Seroepidemiological study of human brucellosis in the Northeast region of Meghalaya, India

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Abstract

Introduction: Brucellosis is a neglected zoonotic disease of major public health concern. In India, the incidence of brucellosis remains vastly underreported due to its non-specific clinical presentation and sub-optimal sensitivity of existing gold standard tests. Studies in Northeast India have shown high incidences of brucellosis in livestock, but the region lacks data on human brucellosis despite its high associated risk. In the present study, we report the seroprevalence of human brucellosis and its associated risk factors in Meghalaya, Northeast India. Materials and Methods: A prospective observational study was conducted in East Khasi Hills and RiBhoi districts of Meghalaya, from July 2018 to July 2020. A total of 1046 suspected patients with febrile illness along with associated risk factors were recruited through camps and various diagnostic laboratories in the defined region as per the pre-specified inclusion and exclusion criteria. Baseline, demographics, and clinical characteristics were recorded of all the consenting participants. Blood samples were analyzed for brucellosis-specific IgM antibodies through enzyme-linked immunosorbent assay (ELISA). Results and discussion: The overall seroprevalence of brucellosis was found to be 11.37% in Meghalaya. Among recruited participants, females were found to be more susceptible than males. Risk factors such as consumption of meat were found to be more significantly associated with brucellosis disease in the study region. Among the clinical presentations, pyrexia of unknown origin, myalgia, and chronic fatigue syndrome were found to be significantly associated with brucellosis disease in IgM-positive cases. Conclusion: Our result suggests further epidemiological investigations for human brucellosis in Northeast India toward improved advocacy for accurate diagnosis, and development of proper response mechanism in areas of high endemicity.

Keywords: ELISA, human brucellosis, Northeast, seroprevalence

Introduction

Brucellosis is a neglected zoonotic disease caused by gram-negative bacteria belonging to the genus Brucella. In humans, occupational contact with infected livestock along with consumption of raw and unpasteurized milk products has been regarded as a significant risk factor for brucellosis.[1,2] The majority of patients with brucellosis suffer from subacute febrile illness and are often misdiagnosed by primary care physicians due to similar clinical manifestation shared with other bacterial and viral diseases.[1,3] As a result, fewer than 10% of human brucellosis in an acute
healthcare setting may be clinically reported or treated.\[^4\] Although most seropositive cases may remain asymptomatic throughout their lifetime, evidence from various studies indicates that brucellosis can lead to severe debilitating illness of the central nervous system known as neurobrucellosis if not adequately undiagnosed and treated.\[^5,6\]

In recent times, an increasing trend of human brucellosis has been reported from several studies across the globe.\[^7\] In India, the occurrence of human brucellosis has been reported from some states with a general prevalence of 17–34%\[^1] and seroprevalence of around 0.9–8.5%.\[^8\] In our own hospital-based studies, we reported a high seroprevalence of brucellosis of around 11\%.\[^9\] Brucellosis has been regarded to be endemic in rural areas due to high agrarian practices and a lack of awareness about the disease in the rural population. Despite the high risk, lack of good epidemiological data in humans exists from several other states with reported high incidences of brucellosis in livestock.

The northeast region of India, particularly Meghalaya, heavily depends on agriculture and livestock farming for the livelihood and economy of the state.\[^9\] Meghalaya has a cattle population of 0.9 million which is reared mainly for milk, meat, and meat products.\[^10\] East Khasi Hills and Ri-Bhoi districts in Meghalaya are regions where livestock farming is the most common and frequent occupation. The expansion of animal industries and the lack of hygienic measures in animal husbandry contribute to brucellosis’ persistence as a public health problem in this region. Studies in livestock from various northeastern states including Meghalaya show a high prevalence of bovine brucellosis of around 9–10\%.\[^11,12\] Despite high livestock prevalence and associated risk factors, till date no studies have been reported on the incidence of human brucellosis in Meghalaya. In wake of an existing pandemic caused by SARS-CoV-2 which has a zoonotic origin, it becomes imperative for clinicians to investigate the epidemiology of zoonotic diseases like Brucella in high-risk region/population for appropriate preparedness and response mechanism for future epidemics.

In the present study, we aimed to determine the seroprevalence and associated risk factors of brucellosis in Northeast India targeting East Khasi Hills and Ri-Bhoi districts of Meghalaya.

**Materials and Methods**

**Ethical statement**

All clinical investigations in the study were conducted according to the principles expressed in the Declaration of Helsinki 1975, as revised in 1983. Written consents were obtained from all participants before recruitment and after oral explanation about the study [Supplementary File 1]. All protocols for blood collection, analysis, and waste disposal were approved by the respective institutional human ethics committees of Central India Institute of Medical Sciences (CIIMS), Nagpur, and North-Eastern Hill University (NEHU), Shillong [Supplementary File 2].

**Study design and participant recruitment**

A prospective observational study was conducted from July 2018 to July 2020 by NEHU, Shillong, and CIIMS, Nagpur. A total of 1240 samples were recruited, suspected of having pyrexia of unknown origin cases from Ri-Bhoi and East Khasi Hills districts of Meghalaya through camps and various diagnostic laboratories. Every participant was enrolled as per pre-specified inclusion criteria which included clinical suspicion based on symptoms and associated risk factors. Baseline, demographic data, and clinical details were obtained through structured questionnaires prepared in the native Khasi language [Supplementary File 1]. Details such as age, gender, details of occupation, exposure with animals, duration of existing illness, history of abortion in females, etc., were specifically recorded through oral interviews.

Out of the total 1240 recruited participants, 50 participants opted out during the collection of blood. Among the 1190 samples, 144 were further excluded from the study due to incomplete clinical data (n = 35) and baseline data (n = 34), hemolyzed blood samples (n = 35), pregnant and lactating mothers (n = 20), and participants below 18 years of age (n = 20). A total of 1046 participants were finally included for analysis which included 378 samples from Ri-Bhoi and 668 samples from East Khasi Hills. Study flow diagram for participant recruitment is provided in [Figure 1].

**Sample collection**

Two milliliters of blood sample from recruited participants was collected in sterile plain vacutainers and allowed to clot. The separated serum was collected in separate 1.5-ml centrifuge tubes and further centrifuged for 6000 g for 5 min to remove any blood cells. The serum obtained was finally transferred to fresh 2-ml cryotubes and immediately frozen until further analysis.

**Brucella IgM ELISA**

Detection of IgM antibodies using ELISA was performed using a commercial kit (NovaTec Immundiagnostica GmbH, Germany) as per the manufacturer's instructions. The IgM titers above 0.7 were considered positive. Sensitivity and specificity, as
given by the manufacturer, were >95% for IgG and >95% for IgM, respectively.

**Statistical analysis**

The data were analyzed using MedCalc statistical software (version 10.1.2.0). The demographics, clinical factors, and the frequencies (percentage) of risk factors were measured on a nominal scale. A comparison between groups was made using the Chi-square test in MedCalc statistical software (version 10.1.2.0). P value < 0.05 was found to be significant.

**Results**

In the present study, the seroprevalence of human brucellosis was studied in the northeast region of India. A total of 1046 blood samples were collected from Ri-Bhoi (n = 378) and East Khasi Hills (n = 668) districts of Meghalaya. The total overall seroprevalence of brucellosis was found to be 11.37% [Figure 2]. The seropositivity of brucellosis among the two districts was found to be 19.04% and 12.42%, respectively [Figure 2].

Age- and gender-wise distribution of IgM positive cases is indicated in Table 1. Among the gender, females (13.89%) were found to be more seropositive than males (8.90%). The age-wise stratification indicated an economically productive age-group of 20–40 years (54.62%) having high exposure to brucellosis followed by an older group of 40–60 years (26.89%).

Among the reported clinical symptoms, pyrexia of unknown origin (89.07%) was one of the significant risk factors recorded during the study (<0.0001) followed by myalgia (83.19%) and chronic fatigue syndrome (81.5%) among Brucella seropositive subjects [Table 2].

Among the various other risk factors, consumption of meat was found to be significantly associated with seropositive Brucella cases in Meghalaya (<0.0001) [Table 3]. Risk factors, commonly reported for brucellosis such as consumption of raw milk and vegetables, were, however, not found to be significantly linked with Brucella disease [Figure 3].

### Table 1: Age- and gender-wise distribution of seropositive population in Meghalaya, Northeast India

| Characteristics | Levels | IgM-positive (n=119) |
|-----------------|--------|----------------------|
| Gender          | Male   | 47 (8.90)            |
|                 | Female | 72 (13.89)           |
| Age in years    | <20    | 14 (11.76)           |
|                 | 20-40  | 65 (54.62)           |
|                 | 40-60  | 32 (26.89)           |
|                 | 60     | 8 (6.72)             |

### Table 2: Clinical history among Brucella IgM-positive subjects in Meghalaya

| Risk factors                  | Brucella IgM-positive (%) | χ² | DF | P      |
|-------------------------------|--------------------------|----|----|--------|
| Pyrexia of unknown origin     | 106 (89.07)              | 71.126 | 1 | <0.0001|
| Myalgia                       | 99 (83.19)               | 51.126 | 1 | <0.0001|
| Chronic fatigue syndrome      | 97 (81.5)                | 46.017 | 1 | <0.0001|

**Discussion**

In the present study, the seroprevalence of human brucellosis was studied in Meghalaya, Northeast India. Our findings showed an overall seroprevalence of 11.37% in the region. Among the two regions, Ri-Bhoi and East Khasi Hills, the seropositivity of brucellosis was found to be 19.04% and 12.42%, respectively. The present study represents the first report of human brucellosis in the northeast region of Meghalaya.

The seroprevalence of brucellosis in the region was carried out using a commercial IgM ELISA assay. ELISA is one of the most specific and reliable diagnostic tools for brucellosis. The technique does not require any advanced infrastructure and can be used in very limited settings. Since this technique is used to identify the individual IgM and IgG antibodies to the surface antigens, which provides a better clinical correlation for diagnosing the early stages of brucellosis, the technique can be further used for the mass screening in suspected and confirmed cases.\[7,13\]

In the present study, risk factors associated with the seropositivity of brucellosis were pyrexia of unknown origin, chronic fatigue syndrome.
syndrome, and myalgia. Our results are in line with several other reports which suggest these risk factors to be predominantly linked with Brucella exposure.[3,7] Based on gender-wise stratification, seroprevalence was found to be predominantly higher in females (13.89%) compared to males (8.90%). Our reports are in contrast to other studies, which suggest that males owing to frequent exposure are at high risk for brucellosis.[14,15] However, due to the close proximity to the animals in domestic chores and vendors being primarily females in Meghalaya, they are found to be at higher risk of exposure for brucellosis in the study.

We also studied different age-groups which are more vulnerable to Brucella infection. The study shows us that the younger age-group between 20 and 40 years is more frequently affected by brucellosis, followed by the age-group of 40–60 years. Higher exposure of brucellosis in economically productive age-groups is primarily linked with various agrarian practices, slaughtering of pigs, and livestock for meat which is generally the predominant occupation of the majority of population in Meghalaya. These risk factors have been linked to increased exposure to brucellosis and high seropositivity from previous studies.[16,17]

Among the various risk factors analyzed, we found that meat consumption was significantly associated with brucellosis in the Meghalaya population compared to other common risk factors like consumption of dairy products and raw vegetables, which is generally reported from several studies.[8-11] Due to the cold temperatures and Shillong being the hub for the beef market, consumption of beef and other meat products is very high in the population which can be linked with higher seropositivity among the population. Our study reported that 50% of the population recruited under the study were seropositive for brucellosis but were not found significantly associated with any risk factors.[15,16]

Table 3: Risk factors associated with seropositive cases for brucellosis in Meghalaya

| Risk factors                                      | Seropositive (%) | $\chi^2$ | DF | P        |
|--------------------------------------------------|------------------|----------|----|----------|
| Consumption of meat                              | 110 (92.4)       | 84.034   | 1  | <0.0001  |
| Exposure to animals (on the basis of occupation) | 73 (61.3)        | 5.681    | 1  | 0.0172   |
| Consumption of raw milk and dairy products       | 60 (50.4)        | 0.0      | 1  | 1.000    |
| Consumption of raw vegetables                    | 55 (46.2)        | 0.538    | 1  | 0.463    |

Figure 3: Risk factors-wise distribution of Brucella diagnosis

Our study also comes with some limitations. Our study focused on only a few northeast regions in Meghalaya. Secondly, the lack of parallel sampling from animals and the occupationally exposed population was not able to establish the exact risk factors associated with brucellosis. However, our initial study provides epidemiological insights on the high prevalence of human brucellosis in Meghalaya. Brucellosis is an endemic disease in India.[18] In humans, controlling this neglected zoonotic disease can be done by different vaccination policies and a proper clinical diagnosis among the fever with unknown origin cases for Brucella. Prevention of the disease, awareness programs, and safe livestock practices should be initiated to lower the brucellosis cases.

**Conclusion**

In conclusion, we report a high seroprevalence of human brucellosis as 11.37% in the northeast part of the Meghalaya State. Risk factors such as meat consumption were significantly linked with brucellosis. Further epidemiology studies are warranted in such regions of endemcity to determine accurate estimates of prevalence and risk factors and to study Brucella biovars for appropriate policymaking and advocacy and awareness regarding brucellosis in Northeast India.

**Key points in the manuscripts**

**Take-home message from this manuscript**

The study reported seroprevalence of human brucellosis as 11.37% in the northeast region of Meghalaya which is quite high in the region, since the people residing in the northeast have much animal exposure due to their cold climatic conditions, animal rearing habits, and meat-eating habits. Proper awareness and diagnosis of this disease are very important factors for controlling the disease. We emphasize regular screening of the disease in various places of the northeast to develop proper epidemiological data and initiate appropriate control measures.

**Novelty or new knowledge emerging from this manuscript**

This is the first study to report the seroprevalence of human brucellosis in the Meghalaya region of Northeast India. Risk factors such as meat consumption were found to be significantly linked with brucellosis infection. The study provides useful insights into epidemiology of brucellosis and its associated risk factors in the northeast region for policymaking and further...
advocacy for health strategy and diagnosis of brucellosis in high-risk subjects.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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Annexure 2

Consent Form

Lab Number: .............................................

Name: ......................................................

For the participation in study entitled “Screening of Human Brucellosis Infection in NER regions using point of care Immunological and Molecular tools”

Principle Investigator: 1. Dr. Surya Bhan,
                        Assistant Professor,
                        Dept. of Biochemistry,
                        North Eastern Hill University (NEHU),
                        Shillong, Meghalaya

2. Dr. Rajpal Singh Kashyap, Director, Research
   Biochemistry Research Laboratory
   Central India Institute of Medical Sciences,
   Nagpur-440010 (INDIA),
   Phone: 0712-2233381, 2236441 (ext 262/263)

Consent of the Patient and their contacts

With this background information, if you are willing to participate in study, please give your consent and sign below after reading and understanding the following statements.

- I understand that if I need any further information regarding my rights as a subject for this study, I may contact the doctors at the addresses given to me. I understand that in the event of any injury from the research procedures, management of the injury will be provided without the cost to me, but financial compensation will not be available.
- I understand that my participation in this study is completely voluntary, confidential and I may withdraw from the study at any time without the penalty or loss of benefits.
- I have been given an opportunity to ask all/any questions and I have answered to my satisfaction.
- I agree for providing blood samples that could be required to reach diagnosis of Brucellosis
- I agree to participate in the study.

Name of the patient: ___________________________ Name of the witness: ___________________________

Signature: ____________________ (Date __/__/__)           Signature: ____________________ (Date __/__/__)
            (Thumb impression if illiterate)             (Thumb impression if illiterate)

Name of the Researcher: ___________________ Signature of Project Co coordinator/PI
PROFORMA SHEET FOR BRUCELLOSIS

| Name |  |
|------|---|
| Occupation |  |
| Permanent Address |  |
| Current Address |  |
| Sex |  |
| Age |  |
| Marital Status |  |
| No. of Children | Male: | Female: |
| Length of service in years | Clinical | Extension | Laboratory | Livestock Farm | Administrative/Official | Any other | Total |

1. Have you ever suffered with any of these symptoms during your life?  
(√ Tick the appropriate answer)

| Symptom | Once | More than once | Often | Current status |
|---------|------|----------------|-------|---------------|
| Fever of unknown origin / Chills/ Undulant fever |  |  |  |  |
| Weight Loss |  |  |  |  |
| Chronic Fatigue |  |  |  |  |
| Loss of energy/ weakness |  |  |  |  |
| Arthritis |  |  |  |  |
| Low back pain |  |  |  |  |
| Spine and joint pain |  |  |  |  |
| Swelling of joints |  |  |  |  |
| Headache |  |  |  |  |
2. Have you ever suffered following neurological complications?
(√ Tick the appropriate answer)

| Neurological complications                        | Yes | No |
|--------------------------------------------------|-----|----|
| Impairment of language                           |     |    |
| Hearing Loss                                     |     |    |
| Hemiplegia (paralysis of one side of the body)   |     |    |
| Double vision (Diplopia)                         |     |    |
| Sleep Disturbance                                |     |    |
| Confusion                                        |     |    |
| Unsteadiness of gait                             |     |    |
| Depression                                       |     |    |
| Neck Stiffness                                   |     |    |
1. Have you ever screened yourself for ‘rheumatoid arthritis’ Y/N
   If yes what was the result

2. Is any family member suffering with ‘rheumatoid arthritis’ Y/N
   If yes give details

3. Any other symptoms you wish to specify?

4. Have you ever been investigated for these symptoms? Y/N
   If yes then what was the diagnosis? Give details of the tests performed if possible.

5. Did your physician ever suspected you for brucellosis? Y/N

6. Did you ever suspect yourself for brucellosis? Y/N

7. Did you ever asked your physician to go for brucellosis test? Y/N
   If yes then what was the reaction of your physician?

8. Did your physician ever advise you for the confirmatory test of brucellosis? Y/N
   If yes then which test was performed and what was the outcome

9. What was the treatment given to you for your symptoms?

10. How many days you receive the treatment?

11. Did your symptoms recur after treatment? Y/N
    If yes then what you did for that?

Place: ____________________________
Signature: ____________________________

Date: ____________________________
Supplementary File 2: Institutional ethical committee clearance

**ETHICAL CLEARANCE CERTIFICATE**

The collection of blood samples from participants recruited for the project entitled, "Screening of human Brucellosis infection in Northeast regions using point of care immunological and molecular tools - [24766]" has been approved by the institutional ethical committee of Central India Institute of Medical Sciences (CIIMS), Nagpur.

| Name                | Qualification                                      | Position held in the IEC | Signature |
|---------------------|----------------------------------------------------|--------------------------|-----------|
| Dr. J. Y. Deopujari | B.A,M.S, PhD (Ayurveda), Ayurvedic Physician, Nagpur | Chairman, IEC            |           |
| Dr. Lokendra Singh  | MS, MCh, DNB, FRCS (SN) Director, CIIMS           | Secretary, IEC           |           |
| Dr. H. F. Daginawala| M.Sc, PhD, FMASc CIIMS                             | Convenor, IEC            |           |
| Dr. Rajpal S Kashyap| M.Sc, PhD Director Research and Project Investigator, CIIMS | Member, IEC             |           |
| Dr. V.S.Agrawal     | MBBS, MD (Medicine) CIIMS                         | Member, IEC              |           |
| Mr. N. S. Bhattad   | Chartered Accountant, Nagpur                      | Member, IEC              |           |
| Dr. H. J. Purohit   | M.Sc, PhD Sr. Scientist and Head- Environmental Genomics Unit, NEERI, Nagpur | Member, IEC |           |
| Mrs. Nandini Deshmukh| M.A., M.S.W Social Worker, Nagpur                | Member, IEC              |           |
### North Eastern Hill University

NEHU Campus, Shillong-793022

INSTITUTIONAL ETHICS COMMITTEE FOR HUMAN SAMPLES/PARTICIPANTS (IECHSP)

Date: 02/3/2019

#### CLEARANCE CERTIFICATE

| Title of the project | “Screening of human Brucellosis infection in Northeast regions using point of care immunological and molecular tools” Vide No. IECHSP/2018/51/ |
|----------------------|-------------------------------------------------------------------------------------------------|
| Principal Investigator | Dr. S. Bhan |
| Co-investigator | - |
| Address | Department of Biochemistry  
School of Life Sciences  
North Eastern Hill University  
Shillong-793022 |
| Date of meeting of the committee | 29/01/2019 |
| Decision of the committee | Project is cleared |
| Comments, if any | Nil |

Prof. S. R. Rao  
Member Secretary  
IECHSP, NEHU  
North-Eastern Hill University  
Shillong - 793022 (Meghalaya)