

Original Research Article

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Brucellosis and Associated Risk Factors Analysis in Serum Sample of Bovines

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ABSTRACT

A total of 567 bovine serum samples were taken from four districts of Brij region of U.P. All the samples were processed to detection of prevalence of brucellosis by RBPT, STAT and ELISA. Out of 567 bovine serum sample, the prevalence of brucellosis in 391 cattle were found to be 07.93% (31/391), 08.69% (34/391) and 10.74% (42/391) shows positive by RBPT, STAT and I-ELISA respectively and 176 buffaloes tested serum sample the seroprevalence were found to be 09.66% (17/176), 10.79% (19/176) and 12.5% (22/176) positive by RBPT, STAT and I-ELISA respectively. The seroprevalence of brucellosis to this region may have public health significance. It is recommended that good management and hygienic practices shall be performed during handling of cattle and buffaloes.

Keywords

Bovine Serum,
RBPT, STAT, I-
ELISA

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Introduction

Brucellosis is one of the world's major zoonotic problems. Brucellosis is caused by members of genus *Brucella*. These are small, non-motile, aerobic, facultative intracellular, Gram-negative coccobacilli. The ability of

Brucella to replicate and persist in host cells is directly associated with its capacity to cause persistent disease and to circumvent innate and adaptive immunity (Fichi, 2003). The species of *Brucella* and their major hosts are *Br. abortus* (cattle), *Br. melitensis* (goats), *Br. suis* (swine) and *Br. ovis* (sheep).

Br.abortus also causes infection in horses and is commonly found in chronic bursal enlargements as a secondary invader rather than a primary pathogen (Radostits *et al.*, 2000). Brucellosis is a zoonotic disease that exists worldwide and is more or less endemic in most African countries and still exists in some southern European countries (Refai, 2002). Brucellosis is transmitted from mother to offspring before or at the birth, through milk, by sexual contact, direct physical contact, from polluted environments and from eating spoiled raw meat mainly placenta and birth products. Brucellosis is clinically characterized by metritis, mastitis, repeat breeding, abortion in the last trimester of pregnancy, retention of placenta and reduced milk production in the female whereas epididymitis, orchitis and sterility in male (Sundar *et al.*, 2015). Human infected with *Brucella* may suffer from a debilitating nonspecific illness which involves in of various organs. Some agents identified recently in human brucellosis include *B. neotomae* which was previously thought not to be zoonotic, and the novel organism *B. inopinata*. brucellosis is considered to be an occupational disease that mainly affects slaughter house workers, butchers and veterinarians. Symptoms in human brucellosis can be highly variable, ranging from nonspecific, flu-like symptoms to undulant fever, arthritis, orchitis and epididymitis (Hassan *et al.*, 2012). *Brucella abortus*, is an important zoonotic disease which leading to several public health and economic problems in all over the world. The prevalence of brucellosis in India was first established early in the previous century and since then it has been reported from almost all the states (Sehgal and Bhatia, 1990; Renukaradhya *et al.*, 2002). It is a highly infectious zoonotic disease, with a great economic impact on cattle farming with the estimated loss of US\$ 58.8 million per year in India (Kollannur *et al.*, 2007).

Materials and Methods

Sources and places of sample collection

The present study was conducted in four districts viz., Mathura, Agra, Hathras and Kasganj from august, 2017 to march, 2019. These districts were selected because of the high numbers of smallholder dairy farmers, gaushalas and good animal husbandry practices. During the present study, about 10 ml of blood from each animal and total 567 samples (391 cattle and 176 buffalo sera sample) were collected in aseptic conditions using labeled sterile disposable syringes (Dispovan) or vaccutainers (BD, USA). Distribution of samples collected from different districts with their associated risk factors viz. species, area and health status. The serum was separated after clotting of blood and transported to laboratory on ice. All the serum samples were stored at -20°C till tested.

Prevalence based on different serological tests

RBPT & STAT antigen were procured from Indian Veterinary Research Institute, (IVRI), Izatnagar, India and I-ELISA, kit was procured from Svanova (Biotech-AB), Uppasala, Sweden Briefly, each of the 96 wells of flat bottom polystyrene antigen precoated with *Brucella abortus* antigen. RBPT antigen is an 8% suspension of pure smooth killed cells of *Brucella abortus* strain 99 phenolised and stained with rose bengal dye. Rosebengal plate test is a single dilution serum agglutination test. It was performed on glass slides according to the method presented by Alton *et al.*, (1998). To detection of STAT in a serum samples, *Brucella abortus* plain antigen were taken which was heat killed phenolised suspension of *Brucella abortus* strain 99 and it show 50% agglutination at 1/500 final dilution of serum with Indian standard.

Results and Discussion

Species wise seropositivity of bovine brucellosis

The overall prevalence of bovine brucellosis in serum by different serological test viz. RBPT, STAT and I-ELISA was 08.46% (48/567), 09.35% (53/567) and 11.28% (64/567) respectively. Out of 391 tested cattle serum sample, the seroprevalence of brucellosis was found to be 07.93% (31/391), 08.69% (34/391) and 10.74% (42/391) shows positive by RBPT, STAT and I-ELISA respectively. In buffaloes out of 176 tested serum sample the seroprevalence was found to be 09.66% (17/176), 10.79% (19/176) and 12.5% (22/176) positive by RBPT, STAT and I-ELISA respectively. Thus the seroprevalence of bovine brucellosis is significant higher in buffaloes as compare to cattles. The seroprevalence of brucellosis was nonsignificantly higher in buffaloes as compare to cattles ($p=0.541$). In present study the sero-prevalence of brucellosis in cattle and buffalo by I-ELISA was 10.74% and 12.5% respectively in serum samples. Our finding were very close to the reports of (Kebede *et al.*, 2008) (11%), Eshetu *et al.*, (2005) (10%) and 9.7% by Aggad and Boukraa, (2006). Higher prevalence rates were also reported in cattle in Nigeria (32.2%) (Junaidu *et al.*, 2008), (42.31%) Berhe *et al.*, (2007), (31.5%) Aggad and Boukraa, (2006) in Algeria and (25.8%) Ahmad *et al.*, (2009) in Jordan. Some lower prevalence rate was reported by Kassahun (2004) for intensive (2.5%) and extensive farms (1.7%) in Southern Ethiopia, Algeria Berhe *et al.*, (2007) (3.19%) in extensive production systems.

Area wise seropositivity of bovine brucellosis

The overall seroprevalence of bovine brucellosis in serum collected from four districts and tested by different serological

test like RBPT, STAT and I-ELISA in bovines was given in (table- 2). On the basis of RBPT district wise seroprevalence in cattle was higher in Hathras (10.00%) followed by Agra (08.42%), Kasganj (07.05) and Mathura (06.87%) and in buffalo the highest percentage in Mathura (12.19%) followed by Hathras (10.90%) Agra (10.00%) and Kasganj (05.00%). On the basis of STAT district wise seroprevalence in cattle was higher in Agra (09.47%) followed by Mathura (09.16%), Hathras (08.75%) and Kasganj (07.05%) and in buffalo the highest percentage in Agra (15.00%) followed by Kasganj (10.00%), Mathura (09.75%) and Hathras (09.09 %). On the basis of I-ELISA district wise seroprevalence in cattle was higher in Hathras (12.50%) followed by Agra (11.57%), Mathura (10.68%) and Kasganj (8.23%) and in buffalo the highest percentage in Mathura (19.51%) followed by Agra (12.50 %), Hathras (10.90%) and Kasganj (07.50%).

In all four districts the seroprevalence of bovine brucellosis was not significantly associated. Districts wise prevalence of brucellosis in animals revealed that it was widely prevalent in an all circumstances and showed great affinity to all environments. Previous reports Neha *et al.*, (2015) also somewhat similar to present study. Difference of brucellosis in different districts might be due to climatic conditions, rearing practices, intermixing of different species animal and sharing same pasture land, interaction of wild animal due to deforestation and livestock movement (Matope *et al.*, 2011; OIE, 2011; Manish *et al.*, 2013). In a present study the nonsignificant association between *Brucella* infection and risk factors such as abortion, metritis, retention of placenta, stillbirth and repeat breeding was reported. Some researchers report was similar to present findings Mugizi *et al.*, (2015) and Asmare *et al.*, (2013) found no significant association between seroprevalence of *Brucella* in respect of abortion and retention of placenta.

(Aulakh *et al.*, 2008) found significant association between brucellosis and abortion and retention of placenta, but not between brucellosis and repeat breeding.

Seropositivity of bovine brucellosis according to animal health

According to reproductive diseased animals

the seroprevalence of bovine brucellosis on the basis of I-ELISA test was higher in case of abortion in both cattle 21.62% and buffalo 22.23%. In cattle the lower seroprevalence was found in case of retention of placenta 06.25% while in case of buffalo no any finding in case of still birth and retention of placenta (table-3).

Table.1 Species wise seropositivity of bovine brucellosis (cattle and buffalo) detected by RBPT, STAT and I-ELISA

S. No.	Species	No. of serum sample	No. of samples positive by RBPT	Percent positivity by RBPT	No. of samples positive by STAT	Percent positivity by STAT	No. of samples positive by I-ELISA	Percent positivity by I-ELISA	p value
1	Cattle	391	31	07.93	34	08.69	42	10.74	0.541
2	Buffalo	176	17	09.66	19	10.79	22	12.5	
	Total	567	48	08.46	53	09.35	64	11.28	

p>0.05 at 5% level of significance

Table.2 Area wise seropositivity of bovine brucellosis (cattle and buffalo) in serum samples detected by RBPT, STAT and I-ELISA

S. No.	Name of District	Source	No. of serum sample	No. of samples positive by RBPT	Percent positivity by RBPT	No. of samples positive by STAT	Percent positivity by STAT	No. of samples positive by I-ELISA	Percent positivity by I-ELISA	p value
1	Mathura	Cattle	131	9	06.87	12	09.16	14	10.68	0.139
		Buffalo	41	5	12.19	4	09.75	8	19.51	
2	Agra	Cattle	95	8	08.42	9	09.47	11	11.57	0.877
		Buffalo	40	4	10.00	6	15.00	5	12.50	
3	Hathras	Cattle	80	8	10.00	7	08.75	10	12.50	0.778
		Buffalo	55	6	10.90	5	09.09	6	10.90	
4	Kasganj	Cattle	85	6	07.05	6	07.05	7	08.23	0.249
		Buffalo	40	2	05.00	4	10.00	3	07.50	
	Total		567	48	08.46%	53	09.35%	64	11.28%	

p>0.05 at 5% level of significance

Table.3 Seropositivity of bovine brucellosis according to animal health

S. No.	Disease condition	Source	No. of serum sample	No. of samples positive by RBPT	Percent positivity by RBPT	No. of samples positive by STAT	Percent positivity by STAT	No. of samples positive by I-ELISA	Percent positivity by I-ELISA	p value
1	Abortion	Cattle	37	6	16.21	7	18.91	8	21.62	0.967
		Buffalo	18	3	16.67	3	16.67	4	22.23	
2	Still birth	Cattle	12	1	08.34	1	08.34	1	08.34	0.394
		Buffalo	9	0	0	0	0	0	0	
3	Payometra	Cattle	24	2	08.34	1	04.16	2	08.34	0.698
		Buffalo	16	1	06.25	2	12.50	2	12.50	
4	Metritis	Cattle	15	1	06.67	2	13.34	1	06.67	0.832
		Buffalo	11	1	09.09	0	0	1	09.09	
5	Retention of placenta	Cattle	16	1	06.25	0	0	1	06.25	0.512
		Buffalo	7	0	0	0	0	0	0	
6	Repeat breeding	Cattle	76	8	10.52	10	13.15	12	15.78	0.630
		Buffalo	34	4	11.76	5	14.70	4	11.76	
7	Apparently healthy (Anoestrus)	Cattle	211	12	05.68	13	06.16	17	08.05	0.197
		Buffalo	81	8	09.87	9	11.12	11	13.58	
Total			567	48	08.46 (48/567)	53	09.34 (53/567)	64	11.28 (64/567)	

In a present study the nonsignificant association between *Brucella* infection and risk factors such as abortion, metritis, retention of placenta, stillbirth and repeat breeding was reported. Some researchers report was similar to present findings Mugizi *et al.*, (2015) and Asmare *et al.*, (2013) found no significant association between seroprevalence of *Brucella* in respect of abortion and retention of placenta. Aulakh *et al.*, 2008; Adesokan *et al.*, 2013 found significant association between brucellosis and abortion and retention of placenta, but not between brucellosis and repeat breeding.

In conclusion, the higher prevalence of the disease in this region increases the risk of zoonotic transmission and it implies a serious threat to the human population as well as the huge impact on economy by losses in productivity of the livestock. Control of brucellosis in bovines through approved projection such as vaccinations either with S19 or RB51 reduces the likelihood of a

transmission process from animal-animal. This strategy reduces the incidence of brucellosis, thus decreasing the spread into human populations. The higher prevalence of the disease over the population such as increases the risk of zoonotic transmission and it implies a serious threat to the human population as well as the huge impact on economy due to loss of productivity as well as loss of livestock population.

Conflict of interests

The authors declare that they have no conflict of interests with any financial organisation regarding the material discussed in the manuscript.

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