



Original Article

Brucella IgM Seropositivity among Febrile Patients at NAMO Medical Education and Research Institute and NAMO Hospital, Silvassa, Dadra and Nagar Haveli, India.

Sakshi Patil¹, Narayan Kamath², Kamaljeet³, Dhruvi Solanki⁴, Namrata Moraiya⁵

¹Intern, M Sc.in Biotechnology, Amity Institute of Biotechnology, Amity University, Mumbai

²Professor in Microbiology, NAMO Medical Education & Research Institute, Silvassa

³Tutor in Microbiology, NAMO Medical Education & Research Institute, Silvassa

⁴Microbiologist, NHM, UT of Dadra & Nagar Haveli

⁵Tutor in Microbiology, NAMO Medical Education & Research Institute, Silvassa

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Corresponding Author:

Narayan Kamath

Professor in Microbiology, NAMO
Medical Education & Research
Institute, Silvassa

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ABSTRACT

Background: Brucellosis is a zoonotic disease that may be under-recognized because its clinical manifestations overlap with those of other febrile illnesses. This study describes Brucella IgM seropositivity among selected febrile patients and the distribution of positive samples by age, sex, and ward of admission.

Methods: A hospital-based observational study was conducted at NAMO Medical Education and Research Institute and NAMO Hospital, Silvassa, from January 2026 to March 2026. Serum samples from 262 febrile patients with negative screening test results for common febrile illnesses, including malaria, dengue, and typhoid, were tested using a commercial Brucella IgM ELISA kit. ELISA positive samples were subsequently assessed using SAT card and SAT tube tests using Brucella abortus and Brucella melitensis antigens. Age, sex, and ward distribution were analyzed descriptively.

Results: Brucella IgM ELISA positivity was detected in 55 of 262 samples, corresponding to 21.0%. The largest share of ELISA positive samples was observed among patients aged 21 to 30 years, followed by those aged 31 to 40 years. Positivity was 17.8% among male patients and 24.4% among female patients. Most positive samples were from inpatient wards, particularly FMW and MMW. Among ELISA positive samples, SAT tube reactivity was observed more frequently than SAT card reactivity for both Brucella abortus and Brucella melitensis antigens.

Conclusion: Brucella IgM positivity was detected in selected febrile patients in our hospital setting. Brucellosis should be considered in the differential diagnosis of patients with compatible clinical features and relevant exposure history. Serological findings should be interpreted with clinical information and, where feasible, supported by culture or molecular testing.

Keywords: SAT, ELISA, Brucellosis, Zoonosis.

INTRODUCTION

Brucellosis is a major zoonotic disease caused by bacteria of the genus *Brucella*. These organisms are small, gram-negative, non-spore-forming, facultative intracellular coccobacilli that can persist within host cells and produce prolonged infection. The disease primarily affects domestic animals, such as cattle, buffaloes, sheep, goats, pigs, and dogs, and humans usually acquire infection through direct contact with infected animals, handling of aborted material, inhalation of contaminated aerosols, or consumption of unpasteurized milk and milk products. *Brucella melitensis* is regarded as the principal cause of human brucellosis worldwide, although *B. abortus*, *B. suis*, and *B. canis* are also important human pathogens^{1,2}.

Brucellosis remains important because it causes both public health and economic losses. More than 500,000 human cases are reported annually worldwide, but the true burden is expected to be higher because clinical features are non-specific and many endemic areas have limited surveillance capacity [1]. In animals, brucellosis is associated with abortion, infertility, reduced milk yield, and poor productivity; in humans, it may present as an acute, subacute, or chronic febrile illness with sweating, malaise, headache, backache, arthralgia, and myalgia. Because fewer than 10% of human cases may be clinically recognized, treated, or reported, laboratory-supported diagnosis is essential in endemic settings³.

In India prevalence of brucellosis varies by region, host population, occupational exposure and diagnostic method. Seroprevalence studies have reported rates of 0.8% in Kashmir, 6.8% in Varanasi, 8.5% in Gujarat, 11.51% in Andhra Pradesh, 19.83% in Maharashtra, 26.6% in Ludhiana, 8.5% in Belgaum, and 0.9% in Delhi⁴. A cross-sectional survey from 60 villages in Punjab reported an age-standardized human *Brucella* infection prevalence of 2.24% (95% CI 1.61–3.11) in the rural population⁵. In rural Ludhiana, Punjab, 15.1% of large ruminants were seropositive, and approximately one-third of dairy farms had at least one seropositive animal; among people in direct contact with livestock, 9.7% were seropositive for *Brucella* spp.⁶. Among abattoir personnel in Delhi, seroprevalence was 20.60% by RBPT, 12.75% by STAT, 50.30% by complement fixation test, and 25.45% by Dot-ELISA⁷. A study of 2,337 occupationally exposed individuals in India reported a seroprevalence of 9.46% by RBPT, 4.45% by SAT, and 3.64% by 2-mercaptoethanol test⁸.

Different diagnostic methods detect different markers of infection and should therefore be interpreted according to the purpose of testing. The Rose Bengal Plate Test is a simple and rapid agglutination test commonly used for screening, especially in laboratories with limited resources. ELISA detects anti-*Brucella* antibodies and can be useful for large-scale screening or confirmation because it is more standardized and can detect specific immunoglobulin classes. Rapid lateral-flow assays are designed for point-of-care use and may support early field-level screening, particularly in resource-limited laboratories^{9,10}. In contrast, real-time PCR detects *Brucella* DNA and can provide faster evidence of infection than culture; however, it requires molecular laboratory capacity and careful quality control; related nucleic acid amplification methods have also been evaluated for rapid *Brucella* detection¹¹. Because serological and molecular tests measure different biological targets, comparing Rose Bengal, ELISA, rapid testing, and RT-PCR can help identify a practical and reliable testing strategy for local surveillance.

The present study aimed to describe *Brucella* IgM ELISA positivity among selected febrile patients at the NAMO Medical Education and Research Institute and NAMO Hospital, Silvassa, and to describe the distribution of ELISA-positive samples by age, sex, and ward of admission.

MATERIALS AND METHODS

This hospital-based observational study was conducted at the NAMO Medical Education and Research Institute and NAMO Hospital, Silvassa, from January 2026 to March 2026. A total of 262 serum samples were collected from febrile patients suspected of having brucellosis after negative screening for common febrile illnesses such as malaria, dengue, and typhoid. Purposive sampling was used. Patients with fever of unknown origin and clinical features such as headache, chills, fatigue, joint pain, and low back pain were included.

Serum samples were tested using a commercial *Brucella* IgM ELISA kit as the primary screening assay. ELISA positive samples were further evaluated by SAT card and SAT tube methods using *Brucella abortus* and *Brucella melitensis* antigens, as available. Age, sex, and ward distribution were analyzed descriptively. The results are presented as frequencies and percentages.

RESULTS

Among the 262 serum samples tested, 55 were positive for *Brucella* IgM by ELISA, yielding an overall seroprevalence of 21.0%. The remaining 207 samples were negative. Age-wise analysis showed that the 21–30-year age group had the highest proportion of positive cases, accounting for 25.5% of all positives, followed by the 31–40-year age group at 21.8%. The lowest positivity was observed in the 0–10-year age group.

The sample population comprised 135 males and 127 females. ELISA positivity was 17.8% among males and 24.4% among females. Ward-wise, the highest positivity was noted in FMW E Block, followed by MMW C Block and FMW C Block. This pattern suggests that many positive patients were admitted in inpatient units rather than being confined to outpatient assessment.

In the serological comparison, ELISA identified all positive cases in the dataset and demonstrated higher detection than the SAT card and SAT tube. The SAT tube detected more positives than the SAT card, indicating better sensitivity than the slide method.

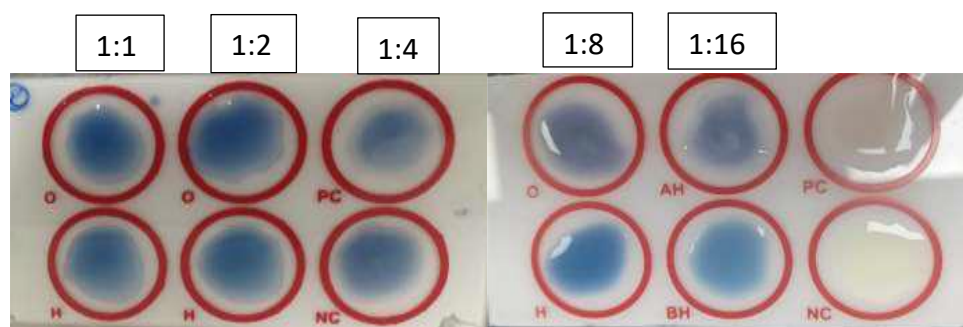


Figure 1: Agglutination was observed in a (SAT) Card test

Semi-quantitative Card test: For every test, 1:1, 1:2, 1:4, 1:8 and 1:16 numbers should be written on a test card. Next, 5 μ l of serum will be placed into each of the five different circles of a card. One drop of positive control (50 μ l) and one drop of negative control should be added to circles, and a well-mixed solution should be placed with the four corresponding sample solutions in each circle and well-mixed with voltage supplied from a stirrer so that the entire surface area of the ring is coated with solution. In a circular motion, rotate the slide at 80-100 rpm for 1 minute and immediately observe agglutination for any positive reactions.

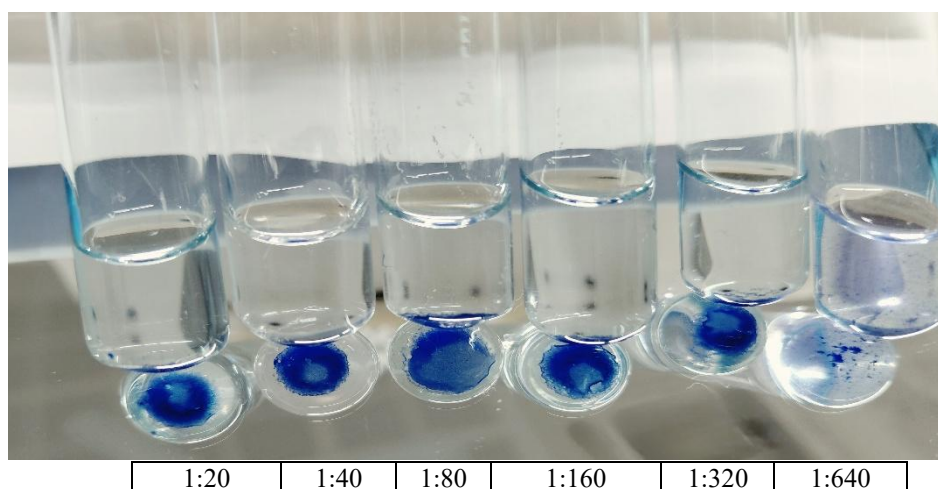


Figure 2: Tube agglutination assay showing a positive agglutination reaction with *Brucella abortus* antigen. Visible blue sediment formation at the bottom of the tube indicated antibody-mediated agglutination in the reaction serum sample.

Table 1: Ward-wise distribution samples from 27 January 2026 to 13 March 2026.

Ward / Unit	Total Samples	Ward share (%)
MMW (C Block)	52	19.8
MMW (E Block)	40	15.3
FMW (C Block)	33	12.6
FMW (E Block)	30	11.5
Gynec Ward-GF	24	9.2
Pediatric Ward	17	6.5
M-ICU	16	6.1
ICU-6th Floor	10	3.8
OPD	6	2.3
Others	34	13.0
Total	262	100.0

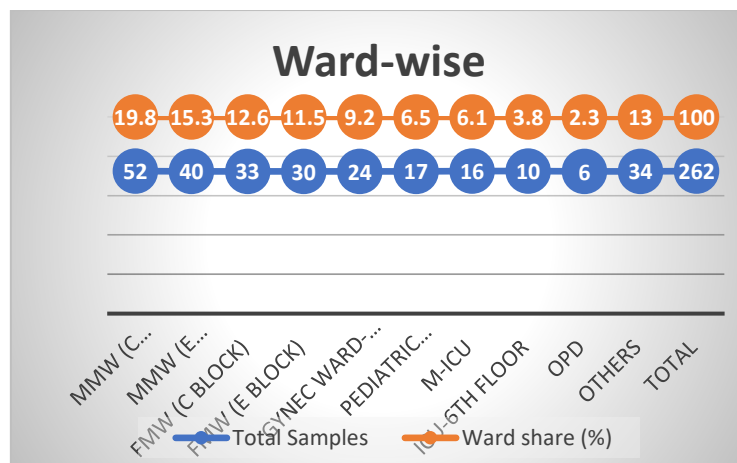


Table 2: Ward distribution of patients with Brucella IgM ELISA positivity from 27 January 2026 to 13 March 2026.

Ward / Unit	Total samples	ELISA positive n (%)
MMW (C Block)	52	12 (23.1)
MMW (E Block)	40	6 (15.0)
FMW (C Block)	33	7 (21.2)
FMW (E Block)	30	15 (50.0)
Gynec Ward–GF	24	3 (12.5)
Pediatric Ward	17	3 (17.6)
M-ICU 5th Floor	16	1 (6.3)
ICU–6th Floor	10	2 (20.0)
OPD	6	1 (16.7)
Other wards	34	6 (17.6)
Total	262	55 (21.0)

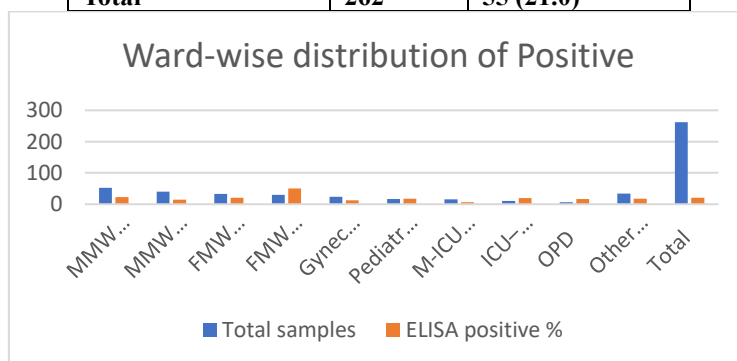
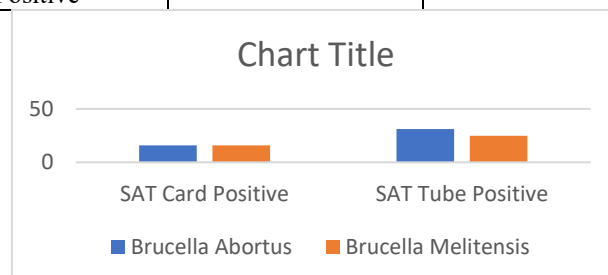


Table 3: Comparison of SAT Card and SAT Tube for all IgM ELISA positive in Brucella abortus and Brucella melitensis patients (N=55).

Method	Brucella Abortus	Brucella Melitensis
SAT Card Positive	16 (29.1)	16 (29.1)
SAT Tube Positive	31 (56.4)	25 (45.5)



DISCUSSION

In this hospital-based study of selected febrile patients, Brucella IgM ELISA positivity was detected in 55 of 262 samples (21.0 %). This finding shows that Brucella IgM reactivity was present among patients with febrile illness in this clinical setting. However, this study used purposive sampling and included clinically suspected patients after negative screening for selected common febrile illnesses. Therefore, the observed percentage should not be interpreted as the population prevalence of human brucellosis in the Dadra and Nagar Haveli district.

Human brucellosis often presents with non-specific clinical features, including fever, fatigue, sweating, arthralgia, myalgia, and low back pain, which overlap with other infectious and non-infectious conditions [2,13]. Studies from India have reported variable serological positivity among patients with pyrexia of unknown origin and among occupationally exposed populations [5,7,8,12]. The present findings support the consideration of brucellosis in the differential diagnosis of selected patients with prolonged or unexplained fever, particularly where a compatible exposure history is present.

The largest share of ELISA-positive samples in this study was observed among patients aged 21–30 years, followed by those aged 31–40 years. These age groups may include individuals with frequent occupational, household, or community exposure to animals and animal products. However, the present dataset did not include detailed information on occupation, livestock contact, consumption of unpasteurized dairy products, or other exposure variables. Consequently, the reasons for the observed age distribution cannot be determined from this study.

Female patients had a higher Brucella IgM ELISA positivity rate than male patients in the present study. This difference should be interpreted cautiously because the study did not evaluate exposure history, clinical severity, occupation, healthcare-seeking behavior, or potential confounding factors. It therefore cannot establish the reasons for the observed sex difference.

Most ELISA-positive samples were obtained from inpatient wards. This distribution describes the location from which samples were collected, but it does not establish disease severity, duration of illness, or hospitalization caused specifically by brucellosis. Clinical correlation and patient-level clinical outcome data are needed to address these questions.

ELISA is useful for detecting class-specific antibody responses and can support the screening of suspected cases, particularly where culture or molecular testing is not routinely available [9,15]. In the present study, SAT tube positivity was more frequent than SAT card positivity among ELISA-positive samples. However, the study design did not permit estimation of the sensitivity or specificity of ELISA, SAT card, or SAT tube because agglutination testing was performed only among ELISA-positive samples and no uniform culture or molecular reference standard was applied. The observed agreement or discordance between tests should therefore be interpreted descriptively rather than as evidence of comparative diagnostic performance.

This study has several limitations. It was hospital-based and used purposive sampling, limiting generalizability to the wider community. Exposure history and detailed clinical outcomes were not available for analysis. In addition, systematic culture or molecular confirmation was not performed for all ELISA-positive samples. These limitations mean that the findings represent Brucella IgM seropositivity among selected febrile patients rather than confirmed brucellosis or community-level prevalence.

CONCLUSION

Brucella IgM ELISA positivity was detected in 55 of 262 selected febrile patients attending the NAMO Medical Education and Research Institute and NAMO Hospital, Silvassa. Positivity was more frequently observed among patients aged 21–40 years and among female patients. These findings support the consideration of Brucellosis in the differential diagnosis of selected febrile patients, particularly in the presence of compatible symptoms and relevant animal or dairy exposure. As serological positivity alone does not establish definitive infection, ELISA results should be interpreted with clinical findings, wherever feasible supported by culture or molecular testing. Further studies using systematic exposure assessment and confirmatory testing are needed to define the burden of confirmed human brucellosis in the region.

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