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Seroprevalence of brucellosis in bovines of Chhattisgarh, India

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ABSTRACT

Brucellosis is an important zoonotic disease causing reproductive problems and abortions in bovines leading to huge economic losses to the dairy industry. Seroepidemiology and risk factors associated with brucellosis were investigated in cattle and buffaloes of Chhattisgarh, India. A total of 374 serum samples from bovines were collected randomly from 94 villages of six districts of Chhattisgarh state with different attributes and were screened by i-ELISA for the detection of antibodies. The overall true seroprevalence for brucellosis was observed to be 14.17%. Brucellosis was significantly more prevalent in animals of organized farms, cross bred, age above 7 years, clinically ailing ones particularly cases of abortion and repeat breeding compared to apparently health animals. The finding represented a major impact on animal health and productivity as well as a high risk to the human population.

Key words: Bovines, Brucellosis, ELISA, Reproductive problems, Risk factor.

INTRODUCTION

Dairy farming plays a substantial role in Indian rural economy. Reproductive problems *viz.,* abortions, repeat breeding, infertility, retained placenta, endometritis are the main impediments in profitable dairy farming and causes direct and indirect huge economic losses to the dairy industry. Most of these problems are of infectious origin due to presence of bacterial or viral infections *viz.*, brucellosis, leptospirosis, listeriosis, camplylobacteriosis, chlamydiosis, salmonellosis, infectious bovine rhinotracheitis, bovine viral diarrhoea which may be responsible for reproductive biotic stresses in dairy animals (Givens, 2006). Some of these also pose a zoonotic risk. These infections have serious economic implications for country like India, which is emerging as the world's biggest milk producer with world's largest cattle and buffalo population. Further in India, artificial insemination using frozen or liquid semen of superior bull is in practice for improvement of productive traits of livestock. At the same time, possible contamination of semen with semen borne pathogens including *Brucella, Leptospira,* and BHV-1 are spreading the disease (Eaglesome and Garcia, 1997).

Bovine brucellosis, a highly transmissible worldwide zoonosis, caused by *Brucella abortus* is an economically important cause of abortions in cattle (Corbel, 2006). It is manifested by reproductive disorders such as abortions, retention of placenta, infertility, stillbirth and calf loss (Radostits *et al.,* 2000). The disease has been eradicated from some countries of the world but is still prevalent in Mediterranean countries of Europe, Northern and Eastern Africa, Mexico, Central and South America, Near East countries, Central Asia and India (Seleem *et al.,* 2010). In India, brucellosis was first recognized in 1887 and thereafter almost all the states of the country have reported its presence. A serological survey performed in 23 states of India revealed the overall prevalence of 1.9% in cattle and 1.8% in buffaloes (Isloor *et al.,* 1998). Bovine brucellosis has appeared to be increasing in recent years, due to increased trade and fast movement of livestock (Renukaradhya *et al.,* 2002). The economic losses occurring due to brucellosis in livestock in India are US $ 3.4 billion in total with loss of US $ 6.8 per cattle, US $ 18.2 per buffalo (Singh *et al.,* 2015).

In order to meet the government national policy, there is a need for serosurveillance of these diseases. However, there exist a paucity of information regarding prevalence of Brucellosis and no systemic study has been carried out on bovine brucellosis in the Chhattisgarh state of India. Therefore, the present investigation was carried out to know the seroprevalence as well as risk factors associated with brucellosis. This study will provide information about the status of brucellosis in cattle and buffaloes in Chhattisgarh and will form the basis for future control strategies for this disease in India and particularly in Chhattisgarh state.

MATERIALS AND METHODS

Study area: This study was carried out in Chhattisgarh, a state geographically situated in central India between 17°46' N to 24°5' North Latitude and 80°15' E to 84°20' East Longitude. The state is divided into three agro-climatic zones *viz.,* northern hill, central plains and southern plateau and subdivided into 27 revenue districts with 146 blocks and 19720 villages. The climate of state is tropical dry sub-humid type with around 1400 mm average annual rainfall and

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temperature between 0-25°C in winter and between 30-45°C in summer. The total geographical area of the state is around 135,192 Km^2 with 25.5 million human population (Anonymous, 2011) with about 80% of the population living in rural areas. Majority of small and marginal farmers rear livestock for their income. The state has about 150.4 lakhs animal population with 9.81million cattle and 1.39 million buffaloes (Anonymous, 2012). Livestock rearing in Chhattisgarh is traditional and subsistence, vast majority being non-descript. Bovine population mainly consists of cross bred (crossed with Holstein Friesian and Jersey) besides Indian milch breeds, Sahiwal and Gir cattle, and Murrah buffaloes. Breeding is done mainly by natural services and artificial insemination both.

Study design and sampling: In this study, random sampling was done in collaboration with the Department of Veterinary Services, Chhattisgarh. Out of 27 districts, six districts namely, Raigarh and Sarguja from northern hills, Dhamtari, Durg and Rajnandgaon from plains and Kanker from southern plateau were selected for sampling based on reports of higher incidences of abortion and reproductive problems in bovines. The total cattle and buffalo population of the six selected districts were 2.49million and 0.33 million respectively. From cattle and buffaloes, a total of 374 sera were collected over six month of period (October, 2015 to March, 2016) and stored at -20 °C. Samples were collected randomly from animals of 94 villages of 6 districts representing the diverse livestock sampling system viz.,cattle (n=345, which included exotic cross bred, n=155; improved Indian breeds, n=65; non-descript breed, n=125 and buffaloes (n=29 including Murrah=27; non-descript=2);male (n=13) and female (n=361); organized (n=155) *vs.* unorganized (n=219) sector; different age group like ≤4years (n=55), between 4-7 years (n=169) and ≥7 years (n=150); apparently healthy animal (n=244)and animal with the history of reproductive disorders (n=130) viz., repeat breeding, infertility, abortion, anoestrous and retention of foetal membrane. The animals sampled in the study were not vaccinated against brucellosis.

ELISA: The serum samples collected were subjected to indirect ELISA for detection of antibodies for bovine brucellosis using commercially available Svanovir Brucella-Ab i-ELISA (Svanova, Sweden). The manufacturer's protocol for ELISA was strictly followed. The manufacturers of *Brucella*-Ab i-ELISA kit quotes 100% sensitivity (Se) and 100% specificity (Sp) relative to complement fixation test.

Statistical analysis: Apparent seroprevalence was calculated by dividing the total number of seropositive samples with the total number of samples. Significant differences within different attributes were calculated using a chi-square test. True prevalence, 95% confidence interval (CI) and chi-square test were evaluated using online available software "Epi tools- Survey toolbox for livestock diseases", and a probability of less than 0.05 was considered statistically significant. The odd ratio (OR) was calculated using a 2x2 contingency table.

RESULTS AND DISCUSSION

Out of 374 serum samples, 53 were positive for antibodies against brucellosis using i-ELISA with an overall apparent seroprevalence of 14.17% (95% CI, 10.64-17.71) in bovines of Chhattisgarh. While, with 100% sensitivity and 100% specificity of test ELISA kit, the true prevalence was 14.17% (95% CI, 10.64-17.71). District wise seroprevalence of brucellosis ranged from 4.76 to 35.82% having the highest in Rajnandgaon and the lowest in Raigarh district. The prevalence of brucellosis was found to be significantly higher in organized farms (23.87%) as compared to unorganized farms (7.31%) ($P \leq 0.05$). Further, all the 53 brucellosis positive samples were from female cattle. Dependency of prevalence on sex and species was found to be non-significant ($P \leq 0.05$). At cattle breed level, the prevalence was significantly higher in exotic cross bred (25.16%) as compared to improved Indian (13.85%) and non-descript zebu cattle (4%) ($P \leq 0.05$). The prevalence was significantly higher ($P \leq 0.05$) in animals with the history of reproductive problems (19.23%) as compared to clinically healthy animals (11.47%). Among various reproductive problems, the *Brucella* antibodies were found in 45.45% of animals with history of abortion, followed by 14.54% positivity among repeat breeders and 7.4% positivity among infertility cases. The seroprevalence was significantly higher in age group of ≥7 years (21.33%) followed by age between 4-7 years (10.1%) and least in age group ≤4 years (7.27%). Thus, the age was significantly influencing the seropositivity ($P \leq 0.05$). In this study, cross bred cattle (OR=4.23, $P \leq 0.0001$) were at higher risk as compared to Indian cattle breeds and non-descript breeds for brucellosis. Similarly animals reared in organized farms (OR=3.98, $P \leq 0.0001$) and above 7 years of age (OR=2.62, P=0.001) were more susceptible than animals of unorganized farms and below 7 years age. While, clinically healthy animals (OR=0.54, P=0.0442) were at less risk than ailing animals with reproductive problems particularly of abortion and repeat breeders for seropositivity of brucellosis (Table 1).

In India, bovine brucellosis is common in most of the states. Long-term serological studies in India indicated 5% of cattle and 3% of buffaloes are infected with brucellosis (Renukaradhya *et al.,* 2002). The avidin biotin ELISA in 973 sera samples from 52 villages of Punjab state revealed prevalence of 9.9% and 13.4% in cattle and buffaloes, respectively with overall prevalence of 12.09% (Dhand *et al.,* 2005) with significantly higher seroreactors in animals with a history of abortion (33.87%) than in those without such a history (11.63%). In Gujarat, seroprevalence of 9.9% was reported in breeding bulls (Kanani *et al.,* 2008). Chauhan

Table 1: Seroprevalence of brucellosis in different attributes and associated risk factors in Chhattisgarh.

Attributes	Sub attributes	No. tested	No. positive	Percent positive	Prevalence 95% CI	Chi square (χ^2) value	P value for χ^2 test	Odds ratio	Odds ratio95% CI	P value for odds ratio
Region	Chhattisgarh	374	53	14.17	10.64-17.71	--	-	-	-	-
District	Rajnandgaon	67	24	35.82	24.34-47.3	36.93*	< 0.0001	5.46	2.34-12.75	< 0.0001
	Dhamtari	97	9	9.28	3.5-15.05			1.72	0.55-5.34	0.412
	Kanker	89	5	5.62	0.83-10.4			0.73	0.17-3.23	0.702
	Sarguja	40	3	7.50	(-) 0.66-15.6			1.62	0.16-16.63	1
	Raigarh	21	1	4.76	(-) 4.35-13.87			0.22	0.03-1.84	0.17
	Durg	60	11	18.33	8.54-28.12			-	-	-
Sector	Organized	155	37	23.87	17.16-30.58	20.48*	< 0.0001	3.98	2.12-7.46	< 0.0001
	Unorganized	219	16	7.31	3.86-10.75			-	-	-
Sex	Male	13	0	0	0	2.224^{NS}	0.13588	0	-	0.2289
	female	361	53	14.68	11.03-18.33			-	-	-
Species	Cattle	345	53	15.36	11.56-19.17	5.191^{NS}	0.0227	infinity	-	0.022
	Buffalo	29	0	0	0			-	-	-
Cattle breed	Cross bred	155	39	25.16	18.33-31.99	23.97*	< 0.0001	2.09	0.95-4.62	0.0742
	Improved Indian	65	9	13.85	5.45-22.24			4.02	1.29-12.53	0.0192
	Non descript	125	5	4.00	0.56-7.44			-	-	-
Clinical condition	Apparently healthy	244	28	11.47	7.48-15.47	4.194*	0.0405	0.54	0.30-0.98	0.0442
	Animals with history of reproductive problems	130	25	19.23	12.46-26.01			-	-	-
Reproductive problems	Infertility	27	2	7.4	(-) 2.47-17.29	21.389*	0.0002	0.47	0.09-2.38	0.4847
	Repeat breeder	55	8	14.54	5.23-23.86			0.20	0.07-0.56	0.002
	Abortion	33	15	45.45	28.47-62.44			infinity	-	0.25
	Retained placental membrane	3	0	0	0			NaN	-	1
	Anoestrous	12	0	0	0			-	-	-
Age of animal	Age ≤4	55	4	7.27	0.41-14.14	10.828*	0.0044	0.701	0.22-2.18	0.60
	Age between 4-7	169	17	10.10	5.52-14.59			0.412	0.218-0.78	0.007
	Age ≥7	150	32	21.33	14.78-27.89			-	-	-

et al. (2000) reported very high overall prevalence of 38.98 per cent in cattle and buffaloes in North Gujarat by using STAT. Plausible explanation for the higher seroprevalence recorded by Chauhan *et al.,* (2000) may be attributed to the inclusion of samples of aborted animals. While, in Maharashtra the prevalence was 40.4% in bovines (Londhe *et al.,* 2011). Higher seroprevalence in cattle from organized farm (76.15%) was reported as compared to unorganized farms (7.02%) in south Bengal (Karmakar *et al.,* 2012). From three states Karnataka, Uttar Pradesh and Uttarakhand, 319 (31.74%) animals were positive for brucellosis includes 138 (27.21%) cattle and 181 (36.34%) buffaloes (Jagapur *et al.,* 2013). In a study of 119 dairy farms with 4,580 female animals from Haryana and Punjab states the overall herd prevalence was 65.54 % and individual animal prevalence was 26.50% (Chand and Chhabra, 2013). Further significantly high risk was observed associated with factors like species, age, awareness of brucellosis among dairymen, entry of newly purchased animals on the farm, abortion in animals and on cow farm while, herd size, use of calving pen on farms, type of breeding and on buffalo and mixed type of farms were insignificant. In an organized farm in Pantnagar, out of 644 samples 33.85% seroprevalence was observed with highest number of seropositive cases in different categories *viz.,* females (36.41%), indigenous breed (21.13%) and 6 to <9 yrs (52.15%), respectively (Kushwaha *et al.,* 2016). In Goa, 27.02% seroprevalence was observed and reported lack of awareness about brucellosis and inadequate floor space were crucial risk factors for transmission of bovine brucellosis (Pathak *et al.,* 2016). In Tamil Nadu, 6.7% prevalence was reported with risk factor associated with the history of abortions (Kumar *et al.,* 2017). Worldwide studies showed reported seroprevalence of 10.3% in Ivory Coast; 1.3% in Niger; 2.5% in Maranhao State, Brazil; 18.1% in Jordan and 6.8% in Katavi-Rukwa ecosystem, Tanzania (Sanogo *et al.,* 2012; Boukary *et al.,* 2013; Borba *et al.,* 2013; Musallam *et al.,* 2015; Assenga *et al.,* 2015).

In this study, the problem of brucellosis is more prevalent in cross bred animals reared in organized conditions. It may be due to the low acclimatization factor of cross bred cattle in Indian environmental conditions and comparatively having lower resistance to diseases. In contrast to this, the Indian non-descript breeds showed less seropositivity to brucellosis which might be due to presence of some genetic resistance to diseases particularly to brucellosis. Similarly, all the bulls were found negative to brucellosis. Thus, the findings support the fact that sexually mature females which are reared intensively are prone to *Brucella* infection. Further, animals with history of reproductive problems should be obtained properly to ensure *Brucella* free herd, as high risk factor and relationship was observed between prevalence and animals with history.

CONCLUSION

The study reported the seropositivity of 14.17% for brucellosis among the bovines in Chhattisgarh region, indicating alarming condition for dairy industry and potential zoonotic risk to farmers and farm workers. There prevalence in apparently healthy bovine indicates the presence of these infectious agents in the animals and environment which may be a potential source of infection for others.

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REFERENCES

Anonymous. (2011). Census of India. http://censusindia.gov.in, 2011 (accessed 16.05.2017).

Anonymous. (2012). 19th Livestock Census, All India report. Ministry of Agriculture, Department of Animal Husbandry, Dairying and Fisheries, Krishi Bhawan, New Delhi, India, 2012. http://dahd.nic.in/sites/default/files/Livestock 5.pdf

Assenga, J.A., Matemba, L.E., Muller, S.K., Malakalinga, J.J. and Kazwala, R.R. (2015). Epidemiology of *Brucella* infection in the human, livestock and wildlife interface in the Katavi-Rukwa ecosystem, Tanzania. *BMC Veterinary Research,* **11**: 189. doi: 10.1186/s12917-015-0504-8.

Borba, M.R., Stevenson, M.A., Goncalves, V.S.P., Neto, J.S.F., Ferreira, F., Amaku, M., Telles, E.O. and Santana, S.S., *et al.,* (2013). Prevalence and risk-mapping of bovine brucellosis in Maranhão State, Brazil. *Preventive Veterinary Medicine,* **110**: 169– 176.

Boukary, A.R., Saegerman, C., Abatih, E., Fretin, D., Bada, R.A., De Deken, R., Harouna, H.A., Yenikoye, A and Thys, E. (2013). Seroprevalence and potential risk factors for *Brucella* spp. infection untraditional cattle, sheep and goats reared in urban, periurban and rural areas of Niger. *PLoS One,* **8**: e83175.

Chand, P., Chhabra, R. (2013). Herd and individual animal prevalence of bovine brucellosis with associated risk factors on dairy farms in Haryana and Punjab in India. *Tropical Animal Health and Production,* **45**: 1313–1319.

Chauhan, H.C., Chandel, B.S., Shah, N.M. (2000). Seroprevalence of brucellosis in buffaloes in Gujarat. *Indian Veterinary Journal,* **77**: 1105-06.

Corbel, M.J. (2006). Brucellosis in Humans and Animals, World Health Organization, Geneva, Switzerland.

Dhand, N.K., Gumber, S., Singh, B.B., Aradhana, Bal, M.S., Kumar, H., Sharma, D.R., Singh, J. and Sandhu, K.S. (2005). A study on the epidemiology of brucellosis in Punjab (India) using survey toolbox. *Rev Sci Tech Off Int Epiz.,* **24**: 879-885.

Eaglesome, M.D. and Garcia, M.M. (1997). Disease risks to animal health from artificial insemination with bovine semen. *Rev Sci Tech Off Int Epiz.,* **16**: 215-225.

Givens, M.D. (2006). A clinical, evidence-based approach to infectious causes of infertility in beef cattle. *Theriogenology,* **66**: 648–654.

Isloor, S., Renukaradhya, G.J. and Rajasekhar, M. (1998). A serological survey of bovine brucellosis in India. *Rev Sci Tech Off Int Epiz.,* **17**: 781-785.

Jagapur, R.V., Rathore, R., Karthik, K. and Somavanshi, R. (2013). Seroprevalence studies of bovine brucellosis using indirect-enzyme- linked immunosorbent assay (i-ELISA) at organized and unorganized farms in three different states of India. *Veterinary World,* **6**: 550-553.

Kanani, A.N., Jain, L., Patel, T.J., Rank, D.N., Joshi, C.G. and Purohit, J.H. (2008). Detection of *Brucella* DNA in semen using the polymerase chain reaction assay. *Indian Journal of Animal Research,* **42**: 222-224.

Karmakar, P.K., Biswas, B.K., Chandra, S. and Das, R. (2012). Seroprevalence of brucellosis in cattle and buffalo in some districts of South Bengal. *Trends in Biosciences,* **5**: 212-213.

Kumar, V.N., Vijaya, B.M. and Porteen, K. (2017). Risk factors associated with seroprevalence of bovine brucellosis. *International Journal of Advcanced Biological Resesearch,* **7**: 182-184.

Kushwaha, N., Rajora, V.S., Mohan, A., Upadhyay, A.K. and Kumar, R. (2016). Comparison of serological tests for detection of *Brucella* antibodies in cattle of an organized dairy farm. *Indian Journal of Animal Research,* **50**: 69-74.

Londhe, S.P., Bannalikar, A.S. and Dighe, V.D. (2011). Serodetection of bovine brucellosis by RBPT and AB-ELISA. *Animal Science Reporter,* **5**: 69-73.

Musallam, I.I., Abo-Shehada, M., Omar, M. and Guitian, J. (2015). Cross-sectional study of brucellosis in Jordan: prevalence, risk factors and spatial distribution in small ruminants and cattle. *Preventive Veterinary Medicine,* **118**: 387–396.

Pathak, A.D., Dubal, Z.B., Karunakaran, M., Doijad, S.P., Raorane, A.V., Dhuri, R.B. and Bale, M.A., *et al.,* (2016). Apparent seroprevalence, isolation and identification of risk factors for brucellosis among dairy cattle in Goa, India. *Comparative Immunology Microbiology and Infectious Diseases,* **47**: 1–6.

Radostits, O.M., Gay, C.C., Blood, D.C., Hinchcliff, K.W. (2000). Diseases Caused by *Brucella* spp. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, Ninth edition, Harcourt Publishers Limited, London.

Renukaradhya, G.J., Isloor, S., Rajasekhar, M. (2002). Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. *Veterinary Microbiology*, **90**: 183–95.

Sanogo, M., Abatih, E., Thys, E., Fretin, D., Berkvens, D., Saegerman, C. (2012). Risk factors associated with brucellosis seropositivity among cattle in the central Savannah-forest area of Ivory Coast. *Preventive Veterinary Medicine,* **107**: 51–56.

Seleem, M.N., Boyle, S.M., Sriranganathan, N. (2010). Brucellosis: A re-emerging zoonoses. *Veterinary Microbiology*, **140**: 392–398.

Singh, B.B., Dhand, N.K., Gill, J.P.S. (2015). Economic losses occurring due to brucellosis in Indian livestock populations. *Preventive Veterinary Medicine,* **119**: 211–215.