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## Prevalence of brucellosis in buffaloes of Bidar district

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### Abstract

The present study was undertaken to determine the seroprevalence of brucellosis in buffaloes in Bidar district of Karnataka. A total of 150 serum and 150 milk samples were collected from five talukas of Bidar district and subjected to RBPT, MRT and i-ELISA for confirmation of brucellosis in buffaloes. The overall prevalence of brucellosis in buffaloes was found to be 12.66%, 8.66% and 21.33% by RBPT, MRT and i-ELISA respectively. Higher prevalence of brucellosis was recorded by i-ELISA followed by RBPT and MRT. On age-wise study, the higher prevalence was noticed in the 4-6 year followed by above 6 year and 1-4 years age groups. Buffaloes with the history of reproductive disorders have higher prevalence of brucellosis. To conclude, i-ELISA test is highly sensitive and better diagnostic tool compared to other tests in the diagnosis of brucellosis in buffaloes.

**Keywords:** Buffaloes, RBPT, MRT, i-ELISA, brucellosis

### 1. Introduction

Brucellosis is an economically significant zoonotic disease with a global distribution, with low-income countries being the most affected. The disease is endemic in India, which has the world's largest cattle and buffalo population and produces the most milk. The disease's prevalence in the country has been reported to range from 1% to 60%. There are approximately 20 different risk factors that have been identified as contributing to or predisposing to the occurrence of bovine brucellosis. The risk factors could be classified into four groups i.e, farmer's factors, host factors, managemental factors and agro-ecological factors (Deka *et al.*, 2018) [3]. The main source of infection is aborted fetuses, placental membranes and uterine discharges. The animals become infected by consuming contaminated milk, feed and water or by coming into contact with aborted fetuses, fetal membranes, and uterine discharges (Khurana *et al.*, 2021) [5]. Inhalation could also be a mode of transmission. Infected bulls may also spread infection from one herd to another through natural service or artificial insemination. (Pedro *et al.*, 2003) [11]. It is a contagious animal disease that causes abortion in females, orchitis an infection of accessory sex glands in males and infertility in both sexes. (Quinn *et al.*, 2015) [12]. Serological tests are the most important diagnostic tests for brucellosis screening and they play an important role in the brucellosis control and eradication program. Serum agglutination test (SAT), Rose Bengal test, complement fixation test and enzyme-linked immunosorbent assay are the most common serological screening tests. In addition to serum, Brucella antibodies are excreted in milk, which is used to screen herds for Brucella infection using the Milk Ring Test (MRT).

### 2. Materials and Methods

#### 2.1 Ethical approval

The study was conducted duly following approved guidelines from the Institutional Animal Ethics Committee.

#### 2.1 Location of the study

The study was conducted in Aurad, Basavakalyana, Bidar, Bhalki and Humnabad talukas of Bidar district.

#### 2.2 Selection of animals

The experimental animals were selected from five talukas of Bidar district. A total of 150 animals were selected with different age groups, lactation stage and reproductive disorders.

### 2.3 Collection and processing of blood samples

Blood samples were collected aseptically from buffaloes using a sterile needle through jugular venipuncture into vacutainers without anticoagulants. Three to four milliliters of blood was collected and allowed to clot with utmost precaution to avoid hemolysis and transported to the laboratory at 4 °C. Collected blood in the clot activator vacutainer was kept in a slant position for 1-2 hours for clotting and centrifuged at 3000 rpm for 3 minutes. Separated serum samples were transferred into the serum collecting tubes (Eppendorf tubes) and stored with proper labeling at -20 °C in a deep freezer until further processing.

### 2.4 Collection and processing of milk samples

The udder of the animal was thoroughly washed and cleaned with potassium permanganate solution (1:1000) and dried with a clean cloth. After discarding a few drops of milk,

approximately 10 ml from each quarter were collected into sterile screw-capped sample collection bottles from 150 animals and transported in an ice pack to the laboratory for Brucella screening.

### 2.5 Statistical analysis

The diagnostic statistics were calculated as per the methods described by Mandrekar and Mandrekar (2004) [7].

### 3. Results and Discussion

The current study sought to determine the prevalence of brucellosis in buffaloes. A total of 150 serum and milk samples were collected randomly from Aurad, Basavakalyana, Bhalki, Bidar, and Humnabad talukas of Bidar district for the detection of Brucella antibodies in serum using the Rose Bengal Plate Test (RBPT), Milk Ring Test (MRT) and Indirect ELISA (i-ELISA).

**Table 1:** Prevalence of brucellosis by RBPT, MRT and i-ELISA in buffaloes

Test	RBPT (n=150)		MRT (n=150)		I-ELISA(n=150)	
	Positive	Negative	Positive	Negative	Positive	Negative
Total no. of positives/negatives	19	131	13	137	32	118
Prevalence of brucellosis (%)	12.66%		8.66%		21.33%	
Chi-square value	10.310					
P- Value	<0.05					

p<0.05 at 5% level of significance

The prevalence of brucellosis in 150 buffalo serum and milk samples was found to be 12.66% (19/150) by RBPT, 8.66% (13/150) by MRT and 21.33% (32/150) by i-ELISA collected and then were screened by RBPT, MRT and I-ELISA respectively. Similar findings were observed by Begam

(2017) [2] who found highest prevalence by I-ELISA (26.50%) followed by RBPT (11.45%) and least by MRT (10.21%). On contrast, Patel *et al.* (2022) [10] reported higher prevalence by MRT (18.65%) followed by RBPT (9.3%) and least by I-ELISA (8.37%).

**Table 2:** Taluk-wise prevalence of brucellosis in buffaloes by RBPT, MRT and I-ELISA

Sl. No	Talukas	No. of serum samples tested	No. of milk samples tested	No. of positives			P-Value
				RBPT (%)	MRT (%)	I-ELISA (%)	
1	Aurad	30	30	02 (6.66%)	02 (6.66%)	04(13.33%)	<0.05
2	Basavakalyana	31	31	02 (6.45%)	02 (6.45%)	03(9.67%)	<0.05
3	Bhalki	30	30	05 (16.60%)	03 (10.00%)	08(26.60%)	>0.05
4	Bidar	31	31	06 (19.35%)	03 (9.67%)	06(19.35%)	<0.05
5	Humnabad	28	28	04 (14.28%)	03 (10.71%)	11(39.28%)	<0.05
Chi-square value				10.301			
P- value				<0.05			

p<0.05 at 5% level of significance

Buffaloes from 5 talukas of Bidar district were randomly screened for brucellosis. The highest prevalence was observed in Humnabad taluka (39.28%) followed by Bhalki (26.60%), Bidar (19.35%), Aurad (13.33%) and Basavakalyana (9.67%) by I-ELISA. Among all the talukas, taluka Aurad, Bidar,

Basavakalyana and Humnabad were found to be statistically significant (p<0.05). This might be due to irregular cleaning, higher levels of exposure to infected or carrier animals at the farm (Khan *et al.*, 2021) [4].

**Table 3:** Age-wise prevalence of brucellosis with different tests in buffaloes

Age (Years)	Total (n=150)	RBPT	MRT	I-ELISA
1-4	25	03 (12.00%)	02 (8.00%)	04 (16.00%)
4-6	81	13 (16.04%)	09 (11.11%)	21 (25.92%)
6 and above	44	03 (6.81%)	02 (4.54%)	07 (16.00%)
Chi-Square value	5.003			
P-value	>0.05			

p>0.05 at 5% level of significance

According to RBPT, MRT and I-ELISA results, the age groups of 4-6-years buffaloes had the highest seroprevalence (25.92%) followed by those more than 6 years age group (16.00%) and those younger than 4 years age group (16.00%).

These findings are in agreement with the observations of Maiti and Mohan (2013) [6] who found higher prevalence in buffaloes of 4-6 years age group (34.2%) followed by buffaloes of >6 years age group (14.2%) and lowest

prevalence in 2-4 years age group (74%) by I-ELISA. On contrary, Shome *et al.* (2014) [16] observed higher prevalence in 3-4 years age group (51.58%) followed by 4-6 years age group (10.89%) and least prevalence in 6-8 years age group (2.57%).

The age group found non significant from each other ( $p>0.05$ ). The results of the present study suggested that animals older than 4 years of age are more likely to become sero-positive to brucella. Similar observations were made by Radostits *et al.* (2007) [14] higher prevalence in animals of > 5

years age group may be due due to sex hormones and erythritol which stimulate the growth and multiplication of brucella organisms tend to increase in concentration with age. Young animals may have lower prevalence because they have stronger resistance and can rapidly eliminate the brucella organism from their body (Walker *et al.* 1999) [17]. The higher prevalence in older animals can be attributed to the chronic nature of the disease, chances of exposure to the pathogen with an increase in age and mating with seropositive animals (Khan *et al.*, 2021) [4].

**Table 4:** Lactation stage-wise prevalence of brucellosis in buffaloes

No. of lactation	Samples tested	RBPT	MRT	I-ELISA
01-2	72	10 (13.88%)	7 (9.72%)	15 (20.83%)
3 and above	78	9 (11.53%)	6 (7.69%)	17 (21.79%)
Total	150	19 (12.6%)	13 (8.66%)	32 (21.33%)
Chi-Square value		0.123		
P-value		>0.05		

$p>0.05$  at 5% level of significance

The prevalence of brucellosis in buffaloes in 1-2 lactation was from 9.72% by MRT to 20.83% by I-ELISA whereas in 3<sup>rd</sup> and above lactations from 7.69% by MRT to 21.33% by I-ELISA respectively. The results were in accordance with Panchasara *et al.* (2015) [9] who observed higher prevalence in >3<sup>rd</sup> lactation than in 1-2<sup>nd</sup> lactation. Contradictory to present

study Aziz *et al.* (2021) [11] observed the higher prevalence in second lactation group than the 5<sup>th</sup> lactation group. Lower prevalence of brucellosis in 1<sup>st</sup> lactation stage of buffaloes due to the resistance of sexually immature animals or because of passive immunization of calves through colostrums from infected dams (Mohammed *et al.*, 2011) [8].

**Table 5:** Prevalence of brucellosis in buffaloes with a history of reproductive disorders

History of the animal	Samples tested	RBPT	MRT	I-ELISA
With reproductive disorders	25	08 (32.00%)	06 (24.00%)	09 (36.00%)
With no disorders	125	11 (8.80%)	07 (5.60%)	23 (18.45%)
Chi-Square value		17.559		
P-value		0.000**		

$p<0.05^*$  at 5% level of significance

In the present study a total of 25 buffaloes were screened with different reproductive disorders (Abortion, retention of fetal membrane, repeat breeders) and revealed higher prevalence rate in buffaloes with history of reproductive disorders (32%, 24% and 36%) than in animals without any reproductive disorders (8.80%, 5.60% and 18.45%) by RBPT, MRT and I-ELISA, respectively. Results were in agreement with Panchasara *et al.* (2015) [9] who reported higher prevalence in animals with history of reproductive disorders (38.09%) than in healthy animals (11.95%). However higher prevalence than our study was reported by Rahman *et al.* (2011) [15] as 60% in buffaloes with reproductive disorders.

In the present study buffaloes with a history of reproductive disorders were statistically highly significant ( $p<0.05^*$ ) than buffaloes with no reproductive disorders and showed major risk factor for brucellosis. The higher incidence of abortion in third trimester may be due to the fact that the uterine environment becomes conducive for growth of brucella due to production of erythritol, which in turn causes damage to placenta and abortion (Radostits *et al.*, 2000) [13].

#### 4. Conclusion

To conclude, the overall prevalence of brucellosis in buffaloes was 21.33% by I-ELISA, higher prevalence of brucellosis in buffaloes (21.33%) was detected by I-ELISA followed by RBPT (12.66%) and MRT (8.66%). The highest prevalence was observed in Humnabad taluka (39.28%) of Bidar district. Buffaloes in the age group of 4-6 years showed a higher prevalence of brucellosis than other age groups of 6 and

above 6 years age group and 1-4 years. Buffaloes with a history of reproductive problems and above 3<sup>rd</sup> lactation group were shown to have a higher prevalence of brucellosis.

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