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Prevalence and risk factors analysis of brucellosis in milk sample of bovines in Brij region of Uttar-Pradesh

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Abstract
A total of 700 bovine milk samples was taken from four districts of brij region of UP. All the samples were processed to detection of prevalence of brucellosis by MRT and ELISA and analysis risk factors associted with brucellosis. Out of 700 bovine milk sample, the prevalence of brucellosis in milk sample of cattle was found to be 05.78% (26/450) and 08.45% (38/450) positive by MRT and I- ELISA respectively. On the other hand in buffaloes milk the prevalence of brucellosis in 250 milk sample was found to be 07.20% (18/250) and 09.02% (23/250) positive by MRT and I- ELISA respectively. The seroprevalence of brucellosis to this region may have public health significane. It is recommended that good management and hygienic practices shall be performed during handling ruminants specially cattle and buffaloes.

Keywords: Milk, MRT, I- ELISA, risk factors

Introduction
Brucellosis is a world's major zoonotic disease that exists worldwide and is more or less endemic in most African countries and still exists in some southern European countries. Almost all domestic species can be affected with brucellosis except cats which are resistant to *Brucella* infection. Brucellosis is transmitted from milk, by sexual contact, direct physical contact, from polluted environments, meat, contact to placenta and birth products. 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). 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 (Radostits *et al.*, 2000) [18]. In humans, brucellosis is considered to be an occupational disease that mainly affects slaughter house workers, butchers and veterinarians. So this considered as an important zoonotic disease leading to several public health and economic problems in endemic areas.

Material and Methods
Data collection
The study was carried out to determine the individual animal and herd level seroprevalence of bovine brucellosis and their association with different risk factors. The data was collected using a structured questionnaire proforma. The questionnaire sought information about species, sex, age, animal rearing practice, geographical location (district), clinical signs and exposure of disease of the animal etc.

Sources and places of samples/materials
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 15 ml of milk sample as each animal and total 700 samples (450 cattle and 250 buffalo milk 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. milk colletion, general characteristic of farms management of farm, owner unconformity and exposure of disease. The milk sample transported to laboratory on ice. All the milk samples were stored at -20 ^{0}C till tested.

Prevalence based on different serological tests

MRT antigen were procured from Indian Veterinary Research Institute, (IVRI), Izatnagar, India. Milk ELISA, kit was procured from Svanova (Biotech-AB), Uppasala, Sweden. The MRT is an agglutination test conducted on fresh milk collected from dairy cattle, but it does not work on pasteurized or homogenized milk (Fleischhauer, 1937) [8]. The MRT, which detects IgM and IgA antibodies bound to fat globules, may have wide acceptability as it is cost effective, easy to perform and can cover a large population in a short time (Cadmus *et al*., 2008) [2]. antibody was detected by Indirect ELISA (I-ELISA) kit procured from Svanova (Biotech-AB), Uppasala, Sweden. Briefly, each of the 96 wells of flat bottom polystyrene antigen precoated with *Brucella abortus* antigen.

Results and Discussion

Species wise Prevalence of bovine brucellosis

Table 1: Species wise Prevalence of bovine brucellosis (cattle and buffaloes) in milk by MRT and I-ELISA

S. No.	Species	Number of animals tested	No. of samples positive by MRT	Percent positivity by MRT	Number of samples positive by I-ELISA	Percent positivity by I-ELISA	p value
1	Cattle	450	26	05.78	38	08.45	
2	Buffalo	250	18	07.20	23	09.02	0.7341
	Total	**700**	44	06.28	61	08.71	

p>0.05 at 5% level of significcnce

Species wise prevalence of brucellosis in 450 cattle milk sample was found to be 05.78% (26/450) and 08.45% (38/450) positive by MRT and I- ELISA respectively. On the other hand in buffaloes milk the prevalence of brucellosis in 250 milk sample was found to be 07.20% (18/250) and 09.02% (23/250) positive by MRT and I- ELISA respectively. Thus the prevalence of bovine brucellosis is significantly higher in buffaloes as compare to cattle.

Risk factors analysis

This study was carried out to determine the prevalence of brucellosis and asses the potential risk facorts, which showed either significantly associated with occurrence of bovine brucellosis or the information of distribution of those risk factors had statistically non-significant association with occurrence of brucellosis which were discussed in respective table.

A. Risk factors Analysis on the basis of milk collection

Table 2: Prevalence brucellosis according to milk collection

S. No.	Source	Types of milk sample	No. of sample	No. of samples positive by MRT	Percent positivity by MRT	No. of samples positive by I-ELISA	Percent positivity by I-ELISA	p value
1.	Cattle	a. Individal	315	18	05.71	21	06.67	
		b. Pooled	135	8	05.92	17	12.59	0.038*
2.	Buffalo	a. Individual	155	11	07.09	10	06.45	
		b. Pooled	95	7	07..36	13	13.68	0.054
	Total		700	44	06.28 (44/700)	61	08.71 (61/700)	

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

In the present finding the prevalence of brucellosis in milk sample of cattle and buffalo was not significantly associated and it was some what similar in individual milk sample (table-2) whereas in pooled milk sample the prevalence of brucellosis non significantly compratively higher in buffalo milk than cattle. In cattle milk sample the prevalence of brucellosis was significantly associated between pooled and individual milk (p=0.038*) but in buffalo the prevalence was not significant associated between pooled milk and individual milk sample.

B. On the basis of general characteristics of farm

Table 3: Prevalence of brucellosis according to general characteristics of farm

S. No.	Particulars	Number of milk samples	No. of samples positive by MRT	Percent positivity by MRT	No. of samples positive by I-ELISA	Percent positivity by I-ELISA	p value
1.	**Types of species**						
	a. Cattle	450	26	05.77	38	08.44	
	b. Buffalo	250	18	07.20	23	09.20	0.734
2.	Herd size						
	a. Small- <10 animals	325	18	05.53	24	07.38	
	b. Medium- >10-20 animals	230	15	06.52	21	09.13	
	c. Large->20 animals	145	11	07.58	16	11.03	0.452

p>0.05 at 5% level of significance

In the present study two risk factors viz. species and herd size were taken with respect to general characteristics of farms and their association with prevalence of bovine brucellosis which were given in (table-3). In the respect of milk ELISA, prevalence of bovine brucellosis in buffalo milk (09.20%) higher than cattle milk (08.44%), the prevalence of brucellosis in milk sample of cattle and buffalo not significantly associated (p=0.734) in previous the prevalence of milk brucellosis in cattle and buffalo was 3% and 8.5% respectively. The highest prevalence (11.03%) was observed in a group of herds with large herd size above 20 animals whereas it was the lowest (7.38%) for a group of herds with less than 20 animals and the prevalence in medium herd size betveen 10-20 animals the prevalence was 9.13%. it indicates that increase in prevalence with increased herd size was observed but it is not significant association (p=0.452). Such finding is in accordance with the earlier report of Tun *et al*. (2007) [25] who reported prevalence was higher when the herd size is greater than 50 animals as it was 28.6% for herd size above 50 and only 3.1% for herd size less than 50 animals. Likewise, non-significant association between herd size and prevalence of brucellosis was also reported by Kebede *et al*. (2008) [11], Tolosa *et al*. (2010) [24], Chand and Chhabra (2013) [5]. On contrary, some researcher had reported significant association of herd size with prevalence of brucellosis (McDermott and Arimi 2002; Al-Majali *et al*., 2009; Mugizi, 2009; Haileselassie *et al*., 2010; Ibrahim *et al*., 2010; Calistri *et al*., 2013; Lindahl *et al*., 2014) [15, 9, 10, 3, 12]

C. On the basis of management system of farm

Table 4: Prevalence of brucellosis according to management system of farm

S. No.	Particulars	Number of milk samples	No. of samples positive by MRT	Percent positivity by MRT	No. of samples positive by I-ELISA	Percent positivity by I-ELISA	p value
1.	**Types of housing system**						
	a. Open	425	24	05.64	32	07.52	0.167
	b. Close	275	20	07.27	29	10.54	
2.	**Types of floor**						
	a. Concreted	230	13	05.65	16	06.95	0.480
	b. Kaccha	175	14	08.00	19	10.85	
	c. Other	295	17	05.76	26	08.81	
3.	**Level of hygiene at farm**						
	a. Good	240	08	03.34	09	03.75	0.001*
	b. Fair	190	11	05.78	18	09.47	
	c. Poor	270	25	09.25	34	12.59	
4.	**Grazing practices**						
	a. Yes	160	14	08.75	17	10.62	0.018*
	b. No	540	30	05.56	44	08.14	
5.	**Disinfection practices**						
	a. Yes	270	12	04.45	15	05.56	0.003*
	b. No	430	32	07.44	46	10.69	

*p<0.05 at 5% level of significance

In this group five different risk factors were studied. In management system the role of housing and types of floor was not significantly associated with prevalence rate. On the other hand level of hygiene, grazing practices and disinfection practices were significantly associated with prevalence rate of brucellosis. Some studies also accordance to grazing practices which showed similarity against significantly association with prevalence of brucellosis. Some studies have found that communal grazing might be a risk factor for the transmission of the disease (Salman and Meyer 1984). The finding in the current study might be attributable to the fact that most farmers keep their cows within the farms instead of on the pasture 1 month prior to and during calving and, hence, might decrease the risk of contaminating the pasture with Brucella bacteria shed in birth fluids and placentas. Though keeping good hygiene at dairy farm (Mugizi, 2009) [15] and zero grazing (Swai *et al*., 2003; Sikder *et al*., 1985) [22] was considered as a protective factor for brucellosis, unhygienic practices were identified as factors that will facilitate the spread of infections (Adesokan *et al*., 2013) [1].

D. On the basis of unconformity of owners

Table 5: Prevalence of brucellosis according to unconformity of owners

S. No.	Particulars	Number of milk samples	No. of samples positive by MRT	Percent positivity by MRT	No. of samples positive by I-ELISA	Percent positivity by I-ELISA	p value
1.	**Breeding methods**						
	a. AI	330	16	04.84	18	05.45	0.004*
	b. Natural services	240	19	07.91	32	13.34	
	c. Mixed	130	9	06.92	11	08.46	
2.	**Milking methods**						
	a. Hand milking	648	42	06.48	58	08.95	0.932
	b. Machine milking	5	0	00.00	00.00	00.00	
	c. Mixed milking	47	2	04.25	3	06.38	
3.	**Owners education**						
	a. Upto 8th class	465	30	06.45	43	09.24	0.536
	b. Above 8th class	160	11	06.87	14	08.75	

	c. Professionals	75	3	04.00	4	05.34	

*p<0.05 at 5% level of significance

In this category three different risk factors were studied. The prevalence of bovine brucellosis was significantly associated with breeding methods (p=0.004*) the prevalence of bovine brucellosis was comparatively higher in natural service (Singh *et al*., 2013) than artificial insemination this may be due to the semen used in AI is fully brucella tested animals.the present study show similarity with other studies (Chatterjee *et al*., 1985; Dias *et al*., 2009; Oliveira *et al*., 2013) [7]. The prevalence of brucellosis was not significantly associated with milking methods and owners education.The results observed in present study are in accordance to the findings of Tun *et al*. (2007) [25], who reported statistically non-significant effects of the risk factor variables (breeding, milking methods). The awareness of brucellosis among dairymen was also reported as significant risk factor (Tun *et al*., 2007; Chatterjee *et al*., 1985; Sikder *et al*., 1985) [25].

E. On the basis of exposure of disease

Table 6: Prevalence of brucellosis according to exposure of disease

S. No.	Disease condition	Number of sample	No. of samples positive by MRT	Percent positivity by MRT	No. of samples positive by I-ELISA	Percent positivity by I-ELISA	p value
1	**History of Abortion**						
	Yes	42	10	23.80	12	28.57	0.000003*
	No	658	34	05.16	49	07.44	
2	**Metritis**						
	Yes	75	3	04.00	4	05.33	0.0221*
	No	625	41	06.56	57	09.12	
3	**Pyometra**						
	Yes	86	2	02.32	3	03.48	0.071
	No	614	42	06.84	58	09.44	
4	**Repeat breeding**						
	Yes	185	8	04.32	14	07.56	0.519
	No	515	36	06.99	47	09.12	
5	**Retention of placenta**						
	Yes	45	3	06.67	5	11.12	0.555
	No	655	41	06.25	56	08.54	
6	**Stillbirth**						
	Yes	32	1	03.12	1	03.12	0.251
	No	668	43	06.63	60	08.98	

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

A total six different risk factors were covered under this group. The distribution of six different risk factors on exposure of disease and their association with prevalence of bovine brucellosis is presented in (table-6). Exposure of disease risk factor were analyze due to its severity to spread infection from one animal to other, if increased prevalence rate of brucellosis were avoid or neglect it may cause huge economic impact in case of both animal and human. In many farms there was no any diseased isolation room for diseased animals. In the present study the risk factors such as pyometra (p=0.071), repeat breeding (p=0.519), retention of placenta (p=0.555) and still birth (p=0.251) was not significantly associated with prevalence of brucellosis, on the other hand history of abortion (p=0.000003*) and metritis (p=0.0221*) had statistically significant effects on prevalence of brucellosis. The results are in accordance to the findings of scientists who had reported significant association with reproductive disorders like abortion, retention of placenta and repeat breeding (Sikder *et al*., 1985, Rahman *et al*., 2012) [19]. Some scientists also found higher prevalence of brucellosis with reproductive disorders but their association with prevalence was non-significant (Panchasara, 2007; Tedele *et al*., 2010; Matope *et al*., 2012) [23].

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.

Competing interests

The authors declare that they have no competing interests.

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