Indian Journal of Animal Sciences **82** (5): 448–450, May 2012

Seroprevalence of bovine brucellosis in Haryana by avidin-biotin serum ELISA and its comparison with RBPT and SAT

S K KHURANA[1], S K SRIVASTAVA[2] and K PRABHUDAS[3]

National Research Centre on Equines, Hisar, Haryana 125 001 India

Received: 30 June 2011; Accepted: 2 November 2011

ABSRACT

Brucellosis is a zoonotic disease of immense economic importance affecting many species of animals. Serosurveillance of animals from 45 randomly selected villages of 11 districts of Southern Haryana was carried out. Representative serum samples were collected randomly from 5% of the total population of cattle and buffaloes. Overall prevalence of brucellosis was 12.13% (142 cases) out of 1170 serum samples screened by avidin-biotin serum ELISA. It was 14.44% (26 cases) and 11.71% (116 cases) out of 180 cattle and 990 buffalo serum samples, respectively. However, 101(8.6%) and 66 (5.64%) serum samples were found positive by STAT and RBPT, respectively. Prevalence of *Brucella* antibodies was higher in female animals (16.02% in cattle and 12.32% in buffaloes) than in males (4.14% in cattle and 4.10% in buffaloes). Similarly, sexually mature animals (>3 years of age) were found to be affected more (16.94% in cattle and 13.68% in buffaloes) than the immature ones (<3 years of age (9.67% in cattle and 6.56% in buffaloes), Jind district with prevalence rate of 22.72% and 25.74% and Faridabad with prevalence rate of 13.88% and 14.62% were affected more against overall prevalence rate of 14.44% and 11.71% in cattle and buffaloes respectively, in Southern Haryana.

Key words: Brucellosis, Haryana, Seroprevalence

Brucellosis, an anthropozoonotic disease affecting multiple animal species, is one of the most neglected endemic zoonotic diseases and often persists in the poorest and most vulnerable populations (FAO 2009). The disease mainly occurs in Mediterranean basin, Middle east, Western and central Asia, Latin America, Africa and India (Maurin 2008). This disease is mainly caused by *Brucella abortus* in bovines. It is the major cause of abortions in countries where national level eradication programmes are not being enforced (Radostits *et al*. 2000). Some countries have eliminated or substantially reduced the disease yet, it remains endemic in many areas of the world (Whatmore *et al*. 2007). The control of brucellosis in animal reservoirs has a corresponding and significant decline in human brucellosis (Mohamed *et al*. 2010). The disease has been reported in India since 1939. The disease is prevalent in organized farms as well as in unorganized areas. Serological evidence of brucellosis has been reported from almost all states of India (Sharma *et al*. 1979, Chatterji *et al*. 1985, Sandhu *et al*. 2001, Rathore *et al*. 2002, Chand and Sharma 2004, Charanjeet *et al*. 2004), however, these reports can not be utilized as epidemiological database for making prevention, control and eradication strategies as the sampling was not statistically representative of animal populations in the particular areas/states and also these reports were not very comprehensive. Therefore to generate a reliable and authentic database for implementing prevention, control and eradication programs for brucellosis a systematic and detailed sero-epidemiological study was undertaken under NATP mission mode project on Animal Health information System through Disease Monitoring and Surveillance (NATP (MM) on AHIS_DMS).

This study provided the database of sero-prevalence of brucellosis in 11 districts of Haryana for development of National Animal Disease Referral Expert System (NADRES) for suggesting prevention and control strategies.

MATERIALS AND METHODS

Bovine serum samples (180 cattle and 990 buffaloes) which form a 5% random representative of the total population were collected from 45 randomly selected villages of 11 districts of Southern Haryana. These serum samples were tested for *Brucella* agglutinins by Rose Bengal plate test (RBPT) and by standard tube agglutination test (STAT) as per Alton *et al*. (1975). The antigens for these tests were procured from Division of Biological Products, Indian

Present address: [1]Senior Scientist (sandipkk2003@yahoo.co.in).
[2]Former Director, 95, Mandal Vihar, Bareilly, Uttar Pradesh 243 122.
[3]Project Director, PD-ADMAS, Hebbal, Bengaluru,Karnataka 560 024.

Veterinary Research Institute, Izatnagar. The same samples were also screened by AB-ELISA (avidin-biotin ELISA) using AB-ELISA kit developed by Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS), Bengaluru, India. Standard protocol of the AB-ELISA kit provided by the manufacturer was followed. There was no history of calf hood vaccination in any of the animals.

RESULTS AND DISCUSSION

Out of 1170 serum samples tested, 12.1% were found positive for *Brucella* by AB-ELISA, 8.6% by STAT, 5.64% by RBPT (Table 1). The seropositivity is highest by ELISA, intermediate by SAT and lowest by RBPT, which is in accordance with respective sensitivity of the tests. The findings are also quite similar to those reported by Trangadia *et al.* (2009), which have reported 22.18% and 13.78% bovines (both cattle and buffaloes) seropositive by ELISA and RBPT respectively. Species prevalence was 6.66% (12/180) by RBPT, 10.55% (19/180) by SAT, 14.44% (26/180) by AB-ELISA in cattle, whereas it was 5.45 (54/990) by RBPT, 8.28% (82/990) by SAT, 11.71% (116/990) by AB-ELISA in buffaloes (Table 1). These findings are in contrast to Charanjeet *et al.* (2004), who have reported prevalence of 2.65% by RBPT, 2.34% by SAT, 1.56% by ELISA in cattle, and 3.42% by RBPT, 2.73% by SAT, 1.36% by ELISA in buffaloes in Himachal Pradesh. Chand and Sharma (2004) observed a prevalence rate of 20.47% by RBPT, 18.89% by SAT and 26.50% by ELISA in organized dairy farms in Haryana, Uttar Pradesh and Madhya Pradesh. The higher prevalence by ELISA is similar to our findings. However higher prevalence by RBPT as compared with SAT is different from our findings. Paul (2000) reported prevalence of 5.0 and 10.23% by RBPT and 5.0 and 17.61% by SAT in buffaloes and cattle, respectively in Himachal Pradesh.

The prevalence was more in female than male (Table 2). The higher susceptibility of female in both the species may be attributed to higher concentration of erythritol in placenta (Pearce *et al*. 1962, Bala and Sidhu 1982) which supplements

Table 1. Overall seroprevalence of brucellosis in cattle and buffaloes employing RBPT, SAT and ELISA

Animal species	Serum samples tested	Seropositive samples		
		RBPT	SAT	ELISA
Cattle	180	12(6.66)*	19(10.55)	26(14.44)
Buffaloes	990	54(5.45)	82(8.28)	116(11.71)
Total	1170	66(5.64)	101(8.6)	142(12.1)

*Figures in parentheses indicate per cent positivity.

Table 2. Species- age- and sex-wise prevalence of bovine brucellosis by AB-ELISA

Species	Age	Male			Female			Both sexes		
		Tested	Positive	% Prevalence	Tested	Positive	% Prevalence	Tested	Positive	% Prevalence
Cattle	Young (<3 years)	8	0	0	54	6	11.1	62	6	9.67
	Adult (>3 years)	16	1	6.25	102	19	18.62	118	20	16.94
Total		24	1	4.14	156	25	16.02	180	26	14.44
Buffalo	Young (<3 years)	26	1	3.84	248	17	6.85	274	18	6.56
	Adult (>3 years)	47	2	4.25	669	96	14.34	716	98	13.68
Total		73	3	4.10	917	113	12.32	990	116	11.71
Grand total		97	4	4.12	1073	138	12.86	1170	142	12.13

Table 3. District-wise prevalence of bovine brucellosis by AB-ELISA

Districts	Cattle			Buffalo		
	Tested	Positive	% Prevalence	Tested	Positive	% Prevalence
Jind	22	5	22.72	101	26	25.74
Rohtak	7	1	14.28	114	15	13.15
Kaithal	3	1	33.3	33	2	6.06
Sirsa	11	0	0	73	14	19.17
Gurgaon	16	2	12.5	31	4	12.90
Mahendergarh	5	0	0	24	1	4.16
Jhajjar	8	2	25	66	4	6.06
Bhiwani	6	0	0	35	0	0
Rewari	13	0	0	109	1	0.91
Hisar	53	10	18.86	110	6	5.45
Faridabad	36	5	13.88	294	43	14.62
Total	180	26	14.44	990	116	11.71

the growth of organisms in gravid uterus (Radostits *et al*. 2000). Higher susceptibility of females than males was also recorded earlier (Folhadela *et al*. 2001). Similarly, sexually mature animals (>3 years of age) were found to be affected more (16.94%, cattle and 13.68%, buffaloes) than the immature ones (< 3 years of age 9.67% in cattle and 6.56% in buffaloes; Table 2). Bovines of all age groups are affected by brucellosis and susceptibility is greatly influenced by the age, sex and reproductive status of the animal but it is the sexual maturity which makes the animal more prone to brucellosis (Radostits *et al*. 2000). Age should always be taken care of while describing the seroprevalence of brucellosis (Martin *et al*. 1993). The detailed species-age- and sex-wise prevalence of bovine brucellosis in Southern part of Haryana is described in Table 2. District-wise prevalence of bovine brucellosis is shown in Table 3. Jind (cattle, 22.72%; buffaloes, 25.74%) and Faridabad (cattle, 13.88%; buffaloes,14.62%) districts were found affected more than other districts. This is probably due to higher density of animals in less space, as it was observed that the owners were having higher number of animals which were densely located in premises as compared to other districts.

ACKNOWLEDGMENTS

The authors are thankful to NATP for providing the financial assistance and gratefully acknowledge the guidance and laboratory facilities provided by Dr S K Dwivedi former Director, NRCE, Hisar.

REFERENCES

Alton G C, Jones L M and Pietz D E.1975. *Laboratory Techniques in Brucellosis*. 2nd edn. WHO, Geneva.

Bala A K and Sidhu N S.1982 Studies on disease resistance vis-à-vis susceptibility in farm animals III, Genetic group differences for incidences of brucellosis in cattle. *Indian Journal of Animal Health* **21**: 61–64.

Chand P and Sharma A K. 2004. Situation of brucellosis in bovines of organized cattle farms belonging to three different states. *Journal of Immunology and Immunopathology* **6**(2): 11–15.

Charanjeet, Sharma M, Katoch R C, Dhar P and Kumar R. 2004. Application of RBPT, SAT and avidin-biotin serum ELISA for detecting brucellosis among livestock in Himachal Pradesh. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases* **25**: 15–18.

Chatterji A, De B N, Bidyanta J, Chakraborty M, Mondal P and Sen G P. 1985. Seroepidemiological studies on bovine brucellosis in organized herds in West Bengal. *Indian Journal of Animal Sciences* **55**(4): 249–52.

FAO. 2009. *Food and Agriculture Organisation: The State of Food and Agriculture*. ISSN 0081-4539. Rome.

Folhadela I M, Jesus V L T, Folhadela D S, Goulat I, Andrade C M and Pinhoiro L P L. 2001. Risk factor for occurrence of bovine brucellosis in cattle farms in Rio de Jeneiro state. *Revista Brasileira de Reproducao Animal* **25**(20): 239–40.

Martin C R, Meek A H and Willeberg P. 1993. *Veterinary Epidemiology* 1st Indian Edition pp 81.

Maurin M. 2008. Brucellosis at the dawn of 21st century. *Medical Malignant Infections* **35**(1): 6–16.

Mohamed N S, Stephan M B and Nammallwar S. 2010. Brucellosis: a re-emerging zoonosis. *Journal of Veterinary Microbiology* **140**: 392–98.

Paul R. 2000. 'Serosurveillance of some infectious diseases in cattle, buffaloes, sheep and goats with special emphasis on high hills temperate dry climate zone of Himachal Pradesh.' M.V.Sc. thesis. CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur.

Pearce J H, Williums A E, Harris Smith P W, Fitzgeorge R B and Smith H. 1962. The chemical basis of the virulence of *Brucella abortus*.II. Erytritol, a constituent of bovine foetal fluids which stimulates the growth of *Brucella abortus* in bovine phagocytes. *British Journal of Experimental Pathology* **43**: 31–37.

Rathore B S, Burman T K, Singh K P, Singh R and Mehrotra M L. 2002. Microbiological and and epidemiologal studies on brucellosis in and organized herd and rural cattle and buffaloes of Uttar Pradesh. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases* **23**(2): 195–96.

Radostits O M, Blood D C and Gay G C. 2000. *Veterinary Medicine*. 9th edn.ELBS, Bailliere and Tindall, London.

Sandhu K S, Filia G, Sharma D R, Dhand N K, Singh J and Saini S S. 2001. Prevalence of brucellosis among dairy animals of Punjab. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases* **22**(2): 160–61.

Sharma V D, Sethi M S, Yadav M P and Dube D C. 1979. Seroepidemiologal investigations on brucellosis in States of Uttar Pradesh (UP) and Delhi (India). *International Journal of Zoonoses* **6**: 75–81.

Trangadia B, Rana S K, Mukherjee F and Srinivasan V A. 2009. Prevalence of brucellosis and infectious bovine rhinotracheitis infections in organized dairy farms in India. *Tropical Animal Health and Production* **42**(2): 203–07.

Whatmore A M, Perret L L and Mc Millan A P. 2007. Characterization of genetic diversity of *Brucella* by multilocus sequencing. *BMC Microbiology* **7**: 34–38.