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ORIGINAL ARTICLE

Human brucellosis: Six years retrospective study on seropositivity in Malaysia

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Abstract

Introduction: Human brucellosis is a zoonotic disease in Malaysia. This study analysed six-year retrospective seropositivity trends of human brucellosis cases from 2014 to 2019. *Methods and materials*: A total of 1,281 serum samples were obtained from suspected brucellosis patients were included. The sera were tested using an enzyme-linked immunosorbent assay for IgM and IgG antibodies for *Brucella* spp. Samples with equivocal or positive antibody index were confirmed with an immunocapture agglutination. *Results*: During the study period, 5.8% (n=74) of suspected cases showed seropositivity for human brucellosis. The central region has the highest seropositivity cases of human brucellosis. Consumption of unpasteurised milk was significantly associated with human brucellosis in this study with adjusted odds ratio ((AOR) = 4.56, 95% CI = 2.6, 8.02, p-value < 0.001). The age group of less than 15 years old was more likely to contract brucellosis ((AOR) = 2.81, 95% CI = 1.01, 7.84 p-value < 0.048). *Conclusion*: Serological tests have been widely used for the diagnosis of human brucellosis. However, diagnosis using serology is often challenging without the presence of a convalescent sample. In conclusion, even though human brucellosis has a low prevalence rate, the disease has serious public health implications. The usage of effective diagnostic tools as well as implementation of 'One Health' approach are the way forward to prevent and control of brucellosis in the country.

Keywords: human brucellosis, Malaysia, serology, seropositivity

INTRODUCTION

Brucellosis is a zoonotic disease caused by *Brucella* spp. It is a gram-negative, aerobic bacteria. Six classical *Brucella* species have been recognised comprising *Brucella abortus*, *Brucella mellitensis*, *Brucella suis*, *Brucella canis*, *Brucella ovis,* and *Brucella neotomae* according to host preference.[1] Recently, two species of *Brucella*, namely *B. ceti* and *B. pinnipedialis* have been recognised in marine mammals as their hosts.[2] The most common *Brucella* spp. associated with human brucellosis includes *B. abortus*, *B. melitensis,* and *B. suis*.[3] Brucellosis can be contracted through the consumption of unpasteurised dairy products and from raw meat of infected animals.[2] Abattoir workers, veterinarians, farmers, and laboratory technicians are at risk of exposure to *Brucella* spp. through direct contact with infectious animal secretions and the inhalation of contaminated aerosols.[4] Brucellosis is manifested in humans as an acute or insidious febrile illness, involving any organ and hence presents with a diverse range of clinical symptoms. If the disease is not treated adequately, acute infections may progress to a chronic stage that lasts from months to years.[5]

The diagnosis of brucellosis in human is usually difficult due to the non-specific clinical manifestation of the disease.[6] Hence, laboratory investigations are essential to make an accurate

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diagnosis to prevent treatment failure and relapse. Identification of the organism from culture is the gold standard method for diagnosis due to its high specificity. However, the limitations to this method include firstly, the need for a prolonged incubation of up to 21 days for *Brucella* spp., which would delay the diagnosis.[4] Secondly, the requirement of a suitable biosafety level 3 laboratory to handle the organism and to reduce the incidence of laboratory acquired infections. Therefore, various serological methods have been utilised to provide rapid and accurate diagnosis in the absence of an isolate.[7]

The standard tube agglutination test (SAT) for human brucellosis is still the reference to which other serological tests are compared.[4] These tests being used include the Rose Bengal test, complement fixation test, indirect Coombs test, enzyme-linked immunosorbent assay (ELISA), and immunocapture-agglutination test (Brucellacapt).[8] Nonetheless, these serological tests, are often hard to interpret especially in areas of a high prevalence of brucellosis where a large proportion of the population has developed brucella antibodies.[9]

There are limited reports on the prevalence of human brucellosis in Malaysia. However, past studies have reported a seroprevalence for human brucellosis of 5.4% and up to 14.29% among veterinary workers and farmers.[10,11] Hence, further research is required to determine the accurate annual incidence of human brucellosis in the Malaysian population. Institute for Medical Research (IMR) is the reference laboratory for *Brucella spp*. detection in Malaysia. This study analysed retrospectively a six-year trend of human brucellosis cases in Malaysia from the year 2014 to 2019.

MATERIALS AND METHODS

Study area

Malaysia is a country in Southeast Asia with a land area of 330,534 km^2.[12] The country consists of thirteen states which include several regions and three federal territories. Eleven states were listed according to regions; the Northern region consists of Perlis, Kedah, Penang, Perak; East Coast region: Kelantan, Terengganu, Pahang; Central region: Selangor, Federal Territories of Kuala Lumpur and Putrajaya; and Southern Region: Negeri Sembilan, Malacca, and Johor. Whilst East Malaysia is separated from West Malaysia consists of Sabah, Sarawak, and Federal Territory of Labuan. The ethnicities were classified as Malay, Chinese, Indian, and Others. Ethnicities from Sabah and Sarawak, Orang Asli, and foreigners were combined as 'Others'. Age groups were categorised according to the Department of Statistics Malaysia, which comprised of young age group (0 – 14 years old), working-age group (15 – 64 years old), and old age (65 years and above). Several associated exposure factors with human brucellosis were also identified in this study, which consisted of consumption of unpasteurised milk, those working or having contact with livestock and people who live in farms. Maternal brucellosis was defined as a breastfeeding child of 2 years old and below whose mother was diagnosed with brucellosis. Those who were working with livestock included veterinary personnel, livestock farmers, and agriculture and veterinary students. No direct contact with animal parturition or animal slaughtering information was given.

Sampling

A total of 1,281 serum samples were obtained from suspected brucellosis patients during the period of 1st January 2014 to 31st December 2019. The records during this study period were retrieved from the Bacteriology laboratory, Institute for Medical Research (IMR), Malaysia. Serum samples for suspected brucellosis (among humans) were the primary criteria sent to the laboratory for testing and confirmation while waiting for the presence of *Brucella* isolates in blood culture.

Laboratory test data

Sera obtained were tested using the ELISA method (Vircell, S.L, Barcelona, Spain) for detection of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies against *Brucella* spp. as described in a previous study.[10] This method is a qualitative assay, and the result was interpreted based on the antibody index, where it is considered negative if the index of < 9; equivocal if the index is between 9 to 11, and positive with index > 11. Any serum samples with equivocal or positive antibody index will be confirmed with an immunocapture agglutination test for the detection of total anti-Brucella antibodies (Brucellacapt, Vircell, S.L, Spain) using the method as described in another study.[13] The serum was considered to be positive for brucellosis when the antibody titre is ≥ 1:320 from Brucellacapt.[14] In this study, only serum samples with complete demographic data were included in the analysis.

Statistical analysis

All data were analysed using SPSS version 26.0 for Windows (SPSS Inc., USA). Descriptive analyses were performed and univariate analysis conducted using binary logistic regression. Variables having a p-value less than 0.25 from the univariate analysis were included in the initial multivariate logistic regression model. Variables were then analysed using the enter method to arrive at the final model. Multicollinearity and interaction terms were checked, and the Hosmer Lemeshow test and classification table were applied to check the model fitness. The strength of association for each risk factor was assessed using crude and adjusted odds ratios (AOR). All results with p-value less than 0.05 was considered significant.

Study ethics

The study was registered with the National Medical Research Register and ethically approved by the Medical Research and Ethics Committee, Ministry of Health, Malaysia (NMRR-21-02223-ZLN).

RESULTS

Throughout the six years, a total of 1281 serum samples from suspected human brucellosis patients were received. Of all serum samples tested for human brucellosis, only 5.8% (74 of 1281) were seropositive. The central region has shown the highest seropositivity cases of human brucellosis in almost all 6 years with 39 cases altogether (Figure 1). However, in 2015 there was an increased number of human brucellosis cases seen in the Southern region (21.6%, n = 8) of Malaysia with all samples coming from the state of Johor (Figure 2). In reference to age groups, those 15-64 years of age 48/74 (64.8%) were predominantly seropositive for brucellosis, followed by those from less than 15 years old in 21/74 (28.3%). Both genders showed an almost equal number of seropositive cases. Malay 53/74 (71.6%) had the highest seropositive cases of brucellosis, followed by Chinese 11/74 (14.9%) and Indians 5/74 (6.8%). Unpasteurised milk was consumed in 20/74 (27%) of cases and 3/74 (4%) cases were working with livestock (Table 1). There were seven suspected cases of maternal human brucellosis, however only two were tested seropositive.

This study observed statistically significant associations for the age group of less than 15 years old (OR = 2.74, 95% CI = 1.00, 7.49) and consumption of unpasteurised milk (OR = 4.60, 95% CI = 2.64, 8.02) (Table 2). The multiple logistic regression analysis showed that consumption of unpasteurised milk was significantly higher in the likelihood of getting human brucellosis (AOR = 4.56, 95% CI = 2.6, 8.02). The odds of having brucellosis by age group of less than 15 years was also significant after the factor was adjusted (AOR = 2.81, 95% CI = 1.01, 7.84) (Table 2).

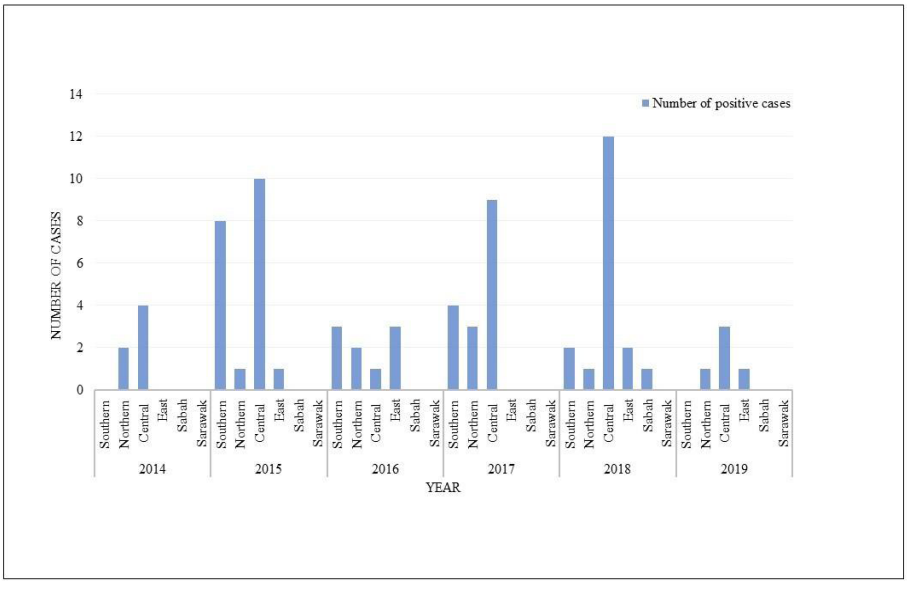

FIG. 1: Trends of seropositivity of the human brucellosis cases in Malaysian regions from 2014-2019.

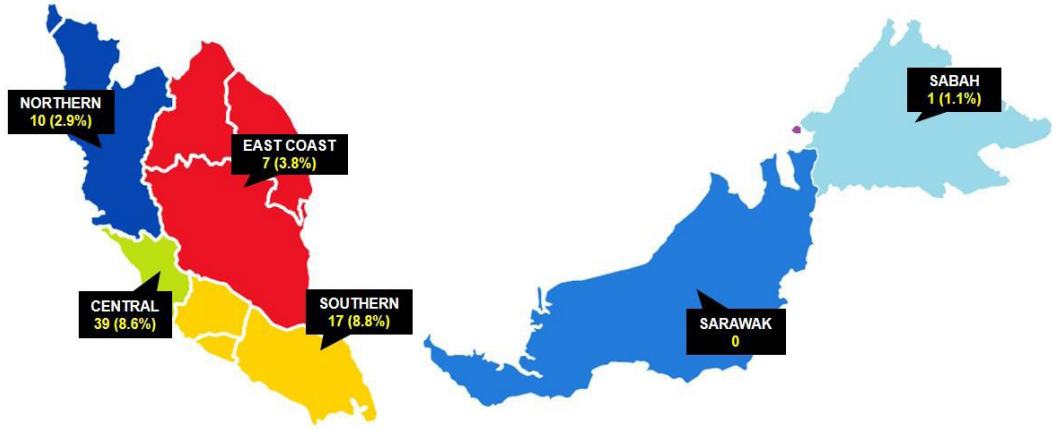

FIG. 2: Total cases and percentage of seropositive human brucellosis in regions of Malaysia 2014-2019.

DISCUSSION

The consumption of unpasteurised milk was reported to be significantly associated with brucellosis in humans, which is in concordance with the previous studies.[9,15,16] The highest antibody titre for the cases in the group was ≥ 1:5120 with 13 out of 20 samples

Table 1: Characteristics of seropositive human brucellosis cases from 2014- 2019

Factor	Positive cases (n=74)		Negative cases (n=1207)	
	n	%	n	%
Race				
Malay	53	71.6	798	66.1
Chinese	11	14.9	142	11.8
Indian	5	6.8	116	9.6
Others	5	6.8	151	12.5
Gender				
Male	36	48.6	633	52.4
Female	38	51.4	574	47.6
Age group (years)				
Less than 15	21	28.3	155	12.8
15-64	48	64.8	951	78.7
65 and above	5	6.7	101	8.4
Consumption of unpasteurized milk				
Yes	20	27	90	7.5
No	54	72.9	1117	92.5
Working with livestock				
Yes	3	4	55	4.5
No	71	95.9	1152	95.4
Contact with livestock				
Yes	4	5.4	66	5.4
No	70	94.5	1141	94.5
Lives at farm				
Yes	2	2.7	9	0.74
No	72	97.2	1198	99.2

Table 2: Logistic regression analysis of socio-demographic factors associated with seropositive human brucellosis cases

Factor	Univariate analysis		Multivariable analysis	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Race				
Indian	1		-	
Malay	1.54 (0.60-3.93)	0.366	-	
Chinese	1.80 (0.61-5.32)	0.290	-	
Others	0.77 (0.22-2.72)	0.682	-	
Gender				
Female	1		-	
Male	0.86 (0.54-1.37)	0.526	-	
Age group (year)				
65 and above	1		1	
15 – 64	1.02 (0.40-2.62)	0.968	1.07 (0.41-2.78)	0.893
Less than 15	2.74 (1.00-7.49)	0.050†	2.81 (1.01-7.84)	0.048^
History of exposure				
Consumption of unpasteurized milk	4.60 (2.64-8.02)	<0.001†	4.56 (2.6-8.02)	<0.001^
Working with livestock	0.89 (0.27-2.90)	0.840	-	
Lives at farm	3.70 (0.78-17.43)	0.098†	2.02 (0.42-9.86)	0.383
Contact with livestock	0.99 (0.35-2.79)	0.982	-	

OR=Odds Ratio

Hosmer & Lemeshow (p-value=0.515), Classification Table, 94.2% and Nagelkerke R Square, 7.7%

† p-value <0.25 from univariate analysis will be selected to be included into multivariable analysis

^ p-value < 0.05 is significant

detected *Brucella* spp. DNA by PCR. The majority of the human brucellosis cases seen in 2015 were from an outbreak caused by drinking unpasteurised milk in the state of Johor, which was located in the southern region of Malaysia. A study conducted in Palestine reported that consumption of unpasteurised milk products showed a greater risk of contracting brucellosis in humans when compared to direct contact with animals, where the latter was found to be at high risk when it is associated with assisting in animal parturition.[9,17]

This study also found that the age group of less than 15 years old were significantly at risk for human brucellosis. In concordance with previous reports, human brucellosis commonly involved school aged children and adolescent, and the incidence was highest in summer.[18] Other group reported was young adults as they were considered the most productive group in the population, resulting in a significant negative impact on the socio-economic situation in the community.[19] Another study reported that brucellosis in children aged between 0-14 years was from shepherd's families.[20] Likewise, younger and middle-aged patients are at higher risk of having brucellosis because they are most predisposed to occupational exposure involving contact with animals, excreta, or its products.[21]

Brucellosis is an infection with multiple presentations and early diagnosis prevents complications. At present, culture and isolation of the bacteria remain the gold standard for the diagnosis of brucellosis.[4] A wide variety of serological assays has been utilised for diagnosing brucellosis in humans. Nevertheless, in order to overcome false-negative results, a combination of at least two serological methods is needed. SAT is commonly used as the first screening, followed by the confirmatory test – the complement fixation or Coombs' test.[22]

As ELISA could measure the specific antibodies (IgM, IgG, and IgA), it has been preferred as a rapid screening test method and give a better interpretation of the clinical situation and would overcome the false negative

or positive that may occur in SAT.[23,24] The tube and slide agglutination tests are conventional serological methods that have limitations in terms of their poor sensitivity during the early stage of the disease, and their specificity is reduced in areas where the disease is highly endemic and where frequent relapses of the disease occur.[25]

There is no perfect test available for the laboratory diagnosis of brucellosis in humans and it is often challenging without the presence of a second sample for serological methods. Molecular techniques like polymerase chain reaction (PCR) have enabled faster and more sensitive *Brucella spp*. detection from clinical samples, and in patients who live in high brucellosis areas as well as for patients with seronegative test results.[25] Another research stated that the PCR-based method was capable of detecting Brucella DNA from samples of all patients with positive IgM antibodies. Therefore, PCR may not be a reliable method in chronic brucellosis as the sensitivity of PCR is reduced.[26] A study carried out by Morata in 2003 showed improvement and resolved the limitations of brucellosis diagnosis by combination use of both PCR and ELISA as a diagnostic method.[27] PCR is a useful tool to identify more brucellosis cases from suspected patients and to reduce isolation time and contamination risks in culture methods.[7]

Patients who presented with fever and a history of unpasteurised milk consumption or having an occupational risk of exposure to *Brucella* spp. should be investigated for brucellosis. A specific diagnosis for human brucellosis according to The Malaysia Ministry of Health (MOH) requires a clinically significant illness with definite laboratory evidence of *Brucella spp*. infection from either the *Brucella spp*. isolation from clinical samples, increase in antibody titre of four-fold or greater between acute and recovery-phase serum sample taken between two or more weeks or detectable *Brucella spp*. DNA in the blood sample by PCR.[5]

Scientific evidence of human brucellosis incidence is needed to assess the global burden of the disease. However, there is limited information on the disease incidence in Southeast Asia, where most of the data come from North Africa and the Middle East regions.[28] The highest annual incidence of human brucellosis globally was reported in Syria with 1,603 cases per million population, followed by Mongolia and Kyrgyzstan.[29] The reason for this is that some parts of the population of these countries were still dependent on agriculture and livestock for livelihood and lack of public health control.

Since 1979, Southeast Asia countries including Malaysia, have implemented a brucellosis eradication program for animals based on the 'test-and-slaughter' protocol.[30] This strategy has managed to keep the prevalence rate to less than 5%. Although the program implied effective control in the number of brucellosis cases, however it failed to eradicate the disease due to several factors which included challenges to locate and control the movement of the affected animals, new importation of breeder animals and lack of involvement by the farmers.

A comprehensive epidemiological study is needed to determine an accurate prevalence of human brucellosis in Malaysian population by targeting the high-risk groups based on occupational exposures as well as the hot-spots area. Screening and surveillance of cases should be implemented using a combination of serology as well as molecular tests. A coordinated and holistic approach is necessary between public health and veterinary personnel at all levels to ensure the success of the brucellosis eradication program. Once eradication of animal brucellosis has been successful, the surveillance program has to be sustained to ensure that the country is brucellosis-free.[31,32]

There are few limitations present in this study. Firstly, we only obtained suspected brucellosis samples from patients who were admitted to hospitals in Malaysia. Further studies need to be conducted to determine the exact prevalence of human brucellosis in Malaysia. Secondly, we received many samples with incomplete clinical history. Thus, they were coded as negative risk cases. Thirdly, the relatively small sample sizes limit statistical power. Therefore, confirmation by future research with a larger sample size is needed. Despite these limitations, our study is the first to provide some insights into the seropositivity of brucellosis using combinations of two serological methods among suspected patients in Malaysia. We utilised the data over 6 years from suspected brucellosis patients who were admitted in the hospital from all over Malaysia and not limited to certain occupational groups. As such, it has provided an accurate and reliable representation of human brucellosis seropositivity cases in this country.

In conclusion, as the disease is of important public health concern, a systematic disease surveillance and control strategy is essential to be in place. The interdisciplinary 'One Health'

approach involves which a collaborative work of veterinary, medical, and public health sectors. This may result in synergistic effects on prevention and control of zoonotic diseases such as brucellosis. In addition, efficient screening and effective diagnostic tools are critical in establishing accurate diagnosis together with a comprehensive surveillance system to ensure effective disease control.

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