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## Occurrence of brucellosis in cows

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**Abstract**

Brucellosis is one of the most serious diseases in developing countries. For epidemiological study a total of 200 lactating cows were screened for brucellosis from private dairy farms of Satna and Jabalpur. The information pertaining to age, parity, history of abortion and vaccination status of individual cow was recorded. For screening of brucellosis, milk and sera samples were collected and tested by MRT, RBPT and STAT. The overall occurrence of the brucellosis in cow was recorded as 13.50% by MRT, 18.00% by RBPT and 26.00% by STAT. Higher occurrence of brucellosis was found in crossbred cows 18.18% by MRT, 22.72% by RBPT and 34.09% by STAT, in comparison to indigenous cows i.e. 4.41% by MRT, 8.82% by RBPT and 10.29% by STAT. Age wise highest occurrence recorded in cows above 6 years of age while no seropositivity was observed in cows up to 2 years of age.

**Keywords:** Brucellosis, occurrence, MRT, RBPT, STAT

**Introduction**

India is an agrarian country. A large proportion of the population, especially in the rural areas, depends on agriculture and primarily on animal production. Animal resource in the country is threatened by reproductive disorders viz., infertility, retained placenta, abortion, endometritis etc., causing considerable economic losses. Brucellosis has been one of the most important reproductive diseases among different livestock species as well as animal handlers.

Brucellosis is found worldwide. It is one of the most serious diseases in developing countries. The rate of infection varies greatly from one country to another and between regions within the country, with highest prevalence in dairy cattle. In India, brucellosis was first reported in 1942 and is now endemic throughout the country [1]. In general, risk factors such as unrestricted trade, movements of animals, use of local cattle yards or fairs for trading, sending dry animals back to villages for maintenance, use of semen from unscreened bulls for artificial insemination and poor farm hygiene probably attribute to the spread and transmission of the infection.

Despite the advances made in the diagnosis and therapy, brucellosis is still wide spread and prevalent in many developing countries. Economic losses by brucellosis in animals are due to abortion, premature births, decrease milk production and due to repeat breeding and may lead to temporary or permanent infertility in infected livestock. Economic losses due to brucellosis in livestock are considerable in India.

The most widely used serological tests for diagnosis of brucellosis in animals are Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT) and Enzyme Linked Immuno Sorbent Assay (ELISA). Since, neither a single serological test nor a combination of tests detects all infected animals and also due to high homology among *Brucella* species, the detection of brucellosis remains a major problem. Keeping the above facts in view, the present study was under taken with the objective to study the occurrence of brucellosis in cows.

**Material and Methods****Animals**

A total of 200 lactating cows were screened for brucellosis from private dairy farms of Satna and Jabalpur. The information pertaining to age, parity, history of abortion and vaccination status of individual animal was recorded. The samples were collected from October 2016 to April 2017.

## Sample Collection

### Milk

The udder was thoroughly washed and cleaned with potassium permanganate solution (1:1000) and dried with clean cloth. Teat opening was disinfected with 70% of ethyl alcohol. After discarding few drop of milk, approximately 5 ml of milk from each animal was collected in sterile screw capped plastic vials and transported on the ice to the laboratory for Milk ring test.

### Serum

About 9 ml of blood was collected aseptically from the jugular vein of properly restrained animal in a vacuette with serum clot activator on day 0 pre treatment and days 15, 30 and 45 post-treatment. The vacuettes were kept in upright position at room temperature for 2 hours. The separated serum was collected in a screw capped plastic vial and transported to the laboratory were stored at -20°C till further use. Collected serum sample were subjected to Rose Bengal Plate Test (RBPT) and Standard Tube Agglutination Test (STAT).

## Testing of samples

### Milk Ring Test (MRT)

It is also known as Abortus Bang Ring Test (ABRT). This test was employed to test milk from *Brucella* infected cattle herd.

The antigen was procured from biological products division, Indian Veterinary Research Institute Izzatnagar (U.P.)

### Standard Tube Agglutination Test (STAT)

*Brucella* SAT antigen is a suspension of a pure smooth culture of *Brucella abortus* strain 99 in phenol saline. The standard tube agglutination test was performed according to Weybridge technique (Alton *et al.*, 1975). All the serum samples were tested up to minimum of nine dilutions. For high titred sera, more dilutions were prepared in order to achieve end point titre. In brief, eleven agglutination tubes were placed in a rack. Further, 0.8 ml of 0.5 per cent phenol saline was taken in a first tube and 0.5 ml in rest of the tubes. 0.2 ml of serum was added in the first tube, mixed well and 0.5 ml of diluted serum transferred to the second tube. The process was continued up to the ninth tube and 10<sup>th</sup> tube was kept for control tube, 0.5 ml was discarded from the last tube after mixing. Then 0.5 ml *B. abortus* plain antigen was added to each tube and mixed thoroughly. This provided a final dilution of 1:10, 1:20, 1:40, 1:80 and 1:160 and so on. Considering the special significance of 50 per cent end point, a control tube was set up to simulate 50 per cent clearing by mixing 0.25 ml antigen with 0.75 ml of 0.5 per cent phenol saline in an agglutination tube. All the tubes were incubated at 37 °C for 24 hour.

**Table 1:** Procedure for standard tube agglutination test

Tube No.	1	2	3	4	5	6	7	8	9	10	11
a. 0.5% Phenol saline	0.8	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
b. Test Serum	0.2	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
<b>Mixed thoroughly and transferred until tube no. 9 discarded 0.5 ml from tube no. 11 i.e. discard tube.</b>											
c. <i>Brucella abortus</i> plain antigen	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
d. Final dilution	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560		

## Interpretation

The results were compared with the control. The highest dilution of the serum which showed 50 per cent agglutination was taken as end titre. The titre so obtained was expressed in unit system by doubling of the serum titre as International Unit (IU) per ml of serum. The antibody titre of 1:80 (160 IU / ml) and above was taken as positive for *Brucella*.

## Results and Discussion

### Overall occurrence of brucellosis

Out of 200 samples, 27, 36 and 52 samples were found positive for brucellosis using MRT, RBPT and STAT, respectively. The overall occurrence of the brucellosis in cows was 13.50% by MRT, 18.00% by RBPT and 26.00% by STAT.

**Table 2:** Overall occurrence of brucellosis in cows

Cows screened	<i>Brucella</i> positive cows		
	MRT	RBPT	STAT
200	27 (13.50)	36 (18.00)	52 (26.00)

Figure in parenthesis ( ) indicate percentage

The results of the study are in agreement with findings of researcher [3] who have reported the overall 12.6% sero prevalence of brucellosis. The overall prevalence of brucellosis as 19.76% by RBPT and 16.57% by STAT [4]. Researcher reported comparatively higher sero-prevalence of brucellosis as 50.35% by STAT and 33.33% by RBPT in West Bengal [5].

In contrast to the present findings, Rahman *et al.* (2012) [6] reported the overall prevalence of brucellosis in dairy cattle as

2.25%. It was also documented lower sero-prevalence of brucellosis as 2.62% by MRT and 2.06% by RBPT [7].

The present findings indicate the presence of brucellosis in the cows. Serological methods of diagnosis are very useful in supporting the diagnosis and are useful particularly in epidemiological investigation. The serological tests employed in the present investigation were MRT, RBPT and STAT, which are simple, easy to perform and less time consuming. The variation in the prevalence rate of brucellosis might be attributed to the fact that diagnostic tests varied between studies. Moreover, there may be difference in managemental conditions, climate study, Designed and study methods used. Due to wide variation in the number of sample tested by different workers in different part of the world, comparison in this regard would be of little value.

### Origin wise occurrence of brucellosis

Out of 132 crossbred cow's milk and sera samples were tested by MRT, RBPT, and STAT and found to be positive for brucellosis 24 (18.18%), 30 (22.72%) and 45 (34.09%) respectively. While in indigenous cows, out of 68 milk and sera samples tested; 3 (4.41%), 6(8.82%) and 7 (10.29%) by MRT, RBPT and STAT respectively showed seropositivity for brucellosis.

**Table 3:** Occurrence of brucellosis in indigenous and crossbred cows

Description	Cows screened	<i>Brucella</i> positive cows		
		MRT	RBPT	STAT
Indigenous cows	68	3 (4.41)	6 (8.82)	7 (10.29)
Cross bred cows	132	24 (18.18)	30 (22.72)	45 (34.09)

Figure in parenthesis ( ) indicate percentage

Origin wise occurrence of brucellosis was comparatively higher in crossbred cows in comparison to indigenous cows. Our findings corroborated the findings of researcher [8] who reported higher prevalence in crossbred cattle (12.50%) in comparison to the indigenous cattle (5.38%) of sub Himalayan Kumaon region. Rahman *et al.* (2012) [6] also recorded the higher prevalence in crossbred cattle (3.6%) than indigenous 1.7 per cent. This shows that the indigenous cows are comparatively resistant to bovine brucellosis and crossbred cows are less adapted to the hot and humid climate including management practices of the particular region. The intensive use of artificial insemination (A.I.) in crossbred animals may be a contributing factor for higher prevalence of brucellosis.

#### Age wise occurrence of brucellosis

Cows up to 2 years of age showed no seropositivity for serological tests (RBPT and STAT). Cows above 6 years age group showed the highest occurrence (24.46% by MRT, 31.91% by RBPT and 46.80% by STAT) followed by 4-6 years age group (8.53%, 12.5% and 16.66% by MRT, RBPT and STAT respectively). Whereas lowest occurrence of brucellosis was reported in 2-4 years age group of cows *viz.* 4.76%, 7.14% and 9.52% by MRT, RBPT and STAT, respectively.

**Table 4:** Age wise occurrence of brucellosis in cows

Age of cows in years	Cows screened	Brucella positive cows		
		MRT	RBPT	STAT
0-2	40	-	0 (0.00)	0 (0.00)
2-4	42	2 (4.76)	3 (7.14)	4 (9.52)
4-6	24	2 (8.53)	3 (12.5)	4 (16.66)
6 and above	94	23 (24.46)	30 (31.91)	44 (46.80)

Figure in parenthesis () indicate percentage

Cows up to 2 years of age showed no sero-positivity for serological tests (RBPT and STAT). Cows above 6 years age group showed the highest occurrence whereas lowest occurrence of brucellosis was reported in 2-4 years age group of cows. These findings correlate with the work of researchers [8, 9, 10] who also reported the higher prevalence of brucellosis in animals in 6-8 years age group. Significantly higher prevalence of brucellosis in above 5 years age group was recorded [7]. On the contrary, [6] it was observed that there is higher seroprevalence of brucellosis in dairy cattle in 2 to 3 years of age group (3.0%) when compared with 4 to 8 years age group (2.0%). Higher prevalence of brucellosis in animals above 4 years might be due to the fact that this is the most suitable age for breeding. It might also be due to the fact that there is a marked decrease in immune status with the advancement of age.

#### Parity wise occurrence of brucellosis

Cows of 6<sup>th</sup> and above 6<sup>th</sup> parity revealed the highest occurrence (35.71% by MRT, 50.00% by RBPT and 71.42% by STAT) followed by 4<sup>th</sup> parity cows (25.00% and 33.33%, 41.66% by MRT, RBPT and STAT respectively). However, the cows of 3<sup>rd</sup> parity showed lower occurrence *i.e.* 20.83% and 25.00% by MRT and RBPT respectively, while the lowest occurrence was reported in the cows of 5<sup>th</sup> parity (14.28% and 19.04%, 28.57% by MRT, RBPT and STAT respectively). Cows of 1<sup>st</sup> and 2<sup>nd</sup> parity did not reveal seropositivity for serological tests (MRT, RBPT and STAT).

**Table 5:** Parity wise occurrence of brucellosis in cows

Parity of cows	Cows screened	Brucella positive cows		
		MRT	RBPT	STAT
1	50	0 (0.00)	0 (0.00)	0 (0.00)
2	32	2 (6.25)	3 (9.37)	4 (12.5)
3	24	3 (12.50)	3 (12.50)	6 (25.00)
4	24	6 (25.00)	8 (33.33)	10 (41.66)
5	42	6 (14.28)	8 (19.04)	12 (28.57)
6 and above	28	10 (35.71)	14 (50.00)	20 (71.42)

Figure in parenthesis () indicate percentage

The parity wise occurrence of brucellosis revealed the highest occurrence in cows of 6<sup>th</sup> and above 6<sup>th</sup> parity. Cows of 1<sup>st</sup> and 2<sup>nd</sup> parity did not reveal seropositivity for serological tests. The results of this study are in partial agreement with the findings of researcher [7], who reported higher prevalence of brucellosis in cattle in 3 to 5 parity comparison to other parity groups. Significantly higher prevalence of brucellosis in cattle of 3<sup>rd</sup> parity [11] was recorded. The higher prevalence of brucellosis in females may be due to the preferential localization of *Brucella* organisms in uterus having erythritol which stimulates growth of these organisms [12].

#### Conclusion

Bovine brucellosis has a worldwide occurrence and according to the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World Organization for Animal Health (OIE), is still one of the most important and wide spread bacterial zoonoses in the world. The prevalence of infection varies considerably among herds, areas and countries. Many countries have made considerable progress with their eradication programs, and some have eradicated the disease. However, in our country, brucellosis is still a serious disease facing the veterinary and medical professions. In the present study higher occurrence of brucellosis was found in crossbred cows in comparison to indigenous cows. Livestock producers in India and in many other developing countries cannot afford the traditional "test and slaughter" approach used in developed countries. So we need to focus more intensive approach towards epidemiology and eradication program of brucellosis in India.

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