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## *Mycobacterium avium* subspecies *paratuberculosis* – an important food borne pathogen of high public health significance with special reference to India: an update

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### ABSTRACT

This review underlines the public health significance of ‘Indian Bison Type’ of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and also its potential as ‘zoonotic infection’. In the absence of control programs, bio-load of MAP is increasing and if we take total population of animals (500 million plus) and human beings (1.23 billion plus) into account, the number of infected animals and human beings will run into millions in India. Our research on screening of over 26,000 domestic livestock for MAP infection using 4 different diagnostic tests (microscopy, culture, ELISA and PCR), during last 31 years has shown that the average bio-load of MAP in the livestock population of India is very high (cattle 43%, buffaloes 36%, goats 23% and sheep 41%). ‘Mass screening’ of 28,291 human samples between 2008–2016 revealed also high bio-load of MAP. It has been proved that MAP is not in-activated during pasteurization and therefore live bacilli are continuously reaching human population by consumption of even pasteurized milk and other milk products. Live bacilli have also been recovered from meat products and the environment thus illustrating the potential of MAP as pathogen of public health concern. However, at present, there is inadequate scientific evidence to confirm a conclusive link between MAP infection and Johne’s disease in ruminants and some cases of Crohn’s disease in human beings.

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## 1. Introduction

*Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a major animal pathogen which has inflicted huge losses to the livestock industry in India and globally (Vinodhkumar et al. 2013; Rawat et al. 2014; Bauman et al. 2016). Johne’s disease (JD) caused by MAP is endemic in domestic livestock population wherever investigated (Singh et al. 2014a). In a population suffering from sub-clinical to clinical disease animals continue to shed MAP bacilli in their milk and feces (Streeter et al. 1995; Shankar et al. 2010). Contamination in the milking parlor is one of the commonest sources of contaminating milk with MAP (Nacy and Buckley 2008). MAP is a major food borne pathogen and has been reported to spread to young animals born to infected mothers of each of domestic livestock species as well as human beings (Waddell et al. 2008; Naser et al. 2009; Kumar et al. 2010; KuKanich et al. 2013; Hussain et al. 2015), through consumption of milk and milk products. It is very difficult to diagnose the MAP infection in the early stage of the disease

(sub-clinical). MAP may colonize in the intestines of infected animals for years without exhibiting overt symptoms (clinical disease). However, sub-clinically infected animals continue to shed MAP bacilli in their milk (Shankar et al. 2010) and feces, thereby contaminating pastures, environment and food chain for long time (Singh et al. 2012b). Of note, live MAP bacilli have also been recovered from pasteurized milk (Ellingson et al. 2005, Shankar et al. 2010), infant formulation made from pasteurized milk products (Hruska et al. 2011, Acharya et al. 2017), surface water and soil samples (Singh et al. 2012b), cow manure “lagoons” that leach into surface water and municipal / tap water (Collins 2003), thus providing MAP multiple routes of transmission to infect human population.

Cow manure in solid and liquid forms is applied as fertilizer in agricultural land (Grewal et al. 2006; Gill et al. 2011), plants (upper greens, roots) and soil (surface and depth of 80 cm from plant roots) of agricultural and grazing areas hold MAP for longer period of time (Kaevska et al. 2014). MAP being thermotolerant

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survives commercial pasteurization (72 °C for 15–30 s) temperature (Grant et al. 1998) as well as high salt concentrations (Gao et al. 2002). Filtration, cold shock, hydrostatic pressure and pulsed electric fields procedures have already been tested on milk (Rowan et al. 2001). This superior survival efficiency and dormancy allows the pathogen to be reach insidiously to human population (Whittington et al. 2005). Present paper reviews the bio-load of MAP in domestic livestock, public health significance and potential threat to human population by presence of MAP in the natural environment. Paper also dwells upon the survival abilities, pathogenicity and possible association with number of human diseases and a potential zoonotic disease with major consequences on human health.

## 2. MAP in dairy products

Majority of MAP based infections occur through food producing domestic livestock. Milk derived from infected animals is generally contaminated before harvesting (milking). During harvesting of milk, there is a huge scope of contamination of milk with fecal matter (Rademaker et al. 2007). Early studies were directed at investigating the presence of MAP in live and dead condition in raw and pasteurized milk using PCR technology (Ellingson et al. 2005). Irene Grant and co-workers have extensively screened milk and milk products in UK and published results in 2002. MAP presence was confirmed by culture in 1.6 and 1.8% of raw and pasteurized milk, respectively. Grant et al. (2002a) concluded that live MAP is infrequently present in commercially pasteurized milk in the UK. The UK Ministry of Agriculture, fisheries and Food has started research that was continued by Food Standards Agency, which reported 1.7% prevalence of live MAP from pasteurized milk on retail sale (Food Standard Agency UK 2000), when pasteurized cow's milk was screened by IS900 PCR. Field studies were done on retail pasteurized cow's milk for screening of MAP infection by IS900 PCR in UK, though it was unable to distinguish between live and dead bacilli. But this study indicated high risk of transmission of MAP to human population through consumption of milk and milk products contaminated with MAP (Miller et al. 1996). Grant et al. (2002a) of Queen's University Belfast screened PCR positive cow's milk samples with optimized decontamination protocols and immune-magnetic capture method where, 11.8% were positive, and by culture 1.8% of samples were positive for MAP. In Switzerland, 19.7% of 1384 samples of bulk-tank milk were positive by IS900 PCR again emphasized the risk of MAP in human food chain (Corti & Stephan 2002).

MAP is not inactivated during pasteurization; therefore live MAP bacilli are present in milk and milk products made from pasteurized milk (Tables 1 and 2). Extensive use of milk products made from pasteurized milk globally, poses serious threat to human life. Initially this problem was restricted to most of the developed countries

due to high dependence on pasteurized milk and milk products. But slowly this problem has started plaguing developing countries like India due to increasing use of milk products like ice-cream, milk coffee and condensed milk made from pasteurized milk. Since the use of milk products made from pasteurized milk increased dramatically in the country, therefore chances of contamination of human population with MAP has also increased. Both globally and in India, bio-load of MAP has been estimated in different types of milk and milk products made from pasteurized milk of domestic livestock species in different parts of world including India.

### 2.1. Bio-load of MAP in milk samples (Raw milk, bulk tank milk, pasteurized milk, pooled milk and human milk)

#### (i) Global scenario

##### Cattle milk

**Raw milk samples:** Reported bio-load of MAP using milk culture was 2.4–28.6%, 6.9, 8.3, 2.0, 0.3 and 35.0% in USA, UK, Argentina, Czech Republic, N. Ireland and Australia, respectively (Taylor et al. 1981; Sweeney et al. 1992a; Streeter et al. 1995; Grant et al. 2002a; Stabel 2002; Pillai & Jayarao 2002; Paolicchi et al. 2003; O'Reilly et al. 2004; Ayele et al. 2005). Bio-load by using IS900 PCR was 33.0 and 14.7–32.0% in USA and Iran, respectively (Pillai and Jayarao 2002; Soltani et al. 2008; Anzabi et al. 2013) and by using microscopy bio-load was assessed as 22.0% (Anzabi et al. 2013).

**Individual milk:** Reported bio-load of MAP by screening of individual raw milk samples using milk ELISA was 3.3–82.4%, 10.7–33.7, 1.7–11.2, 2.5% in USA, Denmark, Ontario and Canada, respectively (Sweeney et al. 1994; Nielsen et al. 2002; Klausen et al. 2003; Hendrick et al. 2005a, 2005b; Lombard et al. 2006a, 2006b; Nielsen and Ersboll 2006; Hendrick et al. 2006).

**Bulk tank milk:** In USA, Jayarao et al. (2004) and Wilson et al. (2010), reported 2.8–20.6% and 13.4–39.0% bio-load of MAP in bulk tank milk samples, using milk culture and IS900 milk PCR, respectively. While using milk PCR bio-load was 22.0, 49.0, 8.6–23.0 and 23.0% in UK, Chile, Iran and Switzerland, respectively (Muehlherr et al. 2003; Haghkah et al. 2008; Botsaris et al. 2013; Kruze et al. 2013; ). By using milk ELISA, the bio-load was, 9.4 and 15.4% in Chile and Denmark, respectively (Nielsen et al. 2000; Salgado et al. 2007).

**Pasteurized milk:** Reported bio-load of MAP by different workers using milk culture was 2.8, 1.8–6.7, 2.7, 2.8, 15.0, 6.9, 1.6% in USA, UK, Brazil, Argentina, Ontario, N. Ireland and Czech republic, respectively (Millar et al. 1996; Grant et al. 2002a; Stabel 2002; O'Reilly et al. 2004; Ayele et al. 2005; Ellingson et al. 2005; Grant 2005; Carvalho et al. 2012a, 2012b; Paolicchi et al. 2012).

##### Goats and sheep

**Pooled milk:** Bio-load of MAP has also been reported in hard and semi-hard cheese, that were

**Table 1.** Global bio-load of MAP in milk: Country-wise.

Species	Countries	Sample type	Tests	Bio-load (%)	References
<b>Bio-load world-wide</b>					
Cattle	USA	Raw milk	Culture	2.4–28.6	Sweeney et al. 1992a; Streeter et al. 1995; Stabel 2002; Pillai & Jayarao 2002
	UK			6.9	Grant et al. 2002a
	Argentina			8.3	Paolicchi et al. 2003
	Czech Republic			2.0	Ayele et al. 2005
	Northern Ireland			0.3	O'Reilly et al. 2004.
	Australia			35.0	Taylor et al. 1981
	USA		PCR	33.0	Pillai and Jayarao 2002
	Iran			14.7	Soltani et al. 2008
	Iran		PCR	32.0	Anzabi et al. 2013
				20.0	
	USA	Individual milk	ELISA	3.3–82.4	Sweeney et al. 1994; Lombard et al. 2006a, 2006b
	Denmark			15.4	Nielsen et al. 2002
				10.7–33.7	Klausen et al. 2003; Nielsen & Ersboll 2006
	Canada			1.7–11.2	Hendrick et al. 2005a; b
				2.5	Hendrick et al. 2006.
	USA	Bulk tank milk	Culture	2.8–20.6	Jayarao et al. 2004
	USA			13.4–39.0	Jayarao et al. 2004; Wilson et al. 2010.
	UK			22.0	Botsaris et al. 2013.
	Chile			49.0	Kruze et al. 2013
	Iran			8.6–23.0	Haghighi et al. 2008
	Switzerland			23.0	Muehlherr et al. 2003.
	Chile		ELISA	9.4	Salgado et al. 2007
	Denmark			70.0	Nielsen et al. 2000
USA	Pasteurized milk	Culture	2.8	Ellingson et al. 2005; Stabel 2002	
UK			1.8–6.7	Millar et al. 1996; Grant et al. 2002a, 2005	
Brazil			2.7	Carvalho et al. 2012a	
Argentina			2.8	Paolicchi et al. 2012.	
Canada			15.0	Grant et al. 2002b.	
N. Ireland			6.9	O'Reilly et al. 2004.	
Czech Republic			1.6	Ayele et al. 2005	
USA			Human milk	–	Naser et al. 2000, 2009; Bannantine et al. 2014
<b>Bio-load in India</b>					
Cattle	North India	Raw milk	Culture	67.0	Shankar et al. 2010
			PCR	6–30.8	Singh et al. 2007a; Shankar et al. 2008, 2010; Kaur et al. 2010
		Individual milk	PCR	16.0	Sharma et al. 2008
		ELISA	28.0		
	Pasteurized milk	Culture	67.0	Shankar et al. 2010	
		PCR	33.0–38.8	Shankar et al. 2008, 2010	
Buffaloes	Rajasthan	Individual milk	ELISA	31.1	Vinodh Kumar et al. 2014
Cattle & buffaloes	Mathura, Agra & New Delhi	Individual milk		38.0	Singh et al. 2016
		Un-pasteurized	d-ELISA	46.2	
		Pasteurized	PCR	100.0	Singh et al. 2007b
Goats	CIRG, Mathura	Raw milk	Culture	20.0–69.8	Singh & Vihan 2004; Kumar et al. 2008
			PCR	7.6–37.7	Kumar et al. 2008; Raghuvanshi et al. 2010
			ELISA	30.7–70.1	
		Individual milk	Microscopy	13.8	Raghuvanshi et al. 2010
		PCR	8.3	Raghuvanshi et al. 2010; Singh et al. 2016	
		ELISA	37.5–38.0		
		Pooled milk	Microscopy	16.6	
			d-ELISA	54.9	Singh et al. 2016
		PCR	5.8	Raghuvanshi et al. 2010	
		ELISA	11.7		
		Microscopy	5.8		

**Table 2.** Presence of MAP in milk products from domestic livestock.

S. No.	Milk products from domestic livestock				
	Animal species	Product Details	Country	Bio-load (%)	Reference
1	Cattle	Semi hard cheese	Switzerland	4.0	Stephan et al. 2007
2		Cheese	USA	23.0	Clark Jr. et al. 2006
3		Curd		23.0	
4		Milk powder	Czech Republic	35.0	Hruska et al. 2005
5		Milk products	India	22.0–100.0	Shankar et al. 2010
6		Ice cream & flavoured milk		56.0–78.0	
7	Goats and sheep	Hard cheese	Czech Republic	5.4	Ikonomopoulos et al. 2005
8		Semi hard cheese		5.0	
9	Goats	Milk paneer	India	0.0–16.6	Raghuvanshi et al. 2013

prepared from pooled milk of goats and sheep using culture and IS900 PCR (Raghuvanshi et al. 2010).

#### Human

**Human milk:** MAP was also reported in human breast milk of Crohn's disease patients in USA (Naser et al. 2000; Bannantine et al. 2014) and its complete genome sequence was recovered and found highly identical with characterized strain recovered from cattle (Bannantine et al. 2014).

#### (ii) Indian scenario

##### Cattle

**Raw milk:** Bio-load of MAP in raw milk was 67.0 and 6–30.8%, by milk culture and IS900 milk PCR, respectively in North India (Singh et al. 2007a; Shankar et al. 2008, 2010; Kaur et al. 2010).

**Individual milk:** The bio-load of MAP was reported as 16.0 and 28.0% using IS900 milk PCR and milk ELISA respectively in North India in a study by Sharma et al. (2008).

**Pasteurized milk:** Bio-load of MAP in pasteurized cattle milk was 67.0, and 33.0–38.8%, using milk culture and IS900 milk PCR, respectively in North India (Shankar et al. 2008, 2010).

##### Buffaloes

**Raw milk:** Screening of raw milk samples by ELISA revealed a 31.1% bio-load of MAP (Vinodhkumar et al., 2014).

##### Cattle and buffaloes

**Pasteurized and unpasteurized milk:** Singh et al. (2007b, 2016), reported 100.0% bio-load of MAP in both pasteurized and un-pasteurized milk samples by IS900 PCR. While using individual milk samples, the bio-load was 38.0 and 46.2%, respectively using milk ELISA and milk dot\_ELISA tests.

##### Goats

**Raw milk:** Screening of the raw milk samples of goats belonging to CIRG farm herds (with herds endemic for JD since 1985 (Singh et al. 2014a)), the percent bio-load of MAP was 20.0–69.8, 7.6–37.7, 30.7–54.7 and 13.8 using milk culture, milk IS900 PCR, milk ELISA and milk microscopy (Singh and Vihan 2004; Kumar et al. 2008; Raghuvanshi et al. 2010).

**Individual milk:** Percent bio-load of MAP was 8.3, 37.5–38.0, 16.6 and 54.9 by IS900 milk PCR, milk ELISA, milk microscopy and milk dot ELISA, respectively (Raghuvanshi et al. 2010; Singh et al. 2016).

**Pooled milk:** Milk samples were collected from pooled milk of 5 farm goat units (each farm unit consisted of 50–150 lactating goats located at CIRG). The percent bio-load was 5.8, 11.7 and 5.8, using IS900 milk PCR, milk ELISA and milk microscopy, respectively (Raghuvanshi et al. 2010).

## 2.2 Bio-load of MAP in milk products made from pasteurized milk

### (i) Global scenario

#### Cattle milk

**Milk Products:** Hruska et al., in 2005 using IS900 PCR reported 35.0% bio-load of MAP in milk powder samples from Czech Republic. Stephen et al. (2007) in semi-hard cheese reported 4.0% bio-load of MAP using IS900 PCR in Switzerland. Clark Jr. et al. (2006) in USA by screening of curd using culture reported 23.0% bio-load. In cheese samples, Clark Jr. et al. (2006) reported 23.0% bio-load using IS900 PCR.

#### Goats and sheep

**Milk products:** In Czech Republic, bio-load of MAP was 5.4% in hard cheese using culture and IS900 PCR, whereas bio-load was 3.0 and 50.0% in semi-hard cheese using culture and IS900 PCR, respectively (Ikonopoulou et al. 2005).

#### (ii) Indian scenario

##### Cattle

**Milk products:** Shankar et al. (2010) reported 22.0–100.0% bio-load of MAP in milk products using culture. Shankar et al. 2010 in another study reported 56.0% bio-load of MAP in ice creams and 78.0% in flavoured milk by using culture and IS900 PCR, respectively.

##### Goats

**Milk products:** Percent bio-load of MAP in paneer (fresh cheese) prepared from pooled goat milk (at CIRG, Mathura) was 0.0 and 16.6% using IS900 milk PCR and milk microscopy, respectively (Raghuvanshi et al. 2013).

## 3. MAP in meat products

MAP primarily affects the intestines of domestic livestock (Kopečna et al. 2008) and is also found systemically in animal body with the advancing stages of disease or JD (Rubery 2002). In case of small ruminants (goats and sheep) the site of predilection is intestinal mucosa at ileo-caecal junction and from there disease progresses towards duodenum, whereas in case of large ruminants, disease progresses towards large intestine, hence it is possible to take rectal pinch. Presence of MAP has been reported in beef from USA (Jaravata et al. 2007), Denmark (Okara et al. 2010) and Italy (Savi et al. 2015). With respect to the scale of slaughter of domestic livestock and other animals for meat production and consumption globally, the reports are few on presence of MAP in meat and meat products or cooked meat. However, Lorencova et al., (2014) and Jaravata et al., (2007), reported MAP from raw meat and meat products in Czech Republic and UK, respectively. In addition to the gastro-intestinal system, MAP has also been detected in lymph nodes (intestinal, pulmonary, supra mammary, etc.), other tissues (milk, blood, semen, mammary glands, reproductive organs (uterus, amniotic fluid, testicles, epididymis, and foetuses in utero) (Larsen et al. 1970; Sweeney et al. 1992b; Collins 1997, Kopečna et al. 2008) (Table 3). MAP can be detected in these tissues of animals with sub-clinical infection. Therefore, it is possible that meat

**Table 3.** Presence of MAP in meat, organs and secretory products of livestock species.

S. No.	Product details	Country	Reference
(1)	Beef (cattle)	USA Denmark Italy	Jaravata et al. 2007 Okara et al. 2010 Savi et al. 2015
(2)	Raw meat products	Czech Republic	Lorencova et al. 2014
(3)	Meat & meat products	UK	Jaravata et al. 2007
(4)	Semen (bull)	UK	Larsen and Kopecky 1970
(5)	Epididymis (bulls)	USA	Collins 1997
(6)	Foetus (in-utero)	USA	Sweeney et al. 1992b
(7)	Intestinal tract, pulmonary lymph nodes, mammary gland, milk, uterus, amniotic fluid, testicles	Czech Republic	Kopecna et al. 2008
(8)	Udder	India USA India	Vohra et al. 2008a Collins 1997 Vohra et al. 2008a
(9)	Kidneys, lungs, liver	USA	Collins 1997
(10)	Supra-mammary lymph nodes	Czech Republic	Pavlik et al. 2000; Vohra et al. 2008a
(11)	Intestine	India	Kumar et al. 2007; Singh et al. 2008b; 2008c; 2008d; Yadav et al. 2008; Chaturvedi et al. 2017
(12)	Mesenteric Lymph Nodes	India	Kumar et al. 2007; Singh et al. 2008c; 2008d; Yadav et al. 2008; Chaturvedi et al. 2017
(13)	Vaginal Secretion	India	Vohra et al. 2008b

can be contaminated with MAP obtained from both clinically infected and apparently healthy animals. Meat could also be become contaminated with faecal material during slaughtering and processing procedures (Verma et al. 2014).

Johne's disease is endemic in the domestic livestock population of India (Singh et al. 2014a). Though there is heavy slaughter of buffaloes for beef production and export from India, information is non-existent on either presence or bio-load of MAP in buffalo beef. With reference to goat meat (chevon), which is preferred meat and liked by people of all communities and religious groups due to tropical climate, there is very heavy slaughter of goats (>42.0%) in slaughter houses, but the majority of goats are slaughtered in homes or clandestinely, but there is no report on the presence of MAP in chevon. Similarly, mutton is preferred in hilly and coastal regions of India but there is no information on the presence of MAP in mutton. It should be realized that the majority of animals (buffaloes, goats and sheep) that are slaughtered for meat production are those which are weak and emaciated and off production. Therefore, chances of recovery of MAP from beef, mutton or chevon are expected to be high. Similar situation exists in other developing and poor countries of Asia and other parts of the world, where JD is endemic and responsible for low per animal productivity unlike animals in the developed countries.

Rossiter & Henning (2001) detected presence of MAP in the intestinal lymph nodes / faeces of 34.0% of healthy thin dairy cows and 3.0% of healthy thin beef cows at slaughter. MAP was also isolated from 3.0% in peripheral lymph nodes and 8.0% in liver of dairy and beef cows, respectively. Information is not available concerning the ability of MAP to survive during meat cooking and processing methods. It is likely that contaminated meat that is cooked at low temperatures or not processed thoroughly may contain live MAP.

Though, the significance of meat as a potential vehicle of food-borne exposure to MAP relative to the potential exposure through milk is not known (Food Standards Australia New Zealand 2004). It has been reported that fecal matter of slaughtered animals is the main source of MAP contamination in meat and meat product (Wells et al. 2009). Gwozdz et al (2000) has reported the evidence of MAP DNA in the blood and / or liver of many sheep with advanced symptoms of JD.

#### 4. Survival of MAP in the environment/during manufacturing processes

During food manufacturing processes, killing of MAP is presumed on pasteurization, specifically high-temperature short-time (HTST) pasteurization (Grant and Lambert, 2002b). Early laboratory studies were carried out with MAP inoculated milk in test tubes. In these studies, the milk was subjected to heat treatments similar to those used for pasteurization in holding tanks (holder pasteurizing) or for HTST pasteurization (Stabel and Lambert, 2004). MAP was recovered from most of the samples of pasteurized milk. Studies with milk heated in apparatus (test tubes, capillary tubes, or laboratory pasteurizers) indicated that both holder and HTST pasteurization could be effective for killing (inactivating) of MAP in milk (Table 4). Measurement of standard thermal tolerance parameters (STTP) for MAP in laboratory studies indicated that it is more heat-resistant than *Mycobacterium bovis* and *Coxiella burnetii* (milk-borne zoonotic pathogens) designed to be killed by pasteurization (Sung & Collins 1998). Corroborating there are three independent retail milk surveys reporting recovery of viable MAP by culture from retail HTST pasteurized milk (Ellingson et al. 2005). Clearly, HTST pasteurization method kills large numbers of MAP bacilli in milk. However, evidences suggest that it does not kill 100% of MAP bacilli and post-

**Table 4.** In-activation of MAP bacilli inoculated into milk during Pasteurization temperature.

Pasteurization Equipments	Pasteurization		Detection level (CFU/ml)	Maximum Inoculum (log CFU/ml)	No. of samples (n)	Presence of MAP		Minimum reduction (log CFU)	Reference
	Temperature (°C)	Time				No. (n)	%		
Laboratory Test tubes	63	30 min	2	5	36	36	100	<1	Chiodini and Hermon-Taylor 1993
	72	15 sec			36	36	100	<1	
	63.5	30 min	2	7	66	63	95	2	Grant et al. 1996
	71.7	15 sec			66	60	91	2	
	65–76	30 min	5	8	16	5	31	2	Stabel 1997
Pasteurizer Capillary tubes	65–75	15 sec	NA	6	12	0	NA	>6	
	63	30 min	4	5	30	0	NA	>5	Keshwani and Frank, 1998
Pasteurizer	72	15 sec	NA	NA	30	0	NA	>5	
Pasteurizer	72	15 sec	0.04	3	117	7	6	<1	Grant et al. 1998
Pasteurizer	≤90	15 sec	2	6	54	18	33	6	Grant et al. 1999
Laboratory test tubes	72	25 sec	NA	NA	9	0	NA	>6	
	63	30 min	3	7	7	0	NA	7	Gao et al. 2002
Pasteurizer	72	15 sec	NA	NA	11	2	18	5	
	63	30 min	0.04	8	36	4	11	4	Stabel and Lambertz 2004
Local pasteurization unit	66	16 sec	NA	NA	36	34	94	1	
	72–74	16 sec	NA	NA	108	8	7	1	
	71.7	15 sec	NA	NA	244	4	1.6	NA	Ayele et al. 2005
Slug flow pasteurization unit			NA	NA	10	1	10.0	NA	
	62.7	30 min	NA	10 <sup>B</sup>	66	13	19.7	NA	Stabel et al. 2004
HTST pasteurization unit	65.5	16 sec	NA	10 <sup>B</sup>	3	3	100.0	4	
	71.7	15 sec	NA	10 <sup>5</sup>	3	1	33.3	4	
	71.7	20 sec	NA	10 <sup>B</sup>	3	2	66.6	>8	
	74.4	15 sec	NA	10 <sup>B</sup>	3	1	33.3	7	
	62.7	30 sec	NA	10 <sup>B</sup>	3	0	0.0	>8	Stabel et al. 2004
HTST pasteurization unit	65.5	16 sec	NA	10 <sup>B</sup>	3	3	100.0	>5	
	71.7	15 sec	NA	10 <sup>B</sup>	3	1	33.3	>8	
	71.7	20 sec	NA	10 <sup>B</sup>	3	1	33.3	>8	
	74.4	15 sec	NA	10 <sup>B</sup>	3	0	0.0	>8	

pasteurization MAP contamination is frequently occurring. This may be happening due to formation of clumps by the bacilli due to high lipid contents during initial heating phase. Within these clumps these bacilli survive the pasteurization temperature. Lamont et al. (2012) gave heat treatment to MAP K-10 spores at 70 and 90 °C for 30 min in addition to 2% lysozyme, proteinase K, kanamycin and anaerobic exposure. Heat treatment served two purposes to: 1) Determine temperature threshold for survival and 2) Eliminate any remaining vegetative cells such that the re-grown culture only originated from spores. Both lysozyme and proteinase K are typically used as a standard DNA extraction protocol that functions by damaging the cell wall of vegetative MAP cells, which causes bacterial lysis. MAP K-10 spores survived exposure to 70 °C, but not at 90 °C. Heat exposed spores treated in combination with either lysozyme, PK or kanamycin were capable of re-growth due to the coat layer resistance to these enzymes. MAP spore survival post exposure to 70 °C may in fact not be extremely surprising since many studies have shown the presence of MAP as a food contaminant in pasteurized (also treated at 70 °C) milk, cheeses and yogurt (Donaghy et al. 2004; Shankar

et al. 2010; Van Brandt et al. 2011). Table 4 summarizes the effect of temperature on survival of MAP by different workers in developed countries. However, such studies are non-existent in other countries. In India over the past 20–30 years there is a shift in the habit of the use of milk in daily diet. Since centuries in our traditional society advocated boiling of milk before consumption. However, due to globalization of world economies India has seen quick popularization of the use of milk products especially ice-creams and cheese with varieties of fast food and this has led to excessive pressure on processing of milk in view of high demand. Therefore, threat emanating from the presence of bugs like MAP in milk of domestic livestock, where Johne's disease is endemic, is recent in Indian context but is fastly increasing due to high demand of fast food, in face of the rapidly growing of human population.

### 5. Risk of human infection from consumption of milk and meat products contaminated with MAP

JD caused by MAP in animals is one of the most difficult diseases to diagnose and control because it has

the ability to resist adverse conditions in the natural environment. MAP is belonging to intracellular bacilli and within JD infected animals, macrophages infected/laden with MAP can be found throughout the body. Most tissues including lymph nodes, spleen, bone marrow, liver, kidneys, testes, fetuses, supra-mammary lymph nodes and lungs might be affected (Kopečna et al. 2008). It has been suggested that meat harvested from old dairy cows and used for making ground beef for human consumption may be an active source of MAP infection (Rossiter and Henning, 2001). Meat can also be contaminated with fecal material loaded with MAP bacilli during slaughter and procedures used for evisceration and processing of meat.

MAP might be present in pasteurized milk (Slana et al. 2008), infant formulation made from pasteurized milk (Lund et al. 2002; Makharia and Singh 2009), breast milk of a woman suffering from Crohn's disease (CD) (Naser et al. 2009). The National Association for Colitis and Crohn's disease (NACC) in UK commissioned a report from an expert review group into the evidence linking MAP and Crohn's disease in 2003 (NACC 2003). This report concluded that: 'both live and dead MAP' were present in human food samples and the strongest evidence for this is in milk and DNA of MAP can be found in the bowel tissue of a proportion of patients with CD but also in lesser quantities in the bowel tissue of human beings having no lesions of CD. International Life Science Institute (ILSI), Europe in 2004 published a report from its Emerging Pathogen Task Force on MAP and the food chain (Gould et al 2005). In its conclusion, it stated that 'survival of MAP having public health concern depends on their possible involvement in human disease, in particular CD.' At the present time, despite substantial research, the possible involvement of MAP in human diseases is still under discussion. This opinion followed a review of MAP survival characteristics in food which noted in particular, studies demonstrating survival of viable MAP in pasteurized milk (Grant et al. 2005).

MAP infection is primarily a disease of food-producing domestic livestock (cattle, buffaloes, sheep and goats). Food products (meat and milk) derived from animals infected with MAP are commonly contaminated much before harvesting, that is during the disseminative stage of JD, when pathogen exist in muscle meat, internal organs (intestines, mesenteric lymph nodes), colostrum and milk, and the un-born fetus (Table 4) (Brady et al. 2008). During harvesting of milk and meat from animals, there is second opportunity for the contamination of food products with MAP by contact with feces of the animal infected with MAP (Grant 2010). Thus raw products obtained from MAP-infected animals are likely to be contaminated with MAP (Eltholth et al. 2009). Counts up to 560 MAP bacteria/ml of raw milk from individual cows have been reported (Grant 2010). The question then becomes

whether manufacturing processes or, cooking by the consumer will reliably kill MAP.

## 6. MAP in the natural environment

It is assumed that the survival of *Mycobacterium bovis* in the environment is limited to days to months (Young et al. 2005). By contrast, MAP is physically more robust than other mycobacteria and is known to survive for months and perhaps years (Table 5). Geographical regions having acidic soils enriched with humic and fulvic acids, boreal forests and areas with heavy rainfall may favor the accumulation and persistence of MAP in the environment (Singh et al. 2012b; Hruska and Kaevska, 2012; Thomson et al. 2013; Rhodes et al. 2014). There are no reported studies to give us detailed understanding of the ecology and providence and presence of MAP in the atmosphere and potential cycling of MAP through human populations. It is very much possible that environmental MAP is taken up into protozoa and adapts in intracellular condition within protozoa present in the environment and in bio-film communities that profoundly influence microbial survival, phenotype and virulence (Barker & Brown, 1994; Hermon-Taylor et al. 2000). MAP grown in vacuoles in *Acanthamoeba castellanii* has developed the capacity of MAP to infect other amoebae, macrophages and human colonic epithelial cells including improved virulence for infection in a beige mouse model (Cirillo et al. 1997). MAP can remain alive for long periods inside the *Acanthamoeba polyphaga* (Steinert et al. 1998). The environmental exposure of MAP and its cycling through unicellular living beings as well

**Table 5.** *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in water supplies and environment.

Sn.	Environmental samples	Country	Reference
1.	Environmental and domestic aerosols	UK	Rhodes et al. 2014
2.	River water	UK	Pickup et al. 2006
3.	Drinking water supply system	India	Singh et al. 2012b
		UK	Pickup et al. 2006
4.	Domestic showers	USA	Pierce et al. 2009b; Beumer et al. 2010
		Czech Republic	Klanicova et al. 2013
		USA	Falkinham et al. 2013
		UK	Aboagye et al. 2011
		Canada	Gill et al. 2011
		Czech Republic	Hruska et al. 2012
		USA	Feazel et al. 2009
5.	Dam water and sediment	Australia	Thomson et al. 2013
6.	Soil	Australia	Whittington et al. 2005
		USA	Reizman et al. 2011
7.	Distilled water	Chile	Salgado et al. 2011
		UK	Pickup et al. 2005
		Australia	Dhand et al. 2009
8.	Surface water	India	Singh et al. 2012b
		USA	Collins et al. 2001
8.	Surface water	USA	Grewal et al. 2006
		UK	Pickup et al. 2006
		Ireland	Whan et al. 2005



as multi-cellular animal and human populations, has potential to have a profound effect on the evolution of these organisms and the evolution of strains with respect to adaptation and enhanced pathogenicity. Previous studies from England, France, Australia and USA reported that soil types and soil composition have influence on maintenance, survival and repeated infection and incidence of JD in livestock herds (Dhand et al. 2009). Singh et al. (2012b) screened 71 samples from soil (51) river and water (20) (natural resources) of three districts of South Uttar Pradesh by microscopy and IS900 PCR, 46.4 and 23.9% samples were found positive. They reported presence of MAP in holy river Yamuna, this may be due presence of large number of goshalas (cow shelters) for religious reasons in the holy city of Vrindavan (where samples were collected) and most of these cow shelters wash off 'cow dung' into the drains leading to river Yamuna, rather than lifting cow dung making compost. However, water samples from river Chambal with little human and animal population on its banks was comparatively free of MAP and other pathogenic micro-organisms. Similarly soil samples from farm area, where goatherds are reared over the years had soil samples positive for MAP as compared to farmer's fields besides heavy load of other microbial contaminants in soil, water and environment (Table 5).

### 6.1. MAP in water supplies and aerosols

Various studies reported survival of robust zoonotic pathogens in the environment (Szewzik et al. 2000). It has been suggested that there is a high risk of transmission of MAP to human population through drinking-water or by aerosols (Hermon-Taylor et al. 2000). Water bodies like lakes and rivers get contaminated by run-off of MAP from heavily grazed planktonic form of pastures, within protozoa or, more likely, both (Pedley et al. 2004). These organisms reaching domestic outlets in high dilution may aggregate in bio-films present in cold and hot household water storage tanks and delivery systems. Mycobacteria are well-known to be present in aerosols and to concentrate in the water droplets (Blanchard and Syzdek 1982; Wendt et al. 1980) (Table 5).

Heavy rain falls from the Atlantic washes off contaminated pastures into rivers in spate. Taff (one of these rivers in spate), goes through the middle of Cardiff city. Research carried out during the 1970s in Cardiff, recorded significantly increased incidence of CD ( $p < 0.001$ ), but not of ulcerative colitis, in 11 of the local electoral city wards (Mayberry & Hitchens 1978). Of these high incidence wards, eight directly touched the river Taff and the three that did not, were closely adjacent to the North and East. This is the direction in which aerosols would be carried off by the usual South-Westerly winds (Hermon-Taylor 1993). Tracheal

and bronchial inflammation with abnormal lung function tests were noticeable in the significant proportion of human population and chronic granulomatous tracheo-bronchitis in children with CD (Naser et al. 2009; Singh et al. 2012a). These are only few studies but much research is required on MAP presence and survival in the environment, in surface and ground waters and in the aerosols (Table 5).

## 7. Bio-load of MAP in domestic livestock species in last 32 years (1985–March, 2017) in India: CIRG studies.

Our study on screening of 26,009 domestic livestock for Johne's disease and / or MAP infection using 4 different diagnostic tests (microscopy, culture, ELISA and PCR), during last 32 years (1985–March 2017) has shown that the average bio-load of MAP in the livestock population of India was high (26.8%). Livestock species-wise average bio-load in goats, sheep, cattle and buffaloes was 22.5, 40.9, 42.7 and 35.6%, respectively. Bio-load was significantly lower in goats ( $P < 0.05$ ) than in sheep, buffaloes and cows. Average bio-load of MAP in 31 years (1985–2016) time period at five yearly intervals; 1985–1990, 1991–1995, 1996–2000, 2001–2005, 2006–2010 and 2011–March 2017 was 11.4%, 13.1%, 11.1%, 24.2%, 28.9% and 44.2%, respectively. It was alarming to note that percent bio-load of MAP in domestic livestock population showed an increasing trend. Time zone-wise, the average bio-load was 11.6%, 27.0% and 44.2%, in the time period of 15 years (1985–2000), 10 years (2001–10) and 6 years (2011–March 2017), respectively. Our study showed that there was four times increase in the bio-load of MAP from initial levels (Table 6). Bio-load increased 3 and 4 times in case of goats and sheep (small ruminants), whereas the bio-load was high in cattle and buffaloes already from the beginning. This increase in bio-load of MAP is despite very heavy slaughter rate of animals in India in order to feed the population of 1.3 billion humans. Bio-load is also increasing since India lacks programs on the control of Johne's disease in domestic livestock at National level.

## 8. Bio-load in human population in last 9 years (2008–March 2017) in India

Bio-load of MAP was estimated in human population from Northern region of the country by 'mass screening' of human samples. Of the total 28,291 serum samples screened in last 8 years (2008 to 2016) using Indigenous ELISA kit, 30.8% samples were positive for MAP infection (Table 7). Percent bio-load of MAP infection was 34.0, 49.2, 31.7 and 14.8% in time period A (2008–2013), period B (2013–2014), period C (2014–2015) and period D (2015–March 2017), respectively. The study showed that in general, bio-load of MAP in human population

**Table 6.** Percent bio-load of *Mycobacterium avium* subspecies *paratuberculosis* in the four domestic livestock species from different regions of India in past 32 years (1985–March 2017).

Species	Period A			Period B		Period C#	Cumulative
	1985–1990	1991–1995	1996–2000	2001–2005	2006–2010	2011–March, 2017	(1985 – March, 2017)
Goats	11.4 (464 / 4057)	13.1 (292 / 2221)	11.1 (443 / 3974)	21.5 (533/2479)	29.7 (443/1490)	39.8 (2394/ 6010)	22.5 (4569/20 231)
Sheep*	ND	ND	ND	25.0 (5 /20)	25.9 (118/454)	75.8 (154/203)	40.9 (277/ 677)
Sub total	11.4 (464 /4057)	13.1 (292/2221)	11.1 (443/3974)	21.52 (538/2499)	28.8 (561/1944)	41.0 (2548/ 6213)	23.1 (4846/ 20 908)
Cattle*	ND	ND	ND	47.0 (80/170)	31.0 (558/1799)	53.0 (1116/2104)	42.7 (1741/ 4073)
Buffaloes*	ND	ND	ND	41.9 (70/167)	22.1 (115/520)	53.0 (181/341)	35.6 (366/1028)
Sub total	11.4 (464 /4057)	13.1 (292/2221)	11.1 (443/3974)	44.5 (150/337)	29.0 (673/2319)	52.5 (1284/ 2445)	41.3 (2107/ 5101)
Grand Total	11.4 (464 /4057)	13.1 (292/2221)	11.1 (443 /3974)	24.2 (688 /2836)	28.9 (1234 /4263)	44.2 (3832/8658)	26.8 (6976/26 009)
		11.6 (1199/10 252)		27.0 (1922/7099)			

Note: Figures in parenthesis are numbers (Positive/Total); #Up to March 2017; ND = Not done; \* Based on 4 tests (microscopy, ELISA, PCR, Culture)

**Table 7.** Percent bio-load of MAP\* in Human Population: 9 years (2008–March 2017): CIRG studies.

Tests	Period A 2008–2013	Period B 2013–2014	Period C 2014–2015	Period D 2015–March, 2017	Cumulative (2008–March, 2017)
Serum ELISA	(34.0) 7887/23 196	(49.2) 190/386	(31.7) 235/740	(14.8) 73/492	(33.7) 8385/24 814
Blood PCR	(8.4) 260/3093	(18.1) 4/22	(17.4) 19/109	(10.7) 9/84	(8.8) 292/3308
Stool PCR	(5.9) 6/101	(55.5) 10/18	(44.7) 17/38	(41.6) 5/12	(22.4) 38/169
Total (%) n	(30.2) 8153/26 390	(47.8) 204/426	(30.5) 271/887	(14.7) 87/588	(30.8) 8715/28 291

\*Figures in parenthesis are percent.

was high (33.7%). Of 3308 blood samples screened by IS900 PCR showed that 8.8% human population was infected with MAP. Time period-wise bio-load of MAP infection was 8.4%, 18.1%, 17.4 and 10.7%, respectively, by blood PCR. The presence of MAP DNA in blood is in favour of its potential role in the pathogenesis of CD. Screening of 169 stool samples by PCR, the overall bio-load was 22.4% and percent bio-load of MAP was 5.9, 55.5, 44.7 and 41.6% in time period A, period B, period C and period D, respectively. Bio-load of MAP in stool samples showed colonisation of intestines and shedding of MAP from the intestinal lesions. Time period wise cumulative bio-load was 30.2, 47.8, 30.5 and 14.7% in period A, Period B, Period C and Period D, respectively (Table 7).

## 9. Association of MAP and crohn's disease

Johne's disease is presently not in focus for the control and eradication either in the country in the domestic livestock population, therefore human population continues to get widely exposed to MAP infection from sub-clinical and clinical shedders of bacilli (Jones et al. 2006). MAP also has neuro-pathological and immune dys-regulatory properties (Scanu et al. 2007). This is why MAP is considered a candidate pathogen having association with CD (Momotani et al. 2012a). Frequent isolation of MAP from CD patients and similarities of complication of CD matched with JD, which further confirmed the involvement of MAP infection with CD (Scanu et al. 2007). MAP has a cell wall that contains muramyl dipeptide (MDP). Some people have mutations in their genes, like NOD2, which regulates ability of the host to respond appropriately to MDP. Hence, persons having gene mutations in NOD2 may be more vulnerable. Circumstantially, these observations appear to make a compelling case of involving MAP in CD

(Faria et al. 2014). Some literature on the pathology of CD and on possible association with MAP infection have suggested that MAP might directly infect endothelial cells and adipocytes and cause them to proliferate, causing focal obstruction within already existing vessels (including granuloma formation), the development of new vessels (neovascularisation and lymphangiogenesis), and the "creeping fat" of the mesentery that is unique in human pathology to CD but also occurs in bovine JD. The walls of fistulas might result from the neovascularisation or lymphangiogenesis that occurs in the bowel wall in Crohn's disease and therefore are also possible sites of large numbers of MAP. The direct visualization of large numbers of MAP organisms in the tissues of patients with Crohn's disease will help establish that MAP causes Crohn's disease (Pierce 2009a).

Identification of MAP in CD patients has been accomplished by several different techniques. MAP has been cultured from the intestines (Sechi et al. 2005) and blood (Naser et al. 2004) of Crohn's disease patients. In addition, MAP has been identified in the intestines and blood of Crohn's patients by IS900 PCR amplification (Naser et al. 2004; Abubakar et al. 2008). IS900 PCR also detected MAP in two tissues of cervical lymph nodes of a 5-year-old child; later he developed CD (Hermon-Taylor et al. 1998). Multiple serological diagnostics have confirmed the presence of antibodies directed to MAP in human beings suffering from CD (Feller et al. 2007). CD shares certain clinical as well as histo-pathological similarities with JD and is fast emerging as disease of public health significance and a potential human zoonotic infection (Singh et al. 2012a). MAP has been detected in the tissues and blood of CD patients with a greater frequency as compared to controls (Naser et al. 2004). In addition,

human breast milk of a patient with CD contained antibodies directed to MAP antigens (Naser et al. 2000). Furthermore, in-situ DNA hybridization method has allowed direct visualization of small numbers of MAP in CD patient intestines by light microscopy (Abubakar et al. 2008). Initially, Dalziel had characterized 9 human cases of chronic intestinal enteritis and found that jejunum, transverse and sigmoid colon, as well as mid-ileum were affected and patients had similar clinical findings as cattle suffering with JD (Dalziel 1989). He therefore speculated that MAP could be a prospective etiological agent for the complications observed in human patients (Dalziel 1989). Chiodini et al. (1984) revived the association of MAP with CD and reported the isolation of un-characterized mycobacteria from tissues of three CD patients and Yoshimura et al. (1987) anticipated that the pathogen present in a cell-wall deficient form was characterized as MAP. In another study, MAP was directly visualized by light microscopy in small numbers in the intestines of CD patients by ZN staining (Jeyanathan et al. 2007), while other pathogens (*E. coli*, *Enterococcus* spp, *Clostridium perfringens*, *Proteus* spp and *Bacteroides fragilis*) have also been detected in the intestines of patients with CD (De Hertogh et al. 2008). Some researchers argued that MAP has met both Koch's postulates (Greenstein 2003) and Relman's criteria (Chamberlin et al. 2007) for microbial causation of CD. However, the causal association of MAP with CD remains controversial (Miller et al. 1996). Momotani et al (2012) proposed a novel mouse model for CD-like colitis and the ability of MAP antigen to induce necrotizing colitis, which may be the key to understanding the relationship between CD and MAP. This model may help clarify the pathogenesis of CD, as well as other diseases with a suspected etiological relationship to MAP, e.g. irritable bowel syndrome (Scanu et al. 2007), multiple sclerosis (Cossu et al. 2011), and type-1 diabetes mellitus (Paccagnini et al. 2009).

At present, there is inadequate scientific evidence to confirm a conclusive link between JD (MAP infection) in ruminants and some cases of CD in human beings. The American Academy of Microbiology published a review on the evidence of pathogenicity in MAP (Nacy and Buckley 2008). It has been noted that 'there is a suspicion, supported by reports of genetic incompetence to interact correctly with certain bacteria and their products in some patients, that CD may have a currently unrecognized infectious origin, possibly environmentally derived.' It has also been noted that 'the option of more than one infectious reason that leads to a similar set of indications confounds the research agenda to find both a cause and a cure for CD.' The report lists having five reasons why MAP has a suspected role in CD including MAP's ability to survive milk pasteurization and the success in some CD patients of antibiotic therapy against *Mycobacteria*.

**Table 8.** Association of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection with different kinds of human diseases/ailments.

Sn	Diseases/ailments	References
1	Crohn's disease	Hermon-Taylor et al. 2009; Naser et al. 2009; Singh et al. 2012a; MCNeese et al. 2015
2	Inflammatory bowel disease	Abubakar et al. 2008; Momotani et al. 2012; Nazareth et al. 2015; Timms et al. 2016
3	Ulcerative colitis	Pierce et al. 2010; Singh et al. 2011; Tuci et al. 2011
4	Irritable bowel syndrome (IBS)	Scanu et al. 2007; Singh et al. 2008a
5	Sarcoidosis	Reid and Chiodini 1993
6	Type-1 diabetes	Naser et al. 2013; Kawasaki et al. 2014; Singh et al. 2014c
7	Autoimmune thyroiditis	Pinna et al. 2014
8	Blau syndrome	Dow & Ellingson 2010
9	Multiple sclerosis	Mameli et al. 2014
10	Autism	Dow 2011
11	Autoimmune arthritis	Blau 1985; Moudgil et al. 1997
12	Auto-immune hepatitis	Miyata et al. 1995
13	Primary biliary cirrhosis	Vilagut et al. 1997
14	Scleroderma	Danieli et al. 1992
15	Kawasaki disease	Yokota et al. 1993
16	Alzheimer's disease	Cossu et al. 2011
17	Behcet's disease	Direskeneli & Saruhan-Direskeneli 2003
18	Takayasu's arteritis	Aggarwal et al. 1996

There is lack of awareness in developing and under-developed countries on the presence and level of MAP infection in animals and human population (Singh et al. 2014a, 2014b). MAP has also been associated with various other human diseases / syndromes besides Crohn's disease (Table 8). It is likely that MAP is the cause of a range of human gastrointestinal diseases including irritable bowel syndrome (Scanu et al. 2007; Olbe 2008) and ulcerative colitis besides Crohn's disease. MAP has also been investigated in the pathogenesis of other so-called autoimmune diseases including sarcoidosis (Reid & Chiodini 1993), type-1 diabetes (Sechi et al. 2008; Paccagnini et al. 2009) and Hashimoto's thyroiditis (Sisto et al. 2010). If we accept that both ulcerative colitis and Crohn's disease are caused by a specific infectious microorganism, MAP, a long overdue transformation will take place in the prevention and treatment of these diseases (Pierce 2010). Information on the association of MAP with different human health problems is yet to be recognized and taken seriously by the medical world globally. Earlier studies from India on random screening of human population from different geographical regions of North India showed 23–34% sero-prevalence of MAP (Singh et al. 2011, 2014b). As studies (Singh et al. 2011, 2014b) reported moderately higher existence of anti-MAP antibodies in human population, this demands urgent measures and programs for reducing the bio-load of MAP in the animal population and in the environment as well.

**Table 9.** Bio-type profile (Molecular Epidemiology) of MAP strains originating from different geographical regions of India in last 12 years (2004–March 2017) using IS1311 PCR\_REA methodology.

Species	State	IS900 positive DNA (n)	MAP bio-type profile (IS1311 PCR_RE)	
			Indian Bison Type (n)	Cattle Type (n)
Goats	1. Uttar Pradesh	141	141	NIL
	2. Himachal Pradesh	5	5	NIL
	3. Madhya Pradesh	7	7	NIL
	4. Assam	3	3	NIL
	5. Rajasthan	6	6	NIL
Sheep	1. Uttar Pradesh	15	15	NIL
	2. Tamil Nadu	20	20	NIL
Cattle	1. Uttar Pradesh	123	119	4
	2. Punjab	13	11	2
	3. Himachal Pradesh	6	6	NIL
Buffaloes	1. Uttar Pradesh	29	28	1
	2. Punjab	13	11	2
	3. Tamil Nadu	2	2	NIL
<b>Sub-total A</b>		<b>383</b>	<b>377 (98.4)</b>	<b>6 (1.5)</b>
<b>Wild Ruminants</b>				
Deer	Uttar Pradesh	3	2	1
Wild Bison	Tamil Nadu	4	4	0
Rabbits	Tamil Nadu	6	6	0
<b>Sub-total B</b>		<b>13</b>	<b>12 (92.3)</b>	<b>1 (7.7)</b>
<b>Human and milk samples</b>				
Human	Multiple states	49	43	6
Commercial milk	Multiple states	26	26	NIL
<b>Sub-total C</b>		<b>75</b>	<b>69 (92.0)</b>	<b>6 (8.0)</b>
<b>Environmental samples</b>				
Soil	Uttar Pradesh	15	15	-
Yamuna river water	Uttar Pradesh	2	2	-
<b>Sub-total D</b>		<b>17</b>	<b>17 (100%)</b>	<b>-</b>
<b>Grand total (A+ B + C + D)</b>		<b>488</b>	<b>472 (96.7%)</b>	<b>16 (3.2%)</b>

<sup>a</sup>Figures in parenthesis are percent.

## 10. Bio-type profile of MAP infection in domestic livestock, milk and milk products, natural resources and human population

Bio-typing of MAP strains isolated from four domestic livestock populations have shown 'Indian Bison Type' to be the dominant bio-type (Table 9). This bio-type was for the first time reported from India by Sevilla and co-workers (2005) and has been identified as major bio-type infecting domestic livestock illustrating the high pathogenic nature of this bio-type. Similarly, 'Indian Bison Type' is the bio-type frequently reported from milk and milk products, environmental samples (soil and water) and also from human population. Same genotype has also been reported from wild ruminants (Table 9). The high bio-load of MAP in domestic and wild ruminants, other animals (rabbits), milk and milk products, environmental samples and also human beings, provide circumstantial evidence without any doubt that MAP is major zoonotic pathogen infecting both animal and human population in India.

## 11. Conclusions

MAP is a very tough bacterium having zoonotic potential and has capacity to infect wide range of hosts. It is secreted in the milk of affected domestic livestock and milk products derived from the milk of infected animals. MAP contaminates water run-off from animal farms that are contaminated with fecal matter of the animals infected with MAP. Use of pasteurization and

low heating conditions (roasting, smoking, etc.) help MAP to survive cooking and food manufacturing processes. Number of studies on MAP infection in human population, has put strong evidences suggesting that MAP is responsible for causation of Crohn's disease. Global studies have shown that meat and milk products and other food products from animals infected with MAP serve as important means for transmission of MAP to human population through animal based food chain. Evidence indicate that MAP is proficient in surviving low temperature food processing and pasteurization. Clinical and pathological similarities of Crohn's disease have been demonstrated with other mycobacterial diseases. Circumstantial evidence proves beyond doubt that MAP is major zoonotic pathogen of this century. Therefore, milk and milk products as well as meat supplies should be guarded with caution considering their human consumption. However, it should be realized that human infection cannot be prevented totally even in the presence of a vigorous control program in livestock.

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