

## Review Article

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# Current status of *Mycobacterium avium* subspecies *paratuberculosis* infection in animals & humans in India: What needs to be done?

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*Mycobacterium avium* subspecies *paratuberculosis* (MAP) has emerged as a major health problem for domestic livestock and human beings. Reduced per animal productivity of domestic livestock seriously impacts the economics of dairy farming globally. High to very high bioload of MAP in domestic livestock and also in the human population has been reported from north India. Presence of live MAP bacilli in commercial supplies of raw and pasteurized milk and milk products indicates its public health significance. MAP is not inactivated during pasteurization, therefore, entering into human food chain daily. Recovery of MAP from patients with inflammatory bowel disease or Crohn's disease and animal healthcare workers suffering with chronic gastrointestinal problems indicate a close association of MAP with a number of chronic and other diseases affecting human health. Higher bioload of MAP in the animals increases the risk of exposure to the human population with MAP. This review summarizes the current status of MAP infection in animals as well as in human beings and also highlights the prospects of effective management and control of disease in animals to reduce the risk of exposure to human population.

**Key words** Crohn's disease - domestic livestock - inflammatory bowel disease - *Mycobacterium avium* subspecies *paratuberculosis* - paratuberculosis

## Introduction

*Mycobacterium avium* subspecies *paratuberculosis* (MAP), the causative agent of paratuberculosis in domestic ruminants, belongs to *M. avium* complex; a heterogeneous group of slow-growing mycobacteria and are pathogenic to both animals and human beings (especially immunocompromised patients)<sup>1,2</sup>. MAP

infection results in the chronic inflammatory condition of intestines commonly known as paratuberculosis or Johne's disease (JD)<sup>3</sup>. Clinical condition is characterized by poor body condition, progressive weight loss with or without diarrhoea, debility and emaciation. Production losses result from reduced milk yield, altered milk constituents, high somatic cell counts, poor feed

conversion rate, increased susceptibility to mastitis, reduced reproductive efficiency, premature culling and reduced slaughter weight and carcass quality<sup>4</sup>. MAP is excreted in faeces, milk and semen of subclinically or clinically infected animals resulting in increased environmental contamination. Newborn animals acquire infection from infected parents through semen, during pregnancy and by consumption of colostrums and milk (vertical transmission) and oral-faecal route (horizontal transmission) from contaminated environment (soil, water, fodder, feed and pasture)<sup>5-7</sup>. After ingestion, MAP may persist in the intestines and other tissues for years without causing clinical disease<sup>8</sup>. If stressed (nutritional, pregnancy, parturition, lactation, environmental or any other concurrent disease), subclinical infection develops into clinical disease<sup>8</sup>. These animals continue to excrete bacilli in their faeces and milk on a regular basis<sup>9</sup>.

MAP has been incriminated as the cause of Crohn's disease (CD) in human beings<sup>10-13</sup>. Role of MAP in causation of CD has been supported by the frequent isolations of MAP from the CD patients as compared to other suspected and ulcerative colitis (UC) patients<sup>14-18</sup>, detection of MAP RNA in biopsies of CD patients<sup>10</sup>, immunological response<sup>19,20</sup> and response to anti-mycobacterial therapy by CD patients<sup>21,22</sup>. However, critics of the above theory claimed MAP to be environmental or normal intestinal commensal, and CD is the result of molecular mimicry between intestine, commensals and MAP antigens<sup>23,24</sup>. Since MAP escapes pasteurization temperature<sup>25</sup>, milk of the infected animals is the most common source of transmission of MAP from animals to human beings<sup>25,26</sup>.

Diagnosis of MAP infection is difficult, and no single test can diagnose all cases with absolute accuracy<sup>27</sup>. Available diagnostic tests may either detect the bacilli or the host's immune response. Microscopy of the samples (faeces, milk, tissues and blood) is the most convenient test, which can be performed within limited resources, but heavily depends on the expertise and training of the worker<sup>9,28</sup>. Culture being 100 per cent specific is considered 'Gold standard' for the diagnosis of MAP infection in animals<sup>28,29</sup>, and requires 12-16 wk of incubation<sup>30</sup>. Use of new techniques (BACTEC system, MGIT system and MB Bactec system) help in the rapid detection of MAP in culture<sup>31</sup>; however, high cost of equipment and biochemicals limits the use of these techniques, especially in resource-poor countries including India<sup>31,32</sup>. Nucleic acid amplification [Polymerase chain reaction (PCR)]

provides a rapid alternative for the specific diagnosis of MAP infection. IS900 element (present in 14-18 copies in MAP genome)-based PCR protocols have been optimized and most frequently used for the specific detection of MAP in clinical samples (blood, faeces and milk)<sup>33,34</sup>. A wide range of serological tests such as enzyme-linked immune sorbent assay (ELISA), agar gel immunodiffusion, delayed type hypersensitivity, interferon-gamma assay, fluorescence antibody test and complement fixation test have been successfully used for the detection of MAP infection<sup>35-37</sup>. However, due to high sensitivity, rapidity and low cost, ELISA has emerged as the most widely used test for the screening of MAP infection in herds and flocks. Indigenous ELISA developed at Central Institute for Research on Goats (CIRG), Makhdoom, Mathura, India, has been widely used indigenous kit for the screening of animals and human samples in India<sup>38-42</sup>. As compared to the kits available in international market, this indigenous, simple, indirect ELISA kit has been found to be cost-effective, sensitive and specific<sup>39,40,43</sup>. Of the four tests, microscopy and ELISA have been found to be good screening tests, and culture and PCR as confirmatory tests<sup>9,27</sup>. For chronic and insidious disease such as MAP infection, it is recommended to use multiple tests as per the purpose and resources of the animal owner<sup>9,31</sup>.

Practically, MAP infection either in animals or in human beings is incurable<sup>44</sup>. Despite removing major parts of intestines in CD patients, the disease has been found to relapse<sup>45,46</sup>. Control of MAP by treatment of sick animals is neither practical nor cost-effective since treatment cost may run over the cost of animal. Traditional method of control and management of disease (JD) in animals, based on 'test and cull' policy, besides being expensive failed to control disease<sup>47</sup> since this disease is transmitted vertically through semen, colostrum and milk to next generation, and becomes endemic in herds and flocks and continues to perpetuate. In case of human infection, the drugs used in the treatment of tuberculosis showed only 50 per cent efficacy on MAP<sup>48</sup>. For the control of the disease in domestic livestock, vaccination has been proved to be effective in the management of MAP infection in herds and flocks both in India and other parts of the world<sup>49-52</sup>. However, vaccination has mainly been used by European countries to reduce bioload and production losses in animals, thereby lowering the risk of human infection. In India, vaccination has been used to salvage the infected animal population suffering from advance stages of the clinical JD leading to reduced or

zero productivity, from culling and slaughter<sup>49,50</sup>. The 'indigenous vaccine' developed at CIRG, Makhdoom, using native strain of MAP has been found to be both 'therapeutic and preventive' in livestock species of domestic ruminants<sup>50,53,54</sup>. The vaccine has since been commercialized and licensed (No. KTK/28D/11/2008) by the Drugs Controller Department, Government of Karnataka (India) for commercial production and veterinary use. The efforts are on to develop a therapeutic vaccine for human beings in the UK and other parts of the world<sup>55,56</sup>.

Several studies have been published from India reporting MAP in different livestock species, animal derived food and food products, natural resources and human beings<sup>18,41,43,57-72</sup> (Tables I and II). The present review documents published information on the status of MAP in animals and human beings in India, highlights major factors which limit the control of infection in the country and proposes suitable strategies and research priorities for effective control of this disease in animals, which may result in minimizing exposure of MAP to human beings.

### Status of MAP infection

#### *In domestic livestock species*

Paratuberculosis was first described in Germany in 1895 by Johne and Frothingham<sup>17,51</sup>. Studies undertaken in the last few decades showed that paratuberculosis is worldwide in distribution and highly endemic in the dairy cattle herds of the developed countries<sup>73-75</sup>. In India, the first case of paratuberculosis was observed in Lahore (undivided India) in 1913<sup>76</sup>, followed by another case in 1918 from a Military dairy farm<sup>77</sup>. Thereafter (1918 to 1990), the disease was investigated on a limited scale in the country. It may be due to a lack of indigenous and cost-effective diagnostic kits and methodologies and ill-equipped infrastructure of laboratories and trained workforce in the country; consequently, variable prevalence has been reported from different parts of the country<sup>62,64,78</sup>.

In view of the advancement of indigenous diagnostic kits (ELISA, PCR, microscopy and culture kits, reagents (purified protein derivatives of bovine tuberculin and Johnin produced at Indian Veterinary Research Institute, Bareilly, India) and several well-established laboratories (Veterinary Microbiology laboratory, CIRG, Makhdoom; Pathology laboratory, Indian Veterinary Research Institute, Bareilly; Department of Microbiology and Molecular Biology, National JALMA Institute for Leprosy and Other

Mycobacterial Diseases, Agra; Department of Veterinary Epidemiology and Preventive Medicine, Madras Veterinary College, Chennai) in the country, many studies were published on the presence of MAP in different wild animals, non-human primates as well as in animal-derived food and food products in the country (Table I). Several workers have reported the moderate to high bioload (20.0-40.0%) of MAP in small and large ruminants in different parts of the country<sup>61,79-85</sup>. However, most of the information was based on a limited number of samples or samples screened in observational studies conducted on farm herds or slaughter houses or based on samples submitted; therefore, true burden of MAP in domestic livestock species is not known at the national level.

Screening of farm and farmer's herds in north India using 'indigenous ELISA kit', showed moderately high prevalence (29.0%) of MAP in bovines (29.8% cattle and 28.6% buffaloes)<sup>81</sup>. State-wise, seroprevalence of MAP was 31.9 and 23.3 per cent in animals screened from Punjab and Uttar Pradesh, respectively<sup>81</sup>. In another study, Singh *et al*<sup>84</sup> screened 829 serum samples from cattle, buffaloes, goats and sheep population in north India using indigenous absorbed ELISA kit and reported 23.1 per cent animals as positive for the presence of MAP. Prevalence of MAP was higher in large ruminants (24.1% in cattle and buffaloes) as compared to small ruminants (22.5% in sheep and goat). Seropositivity was highest in cattle (26.9%), followed by goats (23.9%), buffaloes (20.2%) and sheep (19.0%). Using multiple diagnostic tests, bioburden of paratuberculosis in clinical samples submitted from suspected population of domestic livestock from different regions of the country [north (Himachal Pradesh and Uttar Pradesh), south (Kerala and Tamil Nadu), west (Gujarat and Rajasthan) and central state of Madhya Pradesh] have been investigated and a moderate bioload (23.3%) of MAP has been reported<sup>38</sup>. Species-wise, bioload was 20.1, 32.7, 39.3 and 28.3 per cent in goats, sheep, cattle and buffaloes, respectively. Geographical zone-wise, bioload of MAP was significantly higher ( $P < 0.05$ ) in Central zone (50.5%) as compared to South (33.0%), West (30.2%), East (31.9%) and North (20.6%) zones<sup>38</sup>.

Studies from different parts of the country indicate that paratuberculosis is endemic in domestic livestock species of the country. At present, there is no national policy for the diagnosis and control of paratuberculosis in animals. Control of paratuberculosis in India is hampered mainly due to the lack of indigenous

**Table I.** Presence of *Mycobacterium avium* subspecies *paratuberculosis* in domestic and wild ruminants, other animals, non-human primates, human beings, milk and milk products, soil and water resources in India

Groups	Species/source	Year	Sample source	Number of samples	Test and number of positive (%)	References
Domestic ruminants	Goat ( <i>Capra hircus</i> )	1996	Faecal	1005	Culture: 139 (13.8)	Singh <i>et al</i> <sup>64</sup>
	Sheep ( <i>Ovis aries</i> )	2000	Faecal	-	Culture: NA	Tripathi <i>et al</i> <sup>57</sup>
	Cattle ( <i>Bos</i> sp.)	1980	Faecal	4758	Microscopy: 91 (1.9)	Kulshrestha <i>et al</i> <sup>62</sup>
	Buffaloes ( <i>Bubalus bubalis</i> )	1960	Faecal	205	Microscopy: 8 (3.9)	Mukerjee and Lahiri <sup>58</sup>
Wild ruminants	Blue bulls ( <i>Boselaphus tragocamelus</i> )	2010	Faecal	42	Culture: 10 (23.8)	Kumar <i>et al</i> <sup>59</sup>
	Hog deer ( <i>Axis porcinus</i> )	2010	Faecal	20	Microscopy: 4 (20.0) Culture: 3 (15.0)	Singh <i>et al</i> <sup>60</sup>
	Wild bison ( <i>Bos gaurus</i> )	2011	Faecal	13	Microscopy: 6 (46.1) Culture: 8 (61.8)	Singh <i>et al</i> <sup>61</sup>
Other animals	Rabbits ( <i>Oryctolagus cuniculus</i> )	2012	Faecal	77	Microscopy: 26 (33.7) IS900 PCR: 6 (7.8)	Singh <i>et al</i> <sup>63</sup>
Primates	Indian monkey ( <i>Rhesus macaques</i> )	2011	Faecal	25	Microscopy: 10 (40.0) IS900 PCR: 2 (8.0)	Singh <i>et al</i> <sup>65</sup>
Human beings	CD patients	2008	Stool	5	Microscopy: 0 Culture: 4 (80.0)	Singh <i>et al</i> <sup>18</sup>
			Biopsies	5	Culture: 4 (80.0)	
			Blood clots	3	Culture: 2 (66.6)	
	Animal attendants	2008	Stool	8	Microscopy: 3 (37.5) Culture: 5 (62.5)	
	Healthy human beings	2008	Stool	22	Microscopy: 0 Culture: 6 (27.2)	
Milk and milk products	2004	2010	Raw milk	20	Culture: 1 (0.5)	Singh and Vihan <sup>66</sup>
			Pasteurized milk	27	Culture: 18 (67.0) IS900 PCR: 9 (33.0)	
	2010	Milk products*	9	Milk culture and PCR: 5 (55.5)		
	2013	Fresh cheese	24	Microscopy: 4 (16.6)	Raghuvanshi <i>et al</i> <sup>69</sup>	
Soil and water resources	2012	Soil	51	Microscopy: 27 (52.9) IS900 PCR: 15 (29.4)	Singh <i>et al</i> <sup>68</sup>	
		River water	20	Microscopy: 6 (30.0) IS900 PCR: 2 (20.0)		

\*Ice-cream and flavoured milk. NA, not available; PCR, polymerase chain reaction

diagnostics kits and vaccines. The indigenous ELISA kit<sup>39</sup> and indigenous vaccine<sup>49</sup> for the diagnosis and control of paratuberculosis in domestic livestock have been developed using a novel native strain of 'Indian Bison type' biotype of MAP, recovered from a terminally sick goat. 'Indian Bison Type' biotype has emerged as a predominant biotype infecting domestic and wild ruminants as well as human population of

India<sup>38,60,85</sup>. Indigenous ELISA kit initially developed for goats has been validated for the screening of other animal species (cattle<sup>81</sup>, buffaloes<sup>83</sup>, Goats and sheep<sup>39,40</sup>) as well as human beings<sup>43</sup>. Comparison of diagnostic potential of indigenous and commercially available ELISA kits reported that sensitivity and specificity of indigenous ELISA kit was better as compared to commercial ELISA kits<sup>39,40</sup>. Similarly,

**Table II.** Studies investigated the presence of *Mycobacterium avium* subspecies *paratuberculosis* in human beings in India

Human subjects/ patients	Samples source	Samples	Test	Samples positive for MAP (%)	Reference
Patients with CD	Stool	5	ZN staining	0	Singh <i>et al</i> <sup>18</sup>
			Culture on HEYM	4 (80.0)	
	Biopsies	5	Culture on HEYM	4 (80.0)	
	Blood clot	3	Culture on HEYM	2 (66.6)	
	Serum	5	Indigenous ELISA	5 (100.0)	
Commercial PPA-based ELISA			2 (40.0)		
Animal attendants*	Stool	8	ZN staining	3 (37.5)	
Healthy humans	Stool	22	ZN staining	0	
			Culture on HEYM	6 (27.2)	
	Serum	71	Indigenous ELISA	27 (38.0)	
			Commercial PPA-based ELISA	29 (40.8)	
Animal keepers suspected for CD	Stool	25	ZN staining	9 (36.0)	Shisodiya <i>et al</i> <sup>70</sup>
			IS900 PCR	7 (28.0)	
	Blood	53	Blood PCR	7 (13.2)	
Animal keepers not suspected for CD	Serum	53	Indigenous ELISA	7 (13.2)	
	Stool	14	ZN staining	2 (14.2)	
			IS900 PCR	1 (7.1)	
	Blood	47	Blood PCR	1 (2.1)	
Serum	47	Indigenous ELISA	2 (4.2)		
		Patients with CD	81	Nested PCR targeting IS900 sequence	0
RT-PCR	0				
Healthy individuals**	Mucosal Biopsies from ileum and colon	85	Nested PCR targeting IS900 sequence	0	
			RT-PCR	0	
Patients with CD	Serum	5	Indigenous absorbed ELISA kit	4 (80.0)	Singh <i>et al</i> <sup>43</sup>
Patients with ulcerative colitis	Serum	22	Indigenous absorbed ELISA kit	1 (4.5)	
Healthy blood donors	Serum	13	Indigenous absorbed ELISA kit	2 (15.3)	
Random human subjects	Serum	452	Indigenous absorbed ELISA kit	106 (23.4)	
Animal attendants suspected for CD	Stool	58	ZN staining	16 (27.6)	Singh <i>et al</i> <sup>72</sup>
			Culture on HEYM	29 (50)	
Healthy humans	Stool	40	ZN staining	0	
			Culture on HEYM	5 (12.5)	

Contd...

Human subjects/ patients	Samples source	Samples	Test	Samples positive for MAP (%)	Reference
Random human population***	Stool	101	ZN staining	6 (5.9)	Singh <i>et al</i> <sup>41</sup>
			IS900 PCR	3 (2.9)	
	Blood	3093	IS900 blood PCR	262 (8.4)	
	Serum	23196	Indigenous ELISA	7893 (34.0)	

\*Working in goatherd's endemic for Johne's disease, and with different degrees of symptoms for colitis or inflammatory bowel disease (chronic stomach pain, constipation, bouts of loose motions, weakness, tiredness, anaemia, *etc.*); \*\*Healthy individuals undergoing screening ileocolonoscopy for colorectal cancer; \*\*\*Humans suffering with different diseases complaints and attended the different pathology laboratories located in Mathura and Agra cities from December, 2010 to March, 2013 on a daily basis. ZN staining, Ziehl-Neelsen staining; HEYM, Herrold's Egg Yolk Medium; MAP, *Mycobacterium avium* subspecies *paratuberculosis*; ELISA, enzyme-linked immune sorbent assay; PPA, protoplasmic paratuberculosis antigen; RT-PCR, reverse transcription polymerase chain reaction; CD, Crohn's disease

'indigenous vaccine' has been found to be superior over commercial vaccine (Gudair, CZ Veterinaria, Spain) for the protection of animals in India<sup>49,54</sup>.

Indigenous diagnostic kits and vaccine may play a major role in the management of paratuberculosis at country level. Therefore, indigenous diagnostics and vaccine developed need to be evaluated for efficacies in the different parts of the country (multilocational testing) on a large number of animal population to control the disease at the national level and improve the per animal productivity. In view of the lack of information on the national level bioburden of MAP, low priority has been given for the control of paratuberculosis in animals. In the absence of control measures, bioload of MAP has shown increasing trend in the country<sup>38</sup>. Hence, there is a need for a national survey to estimate the true burden and losses due to paratuberculosis in domestic livestock species in the country. Information derived from national survey will be crucial for the formulation of strategies for the control of paratuberculosis in the country and to minimize the risk of human exposure to MAP in the country.

### ***In humans***

In view of the remarkable similarities between the presentation of JD and CD, MAP was suggested as a possible cause of CD in humans by Dalziel in 1913<sup>86</sup>. However, Dalziel failed to isolate MAP from CD patients. His colleagues classified CD as an autoimmune disease in 1932<sup>87</sup>. In 1984, Chiodini *et al*<sup>88</sup> succeeded in the isolation of MAP from the intestines of patients with CD. Later, other researchers from different parts of the world reported the presence of MAP in clinical samples (biopsies<sup>14,15,89,90</sup>, blood<sup>16</sup> and milk<sup>91</sup>) of CD patients as compared to the patients of ulcerative colitis

and apparently normal human controls. However, some of the researchers reported the absence of MAP in clinical samples of CD patients<sup>92-94</sup>.

Prevalence of CD in the Asia-Pacific region has been estimated to be lower as compared to North America or Europe. The cases of CD have been reported to be increasing in Asian countries (Japan, Hong Kong and Taiwan) including India<sup>95</sup>. Presence of MAP in different clinical samples (intestinal biopsies, blood clot and stool) of CD patients supported possible association between MAP and CD. MAP has also been recovered from animal attendants who exhibited symptoms of inflammatory bowel disease and history of working with goat herds endemic for MAP infection<sup>18,70,72</sup>. Higher prevalence of MAP in animal keepers suspected for CD as compared to animal keepers without symptoms of CD also indicated the association of MAP and CD<sup>70</sup>. In 2009, Sasikala *et al*<sup>71</sup> reported the absence of MAP-specific DNA/RNA in intestinal tissues of CD patients in south India and did not support the association of MAP and CD in the country. The available information weighs more in favour rather than refutes the association of MAP with CD; therefore, further comprehensive studies are needed to clear the confusion on the issue.

Some of the pilot studies reported moderately high seroprevalence (23.4%) of MAP in human population of north India using indigenous absorbed ELISA kit<sup>41,43</sup>. In a mass scale screening of human population using multiple diagnostic tests reported large-scale exposure of human population to MAP (indigenous ELISA test - 34.0%; IS900 blood PCR - 8.4%; stool microscopy - 5.9% and stool IS900 PCR - 2.9%) in Mathura and Agra districts<sup>41</sup>. Biotyping of MAP isolates/DNA of human origin using IS1311 PCR-REA indicated 'Indian

Bison Type' as the predominant biotype in India<sup>72,85,96</sup>. Similar high presence of MAP and 'Indian Bison Type' biotype has been reported from unpasteurized milk, commercially available pasteurized milk and milk products<sup>67,85</sup>. Risk of human exposure to MAP through food chain has been extensively reported in literature<sup>26,67,75</sup>. Recovery of MAP from soil and water samples further indicates the contamination of the environment and natural resources, besides animals and human beings. Therefore, animals can also contract infection from the environment<sup>68</sup>. Shedding of huge quantities of MAP by large population of clinically and subclinically infected domestic livestock continuously contaminate the natural resources, hence increasing the risks of human exposure. The complex epidemiology of MAP clearly shows that for the control and prevention of MAP infection in human population, it is essential to control disease in animals.

### Conclusions

Paratuberculosis is the major problem for livestock health and productivity not only in India but also globally. Some of the studies on serosurvey using indigenous ELISA kit indicated higher bioload of MAP both in animal and human population. True burden of disease and economic losses due to paratuberculosis need to be estimated in the country. Eradication of paratuberculosis in domestic and wild ruminants should be considered as long-term objectives. More research is needed for the development of cost-effective, indigenous diagnostics and vaccine against paratuberculosis in the country. In the absence of a policy for the control of paratuberculosis in animals, human population is at a continued risk of exposure to MAP. Comprehensive studies are needed to establish association of MAP with CD and other human diseases in the country.

**Conflicts of Interest:** None.

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