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Sewage workers: risk of acquiring enteric virus infections including Hepatitis A

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SUMMARY

To determine if sewage workers have an increased risk of acquiring viral infections, 66 workers at a small wastewater plant in north-eastern Italy and 72 control subjects recruited from blood donors were enrolled in a seroprevalence study to determine whether sewage workers are at increased risk of acquiring viral infections.

In order to evaluate various risk factors, a questionnaire was filled out by each worker whereas seropositivity to Hepatitis A virus, Coxsackievirus B2 - B3 - B4 - B5, and Echovirus types 1 and 9 was determined in the laboratory. Anti-HAV antibodies were present in 37.8% of sewage workers and 36.1% of subjects in the control group. The difference was not statistically significant in the two groups, whereas a significant association was observed regarding age (P<0.3). No association was observed with the occupational age, or with number and duration of contacts per day. The lack of evident occupational risk for hepatitis A among sewage workers may be explained by the adult age of the workers (mean age 41.3 years, range 22-58 years), and thus the antibody titre against different enteroviruses was determined. No statistically significant differences were evident with the raw values, but considering the 90° percentile as a dichotomic value for the antibody levels a strong and significant association was present with Coxsackievirus B3 (O.R. 22.85, C.I. 95% 2.93-178.08) and Coxsackievirus B2 (O.R. 14.25, C.I. 95% 1.78-113.87).

Analysis of the data confirms a limited risk of acquiring infection and/or disease but also the evident possibility of silent exposure to the viruses. The shift in HAV epidemiology and increased morbidity and mortality in adult age suggest that active immunization against hepatitis A should be considered.

KEY WORDS: Occupational hazard, Hepatitis A, Enteroviruses, Seroprevalence, Vaccination

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INTRODUCTION

Wastewater plant workers may be exposed to infectious agents that can cause disease or simply an increased antibody titre (Devaux *et al.*, 2001; Krajewski *et al.*, 2004. These infectious agents include enteric viruses, bacteria, parasitic protozoa and helminths. At present it is unclear whether sewage workers have an increased risk of contracting a disease or not, especially a disease preventable by using a vaccine such as that available for Hepatitis A disease (Tooher *et al.*, 2005).

The Consensus Conference held in Rome in 1995 by the Italian National Institute of Health identified several risk groups for which a specific immunization for Hepatitis A was recommended and sewage plant workers were among those groups (Franco *et al.*, 2003; Arankalle *et al.*, 2003). In fact, sewage workers can be exposed to aerosols and direct contact with potentially contaminated materials such as raw wastewater. Several studies have reported an increased risk for employed personnel, whereas other studies have reported non-significant differences in seroprevalence and risk of acquiring the disease (Cadilhac *et al.*, 1996; Trout *et al.*, 2000).

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Hepatitis A disease is a self-limited acute inflammatory liver disease. Fulminant hepatitis and death are rare and always associated with adult age. In Italy, there has been a significant decrease in seroprevalence in children and young adults in recent years (Manfredi *et al*., 2005), with an increased risk of outbreaks in adults. In 1996 a large outbreak involving adolescent and young adults was recorded (Malfait *et al*., 1996) in the south-eastern region of the country. While HAV may be the most significant enteric virus, due to the social costs and endemic nature of the disease, sewage workers are also exposed to other enteric viruses present in sewage. Coxsackievirus B5 (CB5), B4 (CB4), B3 (CB3) and B2 (CB2), and Echovirus types 1 (E1) and 9 (E9) were isolated from sewage samples and used to determine the antibody titres in sewage workers and in a control group of blood donors.
The aim of this study was to evaluate the risk of acquiring HA infections and to assess whether this risk can be determined using other enteric viruses present in sewage.

MATERIALS AND METHODS

Sewage workers and control group

A study was carried out testing the serum samples of sewage workers (66 workers) and the control group (72 blood donors) using the serum neutralization test against CB5, CB4, CB3, CB2, E1 and E9, while anti-HAV antibody presence was determined using the Elisa test with a kit obtained from Adaltis (Bologna, Italy).
The subjects were informed about the aim of the research before agreeing to participate in the study. Blood samples were collected either at the time of blood donation or during the periodic check-up of the sewage workers.

Antibody analysis

The anti-HAV antibody was determined using the Elisa test (Adaltis, Bologna, Italy); the test was performed and the cut-off calculated according to the manufacturer's instructions.
The anti-CB5, CB4, CB3, CB2, E1 and E9 were determined according to the classic serum neutralization test in micromethod using a 96-well plate. Briefly, each virus was produced and titred on BGM (Buffalo Green Monkey) cells according to Reed and Munch method and Spearman-Karber as described by Hierholzer and Killington (Hierholzer *et al*., 1996) and prediluted to have 100 TCID/inoculum/well. A stock was prepared for each virus and divided into vials, each vial was used just once. Each serum dilution, 50ul, from 1:8 until 1:512, was prepared directly in 96 well plates, treated for 5 min under UV lamp to avoid bacterial contamination, and challenged against 100 TCID of each virus in 50ul. After 1h at 37°C with 5% CO_2 with gentle mixing, 100 **u**l of suspended BGM cells were seeded until a 70-80% confluence was reached.
Each dilution-neutralisation test was performed in triplicate, including in each test both infected cells with media alone (negative controls) and infected cells with 100TCID per well (control virus). The neutralisation test was read each day and the reaction was stopped at 3 days post-infection or when the control virus was completely lysed. The titre of the sera was expressed as the highest dilution able to stop the virus and the cytopathic effect.

Enteric virus isolation and identification

Raw wastewater samples were collected and concentrated and aliquots used to infect BGM cell growth in 25 cm^2 flasks in accordance with Divizia *et al.* (1999). After 10-12 days post-infection or at complete cytopathic effect, the viral genome was extracted using the Viral RNA kit (Qiagen, Milan, Italy). The genomic RNA was amplified by molecular method by partial sequencing of the VP1 gene in accordance with Oberste *et al*. (1999) while the sequences obtained were compared with those present in the database at the NCBI site.

Statistical analysis

All statistical analyses were performed using the SPSS programme, whereas the chi-square was determined at www.georgetown.edu and the student's *t*-test at www.physic.csbju.edu.

RESULTS

The mean age of the sewage workers was 41.3 years (range 22-58 years) vs 37.5 years (range 25-51 years) in the control group. Prevalence of anti-HAV positivity in the sewage plant workers was

TABLE 1 - *Statistical analysis of the risk of acquiring enteric virus infections.*

		Sewage workers (66 subjects)				*Control Group (72 subjects)*				*Statistical Analysis*
		HAV pos		HAV neg		HAV pos		HAV neg		
		25		41		26		46		n.s.
	Real age									
	≤30	2		8		6		7		
	31-39	3		16		8		20		
	≥40	20		17		10		21		p<0.02
Real age		45.4		39.3		38.7		39.5		p<0.3
Years occupational		13.0		10.5						n.s.
Contact/day		3.4		2.8						n.s.
Time/contact (h)		2.0		1.9						n.s.
Number per family		3.2		3.0						n.s.
Cut-off ≤8		Pos	%	Neg	%	Pos	%	Neg	%	
CB5		35	53.0	31	46.9	38	52.8	34	47.2	n.s.
CB4		52	78.7	14	21.2	46	63.9	26	36.1	n.s.
CB3		38	57.5	28	42.4	13	18.1	59	81.9	n.s.
CB2		38	57.5	28	42.4	22	30.6	50	69.4	n.s.
E1		65	98.5	1	1.5	1	1.4	71	98.6	n.s.
E9		28	42.4	38	57.5	34	47.3	38	52.7	n.s.
	90° percentile									
CB5	1:64	6	9.1	60	90.9	6	8.3	66	91.7	n.s.
CB4	1:128	14	21.3	52	78.7	7	9.7	65	90.3	n.s.
CB3	1:16	17	25.8	49	74.2	1	1.3	71	98.7	p<0.01
CB2	1:32	11	91.7	55	8.3	1	1.3	71	98.7	p<0.01
E1	1:32	0	100	66	0.0	0	0.0	72	0.0	n.s.
E9	1:32	7	89.4	59	89.3	3	4.1	69	95.9	n.s.

25 out of 66 positive sera (37.9%); in the control group antibodies against Hepatitis A virus were detected in 26 out of 72 subjects (36.1%). The chi-square was not significant.

In the sewage plant workers, mean duration of exposure was 13.0 years (SD 9.31, range 1.0-33.0) for the anti-HAV positive workers and 10.5 years (SD 9.06, range 1.0-36.0) for anti-HAV negative sewage workers. The means did not significantly differ in the student's *t*-test (p=n.s.).

The frequency of contact per day and duration of daily contacts were evaluated in the anti-HAV positive and negative workers. In both cases the chi-square test was not significant (p=n.s.). Anti-HAV positivity can be dependent on the real age of the two groups of workers. Among sewage workers, those who were anti-HAV positive, had a mean age of 45.4 years (SD=8.98, range 23-58 years) while among the anti-HAV negative workers the mean age was 39.4 years (SD=9.07, range 22-57). The analysis of variance showed a significant difference between real age and distribution in the class-age in the two groups (p<0.02).

Since the differences in the overall HAV prevalence between sewage workers and the control group were not significant and considering that the sewage workers are exposed not only to HAV, we investigated whether significant differences were present using other enteric viruses.

Consequently, sewage samples were collected, concentrated and the enteroviruses isolated on BGM cells. The enteroviruses were characterised amplifying a specific portion of the genome according to the method of Oberste *et al.* (1999) and sequencing the amplified products. The sera of both sewage workers and the control group were tested from ≤1:8 until 1:512. The odds ratio performed on the raw data and considering ≤1:8 as negative cut-off were not significant for all the viruses. At the same time, no significant association was evident considering years of occupational activity, time of contact per day and number of contacts per day.

Since no differences were evident among the sewage workers but they still have the possibility of repeated contact with enteric viruses show-

ing higher titre in the seroneutralisation test, we used the 90° percentile as dichotomic value for negative and positive sera. The different 90° percentiles were respectively: CB2 1:32; CB3 1:16, CB4 1:128; CB5 1:64, E9 1:32. The E1 was not considered since all the sera but one were negative. The use of this cut-off determines an Odds Ratio of 22.85 (C.I. 95%, 2.93 - 178.08) for CB3; 14.25 for CB2 (C.I.. 95%, 1.78 - 113.87). The association for all the other viruses was not significant considering years of occupational activity, number of contacts per day and duration of contact per day.

DISCUSSION

Sewage workers face an occupational risk due to the well-documented presence of pathogens in sewage, and this exposure can occur both through wastewater contact and aerosols. Sigari (Sigari *et al.*, 2006) showed the presence of enteric viruses in aerosols collected in the wastewater plant, while Divizia *et al.* (1999) identified HAV in 80% of raw sewage and occasionally in final treated wastewater. Sewage plant workers have a hypothetical risk of Hepatitis A infection due to the endemic presence of this virus in raw sewage.

Glass *et al.* (2001), after analysing 17 published papers concerning Hepatitis A risk, did not confirm an increased risk of symptomatic HA in sewage works. Similar results were obtained by Venczel *et al.* (2003) regarding sewage workers in Georgia (USA). De Serres *et al.* (1995), in Canada, reported an occupational risk for leptospirosis but not for HAV suggesting an abundant circulation of Leptospira in sewage. Conversely, Weldon *et al.* (2000) found an increased risk of acquiring Hepatitis A infection, which was 2.15 times higher in wastewater workers than in those not occupationally exposed. In Singapore (Heng *et al.*, 1994) the seroprevalence of anti-HAV positive workers was 2.2 times higher than in the control group but the correlation was evident only with age and was independent from the occupational work.

Hepatitis A disease, a self-limited infection, is largely endemic throughout the Mediterranean area. The disease is usually asymptomatic in childhood but in adulthood the infection can evolve towards disease and a higher fatality rate is present.

Our study selected sewage workers from a small wastewater plant and the control group consisted of subjects not exposed to this occupational hazard. Raw anti-HAV prevalence was not significantly correlated in the two groups. The results show an increasing risk of antibody presence among the oldest subjects. In fact, the only variable associated with anti-HAV positivity is the older age of the anti-HAV positive sewage workers (45.4 years) against the anti-HAV negative sewage workers (39.4 years) ($p<0.3$). This is not surprising as the percentage of positivity increases in adulthood by cumulative effect in the study group. According to international literature, it is difficult to evaluate the risk of infection for sewage workers, and to better understand the occupational hazard facing sewage workers we considered the antibody titre against CB5, CB4, CB3, CB2, E1 and E9. The results, using the raw data, confirm the limited risk for the sewage workers (p=n.s.). Because of the occupational hazard, sewage workers can have repeated contacts with the enteric viruses present in the wastewaters, causing an increase in the antibody titre evident only when considering the highest titre. Using the 90° percentile as a cut-off between negative and positive sera, a significant association was observed with CB3 (O.R. 22.85, CI 95% 2.93 - 178.08) and CB2 (O.R. 14.25, CI 95% 1.78 - 113.87).

In order to evaluate HA vaccination for the entire population or only for the risk group, Arankalle *et al.* (2003) considers two focus points: the health budget and the epidemiology of Hepatitis A. In recent years, as in other industrialised countries, Italy has shown a shift toward adult age for the earliest age contact with HAV, with increased occurrence of clinical disease. The evident and progressive decline of HAV seroprevalence together with an increased number of susceptible subjects support the proposal of active immunization against Hepatitis A.

REFERENCES

Arankalle V.A., Chadha M.S. (2003). Who should receive hepatitis A vaccine?. *J. Viral Hepatitis.* **10**, 157-158.

CADILHAC P., ROUDOT-THORAVAL F. (1996). Seroprevalence of hepatitis A virus infection among sewage workers in the Parisian area, France. *Eur. J. Epidemiol.* **12**, 237-240.

DE SERRES G., LEVESQUE B., HIGGINS R., ET AL. (1995). Need for vaccination for sewer workers against leptospirosis and hepatitis A. *Occup. Environ. Med.* **52**, 505-507.

DEVAUX I., GERBAUD L., PLANCHON C., ET AL. (2001). Infectious risk associated with wastewater reuse: an epidemiological approach applied to the case of Clermont-Ferrand, France. *Water Sci. Technol.* **43**, 5-60.

DIVIZIA M., PALOMBI L., BUONOMO E., ET AL. (1999). Genomic characterization of human and environmental polioviruses isolated in Albania. *Apple Environ. Microbiol.* **65**, 3534-3539.

DIVIZIA M., RUSCIO V., DEGENER A.M., ET AL. (1992). Hepatitis A virus detection in wastewater by PCR and hybridization. *New Microbiol.* **21**, 161-167.

FRANCO E., GIAMBI C., IALACCI R., ET AL. (2003). Risk groups for hepatitis A virus infection. *Vaccine.* **21**, 2224-2233.

GLAS C., HOTZ P., STEFFEN R. (2001). Hepatitis A in workers exposed to sewage: a systematic review. *Occup. Environ. Med.* **58**, 762-768.

HENG B.T., GOH K.T., DORAISINGHAM S., ET AL. (1994). Prevalence of hepatitis A virus infection among sewage workers in Singapore. *Epidemiol. Infect.* **17**, 162-166.

HIERHOLZER J.C. AND KILLINGTON RA. (1996). Virus isolation and quantitation. In: Mahy BWJ and Kangro HO Editors. *Virology Methods Manual Academic*. Press, London 25-46.

KRAJEWSKI J.A., CYPROWSKI M., SZYMCZAK W., ET AL. (2004). Health complaints from workplace exposure to bioaerosols: a questionnaire study in sewage workers. *Ann. Agric. Environ. Med.* **11**, 199-204.

MALFAIT P., LO PALCO P.L., SALMASO S., ET AL. (1996). An outbreak of hepatitis A in Puglia, Italy. *Eurosurveillance*. **1**, 33-35.

MANFREDI R., CALZA L., CHIODO F. (2005). Changing epidemiology of hepatitis A in the Bologna metropolitan area, northern Italy. *Clin. Microbiol. Infect. Dis.* **11**, 834-855.

OBERSTE MS, MAHER K, KILPATRICK DR, ET AL. (1999). Molecular evolution of the human enteroviruses: correlation of serotype with VP1 sequence and application to picornavirus classification. *J. Virol.* **73**, 1941-1948.

SIGARI G., PANATTO D., LAI P, ET AL. (2006). Virological investigation on aerosol from waste depuration plants. *J. Prev. Med. Hyg.* **47**, 4-7.

TOOHER R., GRIFFIN T., SHUTE E. (2005). Vaccinations for waste-handling workers. A review of the literature. *Waste Manage Res.* **23**, 79-86.

TROUT D., MUELLER C., VENCZEL L., ET AL. (2000). Evaluation of occupational trasmission of hepatitis A virus among wastewater workers. *J. Occup. Environ. Med.* **42**, 83-87.

VENCZEL L., BROWN S., FRUMKIN H., ET AL. (2003). Prevalence of hepatitis A virus infection among sewage workers in Georgia. *Am. J. Ind. Med.* **43**, 172-178.

WELDON M., VANEGDOM M.J., HENDRICK K.A., ET AL. (2000). Prevalence of antibody to hepatitis A virus in drinking water workers and wastewater workers in Texas from 1996 to 1997. *J. Occup. Environ. Med.* **42**, 821-826.