Research Article

Sero Prevalence of Brucellosis in Pregnant Women Visiting Gynaecology Department of Kathmandu Model Hospital, Kathmandu, Nepal

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Abstract: Introduction: Brucellosis is a highly contagious zoonotic disease caused by ingestion of unpasteurized milk or undercooked meat from infected animals or close contact with their secretions.

Subject and Methods: Sero-prevalence of brucellosis in pregnant women was conducted for the first time in Kathmandu, Nepal. A total of 80 sera samples were collected from the pregnant women visiting Kathmandu Model Hospital. The patients were categorized on the basis of age, trimester and ethnic groups. The sera samples were tested by ELISA method.

Results: The sero-prevalence of brucellosis among pregnant women was found to be 11.25%. Madhesi ethnic group showed the highest (16.66%) seropositivity rates followed by Janajati (11.53%) and the lowest was in Brahmin (8.33%) ethnic group. Similarly, the age group 31-35 years showed highest prevalence (29.41%) followed by the age group 26-30 years (13.33%). There is absence of seropositivity among the age group 16-20 years and 21-25 years. The highest sero-prevalence rate (12.76%) was found in the third trimester followed by first trimester (10%) and the lowest was in second trimester (8.69%). About 3% of them consume raw milk directly from milking animals which is one of the risk factor of brucellosis in pregnant women.

Conclusion: The prevalence was found to be high in pregnant women and ELISA was a sensitive and specific test for the detection of IgG antibodies against *Brucella.*

Keywords: ELISA, sero-prevalence, ethnic groups, trimester, brucellosis.

INTRODUCTION

Brucellosis, a chronic granulomatous infection [1] caused by *Brucella* species, a gram-negative, non-motile, non-spore forming, rod-shaped (coccobacilli) bacteria belonging to family Brucellacease and order Eubacterials. It is an infection that mainly affects animals including goats, sheep, pigs, deer, cattle, dogs etc. Brucellosis is a bacterial zoonotic disease transmitted to humans by consumption of infected, unpasteurized animal milk or through direct contact with infected animals, particularly aborted fetuses [2]. Brucellosis in pregnancy is highly associated with adverse obstetric outcomes including abortion (threatened and spontaneous) and fetal/maternal and neonatal death [3]. *Brucella bacteremia* can result in abortion especially during the early trimesters [4]. The incidence of spontaneous abortion and intrauterine death among pregnant women with acute brucellosis is primarily due to *Brucella melitensis* [5].

Although brucellosis in domestic animals has been controlled in most developed countries, it remains endemic in most developing countries [6] including the Middle East [7] particularly where livestock are a major source of food and income. The countries with the highest incidence of human brucellosis include Saudi Arabia, Iran, Palestinian Authority, Syria, Jordan and Oman [8]. Asian countries like India, Bangladesh, Pakistan, China etc. and even in Nepal it has been reported.

Bacteriological method, serological method (Agglutination test, Rose Bengal test, Coomb's test and ELISA test) and Molecular method are the diagnostic technique required for the isolation of *Brucella* from blood, bone marrow or other tissues [9]. Since, brucellosis can result in abortion in pregnant women; the present study was conducted to determine the sero-prevalence of brucellosis among the pregnant women with the hypothesis that the disease is prevalent among pregnant women.

MATERIALS AND METHODS

The study was designed to screen the brucellosis among the normal pregnant women without any sign and symptoms of the disease. The project was approved by the research committee of the Central Department of Zoology. A total of 80 pregnant woment visiting at Gynaecology Department of Kathmandu Model Hospital, Bagbazar, Kathmandu were randomly selected without repeating and irrespective of their trimester. The written consent was taken from all the selected patients according to their willingness to participate in the study. The blood samples were collected twice a month for

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three months from November 2014 to February 2015. The blood samples were collected in sterile, clean and leak-proof vials and labeled properly. The serum separation was done by centrifuging blood sample for 12-15 minutes with the help of centrifuge machine. The separated serum was pipette out in a sterile eppendorf tubes and were frozen at -20oC till analysis. These serum samples were taken to the laboratory of National Zoonoses and Food Hygiene Research Centre (NZFHRC), Tahachal Kathmandu for test. The serum was tested by Enzyme Linked Immunosorbent Assay (ELISA) method for the further diagnosis of brucellosis. ELISA was conducted at NZFHRC, Kathmandu for the detection of IgG antibodies against *Brucella*. It was performed in polystyrene 96- well microplates following the manufacturer's protocol. All unknown serum samples and four positive control samples were tested in duplicate. To obtain the reliable results strict quality control was maintained and the favourable condition was maintained throughout the lab work. The internal control of each test was done by a conjugate control, a substrate control, cut off, negative and positive controls. The result obtained were statistically analyzed calculating the chi-square values to determine the significance difference among different ethnic groups, age groups and trimesters using free online "R" software.

RESULTS

Sero- Prevalence of Brucellosis Among Pregnant Women

Out of the 80 samples tested, 9 (11.25%) were found to be brucellosis positive (Table **1**).

Table 1. Sero-Positivity Distribution of Pregnant Women by Ethnicity, Age and Trimester by ELISA.

Variables	Frequency (n=80)	Positive (%).	Value of χ^2	d.f	P-value
Ethnicity			0.560	3	0.906
Brahmin	24	2 (8.33)			
Chhetri	18	2 (11.11)			
Janajati	26	3 (11.53)			
Madhesi	12	2 (16.66)			
Age			9.930	3	0.019
16-20	8	0 (0)			
21-25	25	0 (0)			
26-30	30	4 (13.33)			
31-35	17	5 (29.411)			
31-35	17	5 (29.411)			
Trimester			0.274	2	0.872
First trimester (1-3months)	10	1 (10)			
Second trimester (4-6months)	23	2 (8.69)			
Third trimester (7-9months)	47	6 (12.76)			

The statistical analysis revealed that there were no significance differences ($p > 0.05$) between seropositivity of brucellosis, ethnicity and trimester of the pregnant women but found significant differences between seropositivity of brucellosis and age of the pregnant women indicating that seropositivity of brucellosis is high among the age group >30 years.

Ethnic Wise Prevalence

Among 80 samples collected, ethnicity had been differentiated into four major groups such as Brahmin, Chhetri, Janajati and Madhesi on the basis of the surname of the respondents. The highest sero-prevalence rates of brucellosis was found among Madhesi (16.66%) followed by Janajati (11.53%) and the lowest was in Brahmin (8.33%) even though the sample size were not equally divided (Table **1**). There was no significance difference between seropositivity of brucellosis and ethnicity of pregnant women.

Age Wise Prevalence

Of the total samples collected the lowest age was 18 and the highest age was 35 so the age group has been classified into four groups with the class interval of five. The highest sero-prevalence rate (29.411%) was found within the age group 31-35 years followed by the age group 26-30 years (13.33%) whereas there was absence of seropositivity among the age group 16-20 years and 21-25 years (Table **1**). The statistical analysis shows that there was significance difference between seropositivity of brucellosis and age of the pregnant women.

Trimester Wise Prevalence

Trimester had been differentiated into three groups (first, second and third trimester) on the basis of the month of pregnancy. The highest sero-prevalence rate (12.76%) was found in the third trimester followed by first trimester (10%) and the lowest was in second trimester (8.69%) even though the sample sizes were not equally divided. The statistical analysis shows that there was no significance difference ($p > 0.05$) between seropositivity of brucellosis and trimester of pregnant women (Table **1**).

DISCUSSION

Brucellosis is transmitted from meat and milk products to human. In Nepal buffaloes contribute about 64% of the meat consumed, followed by goat meat (20%), pork (7%), poultry (6%) and mutton (2%) [10]. Similarly about 88% of urban households consume milk regularly and 7% occasionally and milk products like ghee (45% of households) and yoghurt (33% of households) is also consumed in Nepal [11].

In Nepal, though the countable reports based on human brucellosis has been reported but still there is no report on brucellosis in pregnant women. This study of brucellosis in

pregnant women was conducted for the first time in the capital of the Nepal. In this study the seroprevalence of brucellosis in pregnant women was found to be 11.25%. Previously, it was reported that the seroprevalence of human brucellosis in Kathmandu was 11.95% [12]. Similarly, it was reported as 20% in Surkhet district and 14% in the patients visiting Bir hospital [13]. Likewise other hospital based studies also showed similar results, 0.4% from the hospitals in Kathmandu [14] and 2.7% from the samples collected from Bir hospital and Teku infectious hospital [15]. The overall seroprevalence of human brucellosis in Chitwan district was 1.4% [16], in Dolakha district was 0.5% [17].

About 4.96% prevalence of brucellosis has been reported among PUO and occupationally exposed individuals in Goa [18], 24.5% in Ludhiana, India [19]. Globally several studies showed the similar seroprevalence such as 6.4% in Iran [20], 5.2% in Afghanistan [21], 3.4% in Central Anatolia [22], 19% in Saudi Arabia [23], 53.25% in Mongolia [24], 20.5% in Tanzania [25], 6.26% in Egypt [26], 16% in Kenya [27], 2.15% in Ethiopia [28], 24.1% in abbatoir workers of Abuja [29], 17% in Uganda [30] and 3.8% in Chad [31].

In the present study, the statistical analysis showed that there is significance difference between seropositivity of brucellosis and age of the pregnant women in which the highest seroprevalence rate (29.41%) was found within the age group 31-35 years followed by the age group 26-30 years (13.33%). The seroprevalence of brucellosis was high (2.72%) among the people above 50 years age group in Chitwan [16], 20-29 years (29%) in Surkhet [13] and 6-15 years age group (29.17%) in Kathmandu [12].

The highest prevalence (4.3% & 4.1%) was found in the 35-44 and 15-24 age groups and the lowest prevalence (2%) was observed in 25-34 age groups among the people living in rural area of Central Anatolia, Turkey [22]. Similarly, the most common age of human brucellosis in Azna, Western Iran was 15-24 (27.9%) and about 60.5% of the patients were between 15-44 years old [32]. Brucellosis was most prevalent among people aged 30-49 years (46%) in Serbia [33] and the highest seroprevalence (26.9%) was found in 15-24 years in Albania [34].

CONCLUSION

The present study showed the highest seroprevalence rate in the third trimester (12.76%) followed by first trimester (10%) and the lowest was in second trimester (8.69%). Similarly, the highest seroprevalence rates of brucellosis was found among Madheshi (16.66%) followed by Janajati (11.53%) and the lowest was in Brahmin (8.33%).

AUTHORS' CONTRIBUTION

Seema Thapa and **Mahendra Maharjan** have contributed equally.

CONFLICT OF INTEREST

Declared none.

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