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DETERMINATION OF BASELINE TITRE FOR ANTI-*BRUCELLA* ANTIBODIES IN APPARENTLY HEALTHY BLOOD DONORS IN AHMEDNAGAR, MAHARASHTRA.

Microbiology

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ABSTRACT

Brucellae are Gram negative coccobacilli causing brucellosis. Brucellosis manifests with non-specific symptoms and so remains misdiagnosed and underreported. Therefore laboratory diagnosis becomes necessary. Blood culture provides a confirmation but has certain limiting factors. Hence laboratory diagnosis mainly relies on the Standard Tube Agglutination Test (STAT) which detects antibodies against *Brucella*. Although a titre of ≥ 160 IU/ml is considered to be diagnostic, it is influenced by the local baseline titre. The study therefore aims to know the baseline antibody prevalence of anti-*Brucella* antibodies in apparently healthy people.

Approximately 2 ml of blood was collected from 300 apparently healthy blood donors. The serum was separated and processed by the STAT. Out of total 300 specimens, 87(29%) specimens showed presence of anti-*Brucella* antibodies. A titre of ≥ 80 IU/ml is considered as significant titre for STAT. Out of 87 sera having anti-*Brucella* antibodies, maximum number (mode) i.e. 32 (36.78%) were positive at a titre of 20 IU/ml.

Therefore considering 20 IU/ml as the baseline titre, a titre of ≥40 IU/ml should be considered as a significant titre for the local population in this area. Hence a titre of ≥40 IU /ml for STAT performed on single sera along with consistent clinical features and history of the patient can be considered as an indication of brucellosis in the local population in this area.

KEYWORDS

INTRODUCTION:

Brucellae are Gram negative coccobacilli causing brucellosis. It is a systemic infection presenting with non-specific clinical manifestations (1). Hence though having considerable morbidity it remains misdiagnosed and underreported (2, 3). Therefore laboratory diagnosis becomes important. Laboratory diagnosis can be done by isolating Brucellae from clinical specimens, detection of antigen, antibodies or genome (1). Although blood culture provides a confirmation of disease it requires level 3 bio-containment facilities, a highly skilled technical hand and is frequently negative which limits its utility (1,4). Its sensitivity ranges from 15-90% depending on the methodology used (5). Culture, though being a gold standard, serology remains the mainstay for diagnosis (6, 7). The Standard Tube Agglutination Test (STAT) is the most popular and preferred serological test worldwide for the diagnosis of brucellosis (8). The STAT collectively detects IgM and IgG anti-*Brucella* antibodies in humans (1, 9). Although a titre of ≥ 160 IU/ml is considered to be diagnostic, it is influenced by the local baseline titre (1). The local baseline titre varies from place to place. Hence it becomes important to estimate the prevalence of baseline antibody titre for appropriate interpretation of the STAT. The study therefore aims to know the baseline antibody prevalence of anti-*Brucella* antibodies in apparently healthy people. Also it will help in data generation, as there is unavailability for the same in our set up.

MATERIALS AND METHODS:

The present study is a cross-sectional study conducted in the department of Microbiology, Dr. Vithalrao Vikhe Patil Foundations Medical College and Hospital. It is a tertiary care, 750 beded hospital located in Ahmednagar district of Maharashtra. The study was carried out between June 2016 to March 2018. Study was executed only after obtaining approval of the institutional ethical committee. Prior written consent from the participants was obtained before collecting the specimen. Random sampling method was used.

Inclusion criteria:

1. Apparently healthy participants were included. All participants were given questionnaires to be filled. Donors who did not have any obvious signs and symptoms of infectious diseases were included in the study.
2. Healthy donors in the age group of 18-50 were included.

Exclusion criteria:

1. Serum of participants having inclusion criteria, but tested reactive for anti-HIV antibodies, HCV, HBsAg and RPR were excluded.

Methodology:

Health screening of the participants was done by using a questionnaire. Randomly selected, non-repetitive 300 (n=300) blood samples were selected from healthy blood donors in the age group of 18-50 years of both the genders.

Approximately 2ml. of blood which was not diluted with CPDA-1 inside the bag was taken from the tube of the blood bag. Serum was separated, labeled and stored at 4^0C for further processing. All of the serum specimens were tested by the STAT. All sera and antigens were brought to room temperature prior to testing. Antigen for STAT was procured from the Indian Veterinary Research Institute, UP, India. Antigen used for STAT was an unstained suspension of pure, smooth *Brucella abortus* strain 99 in phenol saline. The test is a tube agglutination test done with double dilutions of serum in 0.5% phenol saline. The test procedure and interpretation of results was done according to the literature furnished by the IVRI along with the antigen (10).

RESULTS:

A total of 300 sera were tested for presence of anti-*Brucella* antibodies. Out of 300, 87(29%) specimens showed presence of anti-*Brucella* antibodies in STAT. Although a titre of ≥ 80 IU/ml is considered as significant titre for STAT, the results mentioned above are irrespective of significant titre.

Out of 87 sera positive for anti-*Brucella* antibodies, maximum number (Mode) i.e. 32 (36.78%) were positive at a titre of 20 IU/ml.

Table no. 1 shows the distribution of No. of sera showing presence of anti-*Brucella* antibodies with their respective titres.

Table no. 1: Distribution of No. of positive sera with their respective titres.

Titre obtained in STAT (IU/ml)	No. of samples tested positive by STAT (n=87), f (%)
20	32 (36.78)

40	27 (31.03)
80	19 (21.83)
160	08 (9.19)
320	01 (1.14)
640	00 (00)
Total	87 (100)

DISCUSSION:

The present study aims to determine the baseline anti-*Brucella* antibody titre for the Standard Tube Agglutination Test (STAT) and to help develop a local recommendation for interpretation of STAT results. To the best of our knowledge no such study done in Ahmednagar district in the past has been published as of now.

As stated in the results out of 300 sera, 87 i.e. (29%) showed presence of anti-*Brucella* antibodies. This finding is very high than Mangalgi et al. and Nagarathna et al. who reported only 5.91% and 1.1% positivity respectively(7, 11). Finding in the present study is also quite high than Vaishnavi Kumar who reported 16.8% positivity by STAT (12).

Maximum no.(Mode) i.e. 32 (37.78%) sera were positive for anti-*Brucella* antibodies at a titre of 20 IU/ml. Therefore considering 20 IU/ml as the baseline titre, a titre of ≥40 IU/ml should be considered as a significant titre for the local population in this area. Hence a titre of ≥40 IU /ml for STAT performed on single sera along with consistent clinical features and history of the patient can be considered as an indication of brucellosis in the local population in this area. The baseline titre reported by Mangalgiet et. al. is 80 IU/ml and Vaishnavi Kumar is 40 IU/ml.(7, 12) The present study shows a shift towards a lower titre of 20 IU/ml as baseline. This may be because 79% population of Ahmednagar district belongs to rural area. And it is known that prevalence of brucellosis is higher in rural areas (12).Higher prevalences may lead to repeated sub-clinical exposure to *Brucella* and perhaps a higher enedmicity in this case.

On the other hand, eight sera were positive at a titre of 160 IU /ml and one at 320 IU/ml are also very outstanding findings in this study. No reference could be found for such high titres in baseline studies for comparison. These unusual findings could not be investigated further as the blood donor could not be followed.

CONCLUSION:

The study concludes the following:

1. The base line titre of anti-*Brucella* antibodies in healthy blood donors in and around Ahmednagar is determined to be 20 IU/ml.
2. Hence a titre of ≥40 IU/ml for STAT performed on single sera along with consistent clinical features and history of the patient can be considered as an indication of brucellosis in the local population in this area.
3. Higher endemicity suggests investigations for brucellosis in Ahmednagar in different populations like high risk group, febrile illness group (commonest presentation), patients with osteoarticular involvement group (commonest complication), at a larger scale.

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