Antibodies in crevicular fluid: an epidemiological tool for investigation of waterborne disease

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SUMMARY

A significant challenge in the epidemiological investigation of recreational waterborne disease is the establishment of a definite association between exposure to a contaminated water and infection. An increase in specific antibodies as a result of infection is a potent measure of disease exposure and its determination would enhance epidemiological studies of waterborne diseases. We report on the automated detection of HAV antibodies in crevicular fluid and its use in a field study. The method is easy to use, non-invasive, could be applied to volunteers of all ages and is comparable in sensitivity to serological procedures. Application to an epidemiological study of water recreationalists demonstrated that surfers were three times more likely to be immune to hepatitis A virus than either wind-surfers or a control group without recreational water contact.

INTRODUCTION

A major problem with establishing an association between a disease outbreak, or the possibility of acquiring infection, and water usage (either by consumption, recreation or aerosol inhalation) is the need to demonstrate unequivocally that infection has taken place. Volunteer studies, such as those carried out in the United Kingdom [1, 2] have relied upon the reporting of symptoms of gastroenteritis. This is an imprecise way to assess occurrence of disease through contaminated water exposure because there is substantial bias due to differing perception of symptoms. Such studies have relied upon the symptomatology of short incubation diseases without laboratory proof that disease had actually occurred, either by demonstration of the presence of the pathogen or seroconversion to that pathogen. Seroconversion is of

particular importance because many volunteers in epidemiological studies may not show evidence of disease despite having been infected. For slowly developing diseases, e.g. hepatitis A, long-term monitoring of volunteers is rarely pursued. The reluctance of subjects in epidemiological studies to give blood samples may make seroconversion monitoring an almost impossible task. Additionally, ethical bodies monitoring such investigations may be wary of invasive procedures, particularly where studies are being carried out on water-related diseases involving children. In such cases, other surveillance approaches are required, such as questionnaires [3].

Recent UK bathing water studies [1, 2] used defined groups of volunteers stratified by exposure to waters that satisfied EC bathing water standards [4]. Such approaches were necessary on ethical grounds to ensure that those most at risk (particularly children) were excluded from the study and that volunteers were not exposed to water of poor quality. Ac-

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cordingly, such studies probably did not reflect the true picture of exposure of recreational water users to coastal water pollution throughout the year. The results of such surveillance can only give, at best, a conservative estimate of risk.

An ideal means of measuring exposure to a target organism for use in studies of waterborne diseases would (a) be non-invasive, (b) reflect the immune status of the subject and (c) allow monitoring of volunteers of all ages at a wide range of sites with varying water qualities. The presence of antibodies in saliva (particularly in crevicular fluid) provides a means for achieving such goals.

Saliva is a mixture of secretions from the salivary glands and transudate from the capillary bed beneath the buccal mucosa. In particular, the transudate (crevicular fluid) constantly flows from the crevice between the gum margins and the teeth. As this fluid reflects the constituents of the plasma it contains significant amounts of antibody comparable with levels in the serum. Crevicular fluid will reflect previous and ongoing humoral response. Immunoglobulin concentrations in crevicular fluid are much higher than in salivary gland secretions [5] and it is important that crevicular fluid is well represented in any salivary sample collected for diagnosis through detection of antibody.

Saliva and crevicular fluid have been used for many years during clinical investigation of diseases caused by a wide range of pathogens. For example, crevicular fluid has been used to investigate infections due to human immunodeficiency virus [6] and to hepatitis C [7] and to determine the immune status of patients against *Helicobacter pylori* [8]. Of particular interest to us was the use of saliva or crevicular fluid for the detection of hepatitis A antibodies [9–15]. Hepatitis A antibody levels in crevicular fluid are known to be comparable to those found in matching serum samples.

The use of saliva or crevicular fluid to demonstrate exposure to waterborne pathogens has been little reported, although the likelihood of acquiring hepatitis A from consumption of sewage-contaminated drinking water [16] and the occupational risk of wastewater workers acquiring the same disease [17] both exploited salivary antibody detection as an epidemiological tool.

In this study we report on the use of crevicular fluid antibody measurement as a means of determining the immune status of recreationalists exposed to waters of different qualities.

MATERIALS AND METHODS

Collection of samples

Saliva specimens were collected using an absorbent foam swab ('ORACOL', Malvern Medical Developments, Worcester, UK). Subjects were asked to use the swab as if brushing their teeth for 30–60 s until the device was very wet. The swab was tubed and returned (in a cool box) to the laboratory where fluid was squeezed out and clarified by centrifugation (5000 g, 2 min).

Antibody detection

Antibodies to hepatitis A were detected using the automated IMx MEIA analytical system (Abbott Laboratories, Illinois, USA) to perform microparticle enzyme immunoassays with methyl-umbelliferyl phosphate (MUP) as the enzyme tracer. By using the HAVAB total assay (Abbott Laboratories) in the above system, it was possible to measure total antibody to hepatitis A virus in a competitive format. Results were measured in a fluorimeter with the amount of fluorescence being inversely proportional to the level of antibody detected. The instrument was calibrated and used according to manufacturer's instructions. Results were validated if the positive and negative controls were within instrument performance and specifications.

Validation of crevicular fluid assay

This was necessary as (a) the relationship between hepatitis A antibody levels in crevicular fluid and serum was not known and (b) the test kit used was designed only for use with serum samples. Patients attending a hospital clinic for hepatitis A screening (serum antibody levels) were asked to provide a matching saliva sample using the previously described procedure. Saliva samples were processed as described previously. Saliva and serum samples were tested using identical analytical procedures.

Application to an epidemiological study

The immune status of two groups of water recreationalists recruited at national sporting events was compared [18]. The first group comprised surfers who used coastal marine waters for their sport and the second group were windsurfers who used only inland

Table 1. *Volunteer groups in hepatitis A immune status study* [*18*]

	Surfer (n = 117)	Wind-surfer (n = 117)	Territorial Army (n = 119)
Male	84%	89%	62%
Female	16%	11%	38%
Male mean age	25·6	35	33·5
(range)	(8–53)	(11–68)	(18–59)
Female mean age	30	37·9	32·5
(range)	(8–51)	(13–54)	(18–55)
Number immunized	5	2	4

fresh water sites. Territorial Army volunteers, who lacked recreational exposure to either marine or fresh water during the study but were subjected to similar environmental stresses as the test groups through their military activities, were included as a control group. The composition of each group is summarized in Table 1. Compliance with sampling was > 99% in all groups emphasizing the advantages of a non-invasive procedure.

RESULTS AND DISCUSSION

Validation of the crevicular fluid assay

The Abbott test system records a positive result as having an instrument cut-off between positive and negative results based on single point calibration; in this study this was an instrument value of 353·4. In the comparison of antibodies in crevicular fluid and serum, the positive control value was 94·0 and the negative 887·2. No attempt was made to quantify antibody levels in either saliva or serum. Results were expressed simply as positive or negative for presence of antibody. Table 2 details the instrument readings for 20 paired samples from clinic patients. Only four of the patients were deemed positive, reflecting the generally low level of hepatitis A circulating in the community at the time. However, the values obtained showed that the test procedure gave clear differentiation between immune and non-immune patients.

Application to an epidemiological study

The epidemiological study has been reported [18]. The use of the crevicular fluid antibody test for assessing the immune status of volunteers in the study who showed immunity to hepatitis A (Table 3) confirmed the low levels of immunity in the population at large during the study period. Despite a low prevalence,

Table 2. *Comparison of hepatitis A immune status in paired serum and crevicular fluid samples from clinical patients – validation of the Abbot IMx system*

Patient	Abbott IMx values		Result*
	Serum	Crevicular fluid	
1	852	796	Negative
2	889	851	Negative
3	924	879	Negative
4	86	167	Positive
5	767	821	Negative
6	925	887	Negative
7	836	840	Negative
8	97	146	Positive
9	856	725	Negative
10	800	760	Negative
11	832	858	Negative
12	899	822	Negative
13	934	913	Negative
14	968	856	Negative
15	668	724	Negative
16	101	189	Positive
17	777	682	Negative
18	92	176	Positive
19	823	860	Negative
20	844	812	Negative

* Cut-off value 353: <353 = positive; >353 = negative.

there were more immune individuals amongst those who surfed as a major recreational pastime than in either the wind-surfing or control groups, though the differences in the numbers of immune individuals was not significant. Only a few study participants had been immunized against hepatitis A (11 out of 353). The crevicular fluid antibody test confirmed their immunity.

This study showed that the use of crevicular fluid for measuring antibody responses to waterborne pathogens that evoke a specific serum antibody response has a number of advantages. The first, and perhaps most important, is the ability to collect

Table 3. *Hepatitis A immune status of volunteer groups in an epidemiological study of water recreationalists*

	Surfers ($n = 117$)	Wind-surfers ($n = 117$)	Territorial Army* ($n = 119$)
Immune	21	8	10
Immunized immune	5	2	4
Non-immunized immune	16	6	6

* Control group.

samples from volunteers, regardless of age, rapidly and effectively. This would ensure that the effect of a water contamination exposure could be monitored over a wide age range of those exposed and not restricted because of ethical reluctance to permit more invasive procedures such as blood sampling. Indeed, the inclusion of Territorial Army volunteers was only agreed by the Commanding Officer because of the use of a non-invasive procedure.

A second advantage is that the procedure allows long-term follow-up of volunteers facilitating identification of seroconversion to longer incubation illnesses such as hepatitis A. For instance, in the UK bathing water studies [1, 2], no such long-term monitoring was carried out with the emphasis being placed on acquisition of gastroenteritis and not on the incidence of all waterborne infections. The facility to carry out longitudinal antibody monitoring would allow more precise assessment of the risk of infection associated with waters of differing microbiological qualities.

A third advantage is that the procedure is extremely cheap and uses an automated analytical system found in many clinical laboratories being used for other purposes. Finally, the detection of hepatitis A antibodies in crevicular fluid is comparable to serum detection thus offering a tool suitable for epidemiological studies.

It is possible that apparently non-immune participants in the recreational water study may have been exposed to either hepatitis A or its vaccine in the distant past. However, evidence from the literature indicates that immunity after exposure either to the virus or the vaccine persists in many individuals for substantial periods, perhaps in excess of 25 years [19–22]. Thus, the use of crevicular fluid offers a rapid, efficient, non-invasive and economical means of determining the true response of individuals to recent exposure to microorganisms present in contaminated water.

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