www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Bio-presence of *Mycobacterium avium* subspecies *paratuberculosis* infection in Indian livestock farms

Shoor Vir Singh*, Naveen Kumar, Kundan Kumar Chaubey, Saurabh Gupta and Krishna Dutta Rawat

Microbiology Lab, Animal Health Division, Central Institute for Research on Goats (CIRG), Makhdoom, PO-Farah, Mathura- 281122, Uttar Pradesh, India

Abstract

In the present study, a total of 1750 faecal and 2057 serum samples, collected from 20 livestock (cattle, goat, sheep) farms located in nine districts of eight states in India were screened for the bio-presence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) using faecal microscopy and indigenous ELISA technique. The results showed that 25.0 and 29.0% of the faecal and serum samples respectively were positive for MAP infection respectively. The study indicated that higher bio-presence of MAP in domestic livestock farms in India may be directly correlated with low per animal productivity of the native livestock breeds. Bio-presence was highest in cattle followed by sheep and goat. Furthermore, this study showed that microscopy was better screening method as compared to ELISA in goat herds endemic for Johne's disease (JD) and ELISA for the screening of cattle herds. There is an immediate need to initiate long term control programs to prevent losses in livestock production and also to prevent transmission of MAP to human population through food chain.

Keywords: Bio-presence; *Mycobacterium avium* subspecies *paratuberculosis;* Johne's disease; Livestock farms; ELISA; microscopy

To cite this article: Singh SV, N Kumar, KK Chaubey, S Gupta and KD Rawat, 2013. Bio-presence of *Mycobacterium avium* subspecies *paratuberculosis* infection in Indian livestock farms. Res. Opin. Anim. Vet. Sci., 3(11), 401-106.

Introduction

Johne's disease or paratuberculosis, a chronic incurable disease of animals caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is endemic in domestic ruminants word-wide and has been associated with a number of chronic ailments in human beings (Singh et al., 2009; Singh et al., 2010). MAP infection leads to severe form of granulomatous gastroenteritis with progressive weight loss and emaciation, weakness, stunting and non-treatable diarrhea causing huge losses to economics of livestock production (milk, meat, fibre and leather industry). Johne's disease (JD) has frequently been reported from farm herds in India (Mathur et al., 1981; Sharma et al., 1987; Kumar et al., 1988; Koul et al., 1989; Singh et al., 2013a); using multiple tests (Barad et al., 2013) with case reports of clinical disease progressively escalating.

India has the highest livestock (cattle, buffaloes, goats and sheep) population (555.2 million) in the world (FAO, 2012) and large section of human population is engaged in trade related to the livestock industry for their livelihood. Though India leads in milk production and ranked second in meat producing countries but average per animal productivity is very low (Barbaruah and Joseph, 2008). This disease greatly contributes to the reduced productivity and is difficult to diagnose and control. Low productivity of domestic livestock is presently a major concern of the Indian livestock industry. No country in the world has claimed freedom from MAP infections but every develop country has National JD control program in place. Multiple tests have been employed to determine

Corresponding author: Shoor Vir Singh, Microbiology Lab, Animal Health Division, Central Institute for Research on Goats (CIRG), Makhdoom, PO-Farah, Mathura- 281122, Uttar Pradesh, India. Phone No: +91 565 2763260; +91 9719072856, 9412828874; Fax: +91 565 2763246

prevalence of MAP in the livestock farms of the western world (Douarre et al., 2010). This study aimed to estimate bio-presence of *Mycobacterium avium* subspecies *paratuberuclosis* infection in 20 farms of three species of domestic livestock (cattle, goats and sheep) in India using multiple tests.

Materials and Methods

Screening livestock for MAP

Bio-presence of MAP was studied in different livestock farm of three species of domestic animals (cattle, goat and sheep) suffering from sub-clinical to clinical and advance clinical Johne's disease. Cattle herds were maintained on intensive management with very little or no supplementary feeding. Whereas, goat herds under study were raised on either grazing alone or were stall fed. Samples from 20 livestock farms (9 cattle, 7 goats and 4 sheep) from nine districts of eight Indian states (Fig. 1) were submitted to the Microbiology Laboratory of Central Institute for Research on Goats (CIRG) between January 2012 and May 2013 to estimate bio-presence of MAP. A total of 1750 faecal samples (422 cattle, 1050 goats and 278 sheep) and 2057 serum samples (546 cattle, 1252 goats and 259 sheep) from adult livestock population were screened by direct microscopy and indigenous ELISA respectively.

Collection of faecal samples

Faecal samples of domestic livestock (422 cattle, 1050 goats and 278 sheep) were collected from 20 farm settlements at different geographical regions of the country. Signs resembling clinical JD (loss of appetite, dullness, weakness, emaciation, rough hair coat, loss of hairs (alopecia), pasty or loose faeces (diarrhoea), with tail hairs stick together) were reported from animals sampled.

Collection of serum samples

Serum samples of cattle (546), goats (1252) and sheep (259) from 20 herds and flocks were submitted to Microbiology laboratory of CIRG, Makhdoom to estimate bio-presence of MAP.

Direct microscopy

Two grams of faecal sample was collected directly from 1750 animals in polyethylene bags. Samples were homogenized, concentrated by centrifugation at 4500 rpm for 45 min at room temperature. Smears prepared from the middle layer were heat fixed and stained with Ziehl-Nielsen's (ZN) method and examined under microscope for pink colour (acid fast) short rods indistinguishable to MAP (Singh et al., 2013b).

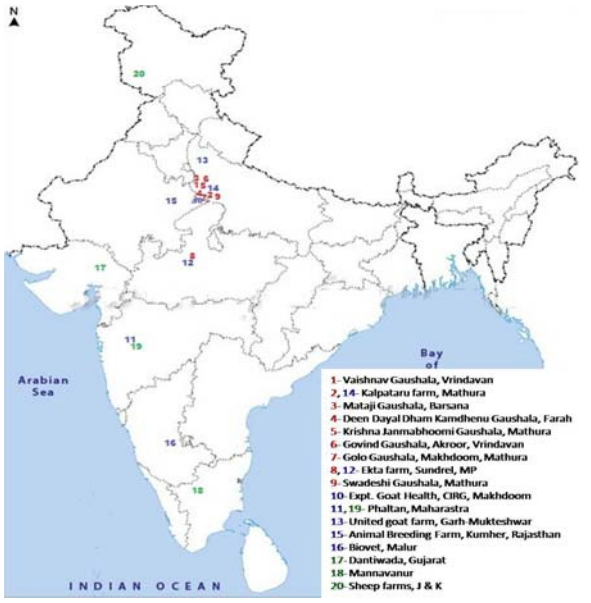

Fig. 1: Geographical distribution of livestock farms for JD in the country

Enzyme-Linked Immuno-Sorbent Assay

Indigenous ELISA kit (i-ELISA) was developed first time for screening of goats against JD (Singh et al., 2007a). Protoplasmic antigen (PPA) was harvested from 'Indian bison type' genotype of MAP isolated from a terminal case of JD in a Jamunapari goat. Whole cell sonicated lysate was centrifuged and standardized at 0.1 µg of protein per well of microtiter plate. Serum samples were used in 1:50 dilution and anti-species horseradish peroxidase conjugate (Sigma) in 1:5000 dilution. Serum samples of culture positive and negative animals were used as positive and negative controls, respectively. Optical densities (OD) were transformed to S/P ratios and animals were considered negative (0.00-0.09), suspected (0.10-0.24), low positive (0.25-0.39), positive (0.40-0.99) and strong positive (1.0-10.00) for the status of JD as per the likelihood ratio method of Collins (2002). Animals in positive and strong positive categories were considered positive for the bio-presence of MAP. Later on this goat based ELISA was adapted to screen bovine serum samples against MAP infection, since MAP 'Indian Bison Type' biotype (the source of antigen for ELISA) was the major bio-type infecting Indian domestic livestock (Singh et al., 2009; Singh et al., 2010).

Statistical analyses

ANOVA was applied on the data obtained from ELISA and compared with mean data of various farm settlements ($P>0.05$). Correlation co-efficient (r) was computed for the MAP from serum (r=67.6%) with MAP from microscopy (r=74.2%).

Results

Screening of MAP using direct microscopic technique

Of the 1750 faecal samples collected from domestic livestock farms, 25.2% (441) were positive. Species-wise bio-presence of MAP was 29.6 (125), 21.6 (227) and 32.0% (89) in cattle, goats and sheep, respectively (Table 2).

Screening of MAP using indigenous ELISA technique

Of the 2057 serum samples collected from domestic livestock farm, 28.9% (595) were positive for ELISA. Species-wise sero-prevalence of MAP was 44.8 (245), 19.8 (248) and 39.3% (102) in cattle, goats and sheep, respectively (Table 2).

Discussion

The results obtained in this study showed moderate to high prevalence of MAP in domestic livestock farms (9 cattle, 7 goats and 4 sheep) using microscopy and ELISA. Clinical signs resembling clinical JD (loss of appetite, dullness, weakness, emaciation, rough hair coat, loss of hairs (alopecia), pasty or loose faeces (diarrhoea, with tail hairs stick together) were reported from animals sampled... Screening of 1750 faecal samples from 20 livestock farms (2012-13) by microscopy showed that shedding of MAP was highest in sheep (32.0%) followed by cattle (29.6%) and goats (21.6%). Shedding of MAP was observed to be high in most of the cattle except those from Mataji gaushala, Barsana and in young cattle of Kalptaru farms Farah. This may be attributable to the fact that samples collected from Gaushalas (community shelters for cattle), where, nutritional inputs were very low and cows were already dis-owned by their owners for being low and poor producers. Low (2.4%) shedding of MAP was observed in the farms at Mataji Goshala due to selective sampling of healthy animals from nearly 14000 cows available. However, two times screening of 201 and 169 serum samples in ELISA showed moderate (25.3%) and too high (49.3%) bio-presence of MAP. However, low (15.1%) bio-presence of MAP in Sahiwal cows of Janmbhumi, Mathura may be due to extreme good physical condition of the cows since cows were maintained on high plane of nutrition, good hygienic conditions and management provided for them. Therefore, screening of cattle using ELISA technique may be preferred over microscopy, whereas in goats microscopy should be the preferred method of choice over ELISA. Shedding of MAP by goats was moderate (20.0 to 29.0%) and this was not commensurate with actual infectivity for MAP. Higher positivity in ELISA titres was seen in goats in farms where nutritional conditions were optimum and mesenteric lymph nodes have not been damaged. Low positivity in microscopy (19.8%) and ELISA (12.9%) was due to good nutrition, better management, keeping them in stall fed conditions away from other animals. In fact such good condition of goats as observed in Phaltan farms was very rare even with 30years working experience by one of the authors. At one of the goat farm (Garh Mukteshwar, UP), despite high shedding of MAP bacilli (64.2%) there was comparatively low positivity (35.1%). This may be due to either stress on animals, since the farm was recently established and animals were purchased and herded from different places and owners and were yet to adapt to new farm conditions and feeding regime. There the disease was in active phase and goat adapted MAP strain was actively multiplying and establishing infection in new animals. However, in the goat farm at Sundrel, MP positivity in ELISA was higher (47.4%) as compared to microscopy (29.0%) due to fact that farm was old one (established 3-4 years back) and goats were on high plane of nutrition and under regular health care. Similarly, Kalptaru farms were newly stated (<1 year old) and sent for grazing in wide grazing land, so infection is yet to be built up. Whereas, goat farms at CIRG, Makhdoom were highly endemic for MAP, since last >30 years, therefore, positivity was high (75.0%) for MAP infection. Similarly, high bio-presence of MAP was seen in farms at Dantiwada and Mannvanur due to endemicity of MAP infection as the farms were very old. Mannavanur farm was established in 1960s and Dantiwada in 1970s. But farms at Phaltan, Maharashtra were new and under good condition of management and high plane of nutrition and were cleanest as far as present status of JD is concerned. JD was a serious problem in all the 3 species of domestic animals, however, due to high slaughter rate, the condition of disease was less severe as compared to cattle, which cannot be slaughtered in the country.

Of the 2057 serum samples, 44.8 (245), 19.8 (248) and 39.3% (102) were positive for seroprevalence of MAP in 546 cattle, 1252 goats and 259 sheep, respectively. Like microscopy, positivity in ELISA was highest in cattle (44.8%) followed by sheep (39.3%) and goats (19.8%). However, population of clinical to advance clinical cases of bovine JD is very high in the country (Singh et al., 2007b&c), which is threatening the existence of cattle, despite being considered 'Holy' in Indian Mythology.

The results of ELISA (either higher or equal to microscopy), correlated well with microscopy in majority of the herds, except three farms; Phaltan, Maharasthra, Kalpataru farm, Farah and Garh Mukteshwar farm (Where condition of nutrition, management and hygiene were good since being in the private sector, whereas farms at CIRG, Dantiwada and Mannavanur were under government system). The low prevalance in goats was mainly due to higher annual

Table 1: Profile of livestock farms and animals screened for the bio-presence of MAP infection

S/No	Profile of livestock farms and animals					Samples screened		
	Geographical locations of the livestock farms	Nutritional Status	Management	Hygiene	Status of animals	Fecal *(n)*	Serum *(n)*	Total *(n)*
A	Cattle Farms							
1.	Vaishnav Gaushala, Vrindavan	Very Good	Intensive	Good	Good	35	12	47
2.	Kalpataru Farm, Mathura A	Fair	Intensive	Good	Fair	4	11	15
	Kalpataru Farm Mathura B	Fair	Intensive	Good	Fair	5	23	28
3.	Mataji Gaushala, Barsana A	Fair	Semi Intensive	Good	Fair	201	201	402
	Mataji Gaushala Barsana B	Fair	Semi Intensive	Good	Fair	NA	160	160
4.	Deen Dayal Dham Kamd -henu Gaushala, Farah	Poor	Intensive	Good	Poor	6	NA	6
5.	Krishna Janmabhoomi Gaushala, Mathura	Very Good	Intensive	Good	Very Good	NA	33	33
6.	Govind Gaushala, Akroor, Vrindavan	Fair	Semi Intensive	Good	Fair	121	63	184
7.	Golo Gaushala, Makhdoom, Mathura	Poor	Semi Intensive	Poor	Poor	8	8	16
8	Ekta Agronomic farm, Sundrel, MP	Optimum	Intensive	Good	Fair	8	1	9
9	Swadeshi Gaushala, Farah, Mathura	Poor	Semi Intensive	Poor	Poor	34	34	68
Sub- Total A						422	546	968
B	Goat Farms							
1.	Goat farm, CIRG, Makhdoom	Very Good	Semi Intensive	Good	Poor	29	48	77
2.	Phaltan, Maharastra	Very Good	Intensive	Good	Fair	948	987	1935
3.	Ekta goat farm, Sundrel,	Good	Intensive	Good	Fair	31	118	149
4.	United Goat Farm, Garh-Mukteshwar	Good	Semi Intensive	Good	Fair	14	37	51
5	Kalpataru farm, Mathura	Good	Intensive	Good	Fair	11	34	45
6	Animal Breeding Farm, Kumher, Rajasthan	Good	Semi Intensive	Good	Poor	5	16	21
7	Biovet, Malur, Karnataka	Intensive	Extensive	Good	Poor	12	12	24
Sub- Total B						1050	1252	2302
C	Sheep Farms							
1	Dantiwada, Gujarat	Poor	Semi Intensive	Poor	Poor	21	52	73
2	Mannavanur, TN	Good	Semi Intensive	Poor	Fair	68	68	136
3	Phaltan, Maharastra	Very Good	Semi Intensive	Very Good	Fair	132	120	252
4	Sheep farms, J & K	Good	Extensive	Poor	Fair	57	19	76
Sub-total C						278	259	537
Grand-total (A + B + C)						1750	2057	3807

removal of animals as compared to sheep and cattle. Between goats and sheep, damage to the mesenteric lymph nodes was higher as compared to sheep. Sheep due to hairy coat and grazing nature has better ability to drive nutrition even from deficient pasture land, whereas goats are basically browsing in nature and loose body energy in maintaining body temperatures (homothermy). Sero-positivity for MAP was moderate (24.1-34.6%) at Phaltan and Dantiwada and high (57.8 to 64.7%) in sheep flocks located in J&K and Mannavanur (TN). Low positivity in Phaltan sheep was better managed and away from other animals. Whereas in Dantiwada and Patanwadi sheep were weak and endemic for MAP infection and depended mainly on grazing in deficient pasture. T heir immune system was weak and were high (71.4%) shedder of MAP, which damaged mesenteric lymph nodes, as revealed at necropsy. High sero-positivity in Mannavanur and J & K sheep flock was due to good pasture land and good health of sheep as both flocks located in the hilly tract of North and South India, where, nutrition conditions were optimum or good.

Earlier studies have reported higher incidence of MAP in domestic livestock (Singh et al., 2008; Gupta et al., 2012; Barad et al., 2013; Singh et al., 2013a, b; Shroff et al., 2013) screened by various diagnostics like microscopy, ELISA and PCR. Among them microscopy was more sensitive because shedding was often seen prior to peripheral immune response (Lybeck et al., 2011). High bio-presence of MAP in livestock was directly associated with low productivity of domestic livestock in India, which increased the chances of spread to human population. Hence, there is immediate need for a national program on the control of Johne's disease in domestic livestock of the country and protect human population from contamination to MAP.

Table 2: Bio-presence of MAP in the livestock farms of the country using direct microscopy and indigenous ELISA

S/ No	Locations of the livestock farms	Physical status of animals	Diagnostic Tests			
			Direct Microscopy		Indigenous ELISA	
			Total *(n)*	Positives *(n)*	Total *(n)*	Positives *(n)*
A	Cattle Farms					
1.	Vaishnav Gaushala, Vrindavan	Good	35	29 (82.8)	12	8 (66.6)
2.	Kalpataru Farm, Mathura A	Fair	4	1 (25)	11	10 (90.9)
	Kalpataru Farm Mathura B	Fair	5	3 (60)	23	21 (91.3)
3.	Mataji Gaushala, Barsana A	Fair	201	5 (2.48)	201	51 (25.3)
	Mataji Gaushala Barsana B	Fair	NA	NA	160	79 (49.3)
4.	Deen Dayal Dham Kamd henu Gaushala, Farah	Poor	6	5 (83.3)	NA	ND
5.	Krishna Janmabhoomi Gaushala, Mathura	Very Good	NA	NA	33	5 (15.1)
6.	Govind Gaushala, Akroor, Vrindavan	Fair	121	51 (42.1)	63	34 (53.9)
7.	Golo Gaushala, Makhdoom, Mathura	Poor	8	6 (75)	8	6 (75)
8	Ekta Agronomic farm, Sundrel, MP	Fair	8	4 (50.0)	1	1 (100.0)
9	Swadeshi Gaushala, Mathura	Poor	34	21 (61.7)	34	30 (88.2)
Sub- Total A			422	125 (29.6)	546	245 (44.8)
B	Goat Farms					
1.	Goat farm, CIRG, Makhdoom	Poor	29	8 (27.5)	48	27 (56.2)
2.	Phaltan, Maharastra	Fair	948	188 (19.8)	987	128 (12.9)
3.	Ekta goat farm, Sundrel,	Fair	31	9 (29.0)	118	56 (47.4)
4.	United goat Farm, Garh- Mukteshwar	Fair	14	9 (64.2)	37	13 (35.1)
5	Kalpataru farm, Mathura	Fair	11	3 (27.2)	34	2 (5.8)
6	Animal Breeding Farm, Kumher, Rajasthan	:Poor	5	1 (20.0)	16	13 (81.2)
7	Biovet, Malur, Karnataka	Poor	12	9 (75.0)	12	9 (75.0)
Sub- Total B			1050	227 (21.6)	1252	248 (19.8)
C	Sheep Farms					
1	Dantiwada, Gujarat	Poor	21	15 (71.4)	52	18 (34.6)
2	Mannavanur, TN	Fair	68	52 (76.4)	68	44 (64.7)
3	Phaltan, Maharastra	Fair	132	11 (8.3)	120	29 (24.1)
4	Sheep farms, J & K	Fair	57	11 (19.2)	19	11 (57.8)
Sub-total C			278	89 (32.0)	259	102 (39.3)
Grand-total (A + B + C)			1750	441 (25.2)	2057	595 (28.9)

Conclusion

Present study reported that JD is endemic in the domestic livestock farms across the country wherein bio-presence of MAP using microscopy and ELISA tests was high (25.2 to 28.9%). The study also underline need for urgent control programs at the national level to improve per animal productivity of native breeds and retain livestock husbandry as a viable option of livelihood and food security to the large sections of human population.

Acknowledgment

Authors are thankful to CSIR, New Delhi and Director, CIRG for funding and extending necessary facilities for the work.

References

Barad, D.B., Chandel, B.S., Dadawala, A.I., Chauhan, H.C., Kher, H.N., Shroff, S., Bhagat, A.G., Singh, S.V., Singh, P.K., Singh, A.V., Sohal, J.S., Gupta, S. and Chaubey, K.K. 2013. Comparative potential of traditional versus modern diagnostic tests in estimating incidence of Caprine Johne's disease. *Advances in Animal and Veterinary Sciences*, 1: 35-40.

Barbaruah, M.I. and Joseph, A.K. 2008. India: diary giant walking bare-foot. FAO-RAP technical meeting. 17-20 Nov, 2008.

Collins, M.T. 2002. Interpretation of a commercial bovine paratuberculosis enzyme-linked immunosorbent assay by using likelihood ratios. *Clinical Diagnostic Laboratory Immunology*, 9: 1367-1371.

Douarre, P.E., Cashman, W., Buckley, J. and O'Mahony, J.M. 2010. Isolation and detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) from cattle in Ireland using both traditional culture and molecular based methods. *Gut Pathogens*, 2:11.

FAO, 2012. FAOSTAT. Food and Agriculture Organization, Rome.

Gupta, A., Rani, S.M., Agrawal, P. and Gupta, P.K. 2012. Sero-prevalence of Paratuberculosis (John's disease) in cattle population of South-Western Banglore using ELISA kit. *Open Journal of Veterinary Medicine*, 2: 196-200.

Koul, G.L., Somvanshi, S. and Biswas, J.C. 1989. Mortality pattern in Pashmina goats. *Indian Veterinary Journal*, 65: 847-849.

Kumar, R., Prasad, M.C. and Paliwal, O.P. 1988. Paratuberculosis in goats. A retrospective study. *Indian Veterinary Journal*, 65: 582-584.

Lybeck, K.R., Storset, A.K., Djonne, B., Valheim, M. and Olsen, I. 2011. Faecal shedding detected earlier than immune responses in goats naturally infected with *Mycobacterium avium* subsp. *paratuberculosis*. *Research in Veterinary Science*, 91: 32-39.

Mathur, P.B., Sharma, M.M., Uppal, P.K., Belwal, L.M. and Lonkar, P.S. 1981. Epidemiological studies on sheep and goat diseases and developing suitable prophylactic control and eradication measures. Annual Report, Central Sheep and Wool Research Institute, Avikanagar, Jaipur, Rajasthan.

Shroff, S., Chandel, B.S., Dadawala, A.I., Singh, S.V., Bhagat, G.B., Chauhan, H.C., Gupta, G. and Chaubey, K.K. 2013. Evaluation of an Indigenous vaccine based on goat adapted *mycobacterium avium* subspecies *paratuberculosis* in patanwadi breed of sheep naturally infected with clinical Johne's disease in north Gujarat. *Research Opinions in Animal & Veterinary Sciences*, 3: 322-329.

Sharma, M.M., Loonkar, P.S., Srivastava, C.P., Maru, A. and Dubey, S.C. 1987. Prevalence and Johne's disease (JD), in sheep and goats. *Indian Journal of Comparative Microbiolology, Immunology and Infectious Diseases*, 8; 48-49.

Singh A.V., Singh S.V., Singh, P.K. and Sohal, J.S. 2010. Genotype diversity in Indian isolates of *Mycobacterium avium* subspecies *paratuberculosis* recovered from domestic and wild ruminants from different agro-climatic regions. *Comparative Immunology, Microbiology and Infectious Diseases*, 33: e127-e131.

Singh, S.V., Singh, A.V., Shukla, N., Singh, P.K., Sohal, J.S., Gupta, V.K. and Vihan, V.S. 2007c. Sero-prevalence of Johne's disease in prospective young bulls of Hariana breed by indigenous ELISA kit, using protoplasmic antigen from 'Bison type' genotupe of *Mycobacterium avium* subspecies *paratuberculosis* of goat origin. *Indian Journal of Animal Sciences*, 77: 659-662.

Singh, S.V., Singh, A.V., Singh, P.K., Gupta, V.K., Kumar, S. and Vohra, J. 2007a. Sero-prevalence of paratuberculosis in young kids using 'Bison type', *Mycobacterium avium* subsp. *paratuberculo*sis antigen in plate ELISA. *Small Ruminant Research*, 70: 89-92.

Singh, S.V., Singh, A.V., Singh, R., Sandhu, K.S., Singh, P.K., Sohal, J.S., Gupta, V.K. and Vihan, V.S. 2007b. Evaluation of highly sensitive indigenous milk ELISA kit with facal culture, milk culture and fecal-PCR for the diagnosis of bovine Johne's disease (BJD) in India. *Comparative immunology, Microbiology and Infectious diseases*, 30: 175-186.

Singh, S.V., Singh, A.V., Singh, R., Sharma, S., Shukla, N., Misra, S., Singh, P.K., Sohal, J.S., Kumar, H., Patil, P.K., Misra, P. and Sandhu, K.S. 2008. Sero-prevalence of Johne's disease in buffaloes and cattle population of North India using indigenous ELISA kit based on native *Mycobacterium avium* subspecies *paratuberculosis* 'Bison type' genotype of goat origin. *Comparative Immunology, Microbiology and Infectious Diseases*, 31: 419-433.

Singh, S.V., Singh, P.K., Singh, A.V., Gupta, S., Chaubey, K.K., Singh, B., Kumar, A., Srivastava, A. and Sohal J.S. 2013a. Bio-burden and Bio-type profiles of *Mycobacterium avium* supspecies *paratuberculosis. International Journal of Current Research*, 5: 1897-1901.

Singh, S.V., Sohal, J.S., Singh, P.K. and Singh, A.V. 2009. Genotype profiles of *Mycobacterium avium* subspecies *paratuberculosis* isolates recovered from animals, commercial milk, and human beings in North India. *International Journal of Infectious Diseases*, 13: e221-e227.

Singh, S.V., Singh, P.K., Gupta, S., Chaubey, K.K., Singh, B., Kumar, A., Singh, A.V. and Kumar, N. 2013b. Evaluation of microscopy ('field laboratory test') with blood-PCR for diagnosis and estimation of Johne's disease in domestic livestock. *Iranian Journal of Veterinary Research* (In press).