

## SEROPREVALENCE OF BRUCELLOSIS IN BUFFALOES OF TELANGANA STATE, INDIA

A. Vijaya Kumar<sup>1,\*</sup>, N. Krishnaiah<sup>2</sup>, L. Venkateswara Rao<sup>3</sup>, Y. Narasimha Reddy<sup>4</sup>,  
K. Kondal Reddy<sup>5</sup> and B.V.L. Narayana<sup>6</sup>

**ABSTRACT**

The present study was undertaken to know the sero prevalence of brucellosis in buffaloes in Telangana State by employing Rose Bengal plate test (RBPT), Lateral flow assay (LFA), Standard Tube agglutination test (STAT) and Enzyme linked immunosorbent assay (ELISA). Out of 920 buffaloes screened, 36 males and 884 females, 32 from Government farms and 888 from private farms and 32 from 1 to 3 years old, 844 from 3 to 6 years old and 44 from above 6 years old. The sero prevalence was 5.65%, 5.76%, 5.43% and 5.98% by RBPT, LFA, STAT and ELISA respectively. The prevalence in males was 5.56% by RBPT, LFA and ELISA, whereas 2.78% by STAT and in females, 5.66%, 5.77%, 5.54% and 6.00% by RBPT, LFA, STAT and ELISA respectively. The prevalence in Government farms was 3.13% by RBPT, LFA and ELISA whereas zero by STAT and from private farms 5.74%, 5.86%, 5.63 % and 6.08% were positive by RBPT, LFA, STAT and

ELISA respectively. The prevalence in 1 to 3 years age was, 3.13% by RBPT, LFA and ELISA, zero by STAT, from 3 to 6 years age, 5.92%, 6.04%, 5.81% and 6.16% by RBPT, LFA STAT and ELISA respectively and from above 6 years age 2.27% by RBPT, LFA and STAT, whereas 4.55% by ELISA. Higher efficacy observed by ELISA followed by LFA, RBPT and STAT. The higher prevalence was observed in females than males, in private than Government farms and in 3 to 6 years age group than other age groups.

**Keywords:** *Bubalus bubalis*, buffaloes, seroprevalence, brucellosis, age wise

**INTRODUCTION**

Bovine brucellosis is found worldwide. Eventhough it has been eradicated from many countries, it is one of the most serious diseases in developing countries. The rates of infection vary

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<sup>1</sup>Department of Veterinary Parasitology and Entomology, College of Veterinary Science, Karimnagar, India,

\*E-mail: vphanumolu@gmail.com

<sup>2</sup>Department of Veterinary Parasitology and Entomology, College of Veterinary Science, Hyderabad, India

<sup>3</sup>Department of Veterinary Parasitology and Entomology, College of Veterinary Science, Proddatur, India

<sup>4</sup>Department of Veterinary Microbiology, College of Veterinary Science, Hyderabad, India

<sup>5</sup>Department of Livestock Products Technology, College of Veterinary Science, Hyderabad, India

<sup>6</sup>Sero Epidemiology Section Disease Investigation Division, Veterinary Biological Research Institute, Hyderabad, India

greatly from one country to another and between regions within a country. The disease is widely prevalent throughout India among the bovine population both in farm and in the village animals causing economic losses to the tune of Rs. 350 million. Economic losses by brucellosis in animals are due to abortions, premature births, decreased milk production and due to repeat breeding and may lead to temporary or permanent infertility in infected livestock. Free grazing and movement with frequent mixing of flocks of sheep and goats also contribute to the wide distribution of brucellosis in these animals, resulting outbreak of brucellosis. Despite the advances made in the diagnosis and therapy, brucellosis is still wide spread and its prevalence in many developing countries is increasing.

The approach to control, prevention, or eradication of brucellosis in a country or region will depend on many factors, such as the level of infection, reliability of diagnostic test, surveillance and monitoring programmes and effective vaccination programmes (FAO, 2003).

Little/scarcely information is available on the prevalence of brucellosis in Telangana State. Hence various types of tests serological methods used and results were compared in the present study for their efficacy.

## MATERIALS AND METHODS

### Collection of samples

A total of 920 buffaloes serum samples were collected from all the 10 districts of Telangana State using adequate equipment, packed in a cooler bag with ice packs and transported from the place of collection to the laboratory (OIE Manual, 2000). 9 ml of blood was collected from the jugular vein

of individual animal in a vacuette with serum clot activator (BD), kept in upright position at room temperature for about 2 h and separated serum was collected in a screw capped plastic vials. In the laboratory the serum samples were heat inactivated at 56°C for 30 minutes and merthiolate (1:10,000) was added as preservative and stored at -20°C till further use.

### Serological tests

Four serological tests i.e., Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT), Lateral flow assay (Immuno-chromatographic assay) and Indirect Enzyme Linked Immunosorbent assay (ELISA) were conducted. RBPT performed using Rose Bengal Plate Test antigen obtained from the Indian Veterinary Research Institute (I.V.R.I.), Izatnagar, Uttara Pradesh. The result was read immediately after four minutes. Definite clumping/agglutination was considered as positive reaction, whereas no clumping/agglutination was considered as negative. The Standard Tube Agglutination Test carried out using the antigen obtained from the I.V.R.I. The titre obtained was expressed in unit system by doubling of the serum titre as International Unit (I.U.) per ml of serum. 40 I.U. per ml or above was considered positive for brucellosis in cattle as well as buffaloes. Lateral flow assay (Immuno-chromatographic assay) was performed using the kits and procedure supplied by Genomix. Positive results can be read as soon as it appears. Negative results may be confirmed in 20 minutes. ELISA (*Brucella abortus* Antibody Test Kit-Brucellosis Serum) was conducted as per the protocol outlined in the user manual of IDEXX CHEKIT., USA.

### Statistical analysis

The statistical analysis (chi-square test)

was done by using SPSS software.

## RESULTS

Sero prevalence of brucellosis in buffaloes assessed by RBPT, LFA, STAT and Indirect ELISA was presented in Table 1. A total of 920 serum samples were screened and an overall prevalence of 5.65% (52), 5.76% (53), 5.43% (50) and 5.98% (55) was observed in the buffaloes by RBPT, LFA, STAT and ELISA respectively. No significant difference was observed in prevalence of brucellosis by different serological tests. Out of 920 buffaloes screened, 36 were males and 884 females. Out of 36 males 2 (5.56%) were positive by RBPT, LFA and ELISA, whereas 1 (2.78%) was positive by STAT. Out of 884 females 50 (5.66%), 51 (5.77%), 49 (5.54%) and 53 (6.00%) were positive by RBPT, LFA, STAT and ELISA respectively. No significant effect was found on prevalence of brucellosis in male and female animals by different serological tests and between male and female animals. Out of 32 samples of Government farms, 1 (3.13%) was positive by RBPT, LFA and ELISA tests whereas none was positive by STAT and out 888 samples of private farms 51(5.74%), 52 (5.86%), 50 (5.63 %) and 54 (6.08%) were positive by RBPT, LFA, STAT and ELISA respectively. The prevalence was not significant different between two types of farms. 920 samples screened, belongs various age-wise groups as 32, 844 and 44 samples from 1 to 3 years, 3 to 6 years and more than 6 years of age respectively. Out of 32 samples of 1 to 3 years age, 1 (3.13%) was by RBPT, LFA and ELISA, whereas no animal was positive by STAT.

Out of 844 samples from 3 to 6 years age 50 (5.92%), 51 (6.04%), 49 (5.81%) and 52 (6.16%) were positive by RBPT, LFA STAT and

ELISA respectively and out of 44 samples from above 6 years age 1 (2.27%) was by RBPT, LFA and STAT, whereas 2 (4.55%) were positive by ELISA. The influence of age on sero-prevalence of brucellosis was not significant among different age groups by four tests employed.

## DISCUSSION

Higher prevalence of brucellosis in buffaloes was noticed by ELISA (5.98%), followed by LFA (5.76%), RBPT (5.65%) and STAT (5.43%). The prevalence by ELISA in the present study (5.98%) was almost similar to the findings of 6.3% (Renukardhya *et al.*, 2002) and 6.92% (Iftikar Hussain *et al.*, 2008). High prevalence than the present study by ELISA i.e., 8.25% (Kanani, 2007), 13.4% (Dhand *et al.*, 2005), 14.5% (Nahed *et al.*, 2014), 15.12% (Islam *et al.*, 2013) and 16.4% (Jagapur *et al.*, 2008) and very high prevalence of 26.63% (Patel, 2007), 39.6% (Ibrahim *et al.*, 2012) and 45.56% (Ramesh *et al.*, 2013) by ELISA were reported. Low prevalence of 2.87% (Rahman *et al.*, 2011), 3% (Rajasekhar *et al.*, 2004), 3.33% (Ajmal *et al.*, 1989) and 4.9% (Sharma *et al.*, 1979) by ELISA than the present study was reported.

The prevalence by RBPT in the present study (5.65%) was almost similar to the prevalence (5.67%) reported by of Kanani (2007) and higher than 1.1% (Nawal and Ahmed 2008), 1.9% (Rahman *et al.*, 2011) 2.4% (Lodhi *et al.*, 1995) and 3.5 % (Samaha *et al.*, 2008). Higher prevalence than the present study i.e., 8.15% (Patel, 2007) 9.58% (Iftikarhussain *et al.*, 2008) and 19.4% (Abdel Hamid *et al.*, 2008), and very high prevalence of 43% (Raheela Akhtar *et al.*, 2010), 44% (Chauhan *et al.*, 2000), 46.6% (Jain *et al.*, 2013), 66.6% (Ibrahim et al 2012) and 88.9% (Kangethi *et al.*,

2000) was reported. The prevalence by STAT in the present study was 5.43%, which was higher than the prevalence of 1.8% (Isloor *et al.*, 1998) and 1.9% (Rahman *et al.*, 2011), whereas higher prevalence of 7.22% (Kanani, 2007), 12.7% (Nahed *et al.*, 2014), 15.76% (Patel, 2007) 33.3% (Jain *et al.*, 2013) and 88.9% (Kangethe *et al.*, 2000) were reported.

The prevalence of brucellosis in buffaloes in the present study (6.00%) by ELISA was similar to 6.32% to 6.8% (Iftikar Hussain *et al.*, 2008) and higher than 2.02% reported by Rahman *et al.* (2011). Higher prevalence of 14.6% (Pandeya *et al.*, 2013) and 22.29% (Islam *et al.*, 2013) was reported. Higher prevalence of 8.69% to 9.2% by RBPT was reported by Iftikar Hussain *et al.* (2008) than 5.69% in the present study, whereas a lower prevalence of 3.3% was reported by Rahman *et al.* (2011).

The prevalence of 5.56% in the buffalo bulls by ELISA in the present study was less than 7.33% to 10% (Iftikar Hussain *et al.*, 2008), 7.14% (Rahman *et al.*, 2011) and 10.6% (Pandeya *et al.*, 2013) whereas higher than 1.81% (Islam *et al.*, 2013).

Generally male and female animals are equally susceptible to brucellosis, a higher seroprevalence of brucellosis in female animals in the present study was supported by various studies (Muma *et al.*, 2007; Tolosa *et al.*, 2008; Bayemi *et al.*, 2009; Islam *et al.*, 2013). The differences observed may be due to the fact that only 36 males were tested in the study, as most of the farmers opt for artificial breeding method. Another aspect is that female animals are kept for longer in a particular herd and are stocked together compared to male animals (individually housed), thereby increasing chances of exposure in females (Mekonnen *et al.*, 2010). Erythritol, a polyhydric

acid found in higher concentration in the placenta and foetal fluids of females than in seminal vesicles and testis of males can be responsible for females being more susceptible than males (Radostits *et al.*, 2007). Females are mostly sent for grazing in free range pastures due to absence of handling problems compared to males, frequent mixing with unknown herds and flocks of Sheep and goat. (Renukaradhya *et al.*, 2002)

The prevalence was high in private farms than Government farms by all the four serological tests performed. Similar higher prevalence in private farms by RBPT (35.40%) and STAT (23.70%), and compared to RBPT (15.38%) and STAT (2.91%) in Government farms (Nasir *et al.*, 2004). Using RBPT, higher prevalence of 10.42% in Government farms (Zahid *et al.*, 2002) and 18.20% in private farms (Sarkar *et al.*, 1987) than the present was reported.

Iftikar Hussain *et al.* (2008) reported prevalence of 6.8% and 7% in private farms and slaughter houses respectively by ELISA, which was slightly higher than the prevalence of private farms (6.08%) in the present study, whereas by RBPT they reported higher prevalence of 9.2% and 9.5% in private farms and slaughter houses respectively than the present study prevalence of 5.74% by RBPT.

The wide distribution and high prevalence of brucellosis in animals at private farms might be due to frequent introduction of new high yielding animals into the farms without proper serological tests and high incidence of abortions (Nasir *et al.*, 2004). Various factors like management, housing, population density, size of farm, type of herd (self raised or purchased from different sources), sanitary condition and method of disposal of infected animals will affect the prevalence of the disease (Radostits *et al.*, 2007).

Table 1. Sero-prevalence of brucellosis in buffaloes sex-wise, age-wise and type of farm.

Category		No. of samples tested	No. of samples positive				X <sup>2</sup> value (df)
			RBPT	LFA	STAT	ELISA	
Sex	Male	36	2 (5.56)	2 (5.56)	1 (2.78)	2 (5.56)	8 (6)
	Female	884	50 (5.66)	51 (5.77)	49 (5.54)	53 (6.00)	12 (9)
X <sup>2</sup> value (df)			2 (1)	2 (1)	2 (1)	2 (1)	
Type of farm	Govt	32	1 (3.13)	1 (3.13)	0 (0)	1 (3.13)	4 (3)
	Private	888	51 (5.74)	52 (5.86)	50 (5.63)	54 (6.08)	12 (9)
X <sup>2</sup> value (df)			2 (1)	2 (1)	2 (1)	2 (1)	
Age	1-3 years	32	1 (3.13)	1 (3.13)	0 (0)	1 (3.13)	4 (3)
	3-6 years	844	50 (5.92)	51 (6.04)	49 (5.81)	52 (6.16)	12 (9)
	>6 years	44	1 (2.27)	1 (2.27)	1 (2.27)	2 (4.55)	4 (3)
X <sup>2</sup> value (df)			6 (4)	6 (4)	6 (4)	6 (4)	
Total		920	52 (5.65)	53 (5.76)	50 (5.43)	55 (5.98)	12 (9)

Percentage in parenthesis, df: Degrees of freedom, Non significant at 99% level (P<0.05).

The prevalence of brucellosis in the animals of age group 1 to 3 years was less and it increased during the age group of 3 to 6 years and then decreased afterwards by all the four serological methods used. Zero prevalence was reported in the animals of 1 to 2 years by Rahman *et al.* (2011), which has been increased afterwards i.e., 2.63% (2 to 4 years) and 3.29 to 4.92% (above 4 years age). Higher prevalence (8.64%) in the age group of less than 3 years, 26.85% in the age group of 3 to 7 years and 39.92% in the age group of above 7 years than the present study using ELISA was reported by Islam *et al.* (2013). Rajesh *et al.* (2003) reported higher prevalence in 3 to 5 years cattle. Usually young animals are resistant (Nicoletti, 1980) due to presence of maternal antibodies, which decreases as age advances and thus the susceptibility will be more in mature animals. Though a congenital infection may occur in calves born to infected dams, they remain serologically negative until their first parturition (Crawford *et al.*, 1986). This explains the reason for lower prevalence in younger animals. As the age of animal increases, animals are more likely to be exposed to the bacteria and contract the disease (Dhand *et al.*, 2005). Although susceptibility to brucellosis increases with age, it seems to be commonly associated with sexual maturity than age (Radostits *et al.*, 2000). However, Kazi *et al.* (2005) reported that high prevalence of brucellosis among old animals might be related to maturity with advancing age, thereby the organism may have propagated to remain as latent infection or it may cause disease. Some older cows may not exhibit detectable antibody titres possibly due to latency, which is common in chronic brucellosis (Matope *et al.*, 2011).

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