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Conventional and molecular characterization of *Brucella abortus* and detection of antibodies against *Brucella abortus* in cattle and buffaloes

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Brucellosis, a chronic infectious disease of livestock, rodents, marine animals and human beings caused by *Brucella,* is economically important and of public health significance. The disease is manifested by abortion, birth of unthrifty calves, retained placenta and repeat breeding in females and epididymitis and orchitis in male animals. In humans it causes undulant fever and if untreated, leads to a chronic debilitating disease (Young 2000). The treatment of brucellosis is tough as the organism infects and replicates within host macrophages (Crasta *et al.* 2008). The main economic impact of infection in animals is because of the reproductive failure and losses incurred due to the death of fetuses (Corbel 1989). In USA the national brucellosis eradication program cost was $3.5 billion between 1934 and 1997 and the cost of reduced milk production and abortion in 1952 alone was $400 million (Acha *et al.* 2003, Sriranganathan *et al.* 2009). Annual loss to the tune of Rs 500 crore due to brucellosis was reported during 1993–94 in India (Rajasekhar 1995). Therefore *Brucella* is a potential threat to our economics and public and animal health. The present study was conducted to isolate *Brucella* from cattle and buffaloes with history of repeat breeding and abortion and their serological diagnosis using RBPT.

Samples (108) from 61 cattle and 47 buffaloes with the history of repeat breeding and abortion were collected from different farms in and around Ludhiana. From cattle the samples were foetal stomach contents (15), foetal membranes (11), uterine discharges (3) and vaginal mucous (32) whereas from buffaloes the samples were foetal stomach contents (14), foetal membranes (6), uterine discharge (1), and vaginal mucous (26). For the primary isolation of *Brucella*, *Brucella* selective medium (BSM) enriched with 5% v/v heat inactivated horse serum and *Brucella* selective supplement was used.

The isolates were initially recognised on the basis of their cultural and morphological features. They were also characterised biochemically as described by Buchanon and Gibbons (1974) and Carter (1995). Suspected colonies were subjected to agglutination by standard *Brucella abortus* antiserum (procured from IVRI Bareilly).

The suspected inoculates were subjected to PCR using *Brucella* genus specific F4/R2 primers for confirmation (Romero *et al.*1995). The PCR reactions were carried out in a gradient thermal cycler in a 25µl reaction mixture. Serum samples (91) were collected from cattle and buffaloes and screened for antibodies against *B. abortus* using rose bengal plate test (RBPT) as per the standard procedure of Quinn *et al*. (1994).

Brucella abortus was isolated from 9 out of 108 samples. *Brucella* isolates were identified on the basis of cultural characteristics, staining characteristics and biochemical parameters. The pinpoint, smooth, glistening and translucent colonies, appearing after 3–5 days of incubation in a microaerophilic environment, were indicative of *Brucella*. These colonies were found to be Gram negative, coccobacillary rods whereas by Modified Ziehl-Neelson MZN staining these appeared to be red coccobacilli with a blue background. All the suspected colonies manifested positive results for oxidase, catalase, urease and nitrate reduction. Out of 9 isolates 3 isolates did not produce H_2S on TSI slants and reaction of all the isolates was alkaline in the medium. All the 9 isolates were found negative for indole.

All the 9 isolates were agglutinated by standard *Brucella abortus* antiserum. On the basis of dye sensitivity test the *Brucella abortus* isolates were identified to belong to biotype 1 as all were found growing in the presence of basic fuchsin (20µg/ml) and not in the presence of thionine dye (20µg/ml) (Table 1).

Prevalence of Brucella *in animals:* From 108 samples 61 cattle and 47 buffaloes (foetal stomach contents, foetal membranes, uterine discharges and vaginal mucous), 9 (8.33%) samples, viz. 5 (8.19%) from cattle and 4 (8.51%) from buffaloes were found positive for *Brucella abortus* through standard isolation procedure (Table 2). Further, the samples from cattle, out of 15 samples of foetal stomach contents 3 (20%) and from 11 foetal membrane samples 2

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Table 1. Biochemical profile of *Brucella abortus* isolates

Isolate No.	Oxidase	Catalase	Urease	Nitrate Reduction	H_2S (TSI)	Indole	Agglutination with antiserum	Phage Lysis (plaques)	Growth in presence of dyes		
									Thionine 20µg/ml	Basic fuchsin 20µg/ml	Biotype
B5	+	+	+	+	+	-	+	+	-	+	1
B6	+	+	+	+	+	-	+	+	-	+	1
B20	+	+	+	+	-	-	+	+	-	+	1
B35	+	+	+	+	-	-	+	+	-	+	1
B50	+	+	+	+	-	-	+	+	-	+	1
B57	+	+	+	+	+	-	+	+	-	+	1
B71	+	+	+	+	+	-	+	+	-	+	1
B76	+	+	+	+	+	-	+	+	-	+	1
B90	+	+	+	+	+	-	+	+	-	+	1

Out of 91 serum samples 39 (32 cattle and 07 buffaloes) were found positive in RBPT testing. Five animals showed positive result in both isolation and RBPT testing. Similarly Kaur *et al.* (2006) obtained 17 isolates of *Brucella abortus* whereas only 10 animals were positive with RBPT.

Table 2. Prevalence of *Brucella abortus* in different samples from aborted cattle and buffaloes

Type of sample	Cattle		Buffalo	
	No. of animals	Positive	No of animals	Positive
Foetal stomach contents	15	3 (20%)	14	3 (21.43%)
Foetal membranes	11	2 (18.18%)	6	0 (0.00%)
Uterine dischrges	03	0 (0.00%)	1	1 (100%)
Vaginal mucous	32	0 (0.00%)	26	0 (0.00%)
Total	61	5 (8.19%)	47	4 (8.51%)

(18.18%) yielded *Brucella* whereas samples from vaginal mucous (32) and uterine discharge (3) did not yield *Brucella.* In buffaloes out of 14 samples of foetal stomach contents *B. abortus* was isolated from 3 (21.43%) samples. The only 1 uterine discharge sample collected was positive for *B. abortus*. *B. abortus* was isolated from none (0.00%) of the 26 vaginal mucous and 6 foetal membrane samples.

Serologically, 32 cattle (41.6%) and 7 buffaloes (50%) were positive in RBPT testing whereas Aulakh *et al.* (2008) observed that the seroprevalence of brucellosis was 20.67% in cattle and 16.41% in buffaloes in Punjab using milk-ELISA. Dhand *et al*. (2005) reported that the overall prevalence of brucellosis in buffaloes was 13.4% and in cattle 9.9%. Gumber *et al.* (2004) reported the prevalence of brucellosis in Punjab at village level was 22.5%. Gill *et al.* (2000) observed the seroprevalence of brucellosis in 11.8% cattle and 10.67% buffaloes in Punjab.

Prevalence of Brucella abortus *with relation to age:* In less than 4 years age group (Table 3), which included 9 cattle and 4 buffaloes, none was found positive for *Brucella* isolation. In the group of 4–6 years age, 4 (12.12%) out of 33 cattle were positive and 4 (15.4%) buffaloes out of 26, were positive. In the age group of more than 6 years, 1 (5.26%) out of 19 cattle was positive whereas none of the 17 buffaloes (0.00%) was positive for *B. abortus*. Thus in the study it could be observed that the prevalence of brucellosis was significantly higher in 4–6 years age group when compared with the other 2 groups in both cattle and buffaloes.

Similar observations were reported earlier too by Kaur *et al.* (2006) who obtained maximum number of *Brucella* isolates in the 4–6 year age group in cattle and buffaloes. Fosgate *et al.* (2002) observed that 5 years of age is the median age for *B. abortus* positive cattle and buffaloes. Also Abubakar *et al.* (2010) observed that the prevalence of *B. abortus* infection increased with the age of animals i.e. 3.27% and 9.52% in groups A (0–2 years) and B (>2 years), respectively, but Mehra *et al*. (2000) observed no difference

Table 3. Prevalence of *Brucella abortus* with reference to age

Age (year)	Cattle				Buffalo			
	Isolation		RBPT		Isolation		RBPT	
	No. of animals	Positive	No. of animals	Positive	No. of animals	Positive	No. of animals	Positive
<4	9	0 (0.00%)	22	09 (40.9%)	4	0 (0.00%)	03	01 (33.3%)
4–6	33	4 (12.12%)	36	17 (47.2%)	26	4 (15.4%)	09	06 (66.7%)
>6	19	1 (5.26%)	19	06 (31.6%)	17	0 (0.00%)	02	0 (0.00%)
Total	61	5 (8.19%)	77	32 (41.6%)	47	4 (8.51%)	14	07 (50.0%)

in prevalence of brucellosis with respect to age.

Confirmation of suspected isolates by PCR: All the *Brucella abortus* isolates were confirmed by using PCR. The DNA extracted from *B. abortus* strain-19 and the suspected *Brucella* field isolates when subjected to PCR using *Brucella* genus specific primer pair F4/R2 revealed the desired amplicon (at 905bp).

Brucella genus specific primer pair F4/R2 can be used for the confirmation of *B. abortus* isolates. Prevalence of brucellosis on the basis of isolation was significantly higher in 4–6 years age group in both cattle and buffaloes. Aborted foetal stomach contents and foetal membranes were the better source for the isolation of the organism as compared to uterine and vaginal discharges. Serologically, the seroprevalence of brucellosis in cattle and buffaloes with the history of repeat breeding and abortion was 41.6 and 50% respectively.

SUMMARY

Brucella abortus biotype-1 (9; 5 from cattle and 4 buffaloes) were isolated from 108 samples comprising aborted foetal stomach contents, foetal membranes and uterine discharges and vaginal mucous from cattle and buffaloes. Out of the 91 serum samples tested for the presence of antibodies against *B. abortus*, 39 were found positive by Rose Bengal plate test (RBPT). Only 5 animals were found positive both serologically and in isolation. In all cattle and buffaloes tested for isolation 5 (8.19%) cattle and 4 (8.51%) buffaloes were found positive for *B. abortus* whereas serologically 32 (41.6%) cattle and 7 (50%) buffaloes were positive. In cattle and buffaloes number of positive cases was the highest (12.12% and 15.4% on isolation and 47.2% and 66.7% serologically, respectively) in 4–6 year age group.

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