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"SEROPREVALENCE OF HUMAN BRUCELLOSIS AMONG VETERINARIANS AND MILKMEN IN WESTERN RAJASTHAN: A CROSS-SECTIONAL STUDY"

Microbiology

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ABSTRACT

Background: Brucellosis is a re-emerging zoonosis with global distribution. In India, it remains common but often neglected. In Rajasthan, where agriculture is prevalent, frequent contact with infected animals like goats, sheep, and cattle leads to brucellosis-like clinical symptoms. This study aims to determine the seroprevalence and clinical-epidemiological profile of Brucellosis in high-risk groups including veterinarians, milk handlers and dairy industry professionals of Western Rajasthan. **Methods:** This cross sectional observational study was conducted on 310 occupationally exposed high-risk populations from January 2019 to December 2019 in Western Rajasthan to detect the seroprevalence of Brucella. Patients with Widal, MP, RA test positive and animal handling for less than 6 months were kept in exclusion criteria. All samples were tested by using screening test Stained Febrile Antigen for Brucella abortus and Brucella melitensis by using Standard tube Agglutination Method (SAT). All samples were processed for ELISA IgM & ELISA IgG titers using Cal biotech kit and results were analyzed statistically. **Results:** Out of 310 samples, 38(12.25%) were positive by STAT while 42(13.54%) positive by ELISA. All 310(100%) cases had histories of animal contact. Raw milk consumption was found 44(20%) in milkmen and 4(4.44%) in veterinarians. Consumption of undercooked or uncooked meat was reported in 31(17.77%) in milkmen and 16(17.77%) in veterinarians. The most common clinical feature was fever in 10 veterinarians and 15 milkmen. ELISA IgM & IgG antibodies were positive in 10(3.22%) & 28(9.03%) patients respectively, and both ELISA IgM & IgG were positive in 4(1.29%) patients. **Conclusion:** Brucellosis is prevalent in Western Rajasthan, due to its dry climatic conditions. It should be considered in occupationally exposed high risk population, with all preventive measures applied. A safe and effective vaccine in human is not yet available. Prevention relies on public awareness, safe livestock practices, and health-veterinary cooperation.

KEYWORDS

RBPT, STAT, ELISA, PCR, RA, MP

INTRODUCTION

Brucella comes under the genus Brucella which is responsible for the development of brucellosis. In natural course of disease man is affected as terminal host. Animals that gather in herds are typically the infection's source of the disease. Both direct and indirect interaction with infected livestock is essential for the disease's transmission to humans.[2] In India, brucellosis is a significant zoonotic issue that causes over 30,000,000 lost men days annually. Brucellosis was initially identified in 1942 and is currently endemic in India.[3]

Epidemiological studies indicate that brucellosis is prevalent among various farm animals in India, including cattle, goats, camels, horses, and pigs.[4] The two primary species of concern are Brucella melitensis and Brucella abortus. Transmission to humans typically occurs through contact with infected domestic animals, with raw milk and dairy products such as clotted cream and inadequately boiled milk being common sources of infection. Several reports from India have documented seropositivity among high-risk occupational groups such as animal handlers, veterinarians, and dairy workers. Due to its non-specific clinical presentation, brucellosis is often misdiagnosed or mistaken for pyrexia of unknown origin (PUO). In many cases, the infection remains subclinical or latent in high-risk individuals, leading to underestimation of its actual prevalence. Despite the widespread occurrence of animal brucellosis in India, the disease in humans remains relatively under-researched.[3,5,6]

Regular serological assays, such as the Rose Bengal plate test (RBPT), Standard tube agglutination test (STAT), Enzyme-Linked Immunosorbent Assay (ELISA) are used to diagnose brucellosis, although each has limitations. It is generally recommended to perform a series of sensitive and specific tests, such as the and Polymerase Chain Reaction (PCR), which amplifies specific sequences of the Brucella genus, species, or biotypes, is the most widely used molecular method for diagnosing brucellosis.[7]

Brucellosis prevention, management, and eradication are essential for any nation to protect both human and animal health. In 2010, the Indian government initiated the Brucellosis Control Program (BCP) for mass testing followed by vaccination in areas with high prevalence and financial loss to livestock and zoonotic significance.

Aim Of Study

The aim of this study is to determine seroprevalence and clinical-epidemiological profile of Brucellosis in high-risk groups including veterinarians, milk handlers and dairy industry professionals of Western Rajasthan.

MATERIAL AND METHOD

This study was cross sectional observational study. Community-based sampling was done equally in both sexes from all age groups in 310 high-risk populations who were occupationally exposed, Veterinarians, paraveterinarian staff, veterinarian's surgeons & milkmen from January 2019 to December 2019 in Western Rajasthan to detect seroprevalence of Brucella. All patients from high-risk populations (veterinarians, para-veterinary staff, veterinary surgeons, and milkmen) who had been involved in animal handling for less than 6 months were excluded from the seroprevalence study. Patients diagnosed other than Brucellosis like positive with Widal test, MP test, RA test. Blood was collected (after obtaining the written consent of the patient and filling predesigned proforma, annexure-1), using sterilized disposable syringes 5 to 10 cc of the patient's blood collected after taking all aseptic precautions. Ethical approval was taken from Institutional ethical committee (IEC) on dated 05.03.2019, Certificate Number SNMC/IEC/2019/34.

All samples were tested for antibrucella antibodies against B. abortus & B. melitensis by agglutination using Stained Febrile Antigen kit by Standard tube Agglutination Method (SAT). All samples were processed for ELISA IgM & ELISA IgG titers using Cal biotech kit. Data was analyzed and interpreted using SPSS statistical software. The Chi-square test was employed to compare the occurrence of brucellosis among cases with PUO across different age groups and genders. Descriptive statistics of the results for ELISA IgM, ELISA IgG were summarized as percentages. A significance level of p-value <0.05 was considered statistically significant

RESULT

Blood samples of 310 high-risk population including veterinarians (n=90) (including doctors and supporting staff) animal handlers, milkmen (n=220) and 100 healthy control were tested in the study. All participants were tested for both B. abortus and B. melitensis. Overall, the prevalence of brucellosis among high-risk group in our study was found to be 42(13.54%) by ELISA followed by STAT 38(12.25%), RBPT 30(9.67%), and RSAT 25(8.06%). Among the ELISA results, IgM showed seropositivity in 10 cases (3.22%), IgG in 28 cases (9.03%), and both IgM and IgG in 4 cases (1.29%). Among the 4 veterinarian doctors, 2(50% each) cases were seropositive by STAT and ELISA IgG while 14(16.27%) out of 86 veterinarian staff had the

highest seropositivity with ELISA.In milkmen 26(11.81%) and 25(11.36%) positivity seen by ELISA and STAT respectively. In healthy controls also1% to 2% positivity was observed. RBPT, RSAT, STAT, ELISA, and ELISA IgG showed p value 0.007, 0.0017,0.0022 and 0.033 respectively had statistically significant differences in seropositivity between occupational groups and healthy controls, while ELISA IgM (p value=0.148) and ELISA IgM & IgG (p value=0.578) did not (Table no.1). Among the total 310 high risk study population, all were direct contact with animals as compared to only 3% of the healthy controls were direct contact with animals. Raw milk consumption was found 44(20%) in milkmen and 4(4.44%) in veterinarians. Consumption of undercooked or uncooked meat was reported in 31(17.77%) in milkmen and 16(17.77%) in veterinarians. These findings showed a strong association between occupational exposure and potential risk factors for brucellosis transmission(Table 2). In the total 310 study population, the most common clinical symptom was fever >38°C, observed in 10 veterinarians and 15 milkmen. Other symptoms included headache and back pain, joint pain with febrile fever and myalgia with fever were also observed. 285(91.93%) of participants (veterinarians, milkmen) and 98 healthy control were asymptomatic. Less common symptoms such as fatigue with weight loss, night sweats, neurobrucellosis, splenomegaly, hepatomegaly, and lymphadenopathy were observed among study population. Fever, headache & back pain, joint pain, and fatigue/weight loss showed statistically significant ($p < 0.001$) differences across groups. (Table no. 3) A total of 38 (12.25%) participants were positive by STAT. Among them, 14 cases (4.51%) showed antibodies against Brucella abortus, while 13 cases (4.19%) were positive for Brucella melitensis. 11 individuals (3.54%) showed reactivity to both B. abortus and B. melitensis, suggesting possible co-exposure or cross-reactivity. The most frequent titers observed were 1:80 (22 cases) and 1:40 (13 cases). This showed that the differences in STAT seropositivity among Brucella abortus, Brucella melitensis, and both species are not statistically significant.(Table no.4)

Table 1:- Seroprevalence Of Brucellosis Among Milkmen & Veterinarian Using Various Diagnostic Tests

Distribution of cases	RBPT	RSAT	STAT	ELISA	ELISA IgM	ELISA IgG	ELISA IgM & IgG
Veterinarian doctors (N=4)	1 (25%)	1 (25%)	2 (50%)	02 (50%)	0 (0%)	2 (50%)	0 (50%)
Veterinarian staff (N=86)	9(10.46%)	8(9.30%)	11(12.79%)	14(16.27%)	4 (4.65%)	8 (9.30%)	2(2.32%)
Milkmen (N=220)	20 (9.09%)	16 (7.27%)	25 (11.36%)	26 (11.81%)	6 (2.72%)	18 (8.18%)	2 (0.90%)
Healthy control (N=100)	1 (1%)	0 (0%)	1(1%)	2 (2%)	0 (0%)	2 (2%)	0 (0%)
Total (Milkmen & veterinarian (N=310)	30 (9.67%)	25 (8.06%)	38 (12.25%)	42 (13.54%)	10 (3.22%)	28 (9.03%)	4 (1.29%)

Table 2:- Overall Distribution Of Cases For Brucellosis According To Exposure Of Subject Based On Contact With Animals, Raw Milk Consumption& Undercooked Or Uncooked Meat Consumption Among Study High Risk Population

Risk factor	Veterinarians (N=90)	Milkmen (N=220)	Healthy control (N=100)	P value
History of contact with animals	90 (100%)	220 (100%)	3 (3%)	**(p < 0.005)**
History of Raw Milk Consumption	4(4.44%)	44 (20%)	1 (1%)	**(p < 0.005)**
Undercooked or uncooked meat consumption	16(17.77%)	31 (14.09%)	1 (1%)	**(p < 0.005)**

Table 3:- Overall Clinical Features Among Study Population

Clinical sign and symptoms	Veterinarians (n=90)	Milkmen (n=220)	Healthy control (n=100)
	No.	**No.**	**No.**
Fever>38°C	10	15	0
Headache & back pain	7	13	2
Joint pain & febrile fever	7	12	0
Myalgia & febrile fever	3	5	0
Asymptomatic	80	205	98
Others -Fatigue & weight	7	13	0
loss	2	5	0
Night sweating	2	4	0
Neurobrucellosis	0	0	0
Splenomegaly	1	0	0
Hepatomegaly	1	2	0
Lymphadenopathy	1	2	0

Table 4:- Seroprevalence Of Brucella Species Antibodies In Milkmen And Veterinarians Using Stat

Brucella species	STAT Titre					Total positive (>/=1:80)
	1:20	1:40	1:80	1:160	1:320	
Brucella abortus	5	6	8	4	2	14 (4.51%)
Brucella melitensis	4	5	7	4	2	13 (4.19%)
Brucella abortus and B. melitensis	2	2	7	3	1	11 (3.54%)
Total	11	13	22	11	5	38(12.25%)

Chi-square value: 1.26, **p-value**: 0.53

DISCUSSION

In the present study, the overall seroprevalence of brucellosis among the high-risk population, including veterinarians and milkmen in Western Rajasthan, was found to be 13.54% by ELISA, followed by 12.25% by STAT, 9.67% by RBPT, and 8.06% by RSAT**(Table no. 1).**

These results suggest that ELISA is the most sensitive among the diagnostic tools used, particularly for detecting chronic or subclinical cases. Pathak et al. (2016)[8] in Maharashtra reported 12.7% seropositivity using ELISA in high-risk occupational groups. Similarly, Sharma et al. (2018)[9] in Himachal Pradesh documented 13% seroprevalence among animal handlers using STAT and ELISA. However, lower seroprevalence was reported by Singh et al. (2013)[10] in Punjab (7.5%), possibly due to differences in regional prevalence, diagnostic protocols, and occupational safety practices.

In our study, ELISA IgG showed the highest seropositivity (9.03%) compared to IgM (3.22%) and combined IgM and IgG (1.29%), indicating predominantly chronic or past infections **(Table no.1).** This trend of higher IgG positivity is consistent with Sharma et al. (2018)[9], who also found chronic exposure to be more common in veterinary professionals and handlers. In contrast, Singh et al. (2013)[10] reported a relatively balanced IgM and IgG distribution, suggesting more recent or acute infections in their cohort, possibly due to an outbreak setting or less chronic occupational exposure.

When comparing different occupational groups, 16.27% of veterinary staff and 50% of veterinarian doctors tested positive by ELISA, whereas milkmen showed 11.81% positivity **(Table no. 1)**. This indicates a higher risk among veterinarians, especially doctors, possibly due to their direct involvement in animal treatment, handling of infected materials, or lack of personal protective measures. Similar higher risk in veterinarians was documented by Mantur et al. (2007)[11] in Karnataka, who reported 14.2% positivity among veterinary and laboratory workers. However, Yadav et al. (2018)[12] in Haryana reported only 4.9% seropositivity among veterinary staff, likely due to improved protective practices and better awareness in their region.

In comparison to the occupational groups, only 1% to 2% of healthy controls tested positive, and this difference was statistically significant across all tests **(Table no.1)**. This supports the occupational nature of the exposure and transmission, a finding similarly reported by Kumar et al. (2012)[13] in Tamil Nadu, who found negligible seropositivity in healthy controls. Pappas et al. (2006)[14] in a global meta-analysis, noted brucellosis prevalence to be generally under 5% in non-endemic countries, underlining the endemic nature and higher occupational risk seen in India.

Risk factor analysis revealed that all individuals in the high-risk group had direct contact with animals, in contrast to only 3% of healthy controls. Moreover, 20% of milkmen and 4.44% of veterinarians reported raw milk consumption, while 17.77% of veterinarians and

14.09% of milkmen consumed undercooked or raw meat. **(Table no. 2)** These behaviors were found to be significantly associated with brucellosis in our study. Similar findings were reported by Pathak et al. (2016)[8] who identified raw milk consumption and poor hygiene practices as key transmission factors. In contrast, Singh et al. (2013)[10] in Punjab found lower prevalence of such risk factors in their population, which may explain their lower seropositivity rates. Clinically, fever >38°C was the most frequently reported symptom, present in 10 veterinarians and 15 milkmen **(Table no. 3).** Other common complaints included headache, back pain, joint pain with febrile episodes, and myalgia. However, 91.93% of the high-risk population remained asymptomatic, suggesting subclinical infection. Our findings are consistent with those of Mantur et al. (2007)[11] who also documented a large proportion of asymptomatic cases among laboratory and veterinary staff. On the contrary, Singh et al. (2013)[10] observed a higher proportion of symptomatic individuals in Punjab, possibly due to active clinical cases or recent exposure.

Serological testing by STAT showed that 14 individuals (4.51%) were positive for Brucella abortus, 13 (4.19%) for B. melitensis, and 11 (3.54%) showed antibodies to both, suggesting possible co-exposure or cross-reactivity. The most common antibody titers observed were 1:80 and 1:40. The difference in seropositivity between the species was not statistically significant ($p = 0.53$), **(Table no. 4)** similar to findings by Kumar et al. (2012)[13] who also noted overlapping responses to B. abortus and B. melitensis. Yadav et al. (2018)[12] reported a much lower dual-reactivity rate, likely due to improved veterinary control and livestock management practices in Haryana.

Our findings indicate that brucellosis remains an important occupational hazard in Western Rajasthan, particularly among individuals with direct and prolonged animal contact. ELISA, especially IgG-based testing, remains a reliable tool for detecting chronic infections. The similarities and discrepancies with other Indian studies highlight the influence of diagnostic methods, regional livestock infection rates, personal hygiene, and preventive practices on disease prevalence.

CONCLUSION

The present study demonstrated a high prevalence of brucellosis among veterinarians, highlighting the occupational risk associated with their profession. To effectively manage brucellosis as an occupation-related disease, regular surveillance should be incorporated into both local and national control and prevention programs. Additionally, a clear understanding of the risk factors is essential for designing effective strategies to prevent and control the spread of brucellosis in high-risk populations. STAT is an efficient screening test for brucellosis. Different diagnostic tests for brucellosis like serology, blood culture and PCR should be conducted to determine sensitivity and specificity in the Indian population.

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REFERENCES

1. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis.Lancet Infect Dis. 2006;6:91–9. https://doi.org/10.1016/ S1473-3099(06)70382-6
2. Refai M. Incidence and control of brucellosis in the Near East region. Vet Microbiol 2002; 90 : 81-110.
3. Renukaradhya GJ, Isloor S, Rajasekhar M. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. Vet Microbiol 2002; 90 : 183-95.
4. Deka RP, Magnusson U, Grace D, Lindahl J. Bovine brucellosis: prevalence, risk factors, economic cost and control options with particular reference to India – a review. Infect Ecol Epidemiol. 2018;8(1):1556548. doi:10.1080/20008686.2018.1556548
5. Thakur SD, Thapliyal DC. Brucellosis: An overview. Int J Med Med Sci. 2002;4(3):23-26.
6. Agasthya AS, Isloor S, Krishnamsetty P. Seroprevalence study of human brucellosis by conventional tests and ELISA. ScientificWorldJournal. 2012;2012:104239. doi:10.1100/2012/104239
7. Da Silva Mol, J.P., De Araujo Franca, S., Da Paixao, T.A. and Santos, R.L.(2012) Laboratorial diagnosis of brucellosis. R. Bras. Ci. Vet., 19(3): 117-126.
8. Pathak, A. D., Dubal, Z. B., Doijad, S. P., Raorane, A. V., & Chakurkar, E. B. (2016). Seroprevalence, isolation, and molecular characterization of Brucella isolates from bovines in Goa, India. Veterinary World, 9(10), 1089-1094.
9. Singh, B. B., Dhand, N. K., & Gill, J. P. S. (2015). Economic losses occurring due to brucellosis in Indian livestock populations. Preventive Veterinary Medicine, 119(3-4), 211-215.
10. Sharma, V., & Sharma, R. (2018). Seroprevalence of brucellosis among patients attending a tertiary care hospital in North India. Journal of Clinical and Diagnostic Research, 12(8), DC01-DC03.
11. Mantur BG, Amarnath SK, Shinde RS. Review of clinical and laboratory features of human brucellosis. Indian J Med Microbiol. 2007;25(3):188–202. doi:10.4103/0255-0857.34758
12. Yadav, J. P., Verma, S., Dahiya, P., & Malik, A. (2018). Seroprevalence and associated risk factors of brucellosis among veterinarians and animal handlers in Haryana, India. Veterinary World, **11**(4), 558–563.
13. Kumar, A., Rajagunalan, S., Barbuddhe, S. B., & Malmarugan, S. (2012). Seroprevalence of brucellosis among livestock handlers in Tamil Nadu. Indian Journal of Animal Sciences, **82**(10), 1083–1085.
14. Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., & Tsianos, E. V. (2006). The new global map of human brucellosis. The Lancet Infectious Diseases, **6**(2), 91–99.