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SEROPREVALENCE OF ANTI-HAV TOTAL ANTIBODIES AMONG WORKERS IN WASTEWATER TREATMENT PLANTS

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Abstract
Objectives: Data on high frequency of hepatitis A virus (HAV) antibodies for wastewater treatment staff is contradictory. Literature lacks data on the seroprevalence of antibodies to HAV (anti-HAV) among workers in wastewater treatment plants (WWTPs) in Bulgaria. The aim of this study is to establish a specific humoral immune response to hepatitis A virus – anti-HAV total antibodies among staff in WWTPs. **Material and Methods:** A complex study of health and working conditions included 110 subjects working in 3 WWTPs in Bulgaria (74% of all workers in the 3 studied WWTPs and 20% of all employees in Bulgaria registered in 2014 under the wastewater collection, discharge and treatment code of economic activity). Workers had been differentiated in 3 groups on the basis of their occupational work: operators, support staff and other workers exposed to biological agents. Venous blood from all 110 subjects was tested once for carriers of HAV antibodies. **Results:** Anti-HAV total antibodies were found for 52.7% of workers in WWTPs. There is a positive association between activity performed in WWTPs (operators, maintenance personnel and others exposed) and a positive one for the presence of anti-HAV ($Chi^2 = 6.882$, df = 2, p = 0.032). Odds ratio (OR) for hepatitis A increases 2.9 times in the group of operators vs. others exposed to biological agents in WWTPs (OR = 2.914, 95% confidence interval (CI): 1.149–7.393, Fisher's p = 0.039). Odds ratio for hepatitis A increases 4.3 times in the group of support staff from WWTPs vs. others exposed to biological agents in WWTP (OR = 4.295, 95% CI: 1.075–17.167, Fisher's p = 0.049). **Conclusions:** Higher frequency of anti-HAV antibodies among operators and maintenance personnel at WWTPs has been established as compared to other workers exposed to biological agents in WWTPs. There is a positive association between increasing age of the workers and the presence of anti-HAV. Int J Occup Med Environ Health 2018;31(3):307–315

Key words:
Anti-HAV total antibodies, Immunization, Hepatitis A, Seroprevalence, Wastewater, Workers

INTRODUCTION

A limited number of viruses able to cause diseases are found in wastewater [1]. This group includes various types of enteroviruses (including poliomyelitis virus), ECHO (enteric cytopathic human orphan), Coxsackie A and B viruses, the causative agent of viral hepatitis A, adenovirus, reovirus, rotavirus, etc. [2–4]. Viruses may be proven by cultivation on cell culture or by molecular biological methods (polymerase chain reaction – PCR). A study of wastewater in Thailand using PCR and enzyme-linked immunosorbent

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assay (ELISA) for the presence of hepatitis A virus (HAV) has found that 15% of the water samples were positive for HAV [5]. Bacteriophage analysis is a considerably simpler and cheaper method for detection of intestinal viruses [6]. Epifluorescence microscopy is the method of choice in determining the diversity, distribution and viral density (number) in wastewater and sludge. This is the method of determining concentrations of viral particles in incoming for purification wastewater, primary sludge, secondary sludge and the process of anaerobic decomposition and purified water [7].

However, so far contemporary methods for detecting viruses have not been applicable to routine water testing. To assess the health risk of exposure to hepatitis A virus it is necessary to identify virus presence in the workplace. Bulgaria has not introduced standardized testing methods proving the existence of this biological agent in water, food, objects from the environment or working tools.

Hepatitis A prevails in terms of percentage against other kinds of viral hepatitis worldwide [8]. Hepatitis A virus infection remains a public health issue in many countries, with approximately 1.5 million clinical cases reported annually worldwide – a figure that is lower than the actual incidence of infections [9]. In the USA figures read 47%, with more than 6000 cases per year, and data is considered strongly underrated [10]. In developed countries (Western European, Scandinavian, etc.) the incidence of viral hepatitis A (VHA) is low and continues its descent, most likely due to the fact that in those countries water is not the main route of infection spread. The lowest levels of VHA morbidity in Europe are recorded in Finland and Denmark (less than 1 out of 10 000 in recent years), in Southern Europe (Portugal, Spain, Italy) it varies between 2.05 and 4.83 out of 10 000. In Bulgaria VHA is the most common among other types of viral hepatitis [11]. Asymptomatic forms are important in terms of epidemiology as the only change in the body of the infected is building immunity, evident in the presence of antibodies [12–14].

Data on high frequency of hepatitis A virus antibodies for wastewater treatment staff is contradictory. Some studies report increased seropositivity prevalence of hepatitis A virus among workers, while other prospective studies do not show higher rates of incidence of hepatitis A among this group [15,16]. Nevertheless, Bonanni et al. believe that the existing recommendation for immunization of workers from sewage wastewater treatment for hepatitis A should be in force [17]. Literature lacks data on the seroprevalence of antibodies to HAV (anti-HAV) among workers in wastewater treatment plants (WWTPs) in Bulgaria.

The aim of this study is to establish a specific humoral immune response to hepatitis A virus – anti-HAV total antibodies among staff in wastewater treatment plants.

MATERIAL AND METHODS

A complex study of health and working conditions carried out between November 2014 and February 2015 included 110 subjects working in 3 WWTPs in the East Aegean Sea Region of Bulgaria. This sample represents 74% of all workers in the 3 studied WWTPs and 20.2% of all employees in Bulgaria registered in 2014 under the "Wastewater collection, discharge and treatment" code of economic activity [18]. Grounds of choosing these WWTPs include:

- compactness of workers;
- these are the 3 WWTPs with the highest design capacity of the number of serviced population equivalents, which is not exceeded at present, and all 3 WWTPs are designed to purify the fecal-urban, industrial and rainwater from the respective towns as sewage in them is of mixed type;
- technological processes in the 3 WWTPs follow the general rules regarding the primary (mechanical) treatment and the biological step that underlie similar working conditions, work organization and work environmental factors with a slight difference regarding only the final stage of processing sludge;

- similarity in specificity of the measures needed for health risks management in WWTPs;
- criterion for inclusion in the study: workers in WWTPs to be exposed to biological agents while performing their duties and to have given a written consent to participate in the research (exclusion criteria: worker refusal for participation in the course of the study or termination of employment of a test person).

Workers had been differentiated in 3 groups on the basis of their occupational work:
- operators of water treatment plant installations,
- support staff – fitters, mechanical fitters for WWTPs, operators-electricians, electricians,
- other workers exposed to biological agents – technologists, samplers, laboratory technicians, launderers, distributors of materials, drivers.

All respondents gave details either on history of hepatitis or vaccination for hepatitis type A. Venous blood from all 110 subjects working for WWTPs was tested once for the presence of HAV antibodies. In the Laboratory of Virology at the University Hospital of Plovdiv, Bulgaria, sera were separated and stored at –80°C before testing. Anti-HAV total antibodies detection was performed by the enzyme immunoassay method (ELISA) using commercial diagnostic kit (DiaPro, Italy) in strict accordance with the manufacturer's instructions.

Ethics

The study is consistent with the requirements of the Helsinki Declaration of 2013 on ethics in science, Principles of good clinical practice, Bulgarian Health Act of 2004 [19] and it has been approved by the Commission on Ethics in Science at the Medical University of Plovdiv (Protocol No. 3/06.26.2014).

Statistics

Processing and analysis of data and results were performed on a computer using routine statistical programs (MS Excel, SPSS v 17.0). Data is expressed as mean ± standard deviation (M±SD). The statistical analysis was performed with the Chi^2 test, with Fisher's exact test (two-sided hypothesis) for variations between the 3 groups, and with odds ratio. A $p < 0.05$ was accepted as the level of significance in rejecting/accepting the null hypothesis, with confidence interval of 95%. Variations in results were interpreted as significant ($p < 0.05$), significant at a high reliability level ($p < 0.01$) and significant at a very high level of reliability ($p < 0.001$).

RESULTS

Characteristics of studied worker staff

The distribution of surveyed workers is presented in the Table 1. There were not statistically significant variations among the 3 groups of employees by age ($Chi^2 = 12.558$, $df = 8$, $p = 0.128$) and by the length of service ($Chi^2 = 6.642$, $df = 8$, $p = 0.576$).

Laboratory test findings of workers for the presence of hepatitis A virus markers (anti-HAV total)

Out of 110 workers in WWTPs anti-HAV antibodies were found for 66 individuals (60%), all without clinical symptoms at the time of the study whereas 17 out of the 17 workers (100%) in one of the studied WWTPs were positive for anti-HAV antibodies. The inquiry among them showed that this was the result of post-vaccination immune response. Immunization for HAV was carried out without prior testing for specific antibodies (one of vaccinees reported a history of past hepatitis A). These participants were excluded from the subsequent processing of data. Out of the remaining respondents (N = 93) anti-HAV total antibodies were found for 49 individuals (52.7%), with a significant variations among work groups: 29 operators for WWTPs (60.4%), 9 maintenance workers for WWTPs (69.2%), and 11 from the group of other WWTP-exposed employees (34.4%). There is a positive association between activity performed in WWTPs

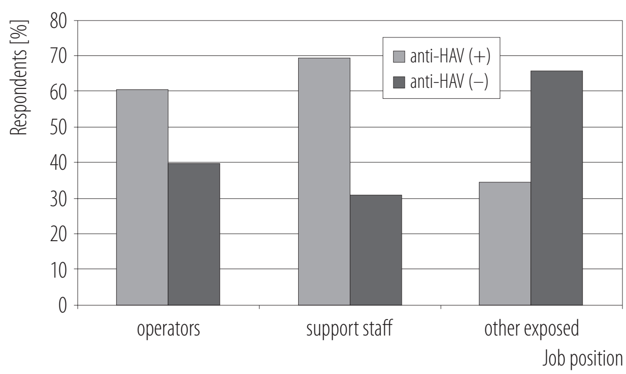
Table 1. Characteristics of the workers of wastewater treatment plants (WWTPs), Bulgaria, 2014–2015

Characteristics	Respondents (N = 110)		M±SD	Min.–max	SE
	n	%			
Age			47.53±10.16	21–72	
20–30 years	6	5.45			2.17
31–40 years	22	20.00			3.81
41–50 years	33	30.00			4.37
51–60 years	40	36.36			4.59
> 60 years	9	8.18			2.61
Seniority			11.64±9.06	0.08–30.25	
< 1 year	9	8.18			2.61
1–5 years	20	18.18			3.68
6–10 years	30	27.27			4.25
11–20 years	22	20.00			3.81
> 20 years	29	26.36			4.20
Sex					
male	87	79.09			3.88
female	23	20.91			3.88
Job position					
operators	55	50.00			4.77
support staff	18	16.36			3.53
other exposed	37	33.64			4.50

M – mean; SD – standard deviation; min. – minimal value; max – maximal value; SE – standard error.

(operators, maintenance personnel and others exposed) and the presence of anti-HAV antibodies ($Chi^2 = 6.882$, df = 2, p = 0.032) (Figure 1).

Out of all anti-HAV positive, 33 persons (67.35%) reported no evident HAV infection in the past (t = 3.355, p < 0.001), without any significant difference in group distribution ($Chi^2 = 3.048$, df = 2, p = 0.218) – operators (N = 17, 51.52%), support staff (N = 8, 24.24%) and others exposed (N = 8, 24.24%). Sixteen employees (32.7%) reported that they had suffered from hepatitis A, as the questionnaire gave the year (period) of illness and social status during that period: childhood (6 operators, 37.5%, and 3 of others exposed, 18.75%), working (6 operators, 37.5%),



HAV – hepatitis A virus; HAV(+) – antibodies to HAV positive; HAV(–) – antibodies to HAV negative.

Fig. 1. Occurrence of antibodies to HAV for workers of wastewater treatment plants (WWTPs), Bulgaria, 2014–2015, by job position

unemployed (1 out of support staff, 6.25%). Among the operators, 5 people (6.41% of workers in this WWTP) reported suffering from hepatitis A during work for this WWTP: 2 in 2000, 2 in 2004, and 1 in 2008. There were outbreaks of hepatitis A between 2000–2006 in the 3 administrative districts harboring the studied water supply and sewerage companies.

We found out an increase in the frequency of positive tests for anti-HAV corresponding to increased age of the WWTP employees ($Chi^2 = 11.658$, df = 4, p = 0.020). Their distribution is presented in the Table 2.

There is no significant difference between the length of service and the presence of anti-HAV antibodies ($Chi^2 = 12.096$, df = 8, p = 0.147) among operators, maintenance personnel and other exposed subjects (Table 3).

When calculating the odds ratio (OR) for hepatitis A in the workplace for employees from the 3 groups of staff (N = 93), we found that the group of operators compared with the group of support staff showed no increase in the risk of suffering from hepatitis A (OR = 0.678, 95% confidence interval (CI): 0.183–2.520, Fisher's p = 0.404). Odds ratio for hepatitis A increases 2.9 times in the group of operators vs. others exposed to biological agents in WWTPs (OR = 2.914, 95% CI: 1.149–7.393, Fisher's p = 0.039, two-sided hypothesis). Odds ratio for hepatitis A increases 4.3 times in the group of support staff from WWTPs vs. others exposed to biological agents in WWTPs (OR = 4.295, 95% CI: 1.075–17.167, Fisher's p = 0.049, two-sided hypothesis).

DISCUSSION

We were not able to confirm the presence of hepatitis type A in the work environment, as Bulgaria had not introduced standardized methods of research proving its presence in water, food and objects from the environment or the working tools. Literature gives evidence that such techniques are

Table 2. Occurrence of antibodies to HAV (anti-HAV) for workers of wastewater treatment plants (WWTPs), Bulgaria, 2014–2015, by age groups

anti-HAV	Respondents by age groups [n (%)]					Total
	20–30 years	31–40 years	41–50 years	51–60 years	> 60 years	
HAV(+)	0 (0.0)	8 (42.1)	13 (48.1)	21 (61.8)	7 (87.5)	49 (52.7)
HAV(–)	5 (100.0)	11 (57.9)	14 (51.9)	13 (38.2)	1 (12.5)	44 (47.3)
Total	5 (5.4)	19 (20.4)	27 (29.0)	34 (36.6)	8 (8.6)	93 (100.0)

Abbreviations as in Figure 1.

Table 3. Occurrence of antibodies to HAV positive (anti-HAV(+)) for workers of wastewater treatment plants (WWTPs), Bulgaria, 2014–2015, by seniority and job position

Job position	Respondents by seniority groups [n (%)]					Total
	< 1 year	1–5 years	6–10 years	11–20 years	> 20 years	
Operators	1 (33.3)	1 (100.0)	8 (53.3)	6 (54.5)	13 (68.4)	29 (59.2)
Support staff	0 (0.0)	0 (0.0)	6 (40.0)	2 (18.2)	1 (5.3)	9 (18.4)
Other exposed	2 (66.7)	0 (0.0)	1 (6.7)	3 (27.3)	5 (26.3)	11 (22.4)
Total	3 (6.1)	1 (2.0)	15 (30.6)	11 (22.4)	19 (38.8)	49 (100.0)

applied to detect the human adenovirus and HAV in wastewater – in South Africa, Eastern Cape in 2012–2013 [20], rotavirus A (RV-A), human adenovirus (HAdV), norovirus genotype I and II (NoV GI/GII) and HAV in hospital wastewater in WWTPs – Rio de Janeiro, Brazil [21]. Introduction of standardized methods for testing the presence of viral biological agents in the work environment would allow for objective and precise detection of the pathway for infection spread. This approach is expected to have far greater economic and prophylactic effect as compared with epidemiological studies, including its introduction as a criterion for confirmation of occupational etiology of the disease. For example, data from viral diagnostics of treated hospital wastewater, entering the WWTP, confirms the possibility of environmental contamination with viruses and may be useful to establish standards policies regarding wastewater management.

In most parts of the developing world, where HAV-infection is endemic, the prevalent rate of morbidity covers subjects infected in early childhood, thus receiving immunity and protected in adulthood. In developed countries, however, HAV infections are less common due to the higher standard of living. Therefore, the number of infected in infancy is limited and the majority of adults remain susceptible to infection, which may result in outbreaks of hepatitis A in the general population [22–24]. Bulgaria is a country falling into the average endemic area for HAV infection [25]. Bad habits, concomitant diseases, risk factors from the social and working environment, travels abroad are relevant to morbidity of HAV [26,27].

Occupational exposure to untreated wastewater is often a significant risk factor for HAV infection (OR = 3.73, 95% CI: 1.48–9.37), independent from other known risk factors – the study conducted by Brugha et al. [28]. The authors found that 60% of the 50 employees who reported occupational exposure to "raw" wastewater for more than a half of working time were anti-HAV positive. In our study we have examined the WWTP workers for the presence of anti-HAV antibodies in blood serum, usually measured as total antibodies because the test does not distinguish whether the positive result is due to anti-HAV immunoglobulin G (IgG) or anti-HAV immunoglobulin M (IgM). According to Martin and Lemon [10] in the absence of clinical symptoms of hepatitis A, a positive result should be interpreted as past hepatitis A infection, i.e., the presence of anti-HAV IgG.

Despite some limitations in our study (the relatively small number of respondents and determination of specific immune response – an indirect approach that reveals exposure to HAV, rather than direct detection of HAV in the workplace), we have found that the frequency of anti-HAV total positivity for the WWTP staff is 52.7%, which is slightly different from a study in Greece in 2002. The Greek authors have found that 65.7% of workers employed in WWTPs have hepatitis A antibodies. Additionally, the presence of anti-HAV is to a great extent associated with increased age, as compared with the control group, unexposed to biological agents, $p < 0.0001$ [29].

In available literature there are several studies on the incidence of anti-HAV IgG for people working in the system of wastewater treatment as some of the authors find a higher incidence in the group of the WWTP workers [30,31], while others [14] find no difference in the anti-HAV IgG seroprevalence among the WWTP workers vs. WT. Trout et al. [32] have tested staff of sewerage services and a control group in the US for the presence of HAV antibodies in saliva. The authors have concluded that work with wastewater is not associated with a significant increase in the presence of HAV antibodies, provided that no statistically significant risk factors for HAV infection are found in the work environment (disturbances of technological processes, using clothing, etc.).

We are of the opinion that during exposure to biological agents, work seniority is not among the leading risk factors for the occurrence of accidents or professional diseases [33,34]. However, employees of shorter service may suffer work accidents, including incidents with biological agents due to the lack of experience. On the other hand, the group of senior workers tend to underestimate the work-place hazards due to experience and routine.

Although the asymptomatic form of hepatitis A is typical for childhood, according to Strashimirov and Cherveniakova [35] in the majority of cases (60–80%) hepatitis A has an anicteric course. A systematic review of Glas et al. [16] criticizes the view of an increased risk of clinically manifested hepatitis A among the WWTP workers, but they do not exclude the risk of subclinical course of the disease, based on a serological study of subjects exposed to wastewater.

This corresponds to our findings concerning the relative percentage share of persons who have history of inapparent form of HAV-infection among the WWTP workers (67.3%) in comparison with the symptomatic form subjects ($p < 0.001$). The inapparent form of HAV infection may very likely be due to disease history – causally related to working conditions in wastewater treatment, suggesting greater exposure to hepatitis A virus.

On the other hand our survey has found that 5 operators (6%) of workers in terms of the longest operating time at the WWTP developed hepatitis type A while working there (during the period 2000–2008). According to Cuthbert [36] hepatitis A is considered an occupational hazard in wastewater treatment in Canada, while in Israel and in the USA it is not accepted as a hazard because of introduced immunization. Immunization against HAV is recommended by a number of authors [37,38] as an effective method for managing the risk of HAV infection.

No research has been carried out for objectifying the presence of HAV-antibodies in Bulgaria. This is due to the lack of legislative regulation-related requirements which would warrant such testing. This study establishes a distribution of HAV-antibodies among workers in WWTPs in Bulgaria for the first time.

CONCLUSIONS

Higher frequency of anti-HAV antibodies in the case of operators and maintenance personnel at WWTPs has been established as compared to other workers exposed to biological agents in WWTPs. There is a positive association between increasing age of the workers and the presence of anti-HAV.

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