Int.J.Curr.Microbiol.App.Sci (2018) *7*(1): 753-761

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 01 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.701.091

Bio-detection of *Mycobacterium avium* subspecies *paratuberculosis* in the Commercial Milk and Milk Products Sold in Bhubaneswar, Odisha, India

Sangram Biswal[1], Shaswati Subhadarsini Pany[2]*, Shoor Vir Singh[3], Niranjana Sahoo[1], Manju Singh[3], Sourabh Gupta[3] and Kundan Kumar Chaubey[3]

[1]Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Science and Animal Husbandry, O.U.A.T., Bhubaneswar, Odisha, India
[2]ICAR-International Centre for Foot and Mouth Disease, Arugul, Bhubaneswar-752050, Odisha, India
[3]Microbiology Laboratory, Animal Health Division, Central Institute for Research on Goats, Makhdoom, PO- Farah, Mathura, India

**Corresponding author*

Keywords

Johne's disease, Milk, Milk products, *Mycobacterium avium subspecies paratuberculosis*, Odisha, Paratuberculosis, Prevalence

Article Info

Accepted:
06 December 2017
Available Online:
10 January 2018

A B S T R A C T

Present study aimed to screen commercial milk and milk products sold in the local markets of Bhubaneswar, Odisha for the presence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP). A total of seventeen (17) samples comprising of commercial milk and milk products (curd, flavoured milk, lassi and milk powder) of leading commercial brands were purchased from the local markets in Bhubaneswar, Odisha. Samples were tested using antigen detection tests [milk microscopy (ZN staining), IS*900* PCR, IS*900* Real Time PCR, Indirect Fluorescent Antibody Test (iFAT)] and antibody detection test [Indigenous plate ELISA test (i_ELISA) by indigenous ELISA kit (provided by Animal Health Division of CIRG, Makhdoom)] in the Microbiology Laboratory, Animal Health Division, CIRG, Makhdoom. None (0%) of the 17 samples tested positive for MAP by any of above the tests, however, 12 were suspected for MAP by i_ELISA. The results indicated that the presence of MAP infection was very low, which sometimes becomes difficult to detect by multiple tests due to less sample size. Present pilot study underlined the need for screening of larger sample size especially raw and individual milk samples to arrive at conclusion as how serious is the threat of MAP in the livestock population of Odisha.

Introduction

Johne's disease(JD) is a chronic wasting disease primarily of ruminants characterised by diarrhoea, weight loss, production loss, emasciation due to protein losing enteropathy caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) (Manning and Collins, 2001). MAP has been incriminated as the cause of Crohn's disease (CD) in human beings (Singh *et al.*, 2016). MAP infection is transmissible to the human beings through consumption of milk and milk products made from the milk of the infected animals.

Pasteurisation has been reported to be ineffective on the bacilli, thus increasing the threat of acquiring the disease from consumption of commercial milk and milk products (Patel and Shah, 2010).

India leads the world in milk production and cattle and buffaloes are the most significant contributors to the produce. So, diseases like JD, affect the economy not only by decreasing the milk production but also by posing a food safety concern rendering the milk unfit for human consumption. Odisha, a coastal state in the eastern starboard of India has never reported JD before (AICRP report 2015-16) but there have been incidences of the disease in various states of India including the neighbouring states of Andhra Pradesh and West Bengal (Ramalakshmi *et al.,* 2016, Bhutediya *et al.,* 2017). Purpose of the present pilot study was to establish presence / absence of *Mycobacterium avium* subsp. *paratuberculosis* in commercial milk and milk products sold in the local market of Bhubaneswar, Odisha.

Materials and Methods

Samples

A total of 17 samples comprising of commercial milk samples (n=6), milk products (Flavoured milk=3, Lassi=1, Curd=3) and milk powder (n=3) from six leading brands (Omfed=6, Amul=4, Milky Moo=3, Pragati Gold=2, Nestle=1 and Mother Dairy=1)were collected from Bhubaneswar, the capital city of Odisha and subjected to multiple tests for detection of MAP in the Microbiology Laboratory of Animal Health Division, CIRG, Makhdoom.

Processing of milk samples

Milk and milk products samples were used as such without centrifugation for separating whey, fat and sediment. Each of the milk and milk products was treated as separate sample and was screened by five tests and a positive result in either of the tests was considered as positive.

Approximately, 2.0 grams of milk powder (dairy creamer) samples were finely grounded in 10–12 ml of autoclaved distilled water to prepare a homogenized solution. This homogenized solution was used as 'test sample'. Commercial pasteurized milk, flavoured milk, lassi (sweetened milk) and curd were used as 'test sample' itself. Commercial milk products samples taken as the test samples were used as such or diluted with distilled water if required during the test.

ZN Staining

Smears of approximately 20 µl of the milk or milk product samples were prepared, air dried, heat fixed, stained by Ziehl Neelson's stain (Singh *et al.,* 2008) and slides were observed under microscope (100X oil immersion lens) for clumps of short pink acid fast bacilli indistinguishable to MAP.

Indirect Fluorescent Antibody Test (iFAT)

Test was performed as per Singh *et al.,* (2016a). Briefly, smears were prepared on clean slides from (20 µl commercial milk and milk products), air dried and heat fixed. Slides were dipped in solution of 30.0% H_2O_2 in 90.0% methanol (3:7 ratio) and incubated for 10 minutes at 37˚C, followed by a second dipping in phosphate-citrate buffer (2.1% citric acid and 3.56% disodium hydrogen phosphate in 100 ml triple distilled water, pH-5). Slides were then heated till boiling in microwave for 30 seconds (15 cycles) with rest of 20 seconds after each heating cycle (total time 10 minutes). Slides were then air dried at room temperature. Primary antibody (whey in ratio of 1:4 and serum in ratio of

1:50) in serum dilution buffer (1% BSA in PBST) was added over the smear and slides were then incubated for 1 hour at 37˚C in BOD incubator, followed by washing of slides in 1X PBS (3 times). Anti-species secondary antibody (FITC conjugate) was added in the ratio 1:750 in 1X PBS (pH-7.6). Slides were incubated in dark for 1 hour at 37˚C followed by washing for 5 times in 1X PBS and air drying in dark at room temperature. Finally, slides were mounted with glycerine and covered with cover slip and then observed immediately under fluorescent microscope. Slides positive for MAP infection exhibited green fluorescence while the slides that were negative were dark and without any fluorescence.

DNA isolation

DNA isolation from commercial milk was carried out as per Van Soolingen *et al.,* (1991) with some modifications. Briefly, to 500 µl commercial milk sample, 100 µl of lysis buffer (50 mM NaCl, 125 mM EDTA, 50 mM Tris-HCl; pH 7.6) was added and incubated at RT for 15 min. After that 100 µl of 24% sodium dodecyl sulfate (SDS) was added and incubated at RT for 10 min., followed by heating at 80°C for 10 min. Then, 325 µg of proteinase K was added to above sample and incubated at 55°C for 2 hrs., followed by addition of 115 µl of 5M NaCl and 93 µl CTAB-NaCl with proper mixing and incubated at 65°C for 30 min. Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added to sample and centrifuged at 15800 g for 5 min. After centrifugation, resulting aqueous phase from sample was transferred to sterilized Eppendorf tube and DNA was precipitated by adding 0.8 volume of chilled isopropanol and kept at -20°C for 2 hours. DNA was pelleted out by centrifuging tube at 15800 g for 10 min at 4°C and then supernatant was discarded. Finally, pellet was washed with 500 µl of 70% ethanol and re-suspended in 30 µl TE buffer/ nuclease free water and stored at -20°C.

IS900 PCR

IS900 PCR was performed on the extracted DNA from the milk and milk products samples according to the protocol of Vary *et al.,* (1999) and the visualisation of a product size of 229 bp by agarose gel electrophoresis of PCR products were considered positive for the presence of MAP bacilli.

IS*900* Real time PCR

Real Time (Rt) PCR was performed on the extracted DNA from the milk and milk products samples using the primers of Vary *et al.,* (1990). The reaction volume of 25µl was prepared with 0.5 µl of 10 pmole/µl forward and reverse primer each, 0.5 µl of Taq Polymerase (5 U/µl), 1.5 µl of $MgCl_2$ (25.0 mM), 2.5 µl of dNTPs (2.0 mM), 2.5 µl of SYBR Green I in Buffer with ROX (10X), 5-10 ng DNA template and the volume was made up to 25µl with HPLC H_2O. The reaction was performed as followed: initial denaturation at 94^0C for 5 min, with 37 cycles of denaturation at 94^0C for 20 sec, annealing at 56^0 C for 30 sec and extension at 72^0 C for 20 sec. Melt curve analysis was carried out by raising the temperature from 65°C to 90°C for 20 minutes with continuous imaging by CCD camera. The positive and negative results were derived from the standard curve generated by graphing the log of the DNA concentration present in specific samples versus the CT value (Cq) for the detection of presence of MAP infection.

Indigenous plate ELISA test (i_ELISA)

Test was performed as per Singh *et al.,* (2016b) and instead of whey, commercial milk and milk products as whole were used as test sample. Briefly, each well of flat bottom 96

well ELISA plate was coated with 0.1 µg of protoplasmic antigen in 100 µl of carbonate-bicarbonate buffer, (pH 9.6) per well and incubated at 4°C overnight. Plates were washed thrice with PBST (PBS with 0.05% Tween 20) followed by blocking in 100 µl of 3.0% skimmed milk in PBS, incubated for one hour at 37°C. Plates were washed three times with PBST and then 100 µl of commercial milk diluted in PBST with 1.0% BSA in ratio of 1:1 was added as sample in duplicate wells and incubated for 2 hrs at 37°C. Plates were washed thrice followed by addition of 100 µl of optimally diluted rabbit anti-bovine (1:6000 in 1X PBS) conjugate and again incubated for one hour at 37°C. Finally after five times washing, 100 µl of freshly prepared OPD substrate was added and incubated till colour developed (3-5 min) at 37°C. Absorbance was read at 450 nm in ELISA reader (i-Mark micro-plate reader, Biorad). Milk whey from weak and culture positive and healthy and culture negative cattle/buffaloes were used as positive and negative controls, respectively. Optical densities (OD) values were transformed and expressed as sample-to-positive (S/P) ratios (Collins, 2002).

Analysis of OD (Absorbance) Values

Sample to positive ratios and corresponding status of JD in animals was determined as per Collins (2002). The samples detected as low positive (LP), positive (P) and strong positive (SP) by S/P ratio were considered as positive for MAP infection in i-ELISA.

Results and Discussion

Johne's disease a chronic intestinal infection of ruminants caused by the acid fast bacilli of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and is detectable by a battery of tests. The gold standard for detection of MAP is bacterial culture which being less sensitive and requiring long incubation is limited in use. The utility of serological tests in diagnosis is limited due to low specificity and sensitivity, as immune response may not be detectable either due to anergy or late appearance in pathogenesis. Thus, no single test can identify all the MAP infected animals in a herd at a given time and the use of multiple tests has been advocated (Sharma *et al.,* 2008). For the first time 17 commercial milk and milk products(flavoured milk, curd, lassi, milk powder) sold in the local markets of Bhubaneswar were screened for the presence of MAP by five different tests i.e. milk microscopy, IS*900* PCR, IS*900* Real Time PCR, Indirect Fluorescence Agglutination test (i_FAT) and i-ELISA in this study.

Present pilot study is the first of its kind for the detection of presence of MAP in the commercial milk and milk products of Bhubaneswar. None of the 17 commercial milk and milk products samples tested positive by ZN staining, iFAT, IS*900* PCR, IS*900* real time PCR or i_ELISA in this study. However, milk i_ELISA classified 12 of the 17 samples as suspected/borderline (Table 1, 2 and 3). The results of the tests were in perfect agreement with each other. ELISA of milk samples proved to be the most sensitive test in detection of MAP infection.

The acid fast staining technique can detect 25% - 40% cases of Johne's disease (Gupta *et al.,* 2012) so probably ZN staining couldn't detect the MAP infection in the current study. The MAP bacilli is shed in milk by 12% of subclinically infected cows in a concentration of 2 to CFU per 50 ml milk (Sweeney *et al.,* 1994), so most likely negligible amount of bacilli present in milk and milk products escaped detection by PCR, Rt-PCR and iFAT. The manual method of DNA isolation used in this study and presence of PCR inhibitors in milk might have also hampered the detection of DNA by PCR and Rt-PCR (Garg *et al.,* 2015). The detection of negligible/less MAP

infection might also be attributed to the spectral nature of the disease with variable bacteriological, immunological and pathological situations which made diagnostic efficacy variable at different points of time during the course of disease (Barad *et al.,* 2014).

The i_ELISA on the commercial milk and milk products samples detected none of the samples as positive, however 12 out of 17 were suspected or borderline. The indirect ELISA though a simple, sensitive and cost effective 'herd screening test' (Sharma *et al.,* 2008) it missed 60.0% of true positives due to anergy (Garg *et al.,*, 2015) which goes in the favour of this study in which none of the samples tested positive for MAP infection in this study.

Although the milk i_ELISA Sample/Positive ratios obtained in the current study doesn't indicate positivity of any samples but the suspected status obtained in 70.59% (12/17) of the samples signifies the probable presence of a small titre of antibodies against the bacilli which might be due to animals in the current study being in stage-1 of infection of JD during which there is no excretion of the organism (Magombedze *et al.,* 2013).

Furthermore, negative results in the other tests do not rule out MAP infection since the success of the test depends upon the number of bacilli present in sample. Moreover the true prevalence of MAP may not be reflected in the results of the tests which having poor sensitivity are able to detect only clinical shedders of MAP (Garg *et al.,* 2015).

Table.1 Product-wise bio-load of MAP in commercial milk samples from Bhubaneswar, Odisha

Sn.	Sample profile	No. of Samples	ZN staining	ELISA	IS*900* PCR	Rt-PCR	I_FAT
1.	Commercial milk	6	0	P=0, S= 83.33%	0	0	0
2.	Flavoured milk	4	0	P=0, S= 100%	0	0	0
3.	Curd	4	0	P=0, S= 50%	0	0	0
4.	Milk Powder	3	0	P=0, S= 66.67%	0	0	0
5.	Total	17	0	P=0, S=70.59%	0	0	0

P=Positive in ELISA, S=Suspected in ELISA

Table.2 Commercial brand-wise bio-load of MAP in commercial milk samples from Bhubaneswar, Odisha

Sn.	Brand	No of Products	ZN staining	ELISA	IS*900* PCR	RT-PCR	I_FAT
1.	Omfed	6	0	P=0, S=66.67%	0	0	0
2.	Amul	4	0	P=0, S=100%	0	0	0
3.	Milky Moo	3	0	P=0, S=66.67%	0	0	0
4.	Pragati Gold	2	0	P=0, S=100%	0	0	0
5.	Nestle	1	0	P=0, S=0	0	0	0
6.	Mother Dairy	1	0	P=0, S=100%	0	0	0
7.	Total	17	0	P=0, S=70.59%	0	0	0

P=Positive in ELISA, S=Suspected in ELISA

Table.3 Determination of bioload of MAP in the commercial milk and milk products of Odisha

Sn.	Sample	ZN staining	I_ELISA	PCR	Rt-PCR	I_FAT
1	Omfed Sweet Curd	Negative	Negative	Negative	Negative	Negative
2	Amul Taaza Toned Milk	Negative	Suspected	Negative	Negative	Negative
3	Amul Spray	Negative	Suspected	Negative	Negative	Negative
4	Amul Kool Kesar	Negative	Suspected	Negative	Negative	Negative
5	Amul Kool Elaichi	Negative	Suspected	Negative	Negative	Negative
6	Elaichi milk	Negative	Suspected	Negative	Negative	Negative
7	Omfed Toned Milk	Negative	Suspected	Negative	Negative	Negative
8	Omfed Premium Milk	Negative	Suspected	Negative	Negative	Negative
9	Milky moo	Negative	Negative	Negative	Negative	Negative
10	Milky Moo Lassi	Negative	Suspected	Negative	Negative	Negative
11	Mother Dairy Milk	Negative	Suspected	Negative	Negative	Negative
12	Pragati Gold Milk	Negative	Suspected	Negative	Negative	Negative
13	Pragati Gold Mishti Dahi	Negative	Suspected	Negative	Negative	Negative
14	Meethi Mishti Dahi	Negative	Suspected	Negative	Negative	Negative
15	Omfed Dairy Whitner	Negative	Suspected	Negative	Negative	Negative
16	Nestle Everyday Milk powder	Negative	Negative	Negative	Negative	Negative
17	Plain Curd	Negative	Negative	Negative	Negative	Negative

In absence of previous reports or vaccinations against JD, the detection of or borderline titre in some samples in this study points towards the possible recent exposure of the animals to MAP or past infection of the animals with the organism which lead to the less titre of antibodies. Gupta *et al.*, (2012) reported that the presence of specific antibodies to MAP in serum and milk samples from diarrheic and/or anaemic animals indicates sub clinical stage of infection in the herd which might have been the case in this study. JD has been reported to be endemic in various regions of India with the recent reporting from the states of Bengal (Bhutediya *et al.*, 2017) and Telengana (Ramalakshmi *et al.*, 2016) bordering Odisha. So the infection might be introduced to the animals due to transboundary movement of the animals. The borderline titre of antibodies against MAP in i_ELISA on the other hand, does not ascertain MAP infection in Odisha as the specificity of ELISA in diagnosing MAP has been found to be low by previous researchers (Collins *et al.*, 2002). The sample size being low in this study also impeded the arrival at a conclusion about the status of MAP infection in Odisha.

The bioload of MAP in the milk and milk product samples from Bhubaneswar, Odisha was detected as negligible in the present study. The results fall in agreement with a previous study in which a total of 157 animals were tested for JD by single intradermal test in Cuttack, Bargarh, Puri and Jagatsinghpur districts of Odisha wherein all the animals were found negative for Johne's disease (AICRP Annual Report 2015-16). Similarly, a low seroprevalence of 2.82% was recorded among large ruminants of Krishna district of Andhra Pradesh (Didugu *et al.*, 2015). Contrarily, Bhutediya *et al.*, (2017) reported that out of 191 animals tested by Delayed Type Hypersensitivity, 57 (29.8%) were found to be positive in comparison to 72 (37.7%) positive samples detected by ELISA for paratuberculosis in unorganised and organised dairy herds in West Bengal. A

higher prevalence of 18.33 % (55/300) was also recorded in milk samples for antibody against Johne's disease by indirect ELISA in the cattle population of south-western Bangalore, Karnataka (Gupta *et al.,* 2012). The present study also disagrees with several workers who have reported the detection of MAP in commercial milk and milk products of India (Stephen *et al.,* 2016; Raghuvanshi *et al.,* 2013). The i_ELISA of milk sample was found to be most sensitive compared to microscopy, IS*900* PCR and Rt-PCR in this study. Garg *et al.*, (2015), however reported sensitivity of microscopy was the highest followed by milk ELISA, serum ELISA, and milk PCR.

The present study detected negligible infection of MAP in the commercial milk and milk products of Bhubaneswar, Odisha. Since, the commercial milk and milk products are prepared from pooled milk, the tests conducted on them are helpful to know the status of the disease in the animal population.

The results of ELISA signal the presence of very low level of antibodies in the sample, thus interplay of the organism is suspected in the border areas of the state. This is the first pilot scale study in Odisha in recent times, wherein unlike other studies in Northern part of India, though the level of MAP infection detected was very low or insignificant, it may be due to low sample size. However, study recommended that to verify the disease free status of the Odisha with respect to JD, there is need for a more extensive and systematic sampling along with the detection by multiple tests including culture and bio-typing of the MAP isolates obtained to know the biotype profile of the MAP in the state of Odisha.

Acknowledgement

The authors are grateful to ICAR-NASF for funding this study, Director, CIRG, Mathura and Dean, College of Veterinary and Animal Science, O.U.A.T Bhubaneswar for granting permission to carry out this study.

References

Annual Report 2015-16. All India Coordinated Research Project on Animal Disease Monitoring and Surveillance (AICRP on ADMAS) ICAR-National Institute of Veterinary Epidemiology and Disease Informatics Yelahanka, Bengaluru – 64. Accessed on 14.7.2017.

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.

Bhutediya, J.M., Dandapat, P., Chakrabarty, A., Das, R., Nanda, P.K., Bandyopadhyay, S. and Biswas, T.K. 2017. Prevalence of paratuberculosis in organized and unorganized dairy cattle herds in West Bengal, India. *Veterinary World*, 10: 574-579.

Collins, M.T. 2002. Interpretation of a Commercial Bovine Paratuberculosis Enzyme-Linked Immunosorbent Assay by Using Likelihood Ratios. *Clin. Diagn. Lab. Immunol.*, 9: 1367-1371.

Didugu, H., Ramanipushpa, R.N., Reddy, C.E.N, Devi, M.A. and Bhaskraramarajusagi, S. 2015. *Mycobacterium avium* subspecies *paratuberculosis* among bovines in Krishna district of Andhra Pradesh, India: a serological survey. *Indian J. Anim. Health*, 54: 149- 154.

Garg, R., Patil, P.K., Singh, S.V., Sharma, S., Gandham, R.K., Singh, A.V., Filia, G.,

Singh, P.K., Jayaraman, S., Gupta, S., Chaubey, K.K., Tiwari, R., Saminathan, M., Dhama, K. and Sohal, J.S. 2015. Comparative evaluation of different test combinations for diagnosis of *Mycobacterium avium* subspecies *paratuberculosis* infecting dairy herds in India. *Bio. Med. Res. Int.*, 2015: 983978.

Gupta, A., Rani, S.M., Agrawal, P. and Gupta, P.K. 2012. Sero-Prevalence of Paratuberculosis (Johne's Disease) in Cattle Population of South-Western Bangalore Using ELISA Kit. *Open Journal of Veterinary Medicine*, 2: 196-200.

Hermans, P.W., de Haas, P.E., Soll, D.R. and van Embden, J.D., 1991. Occurrence and stability of insertion sequences in *Mycobacterium tuberculosis* complex strains: evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. *J. Clin. Microbiol.*, 29: 2578.

Magombedze, G., Ngonghala, C.N. and Lanzas, C. 2013. Evaluation of the "iceberg Phenomenon" in Johne's disease through mathematical modeling. *PLoS ONE*, 8:1-11.

Manning, E.J.B. and Collin, M.T. 2001. *Mycobacterium avium* subsp. paratuberculosis: pathogen, pathogenesis and diagnosis. *Rev. sci. tech. Off. int. Epiz*, 20: 133-150.

Patel, A. and Shah, N. 2011. *Mycobacterium avium* subsp paratuberculosis Incidences in milk and milk products, their isolation, enumeration, characterization, and role in human health. *Journal of Microbiology, Immunology and Infection*, 44: 473-479.

Raghuvanshi, T., Singh, S.V., Sharma, R.B., Gupta, S., Chaubey, K.K., Kumar, N. and Dhama, K. 2013. Identification of *Mycobacterium avium* subspecies *Paratuberculosis* in fresh cheese (paneer) from goat herds endemic for Johne's disease. *J. Inf. Mol. Biol*., 1: 46 – 48.

Ramalakshmi, B., Mukherjee, F., Prasad, A., Bahekar, V. S., Rana, S. K., Sharma, G. K. and Srinivasan, V. A. 2016. Isolation and molecular confirmation of *Mycobacterium avium* subspecies *paratuberculosis* in cattle and buffaloes from three states of India. *Bulg. J. Vet. Med*., 19: 189–199.

Sharma, G., Singh, S.V., Sevilla, I., Singh, A.V., Whittington, R.J., Juste, R.A., Kumar, S., Gupta, V.K., Singh, P.K., Sohal, J.S. and Vihan, V.S. 2008. Evaluation of indigenous milk ELISA with m–culture and m–PCR for the diagnosis of bovine Johne's disease (BJD) in lactating Indian dairy cattle. *Res. Vet. Sci*., 84: 30-7.

Singh A. V., Chauhan D. S., Singh S. V., Kumar V., Singh A., Yadav A. and Yadav V. S. 2016. Current status of *Mycobacterium avium* subspecies *paratuberculosis* infection in animals and humans in India: What needs to be done? *Indian J Med. Res.*, 144: 661-671.

Singh, A.V., Singh, S.V., Makharia, G.K., Singh, P.K. and Sohal, J.S. 2008. Presence and characterization of *Mycobacterium avium* subspecies *paratuberculosis* from clinical and suspected cases of Crohn's disease and in the healthy human population in India. *Int. J. Infect. Dis.*, 12: 190-197.

Singh, S. V., Stephen, B. J., Singh, M., Gupta, S., Chaubey, K. K., Sahzad, Jayaraman, S., Aseri, G. K., Sohal, J. S., Bhatia, A. K., Pachoori, A., Chauhan, J. and Dhama, K. 2016b. Evaluation of new 'indigenous milk dot-ELISA' as 'Field Test' vis-à-vis milk plate-ELISA for the detection of *Mycobacterium avium* sub species *paratuberculosis* infection in

lactating domestic livestock. *Indian Journal of Biotechnology*, 15: 166-171.

Singh, S.V., Stephen, B.J., Singh, M., Gupta, S., Chaubey, K.K., Sahzad, Jayaraman, S., Sachan, T.K., Aseri, G.K., Jain, M., Sohal, J.S. and Dhama, K. 2016a. Evaluation of Indirect Flurorescent Antibody Test (iFAT) as the 'mass screening test' for the diagnosis of *Mycobacterium avium* subspecies *paratuberculosis* infection in the milk samples of lactating domestic livestock. *J. Exp. Biol. Agri. Sci.*, 4:533-540.

Stephen, B.J., Singh, M., Singh, S.V., Gupta, S., Chaubey, K.K., Sahzad, Jayaraman, S., Jain, M., Sohal, J.S., Mukartal, S.Y., Dhama, K. 2016. 'Bio-contamination' estimates of *Mycobacterium avium* subspecies *paratuberculosis* in fresh cottage cheese (Paneer) sold in rural, semi-urban and peri-urban regions of South Uttar Pradesh using multiple diagnostic tests. *Adv. Anim. Vet. Sci.*, 4: 441-448.

Sweeney, R.W., Whitlock, R.H., Buckley, C.L., Spencer, P., Rosenberger, A.E. and Hutchinson, L.J. 1994. Diagnosis of *paratuberculosis* in dairy cattle, using enzyme-linked immunosorbent assay for detection of antibodies against *Mycobacterium paratuberculosis* in milk. *Am. J. Vet. Res*., 55: 905–909.

Vary, P.H., Andersen, P.R., Green, E., Hermon-Taylor, J. and McFadden, J.J. 1990. Use of highly specific DNA probes and the polymerase chain reaction to detect *Mycobacterium paratuberculosis* in Johne's disease. *J. Clin. Microbiol.*, 28: 933-937.

How to cite this article:

Sangram Biswal, Shaswati Subhadarsini Pany, Shoor Vir Singh, Niranjana Sahoo, Manju Singh, Sourabh Gupta and Kundan Kumar Chaubey. 2018. Bio-detection of *Mycobacterium avium* subspecies *paratuberculosis* in the Commercial Milk and Milk Products Sold in Bhubaneswar, Odisha, India. *Int.J.Curr.Microbiol.App.Sci.* 7(01): 753-761.
doi: https://doi.org/10.20546/ijcmas.2018.701.091