ORIGINAL RESEARCH PAPER
Volume - 9 | Issue - 7 | July - 2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SEROPREVALENCE OF ANTI-*BRUCELLA* ANTIBODIES IN FEBRILE ILLNESS IN A TERTIARY CARE HOSPITAL, AHMEDNAGAR, MAHARASHTRA.

Microbiology

Shraddha Prasad Gunjal — Assistant Professor, Dept. of Microbiology, Dr. Vithalrao Vikhe Patil Foundation's Medical College & Hospital, Opposite government Milk Dairy, Post – M.I.D.C., Vadgaon Gupta, Ahmednagar – 414 111.

Dakshayani P. Pandit* — Professor, Microbiology, I/C HICC, Dr. DY Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth, (deemed to be university), Sant Tukaram Nagar, Pimpri, Pune-411018. *Corresponding Author

Prasad N. Gunjal — Assistant Professor, Dept. of Microbiology, Dr. Vithalrao Vikhe Patil Foundation's Medical College & Hospital, Opposite government Milk Dairy, Post – M.I.D.C. Vadgaon Gupta, Ahmednagar – 414 111.

ABSTRACT

Introduction: Brucellosis is a disease caused by bacteria of the genus *Brucella*. It is a zoonosis. Humans acquire the disease through animals. It can also be acquired uncommonly from humans. The disease is predominantly encountered in rural population. The disease commonly takes a chronic and debilitating course with osteoarticular complications to be the commonest. Long term treatment with a combination of antibiotics like doxycycline, streptomycin, rifampicin and ceftriaxone cures brucellosis. Almost 80% of the population of Ahmednagar is rural and hence seroprevalence was determined in this place.

Materials And Methods: Blood was collected from 976 febrile illness patients and processed by the standard tube agglutination test.

Results And Observations: Out of 976 sera, 147 sera showed agglutination in STAT. The titres obtained ranged from 1:20 to 1:320. Out of these 147 (15.06%) sera agglutinating in STAT, 45 (4.61%) sera agglutinated at a titre of ≥ 80 IU/ml. Young and middle age group males are predominantly affected.

Conclusion: In the present study it is observed that the seroprevalence of anti-*Brucella* antibodies is 15.06% over a range of titre of 20 to 320 IU/ml and 4.61% at a titre of ≥ 80 IU/ml.

The young and middle age group males are predominantly infected.

KEYWORDS

Brucella, Brucellosis, Seroprevalence, Rural Population, Ahmednagar District, Febrile Illness.

INTRODUCTION:

Brucellosis is a disease caused by bacteria of the genus *Brucella*. The disease primarily affects livestock and it is a zoonosis in humans (1, 2, 3). Humans acquire the disease through ingestion of contaminated milk, dairy products and meat, inoculation into non intact skin and conjunctiva and inhalation of aerosols. Rarely the disease can also be acquired through blood transfusion, organ transplantation and sexually (4-11).A close association between humans and animals makes humans highly prone to acquire the disease. Eventually the disease is predominantly encountered in rural population (12, 13, 14). The Ahmednagar district of Maharashtra consists of almost 80% of rural population (15). Hence it is more likely that the individuals of this region are at a higher risk of acquiring brucellosis. It should also be considered that the disease becomes chronic and debilitating if not promptly treated and requires a long term treatment with combination of antibiotics often leaving permanent disabilities (10) old synopsis (2, 7, 10, 16-20). Osteoarticular complications are the most common (20). The morbidity ultimately results into a poor quality of life, preceding the working hours which are lost. It should also be noted that although brucellosis results into chronic and crippling disease and complications, it is completely avoidable and curable. Brucellosis is treated with a choice and combination of antibiotics like streptomycin, doxycycline, rifampicin and ceftriaxone over a period of 6 weeks to 6 months (21). Moreover the data regarding the incidence and prevalence of brucellosis in Ahmednagar district is lacking. Although it should be noted that hospital based studies do not effectively reflect the true prevalences, it atleast gives an indication of the qualitative and quantitative magnification of the problem. Having an idea of prevalence of brucellosis in the population may help to effectively rule out the disease amongst other common causes of febrile illness and hence can eventually opt for appropriate treatment. Keeping this background into consideration the present study was designed which aimed to determine the seroprevalence of anti-*Brucella* antibodies in patients presenting with fever and predominantly residing in rural area.

MATERIALS AND METHODS:

Place Of Study:

The study was carried out in the Department of Microbiology, Dr. Vitthalrao Vikhe Patil Medical College, Ahmednagar and Department of Microbiology, Dr. D.Y. Patil Medical College, Pune. Both the institutes lead to foster tertiary care to patients.

Duration Of Study:

The study was executed over a duration of five years from Feb. 2015 to Feb. 2020.

Sample Size:

A sample size of 976 was derived through EPI info version 7, considering the prevalence of 6.7% reported by Joshi et al in 2005(22)

Sampling Method:

A total consecutive sampling method was followed.

Statement Of Ethics:

Clearance of the Institutional Ethical Committee was acquired. A prior written consent was obtained from the patients. Confidentiality of the subjects was maintained.

Study Design:

It is a cross sectional study.

Inclusion Criteria:

The inclusion criteria consisted of patients of both the genders and any age group who manifested with fever of duration of ≥ 7 days.

Exclusion Criteria:

The exclusion criteria consisted of

1.Individuals approaching for medical attention who were already diagnosed with causes of fever other than brucellosis.

2.Patients fulfilling the inclusion criteria but rejecting participation.

Methodology:

Approximately 5 ml. of blood was collected by following standard method under strict asepsis by a paramedic professional. Serum was separated and processed by the Standard Tube Agglutination Test (STAT). STAT is a tube agglutination test. It is a serological test which detects antibodies against *Brucella*. STAT collectively detects IgM, IgG and IgA (21). The test procedure and interpretation is done according to the literature supplied along with the antigen (23). In brief, double dilutions of the test serum in 0.5% phenol saline are done in test tubes. Equal quantity of antigen is added and the set of tubes is incubated at 37^{0}C. A titre of ≥ 80 IU/ml is considered to be the

significant titre. The antigen used for the test was procured from the Division of Biological Products of the Indian Veterinary Research Institute (IVRI), Bareilly, Uttar Pradesh. The antigen is a whole cell, pure and smooth, unstained suspension of *Brucella abortus* strain 99 prepared in phenol saline (23).

RESULTS:

A total of 976 sera were tested by STAT. Out of 976 sera, 147 sera showed agglutination in STAT. The titres obtained ranged from 1:20 to 1:320. Out of these 147 sera agglutinating in STAT, 45 sera agglutinated at a titre of ≥ 80 IU/ml.

Fig 01 describes the distribution of specimens showing presence of anti-*Brucella* antibodies. The sera agglutinating in STAT show variations in age and gender distribution.

Fig. 02 reflects age and gender wise distribution of specimens agglutinating over a range of titre of 20 to 320 IU/ml. Similarly, table no. 01 elaborates exact numbers of specimens showing age and gender wise distribution of specimens agglutinating over a range of titre of 20 to 320 IU/ml.

Fig. 03 reflects age and gender wise distribution of specimens agglutinating at a titer of ≥ 80 IU/ml. Similarly, table no. 02 elaborates exact numbers of specimens showing age and gender wise distribution of specimens agglutinating at a titre of ≥ 80 IU/ml.

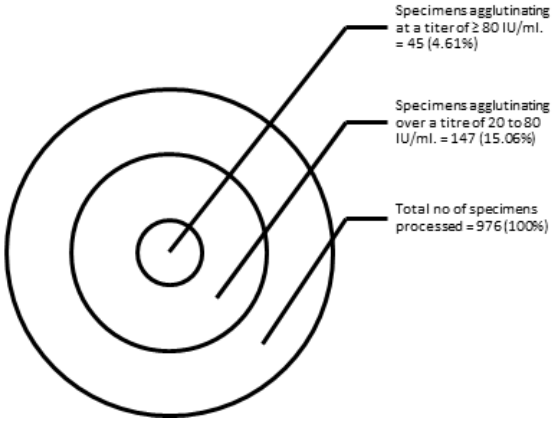


Fig 01: Distribution Of Specimens Showing Presence Of Anti-*Brucella* Antibodies.

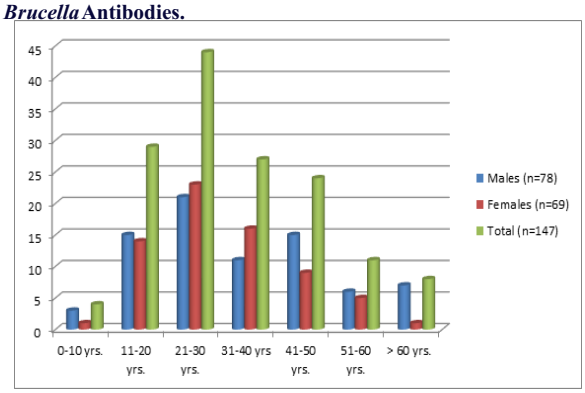
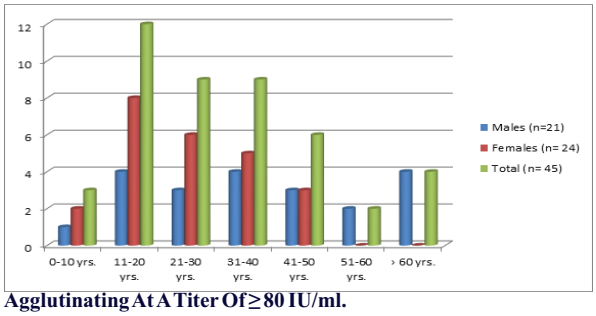


Fig. No. 02: Age And Gender Wise Distribution Of Specimens Agglutinating Over A Range Of Titre Of 20 T0 320 Iu/ml. (n=147).

Table No. 01: Exact Numbers Of Specimens Showing Age And Gender Wise Distribution Of Specimens Agglutinating Over A Range Of Titre Of 20 T0 320 IU/ml. (n=147).

Sr. no.	Age group (Years)	Males (n=78)	Females (n=69)	Total (n=147)
1.	0-10	3	1	4
2.	11-20	15	14	29
3.	21-30	21	23	44
4.	31-40	11	16	27
5.	41-50	15	9	24
6.	51-60	6	5	11
7.	>60	7	1	8



Agglutinating At A Titer Of ≥ 80 IU/ml.

Table No. 02: Exact Numbers Of Specimens Showing Age And Gender Wise Distribution Of Specimens Agglutinating At A Titre Of ≥ 80 IU/ml. (n=45).

Sr. no.	Age group (Years)	Males (n=21)	Females (n=24)	Total (n=45)
1.	0-10	01	02	03
2.	11-20	04	08	12
3.	21-30	03	06	09
4.	31-40	04	05	09
5.	41-50	03	03	06
6.	51-60	02	00	02
7.	>60	04	00	04

In specimens showing agglutination over the range of 20 to 320 IU/ml, males are predominantly affected, whereas in specimens agglutinating at a titre of ≥ 80 IU/ml, females are affected predominantly. Young adults and middle age group individuals are affected predominantly.

DISCUSSION:

Brucellosis in humans presents with non specific symptoms (5). Because the disease does not produce any diagnostic clinical manifestation, laboratory diagnosis plays a key role in defining a case. A positive culture is the direct and confirmatory evidence of the disease (24). Although culture is the gold standard because it is less sensitive and is hazardous, laboratory diagnosis relies on detection of anti-*Brucella* antibodies by serological tests (16). The standard Tube Agglutination Test is the most widely accepted and most popular test for the detection of antibodies against *Brucella (25)*. The sensitivity of culture reported by other workers varies from 10% to 90% (26). Therefore in the present study STAT was used to detect anti-*Brucella* antibodies. Out of 976 specimens processed by STAT, 147 showed agglutination. Agglutination in these specimens was seen at a titre which ranged from 20 IU/ml to 320 IU/ml. Single serum specimen was collected from each patient and subjected to STAT. Testing was not done in paired sera. Specimens agglutinating at a titre below the significant titre (i.e. ≥ 80 IU/ml) are also considered in reporting the seroprevalence. This is because, although they were below the significant titre, the possibility of rise in antibody titre in paired sera cannot be ruled out. Therefore all the specimens agglutinating over a titre of 20 IU/ml to 320 IU/ml are considered to have presence of anti-*Brucella* antibodies. Although the seroprevalence of specimens agglutinating at a titre of ≥ 80 IU/ml is also reported separately. Out of 147 specimens showing agglutination over a titre of 20 to 320 IU/ml, 45 specimens agglutinated at a titre of ≥ 80 IU/ml (at significant titre). So in the present study a seroprevalence of 15.06% (147 specimens in 976) in specimens agglutinating over a range of 20 to 320 IU/ml and 4.61% (45 in 976) in specimens agglutinating at a titre of ≥ 80 IU/ml (significant titre) is observed.

Our results are quite similar to Dias et al. who reported a seroprevalence of 4.4% in cases of Pyrexia of Unknown Origin (PUO) in 2015 from Karnataka (27). Our results are higher than Goel et al. who reported a seroprevalence of 2% in PUO cases in 2015 from Loni, Maharashtra and our results are lower than Purwar et al. who reported a seroprevalence of 8.5% in Acute Febrile Illness (AFI) cases in 2016 from Belgaum, Karnataka (26, 28). Although it should be noted that prevalences are known to change from place to place and time to time. Hence knowing the prevalence of a nearby place or area will not necessarily represent the problem statement in our place at a particular point of time. Therefore it would always be appropriate to practically undertake the determination of prevalence of brucellosis if one wants to know the exact magnitude of it at a particular point of time. Once the magnitude of problem is understood, control measures can be planned, prioritized and implemented accordingly. It is observed that in

specimens showing agglutination over the range of 20 to 320 IU/ml, males are predominantly affected. Whereas in specimens agglutinating at a titre of ≥ 80 IU/ml, females are affected predominantly. It should be considered that specimens agglutinating at ≥ 80 IU/ml are part of specimens agglutinating over 20 to 320 IU/ml. Therefore it can be generalized that over all, males are predominantly affected.

In the context of gender, our findings are similar to Patil et al., Kaur et al. and Agasthya et al. who also reported brucellosis predominantly in males. Patil et al. reported male to female ration of 7:3, Kaur et al. reported 66.66% positivity in males and 33.33% positivity in females. Agasthya et al. reported 2.76% positivity (18 positive specimens in a total of 652) and 0.30% positivity in males and females respectively (02 specimens in a total of 652) (6, 19, 29).

Also it is observed that the young adults and middle age group individuals are affected predominantly. Therefore it can be stated that in the present study febrile individuals from rural background, young and middle age group males are predominantly affected. This may be because young and middle age group males are more associated with outdoor activities which make them more prone to exposure to *Brucella*.

As far as age group is concerned, Sharma et al. shows higher positivity by Rose Bengal Plate Test (RBPT) and STAT in the age group of >20-35years (young adults group)(30). Yohannes et al. also reported highest positivity by RBPT and STAT in the age group of 26-35 years (31). Our findings correlate with these findings. Our findings differ from Reddy et al. who reported highest positivity (23.80%) by RBPT and STAT in the age group of >50 years (32).

CONCLUSION:

In the present study it is observed that the seroprevalence of anti-*Brucella* antibodies is 15.06% over a range of titre of 20 to 320 IU/ml and 4.61% at a titre of ≥ 80 IU/ml.

The young and middle age group males are predominantly infected.

Hence it can be suggested that young and middle age group males in the rural population of Ahmednagar should stringently practice preventive measures for occupational hazards which make them prone to brucellosis. Animal vaccination is more promising option for control of brucellosis in animals and humans as well. So animal vaccination programmes are recommended to be intensified.

REFERENCES:

1. Pappas G, Papadimitriou P, Akritidis N, Christou L. and Tsianos EV. The new global map of human brucellosis. The Lancet infectious diseases 2006;6:91-99.
2. Roth F et. al. Human health benefits from livestock vaccination for brucellosis: case study. Bulletin if the World Health Organisation 2003;81:867-876.
3. Young EJ. *Brucella* spp. Principles and Practice of Clinical Bacteriology, 2nd ed. 265-272 (John Wiley & Sons Ltd, West Sussex, England, 2006).
4. Sathyanarayanan V, Razak A, Saravu K, Ananthakrishna SB, Mukhyprana PM, Vandana KE. Clinical profile of brucellosis from a tertiary care center in southern India. Asian Pac J Trop Med 2011;397-400.
5. Mantur BG, Biradar MS, Bidri RC. Protean clinical manifestations and diagnostic challenges of human brucellosis in adults: 16 years' experience in an endemic area. J Med Microbiol 2006;55:897–903.
6. Kaur A, Soodan PS, Singh VA. Prevalence of Brucellosis in Patients with Fever of Unknown Origin: A Cross sectional Study in a Tertiary Care Hospital. IOSR Journal of Dental and Medical Sciences 2015;14:100-3
7. Kochar DK, Gupta BK, Gupta A, Kalla A, Nayak KC, Purohit SK. Hospital Based Case Series of 175 Cases of Serologically Confirmed Brucellosis in Bikaner. J Assoc Physicians India 2007;55:271-5.
8. Mangalgi S, Sajjan A, Mohite S, Kakade S. Human Brucellosis: Still an Unfamiliar and Misdiagnosed Disease in India. Journal of Krishna Institute of Medical Sciences University 2015;4:59-65.
9. Tyagi A, Verma T, Agarwal S, Gupta RM. Brucellosis as a Cause of Febrile Neutropenia in Acute Myeloid Leukemia: A Case Report. Sch. J. App. Med. Sci 2014;2:1618-20.
10. Available at :https://www.researchgate.net/publication/36447530_Seroprevalence of brucellosis in cattle buffalo and human being in central Gujarat (15 March 2020, date last accessed)
11. Brucellosis in humans and animals, WHO/CDS/EPR/2006. 7, :1-89 Available at: http://www.who.int/csr/resources/publications/Brucellosis.pdf (15 March 2020, date last accessed).
12. Mantur BG, Amarnath SK. Brucellosis in India – A review. J Biosci 2008;33:539-47.
13. Purwar S. Infections of Gastrointestinal System. New Delhi; Jaypee Brothers Medical Publishers (P) Ltd. 2013.
14. Renukaradhya GJ, Isloor S, Rajasekhar M. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. *Vet Microbiol.* 2002;90:183-95.
15. Ahmednagar (Ahmednagar): Census 2011.[Online]. Available from: www.census 2011.co.in/census/district/360-ahmednagar.html [Accessed 16th Sept. 2014].
16. Pandit DP, Pandit PT. Human brucellosis: Are we neglecting an enemy at the backyard? Med J D Y Patil Univ. 2013;6:350-8.
17. Vaishnavi C, Kumar S. Investigation of background prevalence of *Brucella* Agglutinins among blood donors. IJMM 2007;25(3):302-4.
18. Smits HL, Kadri MS. Brucellosis in India: a deceptive infectious disease. Indian J Med Rees. Nov 2005;122:375-384.
19. Agasthya AS, Isroor S, Krishnamsetty P. Seroprevalence study of Human Brucellosis by conventional Tests and Indigenous Indirect Enzyme- Linked Immunosorbent Assay. The Scientific World Journal 2012.
20. Nazir A, Kadri SM, Rukhsana A, Rubina S, Bashir G, Abdoel T, Smits H. Diagnosis of brucellosis in patients with pyrexia of unknown origin. CLI 2006; 8:19-21.
21. Sastry AS, Bhat SK. Essentials of medical microbiology. 1st edition. New Delhi: Jaypee Brothers medical publishers (P) Ltd; 2016. Chapter 35, *Brucella*; p 355-360.
22. Joshi PA, Kulkarni RD, Powar RM. Modified Cold Z-N Staining for presumptive identification of *Brucella.* IJMR 121:108-110, 2005.
23. *Brucella* Serum Agglutination Test (SAT) Antigen [package insert]. Izatnagar, Uttar Pradesh (India): Division of Biological Products, Indian Veterinary Research Institute;2015.
24. WHO/DFID-AHP. The control of neglected zoonotic diseases: a route to poverty alleviation: report of a joint WHO/DFID-AHP meeting. 2005 Sep 20–21. Geneva: WHO Headquarters, with the Participation of FAO and OIE; 2006.
25. Yacoub AA, Bakr S, Hameed AM, Al-Thamery AA, Fartoci MJ. Seroepidemiology of selected zoonotic infections in Basra region of Iraq. East Mediterr. Health J 2006;12:112-118.
26. Purwar S, Metgud SC, Mutnal MB, Nagmoti MB, PAtil CS. Utility of serological tests in the era of molecular testing for diagnosis of human brucellosis in endemic area with limited resources. JCDR 2016;10(2):26-29.
27. Dias M, Dias E. Comparative evaluation of various serological tests in the laboratory diagnosis of Brucellosis. CHRISMED Journal of Health & Research 2015;2(2):136-9.
28. Goel S, Goyal P, Singh A, Goel AK, Gupta A, Sharma A, Bhardwaj A. Incidence and sero epidemiology of brucellosis from a tertiary care centre of rural Maharashtra. IAIM 2015;2(8):71-77.
29. Patil DP, Ajantha GS, Shubhada C, Jain PA, Kalabhavi A, Shetty PC et. al. Trend of human brucellosis over a decade at tertiary care centre in North Karnataka. Indian J Med Microbiol 2016;34:427-32.
30. Sharma HK, Kotwal SK, Singh DK, Malik MA, Kumar A, Rajagunalan, Singh M. Seroprevalence of human brucellosis in and around Jammu, India, using different serological tests. Veterinary World 2016;9(7):742-746.
31. Yohannes M, Gill JPS. Seroepidemiological survey of human brucellosis in and around Ludhiana, India. Emerging Health Threats Journal 2011;4:7361-DOI: 10. 3402/ ehtj. v4io.7361.
32. Reddy AD, Singh DK, Mantur BBG, Kumar A, Kumari G, Rajagunalan S, Vysakh M, Kumar PP. Seroepidemiology of human brucellosis in Karnataka. J. Vet. Pub. Hlth 2014;12(2):113-115.