

**BOVINE BRUCELLOSIS IN REPRODUCTIVE DISORDERS OF DAIRY ANIMALS IN PERI-URBAN AREAS OF GUJARAT**

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**ABSTRACT**

Bovine brucellosis - a significant zoonotic disease is commonly prevalent in dairy animals worldwide with varying magnitude between herds, locations, states and countries requiring sound surveillance to design proper control measures. A surveillance of brucellosis in reproductive disorders of bovines of peri-urban areas of Gujarat, India was therefore conducted using various diagnostic tests. Total 5036 animals in 199 herds of bovines (cattle-1720 and buffaloes-3316) from peri-urban areas were monitored for occurrence of reproductive disorders. Total 16.90% (849) bovines suffered from various reproductive disorders. Of these reproductive disorders cases, 18.30% (155) were found positive for *Brucella abortus* antibodies. Among *Brucella* positive cases of reproductive disorders, significantly ( $p=0.000$ ) higher prevalence of bovine brucellosis was found in cases of abortion (45.50%) than other reproductive disorders. The prevalence of bovine brucellosis in reproductive disorders of bovine in different peri-urban areas varied non-significantly between 12.60 and 23.90% ( $p<0.05$ ). Further, MRT, STAT and RBPT showed 7.27, 14.84 and 15.19% positivity, respectively. Therefore, all reproductive disorders should be monitored using accurate diagnostic tests such as milk-ELISA or I-ELISA for correct and prompt diagnosis as to prevent further spread of infection to apparently healthy animals of the herd and surroundings.

**KEY WORDS:** Bovine Brucellosis, Diagnostic Tests, Peri-urban Areas, Reproductive Disorders**INTRODUCTION**

The economic well being of dairy farmers depends upon healthy, productive and sound reproductive livestock. Among the various prevalent diseases which considerably affect production and reproduction performance of dairy animals, bovine brucellosis is perhaps the most economically important reproductive disease of the rapidly growing Indian dairy industry. In India, brucellosis was first recognized in 1942 and is now endemic throughout the country. The disease has been reported in cattle, buffaloes, sheep, goats, pigs, dogs and humans. Brucellosis in India is yet a very common but often neglected disease (Renukaradhya *et al.*, 2002). Infection may occur in bovines of all ages but persists most commonly in sexually mature animals. An infected cow generally aborts once and then becomes a chronic carrier, intermittently excreting bacteria in the milk and reproductive secretions for many years. Therefore, the most significant feature of bovine brucellosis epidemiology is the shedding of large numbers of organisms during 10 days after abortion or calving of infected cows and the consequent contamination of the environment (FAO, 2003). The prevalence of infection in animal reservoirs provides a key to its occurrence in humans also. That is why, the correct and prompt diagnosis is important in controlling and eradicating the disease in animals. However, the diagnosis of the disease can be challenging and is frequently delayed or missed because the clinical picture may mimic other infectious and non-infectious conditions (Radostits *et al.*, 2000). Therefore, all probable reproductive disorders should be monitored using suitable diagnostic tests. Considering the above facts, the present study on bovine brucellosis in reproductive disorders of dairy animals in peri-urban areas of Gujarat, India was planned.

## MATERIALS AND METHODS

Six selected cities, viz. Ahmedabad, Anand, Surat, Navsari, Valsad and Vapi of Gujarat, India were included in the present study. Five peri-urban areas of each city were randomly selected for the present work. From each peri-urban area, five farmers following intensive production system with herd size  $\geq 10$  milking animals were included in the study. Total 5036 animals in 199 herds of cattle (n=1720) and buffaloes (n=3316) were monitored for occurrence of reproductive disorders. A total of 761 milk and 849 serum samples were collected from bovines having history of reproductive disorders such as abortion, retention of placenta, still birth, repeat breeding and metritis/endometritis during the study period. Milk samples were subjected to milk ring test (MRT) and milk-ELISA, while serum samples were used to detect *Brucella* reactors by Rose Bengal plate test (RBPT), standard tube agglutination test (STAT) and I-ELISA. *Brucella* indirect ELISA test kit was procured from VMRD, Inc., USA and the milk-ELISA and I-ELISA tests were performed from same kit as per the protocol outlined in the user manual. Reagents / antigens for MRT, RBPT and STAT were procured from IVRI, Izatnagar, India and tests were performed following protocol outlined in the user manual.

Data pertaining to prevalence in reproductive disorders of bovines of different peri-urban areas were analyzed on IBM SPSS statistical software version 20.0 using Chi square test (probability at 5% level) as per method described by Snedecor and Cochran (1990). The present study was approved by institutional ethical committee.

## RESULTS AND DISCUSSION

The prevalence of bovine brucellosis in various reproductive disorders of cattle and buffaloes are given in Table 1. Total 849 out of 5036 (16.86%) dairy animals were found to suffer from various reproductive disorders. Considering the results of I-ELISA as gold standard, 155 (18.26%) out of

**Table 1: Species and reproductive disorder-wise prevalence of bovine brucellosis**

Sr. No.	Animal	Reproductive disorders	No. of animals covered	No. of cases reported	No. of cases positive	p value
1	Cattle	Abortion	1720	57 (3.31)	28 (49.12)	0.000*
		Retention of placenta		83 (4.83)	19 (22.89)	
		Still birth		31 (1.80)	05 (16.13)	
		Repeat breeding		56 (3.26)	10 (17.86)	
		Metritis/Endometritis		89 (5.17)	09 (10.11)	
		<b>Sub-total: A</b>		<b>316 (18.37)</b>	<b>71 (22.47)</b>	
2	Buffalo	Abortion	3316	88 (2.65)	38 (43.18)	0.000*
		Retention of placenta		130 (3.92)	21 (16.15)	
		Still birth		74 (2.23)	06 (8.11)	
		Repeat breeding		172 (5.19)	13 (7.56)	
		Metritis/Endometritis		69 (2.08)	06 (8.70)	
		<b>Sub-total: B</b>		<b>533 (16.07)</b>	<b>84 (15.76)</b>	
3	Overall	Abortion	5036	145 (2.90)	66 (45.50)	0.000*
		Retention of placenta		213 (4.20)	40 (18.80)	
		Still birth		105 (2.10)	11 (10.50)	
		Repeat breeding		228 (4.50)	23 (10.10)	
		Metritis/Endometritis		158 (3.10)	15 (9.50)	
		<b>(A + B)</b>	<b>5036</b>	<b>849 (16.86)</b>	<b>155 (18.26)</b>	<b>0.000*</b>

Figures in parentheses indicate percentages; \* indicates significant at  $p < 0.05$

these 849 individual cases of reproductive disorders were found positive for *Brucella* antibodies. The results are in accordance with the findings of Saleem and Fatohi (1993) and Isloor *et al.* (1998) who had recorded significantly higher prevalence (17-18%) in bovines having reproductive problems like abortion, retained placenta and stillbirth than apparently healthy animals. Still higher prevalence of bovine brucellosis (20-60%) have also been reported in cases of reproductive disorders like abnormal termination of pregnancy (ATP), cervicitis, endometritis, repeat breeding and anoestrus (Barman *et al.*, 1989; Dhand *et al.*, 2005; Kebede *et al.*, 2008; Ibrahim *et al.*, 2010; Trangadia *et al.*, 2010; Londhe *et al.*, 2011 and Priyantha, 2011).

Among all reproductive disorders, significantly higher prevalence (45.50%) of bovine brucellosis was observed in cases of abortion, whereas it was the lowest (9.50%) in cases of metritis/endometritis. Further, the prevalence of bovine brucellosis was 18.80, 10.50 and 10.10% in cases of retention of placenta, still birth and repeat breeding, respectively. The reproductive disorder-wise difference in prevalence of bovine brucellosis of cattle and buffaloes was statistically highly significant ( $p=0.000$ ). In accordance to the present findings, Manickam and Mohan (1987) recorded comparatively higher incidences of brucellosis in reproductive disorders (3.49-15.32%) than apparently healthy (1.41-1.94%) cattle and buffaloes. Further, they reported higher incidence in cases of abortion (7.14-15.32%) than other reproductive disorders (3.49-7.46). Barman *et al.* (1989) also reported higher incidence of brucellosis in aborted cattle (60.30%) than in repeat breeding animals (38.80%). The higher prevalence in abortion (55%) than repeat breeding (24%) and retention of placenta (20%) were also reported by Bhattacharya *et al.* (2005). Similarly, higher prevalence in cases of abortion among reproductive disorders of cattle and buffaloes of Gujarat was also reported by Panchasara (2007). Results of significant variation in prevalence of bovine brucellosis in different reproductive disorders are in accordance to the previous reports (Jha *et al.*, 1993; Alam *et al.* 1996; Tripathi *et al.*, 1998; Jeyprakash *et al.*, 1999; Chauhan *et al.*, 2000; Shringi *et al.*, 2002; Aulakh *et al.*, 2008; Ibrahim *et al.*, 2010 and Lindahl *et al.*, 2014).

During the study, comparatively higher prevalence of bovine brucellosis in reproductive disorders was observed for cattle (22.47%) than buffaloes (15.76%). The present finding is in accordance to the report of Manickam and Mohan (1987), who found comparatively higher prevalence of brucellosis in cattle and buffaloes with history of various reproductive disorders like abortion, retention of placenta and infertility. Jha *et al.* (1993) also found 22.22% prevalence of bovine brucellosis in cattle with history of abortion but not a single positive case in aborted buffaloes. On the contrary, Londhe *et al.* (2011) reported higher incidence of bovine brucellosis in reproductive disorders of buffaloes (42.18%) than cattle (37.14%) in dairy farms of Maharashtra under intensive system of production. Whereas, more or less equal prevalence of reproductive disorders in cattle (21.05%) and buffaloes (21.67%) was observed by Panchasara (2007) in Gujarat.

The highest prevalence of bovine brucellosis was observed in peri-urban areas of Vapi (33.89%), followed by prevalence in peri-urban areas of Anand (23.25%), Navsari (17.76%), Ahmedabad (17.39%), Surat (13.33%) and Valsad (12.58%). The difference in prevalence of bovine brucellosis in peri-urban areas was non-significant but difference in prevalence in various reproductive disorders of peri-urban areas was highly significant ( $p<0.05$ ). The results are in accordance with the previous reports indicating non-significant variation in prevalence of bovine brucellosis in different regions under study (Maadi *et al.*, 2011; Ali *et al.*, 2013; Zubairu *et al.*, 2014). Further, the peri-urban area-wise prevalence of brucellosis was also significantly higher in cases of abortion followed by retention of placenta, still birth, repeat breeding and metritis-endometritis. The overall prevalence of brucellosis in cases of abortion ranged between 33.33-54.55%. The values for prevalence of brucellosis in cases of retention of placenta, still birth, repeat breeding and metritis-endometritis were 11.11-32.61, 3.70-25.00, 4.00-12.12 and 5.26-20.00%, respectively.

The sero-positivity in different diagnostic tests is given in Table 2. The overall prevalence of bovine brucellosis in various reproductive disorders by MRT, milk-ELISA, RBPT, STAT and I-ELISA were 7.22, 19.05, 14.84, 15.19 and 18.26%, respectively. Similar to results of present study, the higher positivity in ELISA than other diagnostic test were also reported previously (Barbuddhe *et al.*, 2004; Agrawal *et al.*, 2007; Ghodasara *et al.*, 2010). In accordance to present findings, Trangadia *et al.* (2010) also found 22.18, 13.78 and 12.82% sero-prevalence on ELISA, RBPT and MRT, respectively in animals with history of abortion. Further, Priyadarshini *et al.* (2013) reported comparatively higher sero-positivity in A-B ELISA (8.14%) than RBPT (4.26%) and STAT (2.32%).

**Table 2: Diagnosis of bovine brucellosis in various reproductive disorders by various tests**

Sr. No.	Reproductive disorders	No. of cases reported	Milk			Serum			
			No. of samples	No. of samples positive		No. of samples	No. of samples positive		
				MRT	milk-ELISA		RBPT	STAT	I-ELISA
1	Abortion	145	58	10	58	145	53	55	66
2	Retention of placenta	213	213	18	41	213	35	35	40
3	Still birth	105	104	10	12	105	08	08	11
4	Repeat breeding	228	228	09	21	228	18	19	23
5	Metritis/Endometritis	158	158	08	13	158	12	12	15
	<b>Total</b>	<b>849</b>	<b>761</b>	<b>55</b> <b>(7.22)</b>	<b>145</b> <b>(19.05)</b>	<b>849</b>	<b>126</b> <b>(14.84)</b>	<b>129</b> <b>(15.19)</b>	<b>155</b> <b>(18.26)</b>

Figures in parentheses indicate percentages

From the results, it can be concluded that the cases of reproductive disorders have significant association with bovine brucellosis. Therefore, all reproductive disorders should be monitored using accurate diagnostic tests such as milk-ELISA or I-ELISA for correct and prompt diagnosis as to prevent further spread of infection to apparently healthy animals of the herd and surroundings.

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