weeks 53–104 (1999); weeks 105–156 (2000); weeks 157–208 (2001); weeks 209–260 (2002). Raw sewage sampling started at week 25 with a gap between weeks 133–152. Asterisks denote positive detection of HAV in raw sewage.

cases were reported in 1998, 93 in 1999, 49 in 2000 and only 26 in 2001, representing attack rates per 100 000 inhabitants of 9·1, 6·2, 3·3 and 1·7 respectively. However, in spite of the continued vaccination programme, an abrupt increase was again detected in 2002 with a total of 127 cases (attack rate of 8·0), most of them in the form of small outbreaks among the non-vaccinated population, and mainly affecting school children aged <12 years and men having sex with men (MSM). The decline in the percentage of positive sewage samples appears to be delayed with respect to the decay of clinical cases, being only significant after 2 years of the vaccination programme. An explanation could be the fact that only children aged 12 years were vaccinated, resulting in children <12 years old being susceptible to asymptomatic infections and thus excreting the virus. In contrast, the increase in clinical cases observed throughout 2002 (Fig.) did not correlate with an increase in the percentage of HAV detection in sewage, probably due to the occurrence of several outbreaks, each caused by few cases, without an increase in asymptomatic infections. The unexpected increase in clinical cases after 2002 in Barcelona could be explained, in part, by the high immigration rates. These immigrants, reaching nearly 10 % of the total population, and mainly from North Africa, represent a reservoir for the introduction of viruses with the immigrant

children aged <12 years as potential carriers of the infection. Many of the observed outbreaks are school related, and mostly coincide with the virus incubation period elapsed after the return from the school holiday, and after the immigrant population have returned from visits to their countries of origin.

Data from the occurrence of viruses in raw sewage may provide an overview of the epidemiology of virus infections circulating in the community, and at the same time, reveal the occurrence of asymptomatic infections. However, due to the kind of sample analysed, only those viruses that are more prevalent in the population, and thus excreted in higher numbers, are likely to be detected. This may be the case of the high endemicity of HAV strains in Cairo, with a single strain detected during the entire studied period. Although HAV, as an RNA virus, replicates in quasi-species dynamics [19], it must be pointed out that it presents a clear dominant master sequence, which coincides with the consensus sequence, together with minor sequences. Only the master sequences are likely to be recovered from sewage, which explains the low variability observed. Yet it is remarkable to point out that isolates from a recent outbreak (summer 2004) of hepatitis A involving European citizens returning from vacation at Hurghada in the Egyptian Red Sea, presented VP1X2A regions identical to the ones from

the Cairo strains isolated in the present study (data not shown).

Vaccination against hepatitis A in high-risk groups, including travellers to high endemic areas and MSM, as well as the inclusion of hepatitis A vaccines in mass vaccination programmes in those countries receiving high numbers of immigrants from endemic countries is recommended. However, bearing in mind the quasi-species replication pattern of HAV [19], another source of concern arises from the possibility of the selection of new antigenic variants escaping immune protection induced by vaccination.

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DECLARATION OF INTEREST

None.

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