Seroprevalence and associated risk factors of human *Brucella* infection in selected provinces in Sri Lanka

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**Abstract**

Brucellosis is a systemic zoonotic bacterial infection. We studied the seroprevalence and risk factors for human *Brucella* infection in 1,294 healthy people from 4 provinces: Central, North-Western, North-Central and Western Provinces. Farmers in contact with farm-animals, veterinary staff, abattoir workers, and non-contact urban-dwellers were tested against *B. abortus* and *B. melitensis* antigens by SAT. Seroprevalence was 8.4% of the study population. Farm-animal owners and working full-time with livestock have a significantly higher risk of acquiring *Brucella* infection. Enhanced laboratory support and surveillance is necessary to control brucellosis in Sri Lanka. This is the first report on human *Brucella* infection.

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**Introduction**

Brucellosis is a systemic bacterial zoonosis. It is transmitted by ingestion through food products, direct contact with infected animals and inhalation of aerosols. Transmission through blood transfusion or tissue transplantation and sexual transmission are rare possibilities. Although the disease has been well known for decades, it continues to be a major public health issue worldwide [1].

The true incidence of human brucellosis is unknown globally, as many countries known to be endemic for brucellosis, such as India and South Africa, do not have reliable data on the disease [2].

*Brucella* are small aerobic intracellular coccobacilli, that live mainly in the reproductive organs of wild and domestic animals. There are 8 species identified, but *B. melitensis*, *B. suis*, *B. abortus* and *B. canis* cause disease in humans. Although *B. melitensis* is thought to be the most virulent species, *B. abortus* is the most commonly distributed species worldwide [1]. Livestock farmers, slaughterhouse workers and veterinarians are at high risk of developing the disease. Travelers and urban residents usually acquire the infection through the consumption of contaminated food especially raw milk or milk products [3]. The disease was introduced to Sri Lanka during the second world war, through the importation of cattle. The first clinical outbreak in livestock was reported in 1956 [4,5]. The North-Western and North-Central Provinces with the highest density of cattle, were implicated as endemic areas for brucellosis[4]. However, a meta-analysis in 2013 has shown that data is not available for human brucellosis in Sri Lanka[6].

We studied the seroprevalence of human *Brucella* infection and associated risk factors among high-risk occupations such as farmers, veterinarians and abattoir workers in Central (CP), North-Western (NWP), North-Central (NCP) and Western (WP) Provinces.

**Methods**

The study was conducted in 2014/2015 among 9 districts in the 4 Provinces. The 41 veterinary divisions and their farms were randomly selected by probability-proportional-to-size sampling technique. Veterinary staff, abattoir workers and non-contact urban-dwellers were recruited by convenient sampling.

A total of 1,294 blood samples were collected from farmers in contact with farm animals (818), veterinary staff (190), abattoir workers (137) risk categories and non-contact urban-dwellers (149). The study used a non-random purposive sampling method. Standard tube Agglutination Test (SAT) was performed using *B. abortus* and *B. melitensis* antigens (MAST™ Assure febrile stained antigen, UK) according to manufacturer’s guidelines at clinical bacteriology laboratory, Medical

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Research Institute. A titer of 1:80 was considered for seropositivity (past infection). Socio-demographic data were collected using an interviewer-administered questionnaire.

Statistical analysis

Data entry and statistical analyses were performed using SPSS version 21. Quantitative data were obtained in the form of percentages and figures. Cross tabulations were generated between seroprevalence and socio-demographic factors, and the Pearson $\chi^2$ test was used to express the statistical significance of any associations. P-values of 0.05 or less were considered to be significant. The potential risk factors for seropositivity were also assessed using bivariate analyses and presented as Odds Ratio (OR).

Results

A total of 1,294 healthy adults were screened. Sample size was sufficient to estimate sero-prevalence of brucellosis at 50% in the study population. The level of precision is 95% with confidence interval 3% and the non-response rates was 15%. Males constituted 81.9% and the mean age was 45.6 years (Table 1). SAT was negative in 1,185 (91.6%) of the study population and positive in 104 (8.4%). The overall seroprevalence for human *Brucella* infection was 8.4%. SAT showed seropositivity of 7.5% for *B. abortus* and 3.2% for *B. melitensis*. The risk occupations had a significant seropositivity compared to non-contact residents (p=0.04). Farm animal owners showed a significant seropositivity, in comparison to non-contact residents (OR=3.0, p<0.05) [Table 2,3]. Analysis of specific high-risk behaviors such as assisting in animal delivery, duration and degree of animal contact, and consumption of raw milk did not show significant associations with seropositivity. Individuals with full-time exposure to animals (p=0.01) had a significantly higher risk of developing the *Brucella* infection than part-time workers. Being a livestock farmer with animal contact (p=0.03) yielded a significantly higher risk than for veterinary staff or abattoir workers [Table 2,3].

| Description | Number (n) participated | % |
|-------------|-------------------------|---|
| Province    |                         |   |
| WP          | 403                     | 31.1 |
| CP          | 166                     | 12.8 |
| NWP         | 356                     | 27.5 |
| NCP         | 369                     | 28.5 |
| Risk categories |                 |   |
| Animal-husbandry owners | 596 | 46 |
| Part-time animal handlers | 216 | 16.6 |
| Veterinary staff | 192 | 14 |
| Abattoir workers | 141 | 10.8 |
| Urban-dwellers | 149 | 11.5 |

(Continued)
| Characteristic                  | Seropositivity (n) | %  | Total | Chi-square | P value |
|-------------------------------|--------------------|-----|-------|------------|---------|
| Duration of risk occupation   |                    |     |       |            |         |
| 0-10 years                    | 44                 | 9.6%| 460   | 1.6        | 0.813   |
| 11-20 years                   | 37                 | 7.7%| 483   |            |         |
| 21-30 years                   | 19                 | 8.0%| 238   |            |         |
| 31-40 years                   | 8                  | 8.7%| 92    |            |         |
| 41-50 years                   | 1                  | 4.8%| 21    |            |         |
| Risk Occupation vs. Risk occupations | 103             | 9.0%| 1145  | 4.2        | 0.040   |
| Urban-dwellers                | Yes                | 12  | 154   | .090       | 0.764   |
| Working in slaughter house    | No                 | 97  | 1140  |            |         |
| Type of livestock             |                    |     |       |            |         |
| Cattle only                   | 50                 | 8.3%| 602   | 4.5        | 0.337   |
| Water buffaloes only          | 5                  | 14.7%| 34   |            |         |
| Goat only                     | 2                  | 10.5%| 19   |            |         |
| Swine only                    | 1                  | 16.7%| 6    |            |         |
| Mix herd                      | 24                 | 12.6%| 190  |            |         |
| Working with livestock        |                    |     |       |            |         |
| Full time                     | 51                 | 10.8%| 474   | 6.9        | .032    |
| Part time                     | 34                 | 8.3%| 410   |            |         |
| No                            | 24                 | 5.9%| 410   |            |         |
| Degree of contact             | Habitual contact   | 85  | 982   | 3.5        | 0.320   |
| Frequent contact              | 10                 | 10.3%| 97   |            |         |
| Less frequent contact         | 5                  | 11.9%| 42   |            |         |
| No contact                    | 9                  | 5.2%| 173   |            |         |
| Duration of Contact with animals| No contact     | 9   | 163   | 3.8        | 0.705   |
| ≥20 years                     | 34                 | 8.9%| 382   |            |         |
| 15-19 years                   | 14                 | 8.6%| 162   |            |         |
| 10-14 years                   | 11                 | 6.6%| 167   |            |         |
| 5-9 years                     | 22                 | 9.3%| 236   |            |         |
| 1-4 years                     | 16                 | 10.3%| 155  |            |         |
| <1 year                       | 3                  | 10.7%| 28   |            |         |
| Drinking raw milk             | Yes                | 4   | 53    | 0.06       | 0.815   |
| No                            | 105                | 8.5%| 1241  |            |         |
| Study Prevalence              |                    |     |       |            |         |
| 8.40%                         | 1294               |     |       |            |         |
Table 3. Univariate regression analysis for being seropositive to *Brucella* infection:
Odds ratio for socio-demographic and occupational risk factors (n=1294)

| Variable                                      | Unadjusted OR | Lower  | Upper  | P value |
|-----------------------------------------------|----------------|--------|--------|---------|
| **Gender**                                    |                |        |        |         |
| Female                                        | 1.000          |        |        |         |
| Male                                          | .680           | .427   | 1.082  | .104    |
| Age                                           |                |        |        |         |
| <30 yrs                                       | 1.000          |        |        |         |
| 31-40                                         | .568           | .283   | 1.142  | .112    |
| 41-50                                         | .834           | .447   | 1.554  | .567    |
| 51-60                                         | .360           | .169   | .771   | .009    |
| >60                                           | .991           | .463   | 2.123  | .982    |
| **Occupation**                                |                |        |        |         |
| Non-contact persons                           | 1.000          |        |        | .033    |
| Animal husbandry own                          | 2.917          | 1.239  | 6.870  | .014    |
| Handling farm animals part-time               | 1.402          | .514   | 3.822  | .509    |
| Veterinary staff                              | 2.020          | .764   | 5.339  | .156    |
| Abattoir workers                              | 2.017          | .725   | 5.608  | .179    |
| **Duration of risk Occupation**               |                |        |        |         |
| 0-10 years                                    | 1.000          |        |        | .816    |
| 11-20 years                                   | .784           | .497   | 1.239  | .298    |
| 21-30 years                                   | .820           | .467   | 1.439  | .490    |
| 1-40 years                                    | .900           | .409   | 1.982  | .794    |
| 41-50 years                                   | .473           | .062   | 3.608  | .470    |
| **Occupation risk**                           |                |        |        |         |
| Non-risk occupations                          | 1.000          |        |        |         |
| Risk occupations                              | 2.356          | 1.016  | 5.465  | .046    |
| **Risk of animal exposure**                   |                |        |        |         |
| Non-contact                                   | 1.000          |        |        |         |
| Animal contact                                | 2.497          | 1.067  | 5.841  | .035    |
| Vet staff                                     | 2.020          | .764   | 5.339  | .156    |
| Abattoir                                      | 2.017          | .725   | 5.608  | .179    |
| **Working in slaughter house**                |                |        |        |         |
| No                                           | 1.000          |        |        |         |
| Yes                                           | 1.101          | .589   | 2.056  | .764    |
| **Type of livestock**                         |                |        |        |         |
| Mix herd                                      | 1.000          |        |        |         |
| Cattle only                                   | .627           | .374   | 1.050  | .076    |
| Water buffaloes                               | 1.193          | .421   | 3.378  | .740    |
| Goat only                                     | .814           | .177   | 3.744  | .791    |
| Swine only                                    | 1.383          | .155   | 12.351 | .771    |
| **Working with livestock**                    |                |        |        |         |
| No                                           | 1.000          |        |        |         |
| Full-time                                     | 1.939          | 1.171  | 3.211  | .010    |
| Part-time                                     | 1.454          | .846   | 2.499  | .175    |
| **Degree of contact**                         |                |        |        |         |
| No contact                                    | 1.000          |        |        |         |
| Habitual contact                              | 1.727          | .852   | 3.501  | .130    |
| Frequent contact                              | 2.095          | .820   | 5.348  | .122    |
| Less frequent                                 | 2.462          | .780   | 7.776  | .125    |
| **Duration of contact**                       |                |        |        |         |
| No contact                                    | 1.000          |        |        |         |
| >20 years                                     | 1.672          | .783   | 3.570  | .184    |
| 15-19 years                                   | 1.619          | .680   | 3.853  | .276    |
| 10-14 years                                   | 1.207          | .486   | 3.299  | .685    |
| 5-9 years                                     | 1.759          | .788   | 3.926  | .168    |
| 1-4 years                                     | 1.970          | .843   | 4.600  | .117    |
| <1 year                                       | 2.053          | .520   | 8.107  | .305    |
| **Drinking raw milk**                         |                |        |        |         |
| No                                           | 1.000          |        |        |         |
| Yes                                           | .883           | .313   | 2.495  | .815    |

*Reference category is underlined*
Brucellosis has been recognized as a re-emerging zoonosis due to growing international tourism, migration and potential use as a biological weapon. In Sri Lanka, animal brucellosis is an endemic disease, causing economic impact to the livestock industry [4].

One clinically suspected case of human brucellosis has been briefly described in a Sri Lankan patient from Monaragala four-decades ago [7]. Since then, no reports were available on human disease in Sri Lanka. Our study shows a seroprevalence of 8.4% for human Brucella infection and the most common species detected serologically was B. abortus.

In animal brucellosis, B. abortus has been reported as the common species in Sri Lanka [4]. In the 1980s, cases of caprine brucellosis due to B. melitensis were reported and more recently, in swine in Sri Lanka [6,8]. High incidence of animal brucellosis was reported from Eastern, NCP and NWP [5].

Close contact with animals and raw milk ingestion has been attributed as the major risk factors in Karnataka in India [9]. In our study, farm animal owners and full-time handlers of livestock showed significant seropositivity than part-time livestock handlers and non-contact groups and only 53 persons ingested raw milk [Table 2,3]. This signifies the variations in risk factors for brucellosis in different countries.

In conclusion, the seroprevalence for human Brucella infection is 8.4%. Farm-animal owners and those working full-time with animal husbandry have a significantly high risk of acquiring the infection. This is the first report on human Brucella infection in Sri Lanka.

Awareness of the disease among clinicians, and enhanced laboratory diagnostic support and surveillance in human and animal brucellosis are recommended to control brucellosis in Sri Lanka.

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Conflicts of interests

The authors declare that they have no conflicts of interests.

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Declarations

Ethics approval and consent to participate:

Ethics approval was obtained from the Ethics Review Committee, Medical Research Institute, Sri Lanka (32/2011). Background data on farms were obtained from the District Veterinary Surgeons through the Director General of Animal Health and Production. Written consent was obtained from participants.