ORIGINAL

Prevalence of Antibodies to Legionella Pneumophila in Bahrain*

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ABSTRACT

Formalised yolk sac antigen of Legionella pneumophila serogroup I was used in the indirect immunofluorescent antibody test to determine the prevalence of antibodies to L. pneumophila in Bahrain. Sera from 510 blood donors were randomly chosen for this study. Positive reactions with titres of 1:16 were detected in the case of 12 sera, 1:32 in 5 sera, 1:64 in 2 sera and 1:128 in 1 serum. When a titre of 1:16 is taken as positive, the prevalence of antibodies is approximately 4%. In comparison to surveys conducted elsewhere, the results show that Bahrain is not an area of high exposure to the Legionnaires' disease bacillus.

In the summer of 1976 Legionnaires' disease was described as the cause of an explosive outbreak of febrile respiratory illness with pneumonia in a group of American war veterans attending an annual Legion Convention in Philadelphia[1]. There were 29 deaths from 247 people admitted to hospital. Six months of intensive investigations identified the causative agent as a gram negative bacterium,[2] which was subsequently given the name Legionella pneumophila[3]. Since the original outbreak, several others have been described in other parts of world, many of which have been associated with hotels and hospitals[3,4].

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The disease is known to occur in two forms : (a) Pontiac fever, a relatively mild self-limiting respiratory illness without pneumonia and not requiring hospitalisation, (b) Pneumonic, varying in severity and affecting mainly the immunocompromised, but also healthy individuals, and requiring prompt medical attention.

The disease is transmitted by the airborne route through the inhalation of droplet nuclei[3]. Air conditioning cooling towers, excavation of earth as well as hot and cold domestic water systems in hotels and hospitals have been identified as a source of infection [3-5]. In sporadic cases the mode of transmission is still unknown. Surveys of antibody prevalence to L. pneumophila in the United States,[3] have shown that there exists areas of high exposure to L. pneumophila. The hot and often dusty climate of Bahrain, coupled with the extensive use of air cooling devices seem to provide ideal conditions for the spread of the disease. However, although a decade has elapsed since the description of the original Philadelphia outbreak, the prevalence of the disease in Bahrain and the Gulf region remained unknown. This study was undertaken to determine the prevalence of the disease in Bahrain.

METHODS

Legionella antigen : was supplied by Dr. Taylor, Division of Microbiology Reagents and Quality Control, Public Health Laboratory Service, Colindale, U.K. It consisted of formalin-killed yolk sac

antigen prepared from infected hens' eggs yolk sac homogenised in equal volumes of Dulbeco "A" phosphate buffered saline containing a fixed concentration of 1% formalin, and further diluted 1/50 in phosphate buffered saline containing 0.08% sodium azide.

Test Sera : these were randomly chosen from blood donors attending the Blood Bank at Salmaniya Medical Centre.

Positive control serum : was supplied by Dr. Taylor. It had a titre of 1:128 giving (+) fluorescence intensity. Control sera were obtained from healthy adults.

FITC conjugated sheep anti-human globulin was Wellcome MF 01, used at a dilution of 1/80. It gave a (+) intensity at the stated titre of the positive control serum.

Buffers : P.B.S. pH 7.2 was used for dilution of sera. The mounting medium was buffered glycerol pH 8.5.

Microscope slides coated with paratetrafluorethylene with 12x3 mm clear spot wells (Hendly & Co., Essex, U.K.) were used. To each well was added 0.005 ml of the yolk sac antigen which was allowed to dry at room temperature, then fixed in acetone for 15 minutes.

Test proper : The indirect immunofluorescent technique recommended by the Association of Clinical Pathologists[6] was followed. To each antigen spot 0.01 ml of the appropriate 1/16 serum dilution was added. Positive and negative control sera were included in each slide. The slides were incubated in a moist chamber at 37°C for 30 minutes, then washed in three changes in PBS (pH 7.2) with a final rinse in distilled water then drained. To each well 0.01 ml FITC conjugate, at a dilution of 1/80 was added, and after a further 30 minutes incubation at 37°C, the slides were washed, drained and examined using a fluorescent microscope system equipped for EPI illumination. The slides were initially examined using x 40 objective and x 10 eye piece. Positive sera were retested to determine their titre. They were mounted using buffered glycerol and examined under the oil immersion lens. The results were scored for intensity of fluorescence, the end point being the dilution giving (+) intensity of fluorescence.

RESULTS

Between November 1984 and September 1985, 510 blood donor sera were tested by the indirect immunofluorescent technique. These sera gave negative results for VDRL and Hep. Bs. antigen. 96% of the donors were Bahraini nationals, 4% were non-Bahrainis. The age distribution was 25 to 45 years. Initially sera were tested at 1:16 dilution. Sera giving positive reaction were retested to determine their titre.

From 510 sera tested, 12 gave positive reaction at 1:16 dilution, 5 had titres of 1:32, 2 titres of 1:64 and one a titre of 1:128 (Table 1). A total of 20 sera showed positive results making antibody prevalence to L. pneumophila approximately 4%. The majority of sera (60%) had titres of 1:16, 25% a titre of 1:32, 10% a titre of 1:64 and 5% a titre of 1:128 (Figure 1).

TABLE No. 1

Distribution of antibody titres in 20 positive sera

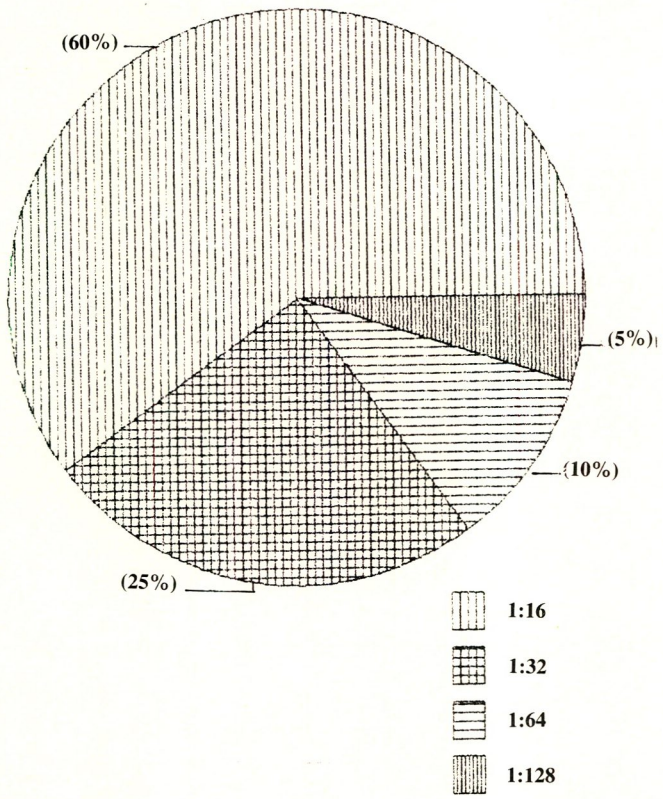
Number of sera	*Antibody titre*	*Percentage*
12	1:16	60
5	1:32	25
2	1:64	10
1	1:128	5
Total 20		**100**

DISCUSSION

The hot, humid and often dusty climate of Bahrain and the Gulf region combined with the preponderance of air cooling devices seem to provide ideal conditions for the spread of Legionnaires' disease. But, despite the fact that a decade has elapsed since the discovery of the original Philadelphia outbreak, the prevalence of the disease remained unknown. This paper is the first part of a study undertaken to fill this gap in our knowledge of this important disease in Bahrain.

FIGURE 1

Distribution of antibody titres as a percentage of the total positive sera



Antibody prevalence in a community can be taken as an indication of the presence of the disease. High antibody prevalence on one hand usually indicates that a disease is endemic while low prevalence on the other indicates that a disease is rare. Values of antibody prevalence to L. pneumophila vary accordingly to the type of antigen used and the cut-off point at which the titre is taken as positive[5]. In this survey a titre of 1:16 was taken as positive. This is in accordance with ACP recommendations. There are two main types of antigen in general use. A heat killed antigen which is widely used in the United States and a formalised yolk sac antigen used in the United Kingdom. The former appears to give higher titres and is less specific than the latter.

Results of this survey has shown that 4% of the population possess antibodies to L. pneumophila when a dilution of 1:16 was taken to indicate significant exposure to Legionella pneumophila. When a dilution of 1:32 is taken as positive the prevalence is only 1.9%. The majority of sera had titres of 1:16 and only one serum had a high titre of 1:128.

Comparison to surveys of prevalence conducted elsewhere shows that Bahrain is among the lowest (Table 2). United States surveys are generally higher than Bahrain but United Kingdom surveys are lower. Investigators in Nottingham and London found IFA titres of 1:128 in only 0.1% and 1:32 in 1.5% of 2023 sera tested[5]. These low levels of United Kingdom surveys are a reflection on the specificity of the formalin killed antigen.

TABLE No. 2

The Prevalence of Antibodies to L. pneumophila in different regions

Type of antigen	*Region*	*Prevalence*
Heat killed[3]	Bristol, Tennessee	5 – 6%
	Burlington, Vermont	8 – 26%
	Los Angeles, California	4 – 17%
	Bloomington, Indiana	15 – 28%
	New York	16 – 26%
Formalised yolk sac	Bahrain	4%
	Saudi Arabia[7]	28%

What is perhaps a little perplexing is the recent study of antibody prevalence conducted in Riyadh, Saudi Arabia,[7] where a level of 28% was recorded. This is a much higher value than Bahrain and is difficult to explain. The low titres obtained with most sera in this study could be due to the monovalent antigen. If these sera were tested against individual type antigens, higher levels might have been recorded.

Since the discovery of the role of air-conditioning systems in the transmission of the disease, measures to combat the growth of Legionnella pneumophila in domestic water and air-conditioning cooling towers

by chlorination or injection of biocides have been implemented in some industrialised countries. It is difficult to recommend such measures in the absence of the disease, however, these measures would be justified in big institutions such as hospitals to prevent the occurence of explosive outbreaks. It may be pleasing to know that such measures have been applied at Salmaniya Medical Centre since it was first opened in 1978.

CONCLUSION

The prevalence of Legionella antibodies in the Bahraini community is 4%. This is a relatively low prevalence which indicates that Bahrain is not an endemic area for Legionnaires' disease.

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