ORIGINAL ARTICLE

Seroprevalance of brucellosis among suspected cases in Malaysia

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Abstract

Brucellosis is a zoonotic disease which can be transmitted by direct or indirect contact with infected animal or their products. It is an important public health problem but little is known on brucellosis in the Malaysian population. The aim of this study was to determine the presence of Brucella antibodies using commercial Brucella IgG and IgM ELISA kits (Vircell, SL, Barcelona Spain). A total of 184 sera from suspected patients were received from 16 hospitals in Malaysia over the years 2004 to 2009. Only 10 serum samples (5.4%) were positive for Brucella antibodies in which 5 showed the presence of both IgM and IgG. Most of the positive patients were occupationally involved with animals. This study suggests the seroprevalance of brucellosis among individuals who have contact with infected animals in Malaysia is low.

Keywords: Brucellosis, brucella, brucella ELISA, brucella serology.

INTRODUCTION

Brucellosis is an important public health problem in many developing countries. The Mediterranean Basin, South and Central America, Eastern Europe, Africa, Asia, The Caribbean and the Middle East are considered as high-risk countries. In the United States, every year 100 to 200 cases of brucellosis are reported. The resemblances of brucellosis with other diseases often lead to incorrect diagnosis and under reporting of the disease. The World Health Organization (WHO) reported 500,000 cases of brucellosis each year from around the world. The incidence and prevalence of the disease vary widely from country to country. In the USA and Northern Europe, B. abortus is more prevalent whereas B. melitensis is more common in Latin America, Mediterranean countries and the developing countries.

Brucellosis in human are caused by Brucella abortus, Brucella suis, Brucella melitensis, Brucella neotomae, Brucella ovis and Brucella canis. The differentiations of these variants are important as the epidemiology of disease in humans is influenced by the type of organism and its source. B. abortus and B. melitensis are the most common cause of brucellosis in humans and B. melitensis is more virulent than B. abortus. However, Brucella suis, Brucella canis, Brucella ovis and Brucella neotomae are occasionally associated with the human brucellosis.

Brucellosis can occur in any age group and the majority of cases are males between the ages of 20 to 45 years. The disease is also related to occupational hazard in young men. The diagnosis of this disease is usually confirmed by isolation of the bacteria or detection of anti-Brucella antibodies in the blood. Blood culture is the gold standard in the diagnosis of Brucella infection but require long incubation periods and good laboratory facilities. The smooth lipopolysacharide (S-LPS) of the outer membrane and the internal cytosolic proteins are the major brucella antigens that are useful in serological diagnosis of human brucellosis. Serological tests that are currently available are standard agglutination test (SAT), rose bengal test, coomb test, compliment fixation test, indirect immunofluorescent antibody test (IFA) and enzyme-linked immunosorbent assay (ELISA). SAT and coomb test are commonly used for detection of Brucella antibodies, however the IFA and ELISA are simple and easy to perform and detect IgM and IgG. This study was conducted to detect IgM and IgG in suspected human brucellosis in Malaysia using an ELISA method.
MATERIALS AND METHODS

Serum samples
A total of 184 serum samples received from patients with suspected brucellosis from 16 hospitals in Malaysia in the years 2004 to 2009 were included in this study. In addition, 100 sera from blood donors were included in this study as negative controls. The sera were received in plain sterile tubes and stored at -20°C until used.

ELISA Method
The sera were tested for immunoglobulin G (IgG) and immunoglobulin M (IgM) for the presence of antibodies against Brucella spp using Vircell ELISA (Vircell, SL, Barcelona). Briefly, 5 μl of each serum was added into 100 μl of serum dilutions to each microplate wells coated with S-LPS antigen of Brucella abortus. Twenty-five μl of IgG human sorbent is included in preparation serum diluents for detection of IgM antibodies only. The use of human IgG sorbent is to avoid false positivity caused by the presence of rheumatoid factor and also to prevent false negative result to the excess of IgG antibodies. The microplates were covered with sealing sheets and incubated at 37°C for 45 minutes. After washing 5 times with phosphate buffer saline containing Tween-20, 100 μl of anti-human peroxidase conjugate IgG and IgM was applied into all microplate wells and incubated at 37°C for 30 minutes. In each run positive, negative and cut off calibrator controls from Vircell ELISA IgG and IgM were included.

The Vircell IgM and IgG ELISA positive or negative qualitative assays used a screening dilution of 1:20. The interpretation of results as in the product insert is any antibody index of < 9 at dilution titre 1:20 is negative, 9 to 11 is equivocal and > 11 at dilution ≥ 1:20 is positive.

RESULT
The results were divided into four groups. Group A is when both IgG and IgM antibody is positive, Group B is when only IgM positive, Group C for only IgG positive and Group D is negative for IgG and IgM.

From suspected brucellosis cases, only 5.4% (10 of 184) were positive and most of the seropositive sera were in group A (Table 1). The detection of both IgM and IgG in the patients’ sera showed that the patients may have been recently infected while those with only IgM antibodies were probably having acute or recent infection. The patient with only IgG positive serum was considered to have past exposure to Brucella spp. There was absence of brucella antibodies in 94.6% of suspected cases. On screening blood donor samples we did not detect any seropositivities.

The seropositive sera were mostly obtained from veterinarians and farmers (Table 2). Two of the seropositive veterinarians have both IgM and IgG brucella antibodies, which suggest that they may have been recently infected with Brucella spp. This was also observed among 4 farmers. One veterinarian had positive IgG which suggested that he had past exposure to Brucella spp. Out of 184 samples, 101 were from males and 83 were female patients with the age ranging from 4 to 73 years. Nine of the seropositive cases were males and 1 was from a female patient, with the ages ranging from 18 to 53 years. All 100 sera from blood donors were negative for both IgM and IgG.

DISCUSSION
The major Brucella antigen that is useful for diagnosis of human brucellosis is the smooth lipopolysaccharide (S-LPS) of the outer cell membrane. The antibodies against S-LPS in serological response of human brucellosis can stay elevated for years after the disease has

<table>
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<tr>
<th>Serodiagnosis Group</th>
<th>ELISAs test Result</th>
<th>No (%) of sera</th>
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<tbody>
<tr>
<td>A</td>
<td>+</td>
<td>5 (2.7)</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>4 (2.2)</td>
</tr>
<tr>
<td>C</td>
<td>–</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>D</td>
<td>–</td>
<td>174 (94.6)</td>
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been cured. Serological tests such as serum agglutination test, rose bengal test, compliment fixation test and ELISA are the commonly used method for diagnosis of human brucellosis.

ELISA has become an increasingly popular assay for diagnosis of brucellosis as it is able to detect specific IgM antibodies and IgG antibodies. In the Brucella Unit (BRU) at Liverpool, IgM and IgG brucella antibodies seropositivity of among brucellosis samples using ELISA, Vircell were shown to be 47% (68 of 143) and 55% (79 of 143) respectively. In our study, the control group sera from the healthy population were all negative whereas from the suspected Brucella patients, 5.4% was positive. Most of the samples (94.6%) sent for brucellosis serology were negative and we also did not detect any brucellosis antibodies in healthy blood donors. This finding suggests that brucellosis may not be a common infection in our local population.

In Malaysia, the National Surveillance Program for Animal Brucellosis has been ongoing since 1978 and 2.4% positive brucellosis in animals was reported in 2001. However, limited information is available for human brucellosis in Malaysia. Our study showed that the positive samples were mainly from veterinarians and farmers. These occupations have been associated with high risk of infection with brucellosis. The four seropositive farmers worked with different farm animals namely cattle and sheep in Seremban, Penang and Selangor states of Malaysia. Three of the positive veterinarians worked with sheep and cattle in Seremban and Perak states of Malaysia. One of them worked with infected sheep during Q fever surveillance in Perak. People who work with farm animals, especially cattle, sheep and goats get exposed through direct contact with infected animals or heavily contaminated environment. They get infected through cuts and wounds or splashing of infected blood or other animal fluids through the respiratory, oral and conjunctiva routes.

Abattoir worker, veterinarians and farmers are among the high risk groups. In Pakistan and Turkey, the seroprevalence of brucella antibodies among high risk groups were shown to be 21.7% and 17.9% respectively. In 2006, the World Health Organization reported that 85% of human brucellosis had high titre of IgG of Brucella antibodies even after 18 months of clinical recovery. The IgM antibodies were shown to persist for 1 year after treatment in 50% of acute brucellosis. Brucella disease affect all age groups and both sexes. Our study showed that positive Brucella serology was mainly in males (90%) compared to females (10%). Those within the age range of 20 and 45 years were mostly affected with brucellosis. In this study 70% (7 of 10) of those with positive serology were more than 40 years old and only 2% of the seropositive cases were less than 20 years of age.

In conclusion a small percentage of seropositivity was observed among patients of suspected brucellosis in Malaysia. The seropositivity was associated with occupational exposure to animals. The information on patient’s occupation is therefore important as a guide for diagnosis of brucellosis.

ACKNOWLEDGEMENT

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