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Letter to the Editor

Brucellosis among fever patients attending a primary health centre in rural South India

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Dear Editor,

Brucellosis is one of the major zoonoses associated with livestock farming, dairy, and meat industries. Human brucellosis has a wide spectrum of clinical manifestations and usually presents as an undifferentiated febrile illness [1]. Consumption of unpasteurized dairy products, contact with livestock, and occupational exposure among veterinarians and abattoir workers are the important risk factors for human infection [2]. India has the world's largest livestock inventory and indigenous farming practices. This involves close interactions between humans and livestock with a risk of possible zoonotic spillover. *Brucella* infection is enzootic in the Indian livestock population [3]. The neglected nature of *Brucella* infection contributes to underdiagnosis and under-reporting of brucellosis in endemic countries, including India [4]. Nevertheless, human brucellosis has been documented in different parts of India [5-8].

In the absence of any documentation from Tamil Nadu State in Southern India, we investigated brucellosis among fever cases attending a primary health care facility (PHC) in Tirunelveli, Tamil Nadu. This study was approved by the Institutional Research Ethics Committee of Tirunelveli Medical College, Tirunelveli. The catchment area of the PHC facility is predominantly rural, with livestock farming as a major source of livelihood.

Using a structured questionnaire, demographic and clinical data were collected. Five mL of blood was collected in plain (red-top) and EDTA vacutainers from the enrolled patients. EDTA blood was used to evaluate the hemogram, and serum was used for *Brucella* antibody detection using multiple serological assays. Rose Bengal Plate Test (RBPT) was carried out using *B. abortus* S99 colored antigen obtained from the Institute of Animal Health and Veterinary Biologicals (IAHVB), Hebbal, Bengaluru, India. RBPT was performed as described by Diaz *et al* [9]. An equal volume of serum (30 µL) and RBPT antigen was mixed and observed for eight minutes with continuous swirling. Positive samples characterized by the appearance of agglutination or thick rim were scored (1 + to 3 +) according to the strength of agglutination. Microagglutination test (MAT), a variant of Wright's serological agglutination test [11], was performed using commercial *B. abortus* antigen (IAHVB) and safranin O at a final concentration of 0.005%. Test and control sera with an equal volume of antigen were tested at 1:10 to 1:2560 dilutions (in phenol saline) under 37°C incubation for 20 hours. Agglutination was graded as 4 + (complete agglutination) to 1 + (mild agglutination). Indirect ELISAs (NovaTec Immundiagnostica GmbH, Dietzenbach, Germany) were performed per the kit protocols to detect Brucella-specific IgM and IgG. Absorbance values of samples and controls were converted into NovaTec Unit (NTU) and reported as positive (> 11 NTU), equivocal (9-11 NTU), and negative (< 9 NTU). A fever case that tested positive for both agglutinating antibodies (RBPT or MAT) and

soluble antibodies (IgG/ IgM ELISA) was considered a confirmed case of brucellosis [1,10]. Descriptive statistics viz., percentage, and median, with inter-quartile range (IQR) was used to describe clinical, epidemiological, and hematological characteristics.

A total of 349 fever cases were enrolled in the study, of which 168 (48%) were male. The median age of the participants was 17 years (IQR 9.00-30.50). The median duration of fever was four days (IQR 3.00-6.00). The commonest symptoms were cough (71%), headache (55%), loss of appetite (51%), chills (39%), myalgia (35%), and arthralgia (30%) (Table 1). Eighty-three (24%) participants reported having a household member with a similar illness. Close contact with animals was reported by 14% of participants.

Twenty-eight (8%) fever patients were diagnosed as cases of brucellosis with a median age of 15 (IQR 9.25-30.75) years (Table 2). The median duration of fever among brucellosis cases was 5.5 (IQR 3.00-7.00) days. The major symptoms among positive cases were cough, loss of appetite, headache, chills, sore throat, myalgia, and arthralgia. One-fourth of them had thrombocytosis and four had leukocytopenia.

We documented brucellosis infection in one out of every 12 persons with febrile illness examined in the study. We did not find any specific clinical manifestation or exposure among the brucellosis cases.

Table 2. Serological testing for Brucellosis among fever cases (N = 349) in a primary health care facility in Tirunelveli, Tamil Nadu, 2018-19.

Brucella antibody tests	Positivity n (%)
RBPT	166 (47.6)
MAT	8 (2.3)
IgM ELISA	6 (1.7)
IgG ELISA	8 (2.3)
RBPT + MAT	7 (2.0)
RBPT + IgM ELISA*	4 (1.1)
RBPT + IgG ELISA*	18 (5.2)
MAT + IgM ELISA*	2 (0.6)
RBPT + MAT + IgM ELISA*	2 (0.6)
RBPT + MAT + IgG ELISA*	1 (0.3)
RBPT + IgM ELISA + IgG ELISA*	1 (0.3)

*Cases confirmed as Brucellosis

We recruited and tested all the fever cases at the health facility. The higher percentage of participants with a cough could be attributable to other infectious etiologies. However, our estimate of brucellosis was similar to what is reported elsewhere in India [5–8]. These reports are either from occupationally exposed healthy individuals in the community or pyrexia of unknown origin (PUO) cases in tertiary care facilities. This is possibly the first report of brucellosis among febrile patients from a PHC in India. Though brucellosis is usually part of the diagnostic panel for differentiation of PUO, it is mostly not tested due to the non-availability of a reliable diagnostic assay. A single

Table 1. Demographics, clinical, and epidemiological characteristics of fever cases (N=349) in a primary health care facility in Tirunelveli, Tamil Nadu, 2018-19.

	All Fever cases (N = 349)	Brucellosis cases (n = 28)
Characteristics	**n (%)**	**n (%)**
Age (years) [Median (IQR)]	17 (9.00-30.50)	15 (9.25-30.75)
Male gender	168 (48)	10 (36)
Clinical symptoms/ signs		
Duration of fever (days) [Median (IQR)]	4 (3.00-6.00)	5.5 (3.00-7.00)
Common cold	107 (31)	7 (25)
Rhinorrhoea	86 (25)	6 (21)
Sore throat	120 (34)	9 (32)
Cough	246 (70)	22 (79)
Difficulty in breathing	33 (9)	3 (11)
Loose stools	26 (7)	2 (7)
Abdominal pain	92 (26)	7 (25)
Vomiting	95 (27)	7 (25)
Chills	136 (39)	9 (32)
Myalgia	121 (35)	8 (29)
Headache	191 (55)	13 (46)
Retro-orbital pain	40 (11)	5 (18)
Arthralgia	106 (30)	8 (29)
Neck rigidity	17 (5)	1 (4)
Drowsiness	89 (25)	8 (29)
Loss of appetite	177 (51)	16 (57)
Exposure factors		
Close contacts with cattle	48 (14)	4 (14)
Presence of similar case in the house	83 (24)	10 (36)
Presence of similar case in the village / locality	54 (15)	4 (14)
History of travel	48 (14)	2 (7)

assay that is easy to perform and reliable will help improve brucellosis testing. The high positivity observed with RBPT could be due to repeated exposures in an animal brucellosis endemic area as well as cross-reactivity with other bacterial species. Surprisingly, only 14% of confirmed brucellosis cases reported close contact with cattle. This is despite livestock farming being a common source of livelihood in the study area. Therefore, the low proportion of animal contact can be on account of information bias as the data on brucellosis or other zoonosis-related exposures was not specifically collected.

The findings from a single centre may not be generalizable to the entire study district/ region. Identification of risk factors for brucellosis was not possible as details on potential zoonosis-associated risk factors and occupation of the participants were not collected. The performance of blood culture or PCR would have enabled the detection of active infections. However, we used a combination of serological assays to enhance the specificity of brucellosis diagnosis. Failure to test for other etiologies of acute febrile illness could have led to underdiagnosis.

The present study documents brucellosis to be an important etiology of febrile illness in rural settings, where a large section of the population is involved in livestock-related activities and animal brucellosis is endemic. A large multi-site study with adequate geographic representativeness will provide a reliable estimate of brucellosis seroprevalence in India. This may enable the identification of *Brucella* hot spots or hyper-endemic areas. Targeted diagnostic testing and reporting of brucellosis should be implemented in those high-risk settings.

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