



Assessment of lipid profile and acute phase protein in *Mycobacterium avium* subspecies *paratuberculosis* infected and healthy goats

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ABSTRACT

Present study is based on 24 goats that were located in goat herds endemically infected with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection. Objective of the study was to access the variations in the lipid profile and acute phase proteins in the serum samples driven from non-infected (negative and physically healthy) and infected (positive and physically weak) goats with MAP infection, cause of incurable Johne's disease (JD) in domestic livestock. Infected goats had significantly higher cholesterol and albumin levels and significantly 'reduced level' of high density lipoprotein (HDL) and 'reduced level' of the density of lipoproteins (LDL) in comparison to non-infected goats. Lipid profile and acute phase proteins could be further explored for their significance in pathogenesis and diagnosis of JD in domestic livestock including goats.

Key words: Acute phase proteins, Johne's disease, Lipid profiling, *Mycobacterium avium*, Paratuberculosis

Johne's disease (JD) is chronic granulomatous enteritis caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) that can infect both animals and human population. Human population is acquiring infection through milk and milk products made from pasteurized milk as MAP is not in-activated during pasteurization and pose a serious public health problem (Grant *et al.* 2008, Singh *et al.* 2019).

Acute phase proteins are a class of proteins such as albumin, C-reactive protein, ceruloplasmin, transferrin, etc, whose plasma concentrations increase or decrease in response to various inflammatory and neoplastic conditions (Jain *et al.* 2011). Binding of C-reactive protein (CRP) to pathogen also interacts with specific receptors on phagocytes, induces anti-inflammatory cytokine production, and modulates neutrophil function. Ceruloplasmin levels increase in the inflammatory, and neoplastic diseases and could have a prognostic role in the diagnosis of the infections that are persistent or recurrent (Natesha *et al.* 1992).

Cholesterol is known to regulate hormones and basic

cellular metabolism in the body (Hu *et al.* 2010). LDL and HDL, transport, cholesterol back and forth between tissues and liver (Chien *et al.* 2005). Ability of macrophages to uptake *Mycobacterium* is compromised due to a low cholesterol content of their cell membrane and thus represents a key defect in the host defence system against tuberculosis (Kaul *et al.* 2004). Lipids and lipoproteins play a role in host defence (Khovidhunkit *et al.* 2004). Cholesterol, HDL, and LDL levels are known to have potential for predictors of clinical outcome (Van Gorp *et al.* 2002). Despite ability of lipoprotein profile to be able to serve as predictor of clinical outcome of infections; its role has not yet been elucidated in goats afflicted with JD. We speculate that assessment of acute phase proteins and lipid profiling in host sera could aid in assigning clinical stage of JD.

Keeping in view all these facts, present study was conducted to compare the levels of serum lipid profile and acute phase proteins in goats infected with MAP to those of apparently healthy goats (Nielsen and Toft 2008).

MATERIALS AND METHODS

Compliance with ethical standards: All animal studies have been approved by the Institute Animal Ethics Committee (Registration number is 207 with CPCSEA) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments (BMJ 1996). All the persons involved in the study gave their consent to their inclusion in the study.

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Sample collection: Of the 24 goats sampled, 12 were apparently healthy and 12 were infected suffering from clinical JD and were also confirmed by ELISA, PCR and Microscopy tests. The age groups of these goats were 12 months and above (adults). Goats in our animal health livestock shed were received from different goat units (6) on regular basis, whenever goats were not responding to primary treatment against diarrhoea or weakness, since JD was endemic in these six goat units. Goat herds (24) sampled were located at Central Institute for Research on Goats (CIRG), Makhdoom (Uttar Pradesh) and Mhow, Madhya Pradesh (India) (Table 1). Adult goats of Barbari, Sirohi and Jakhrana, Non-Descript breeds were sampled and were clinical to advance stage of the disease (JD) with respect to physical condition. Goats located at CIRG were fed intensively by providing concentrate pellets, 6 h grazing, dry fodder (*ad lib.*), tree lopping and harvested green fodder. Despite intensive feeding of goats, some of the goats were getting weak and emaciated with or without diarrhoea on regular basis and were either culled or shifted to shed belonging to Health division. Serum were separated and stored at -20°C till further analysis. 'Indigenous ELISA kit' was used to estimate sero-status (negative or JD positive). Blood and faeces were screened by PCR and microscopy for the confirmation of JD (Singh *et al.* 2013). Goats positive in the above three tests, twice at one month apart were considered positive for MAP infection. Serum samples from apparently healthy goats were found negative in 3 tests, were taken as negative controls.

Microscopic examination (Acid Fast Staining): Test was performed as per Singh *et al.* (2013). Representative MAP positive and negative samples are depicted in Fig. 1.

ELISA: ELISA was performed as per method of Milner (1987) using plasma as samples. Analysis of OD values: S/P ratio value = [(Sample OD – Negative OD) / (Positive OD – Negative OD)]. Sample to positive ratios were derived to estimate corresponding status of JD in goats as per Collins

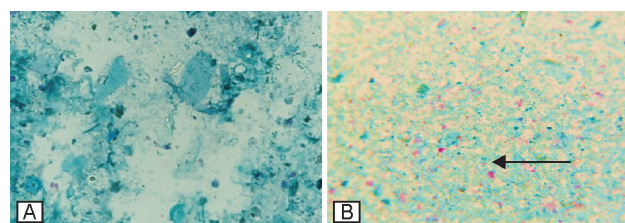


Fig. 1. Microscopic examination of acid fast bacilli (MAP) under oil immersion $\times 100$. (A) Absence of MAP bacilli (B) Arrow indicates the presence of MAP bacilli.

Table 2. Animals in positive and strong positive categories of S/P ratio were taken as 'Positive'

Calculated value of S/P Ratio	Status of Johne's disease in animals	Status
0.00–0.09	Negative (N)	Negative
0.10–0.24	Suspected or Borderline (S)	
0.25–0.39	Low Positive (LP)	
0.4–0.99	Positive (P)	Positive
1.0–10.0	Strong Positive (SP)	

(2002).

Isolation of DNA from faeces and blood and IS900 PCR: Isolation of DNA from faecal and blood samples was done as per the method of Singh *et al.* (2013) and Singh *et al.* (2010), respectively. IS900 PCR was performed as per Marsh *et al.* (1999) (using P-90B and P-91B primers (Singh *et al.* 2013)). 'MAP strain' positive control DNA was provided by the Microbiology Laboratory, Central Institute for Research on Goats (CIRG, Makhdoom, India). Presence and yield of specific PCR product (413 bp) was analysed by 1.0% agarose gel electrophoresis

Ceruloplasmin, Albumin, HDL, LDL, Cholesterol, C reactive protein estimations: Ceruloplasmin was estimated as per the method of Wolf *et al.* (2006). MAP Positive and negative serum samples were assayed photometrically by

Table 1. Data represent the animal specification and health status of MAP positive and negative animal

MAP positive goats			MAP negative goats		
Animal no.	Animal physical status and place of sampling	Breed	Animal no.	Animal physical status and place of sampling	Breed
S8669	2+, CIRG, Mathura	Sirohi	117	4+, CIRG, Mathura	Barbari
BE248	2+, CIRG, Mathura	Barbari	435	3+, CIRG, Mathura	Non-Descript
B1530	3+, CIRG, Mathura	Barbari	153	4+, CIRG, Mathura	Barbari
SE842	2+, CIRG, Mathura	Sirohi	175	4+, CIRG, Mathura	Barbari
BE250	3+, CIRG, Mathura	Barbari	182	3+, CIRG, Mathura	Barbari
1593	3+, Mhow, MP	Non-Descript	116	3+, Barbari	Barbari
1555	2+, Mhow, MP	Non-Descript	123	4+, CIRG, Mathura	Barbari
1604	2+, Mhow, MP	Non-Descript	181	4+, CIRG, Mathura	Barbari
1558	3+, Mhow, MP	Non-Descript	180	3+, CIRG, Mathura	Barbari
1579	2+, Mhow, MP	Non-Descript	166	4+, CIRG, Mathura	Barbari
Goat-1 PC(GHB216)	2+, CIRG, Mathura	Barbari	Goat-1 NC	4+, Kurkunda, Mathura	Non-Descript
Goat-2 PC(GHB126)	2+, CIRG, Mathura	Barbari	Goat-2 NC	4+, Kurkunda, Mathura	Non-Descript

4+ (Healthy); 3+ (Weak); 2+ (Weak and Suspected for JD); 1+ (Clinical cases of JD).

semi-automatic 'Biochemical Analyzer' (AGAPPE) using the commercial kits purchased from ERBA diagnostics Mannheim GmbH, Germany.

Statistical Analysis: Statistical analysis was done by Prism Pad software, USA

RESULTS AND DISCUSSION

Study was undertaken with respect to reports that lipids and lipoproteins play important role in host defences and immune responses against infections (Netea *et al.* 2009, Aora and Borah 2017). We attempted to assess differences in the concentration of lipid profiles (Cholesterol, LDL, and HDL) and acute phase proteins (Albumin, ceruloplasmin, C-reactive protein) between goats infected and un-infected with MAP.

Mean $\pm$ SEM values of cholesterol (Fig. 2A), LDL (Fig. 2B), HDL (Fig. 2C), albumin (Fig. 2D), C-reactive protein (Fig. 2E) and ceruloplasmin (Fig. 2F) are shown.

Significantly higher concentration of cholesterol (51.08 ± 4.22 mg/dL) was recorded in MAP positive animals compared to MAP negative goat herd which was 27.93 ± 3.55 mg/dL. In line with this, hypercholesterolemia impairs the initiation of adaptive immune response to tuberculosis and increased lipid levels aids to *M. tuberculosis* infections (Martens *et al.* 2008). In the battle between pathogen and the host, microbes utilize the lipids / lipo-proteins derived from the host to facilitate their survival and proliferation. For example, *M. tuberculosis* is known to catabolise cholesterol as energy source, which might facilitate the ability of this microbe to survive in macrophages (Feingold

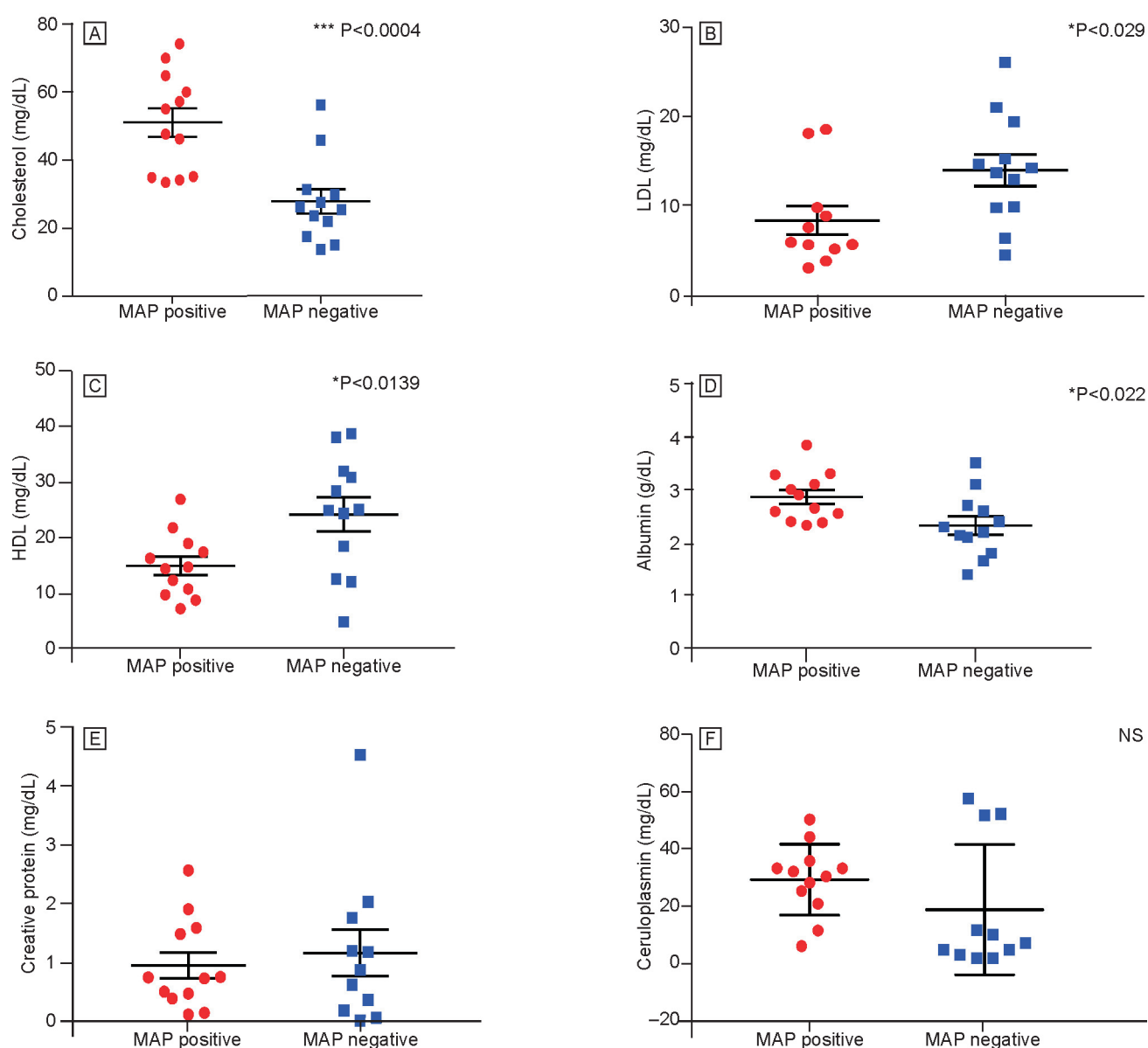


Fig. 2. Lipid profile and acute phase proteins in MAP positive and negative animals.

and Grunfield 2012). Hypercholesterolemia led to increased mortality of mice infected with *M. tuberculosis* (Martens *et al.* 2008). Significantly high triglyceride levels in MAP positive goats have been reported by our group (Sharma *et al.* 2017). Thus by identifying these interactions, it may be possible to alter lipid/lipoprotein metabolism and generate favourable conditions for the host.

Interestingly levels of LDL (8.32 ± 1.61 mg/dL) and HDL (14.90 ± 1.66 mg/dL) were significantly lower in goats infected with MAP as compared to negative goats where the values were 13.97 ± 1.77 mg/dL and 24.15 ± 3.03 mg/dL respectively.

Several studies have reported that inflammation and infection are associated with decreased LDL (Khovidhunkit *et al.* 2004). In the present study also, we observed significant reduction in LDL levels in goats infected with MAP. Precise mechanism by which inflammation and infection decreases LDL and HDL levels is uncertain and is likely to involve multiple mechanisms (Khovidhunkit *et al.* 2004). Reduction in apolipoprotein A-I synthesis in the liver occurring during inflammation have been suggested to contribute to the decreased formation of HDL.

Inflammation results in decrease in Lecithin cholesterol acyl transferase (LCAT) which in turn leads to diminished cholesterol ester formation, thus hindering normal HDL formation. This ends up in decreased cholesterol contents of HDL. In addition, following infection, cytokines such as interleukin- 1β , interleukin-6 also play major role in altering the stability and metabolism of lipoproteins. For instance, cytokine mediated enhanced enzymatic activity of secretory phospholipase A2 (sPLA2) and endothelial cell lipase, which metabolize key constituents of HDL, can modify HDL levels. Given the intricacy of lipoprotein metabolism, multiple pathways may be involved in subsiding HDL levels during infection and inflammation. Mice over-expressing sPLA2 have reduced HDL concentrations (De Beer *et al.* 2000) and HDL from these mice is catabolised more rapidly than HDL from normal mice. Thus it is possible that in our study, infection induced cytokine mediate higher lipase activity and thus reduced HDL levels that we have observed in MAP positive animals.

Infection can also mediate adjustments in lipid and lipoprotein metabolism in order to reshuffling of nutrient towards the immune cells involved in host defence and tissue repair (Khovidhunkit *et al.* 2004). For example, decreased reverse cholesterol transport has been reported following infection (both viral and bacterial) that helps in cholesterol conservation in immune cells such as macrophages (Sammalkorpi *et al.* 1988).

Upon infection, cytokines have been linked with the lipoprotein profile (Shaikh PZ). Tumour necrosis factor has been attributed to the cytokine responsible for coordination of both immune and inflammatory responses (Sharma and Thomas 2013).

Albumin levels were significantly higher (2.86 ± 0.113 g/dL) in infected goats compared to non-infected MAP negative goat herd (2.325 g/dL), which is surprising because

albumin concentration (negative acute phase protein) decreases following infections. True over-production of albumin is not known to occur in any animal. Therefore, any rise in albumin is only a relative hyper-albuminemia due to hemo-concentration as a result of water loss and dehydration. Higher albumin levels in JD positive cases might be due to the dehydration resulting from diarrhoea.

In cattle, weight loss despite adequate feeding accompanied by chronic diarrhoea are standard clinical signs of Johne's disease (Singh *et al.* 2016). However, unlike cattle, diarrhoea is not a consistent or cardinal feature of goat paratuberculosis (Marnelln *et al.* 2005). Hyperprotenemia has been shown to occur in JD positive goats (Sharma *et al.* 2017). However, increased albumin levels in JD positive goats warrants further investigations.

In the current study, no differences were observed in C-reactive protein and ceruloplasmin levels among the MAP positive and negative goats. We speculate that since paratuberculosis is a chronic disease, it is conceivable that T cell exhaustion might possibly occur as a result of which diminished cytokine production by exhausted T cells could be responsible for the unaltered levels of the acute phase proteins. It would be worth estimating the levels of pro-inflammatory cytokines in JD positive and negative animals. However, given the fact that at the time of sampling, JD was endemic in domestic goat population and goats were in different stages of disease incubation and moreover age, sex and physical status of the animals might also influence the parameters observed.

Thus it could be concluded that goats infected with MAP had significantly higher cholesterol and albumin levels and significantly lower HDL and LDL levels as compared to the un-infected goats. Further studies with large number of JD positive and negative goats for their physical status, lipid profile and acute phase proteins could be explored for their role in pathogenesis and diagnosis of Johne's disease in domestic livestock.

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REFERENCES

- Bora K and Borah P. 2017. Plasma lipoproteins as crucial components of host defence against infections. *Advances in Lipoprotein Research* 73-92. TurgayIsbir, IntechOpen, DOI: 10.5772/67601.
- Chien J Y, Jerng, J S, Yu C J and Yang P C. 2005. Low serum level of high density lipoprotein cholesterol is a poor prognostic factor for severe sepsis. *Critical Care Medicine* 33: 1688-93.

- Collins M T. 2002. Interpretation of a commercial bovine paratuberculosis enzyme linked immunosorbent assay by using likelihood ratio. *Clinical and Diagnostic Laboratory Immunology* **9**: 1367–71.
- De Beer F C, Connell P M, Yu J, de Beer M C, Webb N R and van der Westhuyzen D R. 2000. HDL modification by secretory phospholipase A2 promotes scavenger receptor class B type I interaction and accelerates HDL catabolism. *Journal of Lipid Research* **41**: 1849–57.
- Feingold K R and Grunfeld C. 2012. Lipids: a key player in the battle between the host and microorganisms. *Journal of Lipid Research* **53**(12): 2487–89.
- Hu J, Zhang Z, Shen W and Azhar S. 2010. Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. *Nutrition and Metabolism (Lond)* **7**: 47.
- Jain S, Gautam V and Naseem S. 2011. Acute-phase proteins: As diagnostic tool. *Journal of Pharmacy and Bio allied Sciences* **3**(1): 118–27.
- Kaul D, Anand P K and Verma I. 2004. Cholesterol-sensor initiates *M. tuberculosis* entry into human macrophages. *Molecular and Cellular Biochemistry* **258**(1–2): 219–22.
- Khovidhunkit W, Kim M S, Memon R A, Shigenaga J K, Moser A H and Feingold K R and Grunfeld C. 2004. Effects of infection and inflammation on lipid and lipoprotein metabolism: Mechanisms and consequences to the host. *Journal of Lipid Research* **5**(7): 1169–96.
- Marsh I, Whittington R and Cousins D. 1999. PCR-restriction endonuclease analysis for identification and strain typing of *Mycobacterium avium* subsp. *paratuberculosis* and *Mycobacterium avium* subsp. *Avium* based on polymorphisms in IS1311. *Molecular and Cellular Probes* **13**: 115–26.
- Martens G W, Arian M C, Lee J, Ren F, Vallerskog T and Kornfeld H. 2008. Hypercholesterolemia impairs immunity to tuberculosis. *Infection and Immunity* **76**(8): 3464–72.
- Milner A R. 1987. Analysis by ELISA and Western blotting of antibody reactivities in cattle infected with *M. paratuberculosis* after absorption of serum with *M. phlei*. *Research in Veterinary Science* **42**: 140–4.
- Netea M G, Joosten L A, Keuter M, Wagener F, Stalenhoef A F, van der Meer J and Kullberg B J. 2009. Circulating lipoproteins are a crucial component of host defense against invasive *Salmonella typhimurium* infection. *PLoS One* **4**(1): e4237.
- Natesha R K, Natesha R, Victory D, Barnwell S P and Hoover E L. 1992. A prognostic role for ceruloplasmin in the diagnosis of indolent and recurrent inflammation. *Journal of National Medical Association* **84**(9): 781–4.
- Sammalkorpi K, Valtonen V, Kerttula Y, Nikkila E and Taskinen M R. 1988. Changes in serum lipoprotein pattern induced by acute infections. *Metabolism* **37**: 859–65.
- Shaikh P Z. 2011. Cytokines and their physiologic and pharmacologic functions in inflammation: A review. *International Journal of Pharmacy and Life Sciences* **2**(11): 1247–63.
- Sharma S and Thomas P G. 2014. The two faces of heterologous immunity: protection or immunopathology. *Journal of Leukocyte Biology* **95**(3): 405–16.
- Sharma S, Singh S V, Sangwan N, Singh V and Chaubey K K. 2017. Metabolic adjustments as signatures for pathogenesis and diagnosis of Johne's disease in goats. *Haryana Veterinarian* **56** (2): 179–83.
- Singh A V, Chauhan D V, 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 Journal of Medical Research* **144**(5): 661–71.
- Singh S V, Singh P K, Kumar H, Sohal J S and Singh A V. 2010. Evaluation of blood PCR with blood culture, ELISA and microscopic examination for the diagnosis of *Mycobacterium avium* subspecies *paratuberculosis* infection in goat kids. *Indian Journal of Small Ruminants* **16**: 67–73.
- Singh S V, Singh P, Gupta S, Chaubey K, Singh B, Kumar A, Singh A V and Kumar N. 2013. Comparison of microscopy and blood-PCR for the diagnosis of clinical Johne's disease in domestic ruminants. *Iranian Journal of Veterinary Research* **14**(4): 345–49.
- Singh S V, Singh P K, Singh A V, Sohal J S, Kumar N, Chaubey K K, Gupta S, Rawat K D, Kumar A, Bhatia A K, Srivastav A K and Dhama K. 2014. Bio-load and bio-type profiles of *Mycobacterium avium* subspecies *paratuberculosis* infection in the farm and farmer's herds/ flocks of domestic livestock: A 28 years study (1985–2013). *Transboundary and Emerging Diseases* **61**: 43–55.
- Singh M, Singh S V, Gupta S, Chaubey K K, Stephan B J, Sohal J S and Dutta M. 2018. 'Nano-immuno test' for the detection of live *Mycobacterium avium* subspecies *paratuberculosis* bacilli in the milk samples using magnetic nano-particles and chromogen. *Veterinary Research Communications* **42**(3): 183–94.
- The Nuremberg Code (1947). 1996. *British Journal of Medicine* **313**:1448.
- Van Gorp E C, Suharti C, Mairuhu A T, Dolmans W M, van Der Ven J, Demacker P N and van Der Meer J W. 2002. Changes in the plasma lipid profile as a potential predictor of clinical outcome in dengue hemorrhagic fever. *Clinical Infectious Diseases* **34**(8): 1150–3.
- Wolf T L, Kotun J and Meador-Woodruff J H. 2006. Plasma copper, iron, ceruloplasmin and ferroxidase activity in schizophrenia. *Schizophrenia Research* **86**(1–3): 167–71.