



REVIEW ARTICLE

Mycobacterium avium subspecies *paratuberculosis*: an Emerging Animal Pathogen of Global Concern

Deepak Kumar Verma*

Department of Biotechnology, Faculty of Natural and Computational Sciences
University of Gondar, P.O. Box. 196, Gondar, Ethiopia

ABSTRACT

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of Johne's disease (JD), which has some pathological similarities with Crohn's Disease in human beings. The role of MAP in the causation of CD is under investigation since last 100 years. Both the disease (JD and CD) have been primarily and thoroughly investigated by western countries, where high prevalence was reported and has been rising since both are incurable. In India, limited information is available on the status on the presence of MAP infection in animal and humans. The live MAP bacilli have been recovered from raw and pasteurized milk and from human population in the country. These reports indicated that MAP infection has been established in animals in India and humans may get the MAP exposure through food chain or contaminated environment. Present review summarized the information on the status of MAP in animal and humans, economic losses and morbidity and mortality due to JD and CD at national and international level.

Keywords: *Mycobacterium avium* subspecies *paratuberculosis*, Johne's disease, Paratuberculosis, Crohn's disease, Zoonosis.

Received 09/08/2013 Accepted 28/10/2013

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INTRODUCTION

Pathogens that are transmitted between environment, animals (domestic or wild) and humans present major challenges for the animal and human health. Among such pathogens, genus *Mycobacterium* is well represented by *M. bovis*, *M. tuberculosis*, *M. avium* ssp. *avium*, *M. avium* subsp. *paratuberculosis* etc that are shared by different species of animal and primates. These pathogens resist adverse environmental conditions and degradation [36]. *Mycobacterium tuberculosis* is the most successful human pathogen, infecting nearly one third of human population and currently number one cause of deaths due to single infectious agent in the world [87]. Recently, *Mycobacterium avium* subsp. *paratuberculosis* (MAP) has emerged as major and successful animal pathogen with significant zoonotic and public health concerns [23]. MAP is an established cause of Johne's disease (JD) in domestic and wild animals and has also been associated with the Crohn's disease in humans. The role of MAP in the etiology of Crohn's disease (CD) has been debated for last nine decades. However, evidences suggesting association of MAP with human CD have provided time to time [29, 52, 71, 79]. While this debate on etiology of CD is going on, MAP is insidiously entering in the human food chain and has been isolated from retailed pasteurized milk supplies, milk and milk products and water [74, 99]. Association of MAP with CD, presence in immuno-compromised patients [63] poses serious threat to human health globally. This review summarized the prevalence of MAP in animals and its association with cases of CD in human beings at national and international levels with special reference to Indian sub-continent and its potential classification as zoonotic disease.

THE ORGANISM (*Mycobacterium avium* subspecies *paratuberculosis*)

MAP is a member of *Mycobacterium avium* complex of *Mycobacteriaceae* family. It is aerobic, short slender rod of 1-2 mm long and 0.5 mm wide, non-motile gram positive acid fast bacteria [18]. MAP is extremely fastidious and primary culture requires more than 8 weeks of incubation. Since MAP fail to synthesize soluble iron chelating compound (mycobactin), cultivation media must be supplemented with mycobactin [93]. MAP like other mycobacteria have thick waxy cell wall containing 60% lipid layer, which confers it properties of acid fastness, hydrophobicity [60], resistance to chemicals e.g. chlorine [99] and physical processes e.g. pasteurization [25]. MAP share some antigenic determinant and has similar characteristic

with phylo-genetically related *Mycobacterium avium* subspecies *avium*. The complete genome sequence of MAP strain K-10 (Genbank accession no. AE016958) has been published in 2004 [41]. Genomic analysis showed that MAP K-10 has a single circular sequence of 4,829,781 bp with 69.3% G + C content and encodes 4350 predicted ORFs, 45 tRNAs and one rRNA operon [41].

Prior to genome sequencing only 3, IS elements were identified in MAP, IS900 [26, 15], IS1311 [16]. MAP K10 genome contains 17 copies of the previously described IS900, seven copies of IS1311, and three copies of ISMav2 [41]. Basic molecular typing technique (IS1311 PCR-REA) targets a point mutation in IS1311 sequences at 223rd (C/T polymorphism) and is able to divide of MAP isolates into 3 groups; 'Cattle type' (Type II), 'Sheep type' (Type I) and 'Bison type' (B type) [100]. Johne's disease in cattle goats, deer, camelids is mainly caused by C type, where as sheep are usually infected by S type. However, in India Indian Bison type genotype is the major genotype infecting domestic and wild ruminants in different agro-climatic region of the country [77]. The presence of 'Indian Bison Type' genotype of MAP has also been reported in patients of Crohn's disease and animal health care workers (suspected for Crohn's disease) from North India [78].

HOST RANGE

MAP is a multi-host pathogen, it has been isolated from variety of animal species, including domestic (cattle, sheep, goats, buffalo, camel, yak etc.), free ranging (rabbits, fox, weasels, stoat etc) and wildlife species (deer, elk, antelopes, blue bulls, bison, llamas, bighorn sheep and alpacas) worldwide [14]. Risk of transmission of MAP from animal to human is higher with domestic ruminants than wild life animals. Domestic ruminants are source of milk and meat therefore these animals may transmit MAP directly or through products. MAP has also been isolated from primates [45, 32]. MAP can also survive in protozoa [58, 67].

JOHNE'S DISEASE OR PARATUBERCULOSIS (MAP INFECTION IN ANIMALS)

JD has become the most important disease of domestic livestock. JD being in list B of OIE, disease free certification is necessary for the export of animals and their products. JD is chronic, granulomatous, enteritis of ruminants. MAP may persist in intestine and other tissues of animals for years without causing clinical disease. However sub-clinical animals develop clinical disease, if stressed (environmental, nutritional, pregnancy, parturition, lactation or any other concurrent disease). Clinically sick animals show; chronic diarrhea, debility, progressive weight loss and emaciation. Decreased serum concentrations of calcium, total protein and albumin have been also reported in cattle and sheep with clinical JD [37]. MAP is frequently excreted in feces, milk and semen of clinical or sub-clinical infected animals thus increasing environmental load of MAP. Newborn animals acquire infection from infected mothers or environment, early in life but symptoms usually develop later at 2-6 years of age in cattle. Bacilli parasites in reproductive and other organs of both males and females [97] and can cross placenta to enter fetus [7]. Vertical and horizontal transmission of disease has been reported in calf [95]. Pathology of disease varies indifferent animal species and between different organs within animals. Affected animals are unable to absorb digested nutrients and fluid from intestinal tract and if do not recover may die but appetite may remains normal until death.

The available diagnostic measures for JD suffer with poor sensitivity and specificity and fail to detect the infection in early stage of disease. Culture considered 'Gold standard' test has limitations. Mostly confirming diagnosis is made after death at postmortem. There is no treatment of JD. Though a variety of antimicrobials have been tried for satisfactory treatment and is not considered a long-term option due to high cost associated with treating entire herd for prolong periods.

In poor and resource constrained countries, status of JD with respect to national prevalence, production losses, geno-types, diagnosis, control measures, prevalence in other animals etc., is not known. In India information regarding prevalence of JD is limited in huge ruminant population (about 500 million domestic ruminants). Some studies, in buffaloes (86, 103], cattle [75], sheep [97] and goats [40, 83] indicated that disease is established in animal herds and flocks in the country and need urgent attention for its control.

Prevalence and geographic distribution of Johne's disease- JD is distributed worldwide [7], and endemic in cattle in most of the developed countries. In a study from Czech Republic, reported 52.5 and 54.7 % JD positive animals in beef and dairy herds, respectively [3]. Herd prevalence of bovine JD has been estimated in; South West of England - 3.5%, Belgium - 8.0%, Czech Republic - 12.0%, Italy 13.3%, Denmark, 70.0%, The Netherlands 31.0-71.0% [11, 5, 38, 35, 51]. In India most of the early studies were based on Johnin and fecal examination [39, 81]. JD in India is studied at limited scale, using different diagnostic material (feces, tissue, milk, and blood). Using serum ELISA as a diagnostic tool, it was reported 28.0% bovine (cattle and buffalo) were positive in North India [85]. Sero-prevalence of JD in

goat kid from farm and farmers herds was 47.9% in Agra and Mathura region [40]. However, 30.9, 50.0 and 11.8% adult goats from South UP, west UP and Punjab were sero-positive, respectively [85]. Limited studies have been carried out in buffalo and cattle. Prevalence of MAP in Mathura region was 20.8 and 28.3% in dairy by ELISA kit (develop using indigenous PPA from MAP 'Bison type' of goat origin) and fecal culture, respectively [48]. Using milk as a diagnostic test, 88.4 and 96.1 cattle from Ludhiana were positive in milk ELISA and culture [85]. Sero-prevalence in buffalo was estimated to be 21.3% in Chennai [86], 40.3 and 25.5% in south and west UP, respectively [85]. However, using tissue culture 48.0% buffalo from Agra region were positive for MAP infection [103]. Pathologically, it was reported that 4.9% buffalo from Bareilly region were MAP infected [86]. In Haryana, using hypersensitivity reaction reported 8.5% of bulls were found infected by MAP using hypersensitivity reaction [62].

Economic losses –JD causes considerable, huge economic losses in developed and developing countries. Most of the losses occur due to sub-clinical stage of disease, in the form of progressive weight loss, reduced milk production, lower slaughter value, and premature culling, and reduced fertility. Economic losses due to bovine Johne's disease (BJD) have been estimated, New England – \$15.4 million, Wisconsin – \$52.3 million, Pennsylvania –\$5.4 million, US- \$200-250million, Australia- \$2.1million [88, 98]. Direct production and treatment losses was studied annually in an average (7%) JD infected cow herd and found highest losses occur due to, JD (USD 2 472) than Bovine viral diarrhoea, neosporosis and enzootic bovine leukosis [12]. Losses due to JD have not been investigated in India.

Morbidity and mortality – MAP has been responsible for increased mortality, reduced reproductive performance and higher susceptibility in dairy herds. Sub-clinical infections and lack of data on prevalence, morbidity and mortality caused by MAP, JD has not received the priority in developing countries. In Australia, average mortality rate of ovine JD (OJD) was estimated in 12 farms as 6.2% (range 2.1 to 17.5%) in 2002 and 7.8% (range 1.8 to 14.6%) in 2003 [8]. In India, information on mortality and morbidity losses due to JD in large ruminant, but some information on goat and sheep are available. In a study 18.7% morbidity and 14 % mortality was estimated in JD suspected Jumanapari goats of Mathura region, North India [82]. In Rajasthan, a 7 years (1990-1997) study showed high morbidity (27.1 – 75.1%) and mortality (8.1 – 19.1%) due to JD in Multan Carpet wool than Chokla breed (Morbidity- 29.0 – 61.7% and Mortality 4.6 – 24.8%) of sheep [4]. In the country there is no proper documentation of morbidity and mortality due to JD and some times misdiagnosed with weakness and other alimentary diseases.

***Mycobacterium avium* subsp. *paratuberculosis* INFECTION IN HUMAN (CROHN'S DISEASE)**

CD is a systemic disorder whose principal clinico-pathological manifestation is chronic inflammation of intestine. It is characterized by weight loss, abdomen pain, diarrhoea and general malaise [13]. Its peak onset is in late teens to early adult life, with a secondary peak in the elderly. Clinical presentation depends on the site of inflammation. Pain is a common feature particularly those with small bowel involvement. Diarrhea, often with bleeding, occurs in colonic CD. Patients with ileal disease may present with an illness very similar to acute appendicitis. Fistulae are characteristic of CD. Approximately 25% of patients with CD develop perianal complications, in the form of fistures [72]. Bowel obstruction is a known complication, which may require surgical resection. It has been reported that up to 80% patients with CD will require surgery at some stage of their illness [72], and approximately 50% of surgical cases require additional surgery within five years because the disease tends to reappear at the site where bowel was rejoined and some patients eventually develop short bowel syndrome which makes it extremely difficult to digest food. Treatment with immunosuppressive drugs and surgical removal of the affected portion of intestine are used to control symptoms of CD, but at present there is no cure for this disease. Generally patients live with chronic pain throughout their lives.

The etiology of CD is mysterious and still controversial, although it is suggested that patients with CD are a heterogeneous group and the etiology may not be same for all patients. There is a genetic predisposition to develop CD, recently 3 independent studies suggested that mutation in a gene on chromosome 16, known as NOD2/CARD 15 are associated with CD [27, 56, 34]. Infectious cause for CD has been sought since the disease was first described in 1932 and number of micro-organism has been suggested as the cause of CD. But MAP has the attention for the causative agent of CD due to the clinical signs and pathological similarity of JD with CD initially and later frequently isolation of MAP from tissues of CD patients [69, 52, 71]. Early studies did not detect MAP in tissues from patients with CD by conventional staining and culture techniques [94, 68]. Failures in detection may be due unusual nature of cell wall deficient MAP in patients with CD and/or challenges in culturing MAP with its fastidious and slow growing characteristic. Problems in culture were overcome by use of MAP specific IS900 insertion sequences for proxy diagnosis of MAP infection [26, 59]. Identification of this insertion sequence from bacterial culture, feces and tissues by PCR technique have made rapid and sensitive detection with

accuracy possible. But PCR fails to differentiate the live MAP or MAP DNA. RT-PCR was used for amplification and confirmed the presence of MAP RNA in the test samples [47]. Using in-situ hybridization by IS900 specific probe, the presence of MAP in biopsy samples of CD was confirmed and provide evidence for role of MAP in the etiology of CD [70]. Sero-reactivity of CD has been examined in ELISA using protein antigen from MAP. Test has shown significant higher proportion of MAP specific antibodies in CD patients than control sera [91, 96]. MAP has been recently isolated from breast milk of lactating mothers [53] and from blood of CD patients indicating that MAP infection is systemic like paratuberculosis in animal [52].

The presence of *Mycobacterium avium subspecies paratuberculosis* (MAP) antibodies was estimated in human serum samples originating from North India using 'Indigenous absorbed plate-ELISA kit' (ELISA-kit) [80]. Seroprevalence of MAP antibodies was estimated as 23%, on the basis of screening of 452 human serum samples (without history) from different geographical regions of North India. Region-wise, 34.0, 33.3, 32.8, 25.0, 23.0, 17.7, and 12.5% samples were positive from the states of Punjab, Uttarakhand, New Delhi, Himachal Pradesh, Haryana, Uttar Pradesh and Jammu and Kashmir, respectively [80].

Prevalence and Geographic distribution of Crohn's disease- Because most epidemiological studies till date have concentrated on specific regions or populations within a country, the full extent to which CD has reached cannot be completely grasped. However, these reports indicate more prevalence in western populations with northern European and Anglo-Saxon ethnic derivation, than in populations of southern Europe, Asia and Africa [50]. The incidence of the disease has increased progressively in the last 40 years with 5±11 cases per 100,000 population in most developed countries including the US [9, 20]. It is estimated that 500,000 CD sufferers in the USA rising by 68.4 patients /day and about 25,000 new cases each year. In some district of Manitoba, USA, this incidence of CD has reached 262 /100,000 /year, the highest reported in the world [30]. It was that reported 1.4 million persons in the US and 2.2 million persons in Europe suffer from these diseases [42]. In northern Europe it is reported as 7/100,000/ year, in southern Europe 4/100,000/year with an overall incidence rate in Europe of 5.6 /1,00,000 / year [76]. In UK, an annual incidence in the range of 6-13/ 100,000 is quoted and in 150 /100,000 were reported [21]. In Asia, the prevalence of CD in Japan and Southern Israel was 5.8 and 50. 6 /10, 000/ year and has been rising with 0.5 and 4.2/ 100000 person per year, respectively [55, 49].

In Asian countries, patients with CD are frequently misdiagnosed as having intestinal tuberculosis due to limited diagnostic facilities and / or lack of awareness, amongst gastroenterologist [57, 17]. But recent reports indicate that incidence of CD is rising in various Asian countries including India [43, 57]. First case of CD in India was reported in 1986 [2]. Now days, CD is more frequently reported from different parts of the country [61, 57, 1]. The stool samples of persons suffering from chronic colitis and working in goats and sheep farms endemic for MAP infection were investigated and 62.5% were found positive for typical MAP colonies by culture and 50% were positive for AFB. Higher MAP sero-positivity, 42.1 and 40.7% using protoplasmic antigen of MAP 'Bison type' strain of goat origin and commercial PPA from MAP 'Bovine' strain, respectively, are reported in the sera collected from Agra region [84, 85]. On the screening of 5 biopsies and 3 blood clots cultured from the chronic patients (Delhi region) of CD, 4 (80.0%) and 2 (66.6%) samples were positive, respectively. This is the first confirmed report of association of MAP with cases of CD, in India [79].

Economic losses- Crohn's disease is important both for the pain and difficulties, it cause and for the huge treatment expenditure. Economic consequences of CD have been investigated from various developed countries but the information from developing countries is scare. There were not a standard criteria for the estimation of economic losses, so the available information is based on different criteria, parameters and calculations and the total findings are difficult to compare. Due to the fact that some of the consequences of this disease cannot be quantified and some of them are only hypothetical, an accurate estimation is impossible. In Germany, the economic cost to society for CD and ulcerative colitis was evaluated. The total medical cost per patients for one month was 1,425 Euro for CD and 1015 euro for UC [89]. The estimated average annual medical cost per US patient with CD was \$6,561 which was estimated in 1990 [28]. The annual cost, \$ 37, 135 for care of hospitalized US CD patients was estimated [6]. In UK individual patients cost was 1652 UK pounds/ six months. In Sweden, CD is estimated to have cost \$41.3 million in 1991 [19]. No such data have been reported from India.

Morbidity and Mortality-CD patients have higher mortality rate than expected [10, 44]. In Canada, a 16 years of study (1971-1986) analyzed hospital discharges and deaths for patients with CD and ulcerative colitis and cause of death data showed 556 deaths directly attributed to CD and 761 attributed to ulcerative colitis. Under 45-age group accounted for 25.0% of deaths due to CD and for 17.0% of deaths due to ulcerative colitis [64]. Time trends for IBD (CD and UC) hospital discharge rates in Canada closely parallel the findings of hospital discharge rates in United States and England-Wales [64]. Recently, the predictive value of phenotypes at diagnosis on overall and disease related mortality in a European group

of CD patients was assessed [102]. Overall and disease related mortality were recorded 10 years after diagnosis in a prospectively assembled, uniformly diagnosed European population based inception group of 380 CD patients diagnosed between 1991 and 1993. Standardized mortality ratios (SMRs) were calculated for geographic and phenotypic subgroups at diagnosis. Thirty seven deaths were observed in the entire group whereas 21 deaths were expected. Mortality risk was significantly increased in both females and males. Patients from northern European centers had a significant overall increased mortality risk, where as a tendency towards increased overall mortality risk was also observed in the south. Mortality risk was also increased in the age group above 40 years at diagnosis for both total and CD related causes. Excess mortality was mainly due to gastrointestinal causes that were related to CD.

PUBLIC HEALTH ISSUES

Number, route, mode of transmission and stability of an infectious agent outside the host determine the infectivity of the pathogen. MAP with strong zoonotic potential is a sharp veterinary pathogen. Infected animals secrete huge amounts of MAP bacilli in their feces and milk. MAP is able to survive pasteurization temperature and is resistant for chlorine treatment. So the opportunity exists for humans to become infected with this organism via milk, milk product, beef and water supply.

Milk and dairy products- Clinical, sub clinical and apparently healthy animals excrete MAP in their milk [90, 75, 40]. Moreover, it is more heat resistant than other *Mycobacterium* species and *Coxiella burnetii* (the current target of pasteurization) and is able to survive at the current pasteurization standards [92]. The first study to confirm the presence of MAP in commercially pasteurized milk was carried out in UK, 1.8% pasteurized milk samples tested positive for MAP culture [24]. MAP has been frequently isolate from pasteurized milk from Republic of Ireland [54], Canada [22], Australia [46], Czech republic [3] including India [74]. In India, first time, the presence of live cultivable MAP was investigated in un-pasteurized milk and commercial brands of pasteurized milk and milk products, marketed in 3 major cities of North India for human consumption. A very high contamination of MAP 43.7 and 55.5% in un-pasteurized and pasteurized milk respectively, were reported [74]. Viable MAP have been also found in milk of human with CD, which increases the risk of MAP exposure to newborn [53]. Presence of viable MAP in dairy products other than liquid milk has not been studied extensively. The presence of MAP DNA in 49.0% infant milk powders samples from 10 manufacturers in 7 European countries by PCR and viable MAP in one samples was reported [33]. In a study 55.5% milk products were positive in culture [74]. Above evidences suggest that consumption of un-pasteurized and pasteurized milk and milk product is not safe and may be preferred route of transmission of MAP to human population.

Beef- MAP cause systemic infection in advance stage of JD and has been frequently isolated from intestine and mesenteric lymph node of clinical, sub-clinical and apparently healthy animals. Besides target tissues MAP was distributed to udder, SMLN, uterus and testis [97]. In a study MAP from large intestine (36.0%) and MLN (34.0%) tissues of slaughtered buffaloes was reported from India [103]. However, MAP was isolated from 43.6% intestine and 40.0% MLN of slaughtered kids [40]. It was suggested that meat from old dairy cows, used to make ground beef for human consumption may represent a source of MAP infection for consumers [65]. These studies indicate that meat obtained from sub-clinical and apparently healthy animals can be contaminated with MAP. Meat could also be contaminated with fecal material during slaughtering and processing procedures. No information is available regarding the destruction of MAP at cooking and meat processing methods. It is possible beef consumption can be the cause of human exposure to this pathogen.

Water- MAP is excreted in feces of symptomatic and asymptomatic JD infected animals [14], and is able to survive in the environment for prolong periods [101]. Water derived from underground sources is unlikely to be affected by pollution from JD infected animals and further low level of contamination can take place through agriculture run-off in catchments area, used for supplying water for domestic use as well as to estuarine the environment. MAP is able to survive digestion by protozoa that are usually bacteriovores. Indeed this, it has been suggested, allow MAP to acquire a phenotype more pathogenic to human beings [31]. Such internalization may also afford MAP added protection against agents such as chlorine that are used in water treatment operations. MAP located intra cellularly was significantly more resistant than free MAP. This work showed that water may be a vehicle for spread of MAP from animal to human. There is need to determine the efficacy of water treatment operations to remove or inactivate MAP. Non-processing or proper disposal of animals and human excreta also help to contaminate the water resources and environment of MAP. Due to high resistance of MAP to environmental degradation help to increase the load of bacilli in the environment, which in-turn gain access to human and animal food chain and thus maintain the cycle of MAP in environment.

CONCLUSION

Rising incidences of Johne's disease, presence of MAP in food chain are alarming signs for public health. Due to high economic losses, long incubation period, difficult diagnosis, strong survival potential, existence in human food chain and possible link to CD, MAP is an emerging pathogen of concern worldwide. There is urgent need to design the strategies to control spread of MAP in animals and prevent its transmission to human from animals, national and internationally.

ACKNOWLEDGEMENT

I would like to thank Dr Ajay Vir Singh, Department of Microbiology and Molecular Biology, National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra, India for proofreading the manuscript.

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Citation of This Article

Deepak Kumar Verma. *Mycobacterium avium* subspecies *paratuberculosis*: an Emerging Animal Pathogen of Global Concern. Adv. Biores. Vol 4[4] December 2013: 01-08