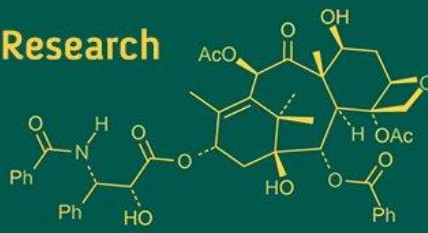
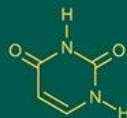
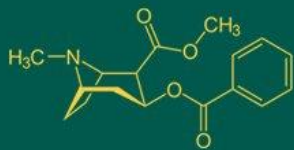


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Bovine brucellosis: Sero-prevalence and risk factor analysis in Indore district of Madhya Pradesh (India)

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Abstract

Bovine brucellosis is one of the most important zoonotic diseases causing significant reproductive losses in livestock and posing a serious public health concern. The present study was undertaken to determine the seroprevalence of bovine brucellosis and to identify associated risk factors in the Indore district of Madhya Pradesh. A total of 509 serum samples were collected randomly from different locations of four blocks i.e. Mhow, Indore, Sanwer, Depalpur of the Indore district of Madhya Pradesh. The collected serum samples were analysed for bovine brucellosis by rose bengal plate test and standard tube agglutination test whereas the milk from seropositive female animals were further subjected to milk ring test. In the present study, the overall sero-prevalence of bovine brucellosis in Indore district was found to be 3.14% by using RBPT and 2.94% by using STAT. In this investigation, a total of 509 serum samples were previously examined, in which 16 serum samples were found to be positive by using RBPT. The milk from these seropositive animals was taken here for further analysis. The results revealed that out of total, 13 (2.55%) milk samples were found to be positive for bovine brucellosis in the studied area. The results showed that 3.30%, 2.50%, 3.52% and 2.70% of serum samples were found to be positive for bovine brucellosis in Mhow, Indore, Sanwer and Depalpur blocks of Indore district, respectively. On application of the Chi-Square test, results observed non-significant ($p > 0.05$) association between blocks and positive samples and negative samples in the Indore district. The study revealed that 3.11% (12/385) and 3.22% (04/124) serum samples were found to be positive for bovine brucellosis in rural and urban areas, respectively. A non-significant ($p > 0.05$) difference was reported for positive and negative samples between various areas. The results also indicated that 1.80% (02/111) and 3.51% (14/398) serum samples were found to be positive for bovine brucellosis in organised and unorganised sectors, respectively. A non-significant ($p > 0.05$) difference was reported for positive and negative samples between various sectors. The potential risk factor investigation indicated that lactation of the animals were found to be non-significantly associated with outcome i.e. bovine brucellosis sero-positivity (OR: 0.98; 95% CI: 0.27, 3.5), while animals with reproductive disorder were found to be significantly associated with outcome i.e. bovine brucellosis sero-positivity (OR: 3.11; 95% CI: 1.14, 8.46). Based on these results, it is recommended that bovines that have reproductive issues should be focused on screening of brucellosis using serological tests. The screening tests ought to be utilized to control brucellosis in dairy animals and to safeguard human health and wellbeing as a whole.

Keywords: Bovine, brucellosis, seroprevalence, risk factors, Madhya Pradesh

Introduction

Brucellosis is an important zoonotic disease with global in distribution. It is caused by proteo-bacteria of genus *Brucella*, which affects a wide range of vertebrate hosts including human beings. The pattern of disease is highly endemic in many states of India and highest prevalence has been reported in dairy cattle (Sharma *et al.*, 2003) [19]. The associated high-risk group for this infection in humans includes shepherds, milkmen (Agasthya *et al.*, 2007) [1], dairy farm workers, veterinarians, veterinary assistants, animal/lab attendants and abattoir workers (Radostits *et al.*, 2007 and Shome *et al.*, 2017) [17, 21].

Bovine brucellosis is clinically characterized by metritis, mastitis, repeat breeding, abortion in last trimester of pregnancy, retention of placenta, infertility, stillbirth, calf loss and reduced milk production in female whereas epididymitis, orchitis and sterility in male (Sundar *et al.*, 2015) [22]. In animals, it can be spread via sexual contact, infected mother to its offspring before or at birth and through milk feeding (Mishra *et al.*, 2020) [13].

Human transmission sources of disease are food (milk, meat), contact with infected animals either in an occupational or recreational contact, airborne or contact infection through environmental contamination (Quinn *et al.*, 1995) [16]. The symptoms of human brucellosis are undulant fever, chills, headache, weakness, loss of appetite, body pain, fatigue, and sometimes endocarditis, orchitis, or arthritis may also develop (Swai and Schoonman, 2009) [23]. There are various assays which are used for diagnosis of brucellosis viz enzyme linked immune-sorbent assay (ELISA), standard tube agglutination test (STAT), antihuman globulin test, radioimmunoassay, rose bengal plate test (RBPT), indirect hemolysis test and western blot (Thakur and Thapliyal, 2002) [24]. RBPT is a rapid and convenient test for brucellosis (Metri *et al.*, 2011) [12] and standard tube agglutination test (STAT) is a most common standard test used for the sero-diagnosis of the disease. Circulating antibodies for *Brucella* can be demonstrated by these serological tests. Further, in any epidemiological investigation risk factors or determinants are the variables associated with an increased risk of disease or infection. There may be any association of bovine brucellosis with physiological state of the animals and reproductive disease condition. Data on what is the strength of association between risk factors and outcome are lacking. Keeping in view above facts and points, present study was undertaken to investigate sero-prevalence and risk factors of bovine brucellosis in Indore district of Madhya Pradesh (India).

Materials and Methods

Study area: The study was conducted in Indore district of Madhya Pradesh in central India. The district of Indore is 3898 square kilometers in size and is located in the Malwa plateau. It is surrounded by the eastern (E) longitudes of 75° 25' and 76° 15' and the northern (N) latitudes of 22° 31' and 23° 15'. Indore district is divided into ten tehsils and four development blocks. Indore has 4 blocks viz. Indore, Mhow, Depalpur and Sanwer. These locations were selected because of large number of small-scale dairy farming in the area.

Collection of the samples: A total of 509 blood samples were collected from different dairy farms situated in 4 blocks as Mhow, Indore, Sanwer and Depalpur of Indore district of Madhya Pradesh, using a random sampling technique (Table 01). Blood samples were collected between the time periods of September 2022 to February 2023. Ten ml of the blood sample from each animal was collected in a sterile non EDTA serum tube. The samples were brought to the laboratory in the ice box and serum were separated by centrifugation at 3500 rpm for 10 min and stored at -20 °C for further testing. Similarly, the milk samples from sero-positive bovine animals were also collected under aseptic conditions into sterilized bottles. All milk samples were immediately transported to the laboratory under low-temperature conditions for further analysis.

Table 1: Bovine blood samples collected from different blocks in the Indore district

S. No.	Blocks of Indore district	No. of blood sample collected
1.	Mhow	271
2.	Indore	80
3.	Sanwer	85
4.	Depalpur	73
	Total samples	509

Rose Bengal Plate Test: The RBPT was performed by the method described by OIE (2008) [15]. A total of 30 µl of serum was mixed with equal volume of *Brucella* antigen on white enamel plate circled approximately 2 cm in diameter with sterile glass or plastic rod. The result was recorded after the mixture was rocked gently for 4 minutes at room temperature. Any sign of agglutination was considered as positive. Definite clumping/agglutination were considered as positive serum samples, whereas no clumping resulted in negative samples.

Standard Tube Agglutination Test (STAT): The STAT was performed as per the method described by Alton *et al.* (1988) [3]. In brief, 0.8 ml of 0.5% phenol saline was taken in the first agglutination tube whereas, 0.5 ml of the same was be taken in remaining further agglutination tubes placed in a rack. Then 0.2 ml of serum sample was added in the first tube and mixed well by shaking. The 0.5 ml of diluted serum was transferred from first to second tube and the process is repeated up to the last tube. The 0.5 ml of diluted serum was discarded from the last tube and 0.5 ml of *B. abortus* plain antigen were added to each tube to get final dilution of 1:10, 1:20, 1:40, 1:80, and 1:160 in first, second, three, four, and fifth tube, respectively. A control tube was set up to simulate 50% agglutination by mixing 0.5 ml antigen and 0.5 ml of 0.5% phenol saline in an agglutination tube. All tubes were incubated at 37 °C for 20 hours before observation. A serum containing 40 or more IU per ml was considered positive for bovine species.

Milk Ring Test: The MRT was performed as per the guidelines of OIE (2008) [15]. MRT was performed on individual milk samples. The milk from all the seropositive animals was screened. The stored antigen and milk samples were brought to room temperature prior to performing the test. About 50 µl of antigen was added to 2 ml of milk in a narrow test tube and mixed thoroughly. The tubes then were incubated at 37 °C for one hour together with positive and negative working standards. A strongly positive reaction was indicated by formation of dark pink ring above a white milk column. The test was considered to be negative if there was uniform pink colour for the milk column and cream layer (Kumar *et al.*, 2016) [10].

The RBPT antigen, plain antigen and abortus bang ring antigen were procured from biological products, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh-243 122.

Results and Discussion

Rose bengal plate test: In the present study, a total of 509 serum samples were examined for the presence of anti-*brucella* antibodies from bovine population (cattle-320 and buffaloes-189). The samples were collected from various dairy farms viz organized and unorganized farms, and also from Gausshalas of the Indore district and screened for brucellosis by using rose bengal plate test (RBPT). Out of total, 16 serum samples were found to be positive (cattle-12 and buffaloes-04) and overall sero-prevalence was recorded as 3.14% (cattle-3.73% and buffaloes-2.11%). The sero-prevalence of brucellosis in bovine population by using RBPT was also observed in various blocks viz. Mhow, Indore, Sanwer and Depalpur blocks, which were found to be 3.32% (9/271), 2.50% (2/80), 3.52% (3/85) and 2.73% (2/73), respectively (Table 02, Table 04).

Table 2: Sero-prevalence of bovine brucellosis in different blocks of the studied area using RBPT (n = 509)

S. No.	Blocks	Number of samples investigated	Total positive	Sero-prevalence (%)
1.	Mhow	271	9	3.32
2.	Indore	80	2	2.50
3.	Sanwer	85	3	3.52
4.	Depalpur	73	2	2.73

The results of the present study are comparable with the results as reported earlier by Basak *et al.* (2019) [4] and Kothalawala *et al.* (2017) [9] as 3.46%, and 2.70% respectively, while low prevalence 2.20% was observed by Gutema *et al.* (2021) [7]. The high occurrence was observed by Shome *et al.* (2015) [20] as 5.22%. The variation in our study may be due to regional differences.

Standard tube agglutination test: In the current study, a total of 509 serum samples were further investigated for the presence of anti-*brucella* antibodies from bovine population (cattle-320 and buffalo-189) by using standard tube agglutination test (STAT). Out of which, 15 serum samples were found to be positive (cattle-11 and buffaloes-04) with overall sero-prevalence was recorded as 2.94% (cattle-3.43% and buffaloes-2.11%) (Table 03, Table 04).

Table 3: Sero-prevalence of bovine brucellosis in different blocks of the studied area using STAT (n = 509)

S. No.	Blocks	Number of samples investigated	Total positive	Sero-prevalence (%)
1.	Mhow	271	9	3.32
2.	Indore	80	1	1.25
3.	Sanwer	85	3	3.52
4.	Depalpur	73	2	2.73

The results of present investigation are found to be comparable with the findings as reported earlier by Lodhi *et al.* (1995) [11] 2.40%, while lower prevalence was observed by Aiyedun *et al.* (2017) [12] 3.30%. The variation in this study might be due to local or climatic differences.

Table 4: Serological test wise status of bovine brucellosis

S. No.	Serological test	Number of samples investigated	Total positive*
1	RBPT	509	16 (3.14)
2	STAT	509	15 (2.94)

*Values within parentheses indicate percentages

Milk ring test (MRT): In this investigation, the female sero-positive animals were further screened for brucellosis by using milk ring test. A total of 509 serum samples were previously examined, in which 16 serum samples were found to be sero-positive by using RBPT. The milk from these sero-positive animals was taken here for further analysis. The results revealed that out of total, 13 (2.55%) samples were found to be positive for bovine brucellosis in the studied area. The results of our study are partially in accordance with the findings of Nofal *et al.* (2017) [14] where MRT were found to be 2.33% and with Shafee *et al.* (2011) [18] who reported brucellosis sero-positivity as 4.60%.

Block wise status of bovine brucellosis: In the present investigation, the results showed that 3.30%, 2.50%, 3.52% and 2.73% of serum samples were found to be positive for

bovine brucellosis in Mhow, Indore, Sanwer and Depalpur blocks, respectively. On application of the Chi-Square test, results observed non-significant ($p > 0.05$) association between blocks and positive samples and negative samples in Indore district (Table 05).

Table 5: Block wise bovine brucellosis status in Indore district (n = 509)

S. No.	Block	Total number of samples analysed	Positive sample*
1	Mhow	271	9 (3.32)
2	Indore	80	2 (2.50)
3	Sanwer	85	3 (3.52)
4	Depalpur	73	2 (2.73)
	Total	509	16 (3.14)

*Values within parentheses indicate percentages

Area wise status of bovine brucellosis: In the present study, the results showed that 3.11% and 3.22% of serum samples were found to be positive for bovine brucellosis in the rural and urban areas, respectively. On application of the Chi-Square test results observed a non-significant ($p > 0.05$) association between areas and positive samples and negative samples in Indore district (Table 06).

Table 6: Area wise status of bovine brucellosis in Indore district (n = 509)

S. No.	Type of areas	Total number of samples analysed	Positive samples*
1	Rural	385	12 (3.11)
2	Urban	124	4 (3.22)
	Total	509	16 (3.14)

*Values within parentheses indicate percentages

The results of the present study are lower than the findings of Deka *et al.* (2021) [6] who reported that sero-positivity was found to be 12.40% and 18.70% in rural and urban areas in Assam, respectively. The variation in our study might be attributed to local differences.

Sector wise status of bovine brucellosis: In this investigation, the results showed that 1.80% and 3.51% of serum samples were found to be positive for bovine brucellosis in organised and unorganised sectors, respectively. On application of the Chi-Square test, results observed non-significant ($p > 0.05$) association between sectors and positive samples and negative samples in the Indore district (Table 07).

Table 7: Organised and unorganised sector wise status of bovine brucellosis in Indore district (n = 509)

S. No.	Type of sector	Total number of samples analysed	Positive sample*
1	Organized	111	2 (1.80)
2	Unorganized	398	14 (3.51)
	Total	509	16 (3.14)

*Values within parentheses indicate percentages

Risk factor investigation of bovine brucellosis in the studied area: In the current study, results showed that on Chi-Square analysis of potential risk factors; Lactation was found to be non-significantly ($p > 0.05$) associated with outcome i.e. bovine brucellosis sero-positivity (Table 08). The lactation positive group has 0.98 times the odds of outcome than the exposure negative group (non-lactating

animals). In other words, the exposure negative group has 1.02 (1/0.98) times the odds compared to the exposure positive group (lactating animals). Further it was 95% confident that the odds ratio in the population would be between 0.27% and 3.5%. In contrast to our study, earlier Islam *et al.* (2021) [8] and Bugeza *et al.* (2019) [5], reported that animals with lactation were significantly associated with a higher risk of being brucellosis sero-positivity. This different pattern might be attributed to nutritional and immune status of the investigated population.

In the present investigation, results revealed that on Chi-Square analysis of potential risk factors; reproductive disorder, was found to be significantly ($p < 0.02$) associated

with outcome i.e. bovine brucellosis sero-positivity (Table 08). The reproductive disorder positive group having 3.11 times the odds of the outcome than the reproductive disorder negative group (odds ratio 95% CI: 1.14, 8.46). The results of present study are comparable with Islam *et al.* (2021) [8] who reported that cows with history of reproductive disorders were having odds ratio 3.2 times in exposure positive group (animal with reproductive disorder) than the exposure negative group (animal without reproductive disorder) (Odds ratio 95% CI: 1.2-10.1). Earlier Ullah *et al.* (2019) [25] also found that reproductive disorder has significant association with brucellosis. Our study is aligned with these findings.

Table 8: Associations between potential risk factors and brucellosis in bovine population of Indore district (M.P.)

S. No.	Variables	Category	Brucella positive	Brucella negative	Odds ratio (OR)	95%CI	P-value
1.	Lactation	Present	13	402	0.98	0.27-3.51	0.976
		Absent	3	91			
2.	Reproductive disorder	Present	8	120	3.11	1.14-8.46	0.020
		Absent	8	373			

Conclusions

In the present study, results revealed that sero-prevalence of bovine brucellosis in Indore district was found as low using screening tests viz RBPT and STAT. Most of the milk samples from seropositive female animals were found positive using MRT. The potential risk factor investigation indicated that lactation of the animals was found to be non-significantly associated with outcome i.e. bovine brucellosis sero-positivity while animals with reproductive disorder were found to be significantly associated with outcome i.e. bovine brucellosis sero-positivity. Based on these results, it is recommended that bovines that have reproductive issues should be focused for screening of brucellosis using serological tests. The screening tests ought to be utilized to control brucellosis in dairy animals and to safeguard human health and wellbeing as a whole.

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