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Review Article

Strain diversity within *Mycobacterium avium* subspecies *paratuberculosis* — A review

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Mycobacterium avium subspecies *paratuberculosis* (MAP), is the etiological agent of Johne's disease (or paratuberculosis) in animals and has also been linked with Crohn's disease of human beings. Extreme fastidious nature of the organism (MAP) has hampered studies on diversity within the organism. Studies based on phenotypic properties like growth rate, pigmentation, lipid profile etc., are unable to provide complete information on diversity of MAP organism in nature. However, with the advent of molecular assays (IS*900* RFLP, PFGE, IS*1311* PCR-REA, SSR typing, VNTR typing etc.) in last 2 decades, progress has been made to differentiate MAP strains. MAP isolates have been classified into various types and subtypes using these molecular tools. Optimization of these typing assays has led to generation of new information about MAP strains, subtypes, their comparative genomics, relative evolution, comparative virulence etc. Knowledge of strain diversity is important for better understanding of molecular and sero-epidemiology, infection and patho-biology, vaccine development and planning control strategies. The present review provides available information on MAP strains, host adaptations, their virulence, comparative genomics, relative genetic evolution and differentiation.

Keywords: Comparative genomics, Host adaptation, Host response, Inter-species transmission, *Mycobacterium avium* subspecies *paratuberculosis,* Paratuberculosis

Introduction

Paratuberculosis or Johne's disease (JD) caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a debilitating chronic granulomatous enteritis of ruminants. MAP is recognized as serious animal health pathogen. MAP is also of public health significance due to its association with Crohn's disease in human beings[1]. MAP has widest host range; including domestic and wild ruminants, free ranging animals, birds, farm animals (rabbits) and human beings. The disease is of great economic significance for animal industry due to negative impact on animal productivity leading to premature culling, reduced carcass value, decreased body weight, increased susceptibility to other infections, reduced fertility and milk production. Nearly 68% of cattle herds in US are infected with JD resulting in estimated loss of USD 250 million annually[2]. Treatment is long, which is not practical and cost effective. Control measures practiced worldwide against JD are - incorporation of hygienic and better management practices, culling of infected animals and vaccination.

So far these control strategies are not effective and burden of disease and micro-organism is continuously increasing in the environment world-wide. In this respect, control strategies based on epidemiological considerations may prove useful in lowering down the increasing burden of MAP and disease. Strain differentiation through genotyping is useful tool in epidemiological investigations, to understand origin of infection, disease transmission, pathogenesis, virulence, evaluation of regional and national control programmes, permitting a rational design of more adequate control measures, improvement in diagnostics and vaccine development. Understanding of genomic diversity may also provide additional insight into mechanism of host-specificity and association of specific genotypes with overt disease versus sub-clinical status. Molecular epidemiological research has long been hampered due to fastidious nature of MAP and only limited numbers of MAP isolates are maintained in available collections. Despite constrains, past two decades have seen increased interest in mycobacterial research and application of molecular strain typing methods has increased for identifying genetic diversity within MAP. Recently, sequencing of genome of MAP (K 10)[3] has helped to identify additional molecular markers for better differentiation of MAP isolates. This has further initiated the work on comparative genomics of MAP strains and identified differences at

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genomic level among diverse strains of MAP[4-7]. To some extent molecular assays has also helped in studying the comparative pathogenicity of different MAP strains in different host systems[8-10]. Earlier studies have also helped in predicting the phylogeny of MAP strains[7]. The present review reveals the current state of knowledge on diversity of MAP strain, their comparative genomics, virulence, host preference and evolution.

MAP strains—Phenotypic and molecular diversity

Phenotypic diversity—Culture characteristics of MAP strains have been utilized to discriminate between different biotypes. Two phenotypes of MAP have been described based on the pigmentation and growth rate[11]. One phenotype has yellow pigment, extremely slow growing (more than 16 weeks of incubation) and usually reported from sheep (Type I or Sheep type). The other phenotype is non-pigmented (Type II or Cattle type), and appear significantly fast growing (6 to 12 weeks) in artificial medium and has been usually reported from cattle goat and other animals. Fastidious nature of MAP has limitation for phenotypic identification. Other methods including serology, biochemical assays, gas-liquid chromatography, and antimicrobial susceptibility are not capable of differentiating MAP isolates[12, 13].

Molecular diversity—Despite difficulties in primary isolation and subsequent maintenance of MAP isolates, past two decades has seen increased interest in application of molecular strain typing methods for identification of genetic diversity within MAP. Molecular techniques used for MAP typing can be divided into 2 basic types — primary genotyping tools; and sub-typing tools. Primary genotyping tools give basic information regarding principal genotype of MAP isolates; and sub-typing tools further divide principal MAP genotypes into various sub-types.

Primary genotyping tools

IS900 RFLP—IS*900* RFLP divides MAP isolates into two principal groups, one group is for isolates predominantly recovered from sheep (Sheep type or Type I), while other group is for isolates recovered from cattle, goats, other animals and human beings (Cattle type or Type II)[14]. Some studies have shown that IS*900* RFLP further divides, 'Sheep type' isolates into a new sub-group called as 'Intermediate type' or 'Type III' [11]. Collectively 'Sheep type' isolates are designated as Type I/III[15]. IS*900* RFLP is time-consuming, laborious and requires large amount of high quality DNA and also suffers with lower discriminatory power. Slow growing nature of MAP further limits the use of RFLP based approaches.

IS1311 RFLP—Like IS*900* RFLP, IS*1311* RFLP also divides MAP isolates into 2 principal groups Type I and Type II[16] and suffers with the same disadvantages as described for IS*900* RFLP.

IS1311 PCR-REA—It' is a PCR-restriction endonuclease analysis (REA) method that targets a point mutation in IS*1311* sequences at 223rd (C/T polymorphism) and is able to divide MAP isolates into 3 groups—'Sheep type' (Type I), 'Cattle type' (Type II) and 'Bison type' (B type)[17]. 'Bison type' genotype of MAP has been reported for the first time from wild bison of Montana, USA[17] and later this genotype has also been reported from domestic ruminants (buffalo, cattle, goat and sheep) and human beings in India[18-20]. Recent studies have shown that 'Bison type' MAP is the predominant genotype infecting livestock population in India[21,22]. 'Bison type' genotype has also been recovered from Crohn's disease patients[21]. 'Bison type' strain has genetic and phenotypic differences with 'Cattle type' and 'Sheep type' strains. Although IS*1311* is not unique to MAP, IS*1311* PCR-REA has the advantage over RFLP tools in terms of simplicity of the test and higher discriminatory potential. Moreover, it can be applied directly on clinical samples to target the strain diversity of non-culturable strains of MAP (mostly of sheep and human origin).

Pulse field gel electrophoresis (PFGE)—Like IS*900* RFLP, PFGE divides MAP isolates in two main groups—'Sheep type' (Type I) and 'Cattle type' (Type II) and is also able to further divide 'Sheep type' isolates into 'Intermediate type' (Type III) [23,11]. PFGE also requires large quantity of good quality DNA and therefore is of limited use in typing of MAP isolates and from clinical samples.

Multiplex PCR for IS900 loci (MPIL)—This method is based on IS*900* locus polymorphism (Presence/Absence of IS*900* at defined locus), and involves a multiplex PCR for different IS*900* loci. MPIL also divides MAP strains into 2 major groups—'Type I' and 'Type II' [24]. Despite some technical advantages over RFLP, MPIL does not provide any additional discrimination.

RDA-PCR—Dohmann *et al.*[6] have identified 'Type I' MAP specific three regions (*pig-RDA10*, *pig-*

RDA20 and *pig-RDA30*) using representational differential analysis (RDA) that have no homology with MAP K10 genome (a 'Type II' MAP). Based on these variations, RDA-PCR has been developed that is capable of differentiating 'Type I' and 'Type II' MAP.

Sub-typing tools

Short sequence repeat (SSR) typing—SSR typing offers a facile and reproducible high resolution typing method for geno-typing MAP isolates. Amonsin *et al.*[25] have initially used SSR typing based on 11 SSR loci and identified 20 distinct SSR types for MAP isolates. They have also shown that G and GGT repeat SSR loci are highly polymorphic among all the loci tested. Following this initial report, G and GGT loci have been widely used to type MAP isolates. SSR typing divides primary MAP genotypes into various subtypes[7, 26-29]. SSR typing has shown that certain sub-types (SSR types) are host restricted, others are shared (interspecies transmission) among different host species[26,28] (Table 1). Most importantly, SSR typing has revealed that of various MAP subtypes, only 7g4ggt and 7g5ggt subtypes have the ability to infect human beings [26]. SSR typing has also shown that specific SSR types are associated with sub-clinical disease and others are associated with clinically overt disease (highly virulent)[26-28].

Variable number tandem repeat (VNTR) analysis—Overduin *et al.*[30] and Biet *et al.*[31] have employed 5 and 8 polymorphic VNTR loci to type MAP isolates and generated 6 and 12 VNTR types, respectively. They have subdivided principal MAP genotypes into various subtypes using VNTR analysis. Thus VNTR typing is a convenient tool for epidemiological surveys.

Large sequence polymorphism (LSP) typing—Semret *et al.* [4] have proposed a simple PCR-based method for typing MAP isolates based on the presence or absence of LSPs. Sohal *et al.* [7] have proposed alternative method to study the distribution of LSPs among different MAP strains. Results have shown the variable distribution of LSPs in epidemiologically different MAP strains.

Single nucleotide polymorphism (SNP) typing—Marsh and Whittington[32] have proposed a method that is capable of differentiating 'Type I' and 'Type II' MAP. This method is based on 11 SNPs in 8 different genes of MAP. Similarly, based on SNP in *gyrB* gene, Castellanos *et al.*[33] have proposed a PCR-REA based tool that can discriminate 'Type III' strains from 'Type I' and 'Type II' strains. Recently, Sohal *et al.* [7] have identified a sequence variation in IS*1311* element that can specifically discriminate 'India Bison type' MAP strains from other non-Indian MAP isolates.

MAP strains: Media requirement and incubation period

Different culture media have been used for isolation and cultivation of MAP. Incubation is usually 16 weeks[34], however in some cases colonies may take years to emerge[35]. Different MAP strains have shown different nutrient preferences. For 'Type II' MAP isolates HEYM (Herrold's egg yolk medium) with mycobactin J has been recommended[36-38]. For 'Type I/III' MAP isolates, LJ (Lowenstein-Jensen), Middlebrook 7H10 and 7H11 agar media with mycobactin J have been recommended[36,39]. Merkal *et al.*[40] have shown that addition of sodium pyruvate to HEYM enhances growth and reduces incubation period. However, it has been confirmed that addition of sodium pyruvate to medium enhances the recovery of 'Type II' MAP strains[15]. There is no difference in the recovery rates of 'Type I/III' MAP by incorporating sodium pyruvate to the medium[38]. HEYM with mycobactin J has also been recommended for isolation of 'Bison type' strains[17, 18]. However, unlike 'Type I' MAP strains, 'Bison type' strain does not prefer sodium pyruvate[41, 18]. Culture phenotypes of MAP can be correlated with the genotypes. 'Cattle type' strain is readily cultivable compare to, 'Bison type' strains

Table 1—Distribution of SSR types in different host species (Ghadiali *et al.*[26])

SSR Type	Host Species
11g5ggt	Cattle, goat
13g5ggt	Cattle, sheep, starling
12g5ggt	Cattle, goat, starling
14g5ggt	Cat, cattle, raccoon, sheep, starling
>15g5ggt	Cattle, goat, mouse, sheep, starling, shrew
7g5ggt	Cattle, deer, goat, human, raccoon, sheep, starling
p3ggt*	Sheep
10g5ggt	Cattle, deer, goat, human, raccoon, sheep, starling
15g3ggt	Sheep
P7g3ggtΨ	Sheep
7g6ggt	Starling
7g4ggt	Cattle, deer, goat, human, raccoon, sheep, armadillo
9g4ggt	Cattle
8g4ggt	Cattle

*Polymorphic GGT Repeat (AGTGGTGGT)
ΨPolymorphic G Repeat (GGCGGGG)

which are difficult to isolate[41]. Among all MAP strains, 'Sheep type' strains are extremely difficult to cultivate[41]. Taking into account the interspecies sharing of MAP strains, de Juan *et al.*[38] have recommended use of four solid media (HEYM with sodium pyruvate and mycobactin, HEYM with mycobactin; LJ with mycobactin; and Middlebrook with mycobactin J).

It has been proved that incubation period depends on type of strain and not on host of origin[38]. 'Type I' and 'Bison type' MAP generally requires 3-4 months[41,42] of incubation period compared with 6 months required by 'Type I/III' MAP[38]. However, MAP isolates from human beings may require more than one year incubation period for appearing in the form of colonies[35] (pauci-bacillary). This prolonged incubation may be due to the fact that MAP in human beings resides as cell wall deficient form (CWD form). Hence, prolonged incubation period of more than 8 months has been recommended to ensure the complete recovery of MAP[38].

MAP strains: Host response

Observations based on phenotype methods and preliminary genotyping studies indicate that 'Type I' MAP infects sheep and 'Type II' MAP infects cattle and goats[43-45]. These findings are also supported by observations on the failure of natural transmission of disease to sheep population exposed to cattle population endemic for paratuberculosis[46]. Later, genotyping studies highlight that interspecies sharing of MAP strains occur in nature. Sheep kept in cattle farm (endemic for paratuberculosis) has been found to be infected with 'Type II' MAP[6]. de Juan and workers[15] have provided first evidence of natural infection of cattle or goats with 'Type I' MAP. Indian studies have shown that 'Indian Bison type' MAP has the ability to infect multiple host species including domestic animals, wild animals and human beings[7, 22]. However, outside India 'Bison type' strains of MAP has only been encountered from wild bison animals [21] and so far there have been no reports from other parts of world on the isolation of 'Bison Type' MAP from domestic animals. Hence, it may be hypothesized that 'Indian Bison type' strains of MAP have accumulated certain variations at genetic level enabling them to infect multiple host species. Recently, it has been shown that 'Indian Bison type' MAP strains have certain genetic differences compared to 'non-Indian' MAP strains[7]. Though not yet proved, these variations may account for the ability of 'Indian Bison type' MAP to infect multiple host species. The highly pathogenic 'Indian Bison type' MAP strains have not been so far reported outside India.

Sub-typing studies based on SSR typing have proved that certain subtypes are host restricted, and others are shared between different host species (inter-species transmission; Table 1). According to earlier studies, 7g4ggt and 7g5ggt subtype have ability to infect multiple host species[25,26,48,49]. Molecular sub-typing of 'Indian Bison type' isolates, reveal 7g4ggt profile, further strengthen the observations on the interspecies sharing of 'Indian Bison type' strains based on primary genotyping studies[7]. Interestingly, only two subtypes (7g4ggt and 7g5ggt) have been observed for human MAP isolates[26]. This restricted variation in human isolates is indicative of ability of few animal subtypes to be associated with pathobiology of Crohn's disease. As both these subtypes (7g4ggt and 7g5ggt) have ability to infect multiple animal species indicating that human beings may have acquired MAP infection from animals[7,26].

In vitro studies have shown that survival and persistence within macrophages is a function of genotype. Janagama *et al*.[9] showed that bovine isolate (B1018) with 7g4ggt SSR type remains in higher numbers within monocyte derived macrophages (MDMs) relative to human isolate (Hu6) with 7g5ggt SSR type and sheep isolate (S7565) with 15g3ggt SSR type. Cells stimulated with bovine isolate (7g4ggt type), up-regulate expression of IL-10 (anti-inflammatory cytokine) and down-regulate the expression of TNFα (pro-inflammatory cytokine), enabling this isolate, to persist for longer period. Compared to B1018, Hu6 and S7565 significantly down-regulate the IL-10 expression and up-regulate the expression of TNFα. Compared to sheep isolate, human isolate persists for longer periods in MDM cells.

Comparative transcriptional analysis of human macrophages exposed to isolates of different origins with different SSR types (cattle-7g4ggt and 15g5ggt, sheep - 15g3ggt and 2gC4g3ggt, human-7g5ggt and bison - 7g4ggt isolates) by Motiwala *et al.*[43], reveals that MAP isolates with different genotypes differentially regulate the expression of immune genes. In general, human (7g5ggt), cattle (7g4ggt and 15g5ggt) and bison (7g4ggt) isolates, down-regulate the pro-inflammatory response genes and un-regulate the genes of anti-inflammatory response and anti-apopototic response, making them a successful pathogen in human macrophage system. Conversely,

sheep isolates (15g3ggt and 2gC4g3ggt) up-regulate the genes involved in pro-inflammatory response, making conditions adverse for its survival. Cattle, bison and human isolates induce anti-inflammatory and anti-apopototic pathways, but the level of expression of different genes of the two pathways differs in these isolates[8]. This differential induction may account for their relative pathogenic ability in particular host.

Gollnick and workers[10] have also shown that survival of MAP in bovine MDMs is affected by genotype. 'Bovine strains' are more successful for survival than 'ovine strain' in bovine MDMs. Bovine MAP strains, 1180 (with 7g6ggt profile) and 1099 (with 7g5ggt profile) are highly successful and bovine MAP strain 1018 (with 7g4ggt profile) is least successful in terms of percentage of infected MDMs and the number of bacteria per infected MDM. The ovine strain, 7565 (with 15g5ggt profile) is the only strain that shows a significant decline in bacteria per infected cell over time.

In recent years, there has been increased interest in studying the host resistance towards susceptibility/resistance to MAP infection. Studies are limited, but have highlighted the breed factor i.e. certain breeds of a particular animal species are relatively resistant to MAP infection compared to others. Studies from India have shown that goat breeds of semi-arid zone (Barbari and Jamunapari) are more susceptible to infection with MAP, whereas arid zone breeds (Sirohi and especially crossbred Rajasthani type) are comparatively resistant[50]. Jersey, Guernsey and Limousin breeds of cattle are considered to be susceptible breeds for paratuberculosis infection[51-53]. Similarly, Scottish Blackface and Shetland sheep breeds are also considered to be susceptible breeds to paratuberculosis[51].

Comparative genomics of MAP

Availability of MAP K10 genome sequence has helped to initiate comparative genomics of MAP with other bacteria and among MAP strains. Comparative genomic hybridization studies reveal that MAV (*Mycobacterium avium*), displays higher genomic diversity compared to MAP, and among MAP isolates those from wildlife animals display higher level of genomic diversity[45]. Comparative genomic hybridization has identified 24 MAV genetic islands (GIs) absent from 95% of MAP isolates and 18 MAP GIs absent from MAV isolates[54]. Compared to MAV, MAP GIs contain mobile genetic elements[54]. Semret *et al*.[4] have also shown that most of MAP specific LSPs contain mobile genetic elements. Mobile elements can play role in genetic rearrangements through simple transposition and integration and may result in inversions of large genomic DNA fragments. Comparative genomic analysis of MAV and MAP has identified 2 large genomic inversions[54]. Such genomic inversions can contribute to reversible phase and variations and because of these inversions it can be predicted that despite high overall sequence identity between MAP and MAV, substantial differences in expression profiles may exist in both the organisms. Compared to MAV, MAP GIs have lower GC percentage, which may reflect the propensity of MAP to acquire genetic elements from other bacteria rich in intestinal micro-environment through lateral gene transfer (LGT)[54]. Marri and workers[55] have provided evidence of LGT in MAP. They have identified 53 genes specific to MAP after divergence from MAV. These genes can be used as specific diagnostic markers for MAP. One of the laterally acquired gene is *HspX* that has role in virulence[55]. Many of laterally acquired genes by MAP belong to proteobacteria and soil dwelling actinobacteria[55]. This is possible, since MAP has ability to survive in soil[56] and also lateral transfers are thought to be influenced by physical proximity rather than phylogenetic proximity[57].

MAP specific *mce* gene cluster has also been identified[4] providing additional reason for the differences in pathogenesis between MAV and MAP. Stratmann *et al*.[58] have identified 3 novel MAP operons (*mpt, fep* and *sid*) present within a 38kb locus and this locus is specific to MAP. Functional genomics have shown that this locus mainly code for cell surface proteins expressed in host and has role in uptake of iron and other essential trace elements. Their role in iron transport is supported by the presence of Fe^{3+} regulated transcriptional control motifs (*Fur* boxes)[59] within putative promoters associated with *sid* and *mpt* operons. Further studies are needed to determine the precise function of MAP specific *feb, mpt* and *sid* operons.

In an effort to analyze the genetic basis of growth rate difference between MAP and MAV, Bannatine *et al*.[60] have analyzed *oriC* regions and region outside *oriC*. Analysis reveals strong synteny and high nucleotide and amino acid identity for these regions between MAP and MAV. They have concluded that genetic differences outside *oriC* in each genome may be responsible for diverse growth rates. Further they

have found cluster of substitutions in region of RNA polymerase A (*rnpA*) gene and each nucleotide substitution results in amino acid change. While mutations in this gene are known to result in dramatic differences in ability of bacteria to respond to environmental stress, the functional significance of these differences between MAV and MAP is unknown at present and requires investigation.

MAP strains exhibit *in vivo* virulence differences at host species level[8]. Microarray analysis shows genome level differences between 'Sheep type' and 'Cattle type' strains[53]. Microarray based comparison by Marsh *et al*.[61], has identified 2 large genomic deletions in sheep MAP strain as well as confirmed a deletion of *mmpL5* gene as identified previously in 'Sheep type' MAP by Marsh and Whittington[62]. A total of ~29kb region of 'Cattle type' strain, involving 24 ORFs has been reported absent from 'Sheep type' strains[61]. The *mmpL* gene required for synthesis or transport across membranes of several components of mycobacterial cell wall including sulfolipid- 1 (SL-1; *mmpL8*), phthiocerol dimycocerosate (PDIM; *mmpL7*)[63] and (GPL; *tmtpC*)[64]. However, putative involvement of *mmpL* genes in fatty acid transport[65] may account for specialized cultural requirements of 'Sheep type' strains[61]. While putative functions have not been assigned to majority genes found absent from 'Sheep type' strains, it remains unclear what effect the presence/absence of these genes that may have the 'Sheep type' and 'Cattle type', phenotypes.

Analysis done by Dohmann *et al*.[6] has identified 3 'Type I' MAP specific regions (*pig-RDA10*, *pig-RDA20* and *pig-RDA30*) those have no homology with MAP K10 genome (Type II MAP), but have 98-99% homology with MAV sequences. Presence of 'Type I' specific regions in MAV and absence of these regions in MAP 'Type II' is consistent with the hypothesis that MAP 'Sheep type' (Type I) is evolutionary intermediate between MAV and 'Type II' MAP isolates[17]. However, Marsh *et al*.[61] have identified 3 large genome fragments specific to 'Type II' MAP and these regions are absent from 'Type I'. Of these, 2 large genome fragments are present in MAV in inverted *et al*.[61]. This finding contradicts with the hypothesis that 'Type I' MAP is evolutionary intermediate between MAV and 'Type II' MAP and demands further investigation.

Semret *et al*.[4] have analyzed 107 isolates of MAP from domestic animals, wild animals and human beings for the presence/absence of certain ORFs of different LSPs and found that these LSPs are heterogeneously distributed among different MAP isolates. However, functional relevance of these differences on phenotype of different isolates has not been addressed. Therefore, it is important to correlate such findings with the strain phenotypes, as presence or absence of certain LSPs. Genomic analysis of 15 MAP strains used for Johnin production by Semret *et al* [4] have shown that 7 strains lack LSPjn region in their genome. LSPjn region codes cell for surface as well as culture filtrate immuno-stimulatory genes. Authors have concluded that Johnin produced from these 7 strains may be less sensitivity compared to other strains.

Using PCR-sequencing based approach Marsh and Whittington[61] have identified 11 SNPs in 8 genes that differentiate 'Type I' and 'Type II' MAP strains. In contrast, results of *in silico* comparisons of each of MAP K10 gene sequences from this study with incomplete MAV 104 genome identify 86 SNPs. However, marked difference has been reported in number of SNPs and the proportion of synonymous substitutions between 'Sheep type' and 'Cattle type' strains of MAP compared to MAP and MAV, indicating that the divergence between MAP and MAV has taken place much earlier than that between the 'Type I' and 'Type II' strains of MAP[61]. Castellanos and workers[33] have analyzed *gyrA* and *gyrB* gene sequences of MAP isolates recovered from different host species and geographical regions and identified 9 SNPs. In the *gyrA* gene, SNPs are located at positions 868, 1653, 1822, and 1986; two of them (at positions 868 and 1653) are implicated in non-synonymous modifications, changing from hydrophilic amino acids into amino acids belonging to the basic group. Sequencing of the *gyrB* gene reveals five SNPs at positions 108, 264, 494, 1353, and 1626; two of them represent a change in coded amino acids (at positions 264 and 494).

Limited data have shown the utility of comparative genomics in providing insight into MAP strains that has immediate applicability and more such studies are required on MAP isolates from different hosts and geographical regions to understand the patho-biology of MAP infection.

Modulation of innate immune response by MAP is one of the seminal event determining the outcome of the infection. Components of MAP cell wall like mannosylated liparabinomannan (Man-LAM) etc., interact with the cell membrane of mononuclear phagocytes and activate the signaling molecules[66].

Toll-like receptor 2 (TLR2) has been incriminated as major signaling receptor that binds to MAP and initiates signaling though mitogen-activated protein kinase (MAPK)–p38 pathway[66]. This pathway induces transcription of interleukin (IL)-10[67]. Early production of IL-10 suppresses pro-inflammatory cytokines, chemokines, IL-12, and major histo-compatability factor class-II expression[66]. Excessive IL-10 expression has emerged as one of the mechanisms by which MAP organisms suppress inflammatory, immune, and antimicrobial responses and promote their survival within host mononuclear phagocytes. The details of the modulation of immune response by MAP, shift from sub-clinical stage to clinical stage and role of various immune factors and cytokines have been discussed by Sohal *et al*.[67]. Hence, the studies directed towards characterizing the host response to different MAP strains will help in designing both, the vaccine and management based control strategies. Limited scale studies carried out on these lines have shown that host behaves differently to different MAP strains (some strains being more pathogenic than others)[9,10]. However, studies so far have not involved MAP strains, which have comparable pathogenicity in multiple host species (eg. 'Indian Bison type' MAP strains). Hence, future studies must be directed to understand the host response to MAP strains capable of infecting multiple host species and suitability of these strains as vaccine candidate for different host species. Also, future studies should be directed in identifying the bacterial targets responsible for differential response in different host species. Once the targets are identified, attenuated MAP strains can be produced and analyzed for vaccine efficacy.

Conclusion

MAP is an important pathogen and a subject of concern in relation to animal health and production and as potential human pathogen as potential human pathogen. Thus, control of MAP infection is extremely important in order to secure animal productivity and to reduce human exposure to MAP. Control strategy based on 'test and cull' policy used world-over has failed in reducing the bio-burden of the disease, instead the load of MAP has increased in the environment over the time. Lack of adequate diagnostic tools is one reason of the failure of this strategy. Other main factor is lack of sufficient epidemiological information while designing control measures. Vaccination offers partial protection and regular practice of vaccination coupled with epidemiological considerations and management change that will help in reducing the bio-burden of the disease. As sufficient information exists on sharing of MAP genotypes between different host species, this factor should be taken into account while designing any control strategy. Also there are sufficient evidences that human beings acquire MAP from animals, so there is an immediate need of control measures in order to eliminate the human exposure to MAP through food chain and other transmission vehicles.

With the advances in the molecular typing and sub-typing studies of MAP isolates, it has now been confirmed that certain types has the ability to infect multiple host species and certain types are host restricted. However, still there are gaps in knowledge and exhaustive studies should be carried out in order to design database for generating information on restricted and shared strains. Also this database should contain the information on the distribution of MAP genotypes and subtypes in different geographical regions. As MAP has the ability to survive in extreme environmental conditions, the load of MAP in the environment (soil/water) should be determined at different locations. Also future studies should address the resistance / susceptibility of animal species/breeds towards MAP infection in general and also towards different MAP strains. All this information can be given in one database for the benefit of scientists, students, farmers and livestock entrepreneurs.

Future studies should address the role of MAP specific gene clusters like *mce* clusters (involved in virulence) and operons like *mpt, feb* and *sid* (involved in iron uptake) during the disease progression. These MAP specific clusters may serve as potential diagnostic targets. Also these clusters have role in virulence, and studies need to be carried out on designing drug targets against these clusters. Also knock-out mutants for these clusters may serve as potential attenuated vaccine candidates. More of such clusters should be identified in order to understand the differences in the pathogenesis of MAP with other mycobacteria. Further studies on analysis of macrophage (derived from different host species) response to MAP isolates (from different sources) supplemented with the clinical observations will help in understanding of seminal events of pathogenesis and progression of paratuberculosis. MAP is

extremely difficult to culture, further studies should be initiated in optimizing *in vitro* culture medium conditions for various genotypes for better understanding of prognosis and progression of paratuberculosis.

References

1 Greenstein R J, Is Crohn's disease caused by a mycobacterium? Comparisons with leprosy, tuberculosis, and Johne's disease, *Lancet Infect Dis,* 3 (2003) 507.

2 Ott S L, Wells S J & Wagner B A, Herd-level economic losses associated with Johne's disease on US dairy operations, *Prev Vet Med,* 40 (1999) 179.

3 Li L, Bannantine J P, Zhang Q, Amonsin A, May B J, Alt D, Banerji N, Kanjilal S & Kapur V, The complete genome sequence of *Mycobacterium avium* subspecies *paratuberculosis*, *PNAS*, 102 (2005) 12344.

4 Semret M, Alexander D C, Turenne C Y, de Haas P, Overduin P, van Soolingen D, Cousins D & Behr M A, Genomic polymorphisms for *Mycobacterium avium* subsp. *paratuberculosis* diagnostics, *J Clin Microbiol,* 43 (2006) 3704.

5 Collins M T, Interpretation of a commercial bovine paratuberculosis enzyme linked immunosorbent assay by using likelihood ratio. *Clin Diagn Lab Immunol*, 9 (2002), 1367.

6 Dohmann K, Strommenger B, Stevenson K, de Juan L, Stratmann J, Kapur V, Bull T J & Gerlach G F, Characterization of genetic differences between *Mycobacterium avium* subsp. *paratuberculosis* type I and type II isolates. *J Clin Microbiol*, 41 (2003) 5215.

7 Sohal J S, Sheoran N, Narayanasamy K, Brahmachari V, Singh S V & Subodh S, Genomic analysis of local isolate of *Mycobacterium avium* subspecies *paratuberculosis*, *Vet Microbiol,* (2009) doi:10.1016/j.vetmic.2008.08.027 (In press).

8 Motiwala A S, Janagama H K, Paustian M L, Zhu X, Bannantine J P, Kapur V & Sreevatsan S, Comparative transcriptional analysis of human macrophages exposed to animal and human isolates of *Mycobacterium avium* subspecies *paratuberculosis* with diverse genotypes, *Infect Immun,* 74 (2006) 6046.

9 Janagama H K, Jeong K L, Kapur V, Coussens P & Sreevatsan S, Cytokine responses of bovine macrophages to diverse clinical *Mycobacterium avium* subspecies *paratuberculosis* strains, *BMC Microbiol*, 6 (2006) 10.

10 Gollnick N S, Mitchell R M, Baumgart M, Janagama H K, Sreevatsan S & Schukken Y H, Survival of *Mycobacterium avium* subsp. *paratuberculosis* in bovine monocyte-derived macrophages is not affected by host infection status but depends on the infecting bacterial genotype, *Vet Immunol Immunopathol,* 120 (2007) 93.

11 Stevenson K, Hughes V M, de Juan L, Inglis N F, Wright F & Sharp J M, Molecular characterization of pigmented and nonpigmented isolates of *Mycobacterium avium* subsp. *Paratuberculosis*, *J Clin Microbiol*, 40 (2002) 1798.

12 Chiodini R J & van Kruiningen H J, Characterization of *Mycobacterium paratuberculosis* of bovine, caprine, and ovine origin by gas-liquid chromatographic analysis of fatty acids in whole-cell extracts, *Am J Vet Res*, 46 (1985) 1980.

13 Chiodini R J, Biochemical characteristics of various strains of *Mycobacterium paratuberculosis*. *Am J Vet Res*, 47 (1986) 1442.

14 Pavlik I, Horvathova A, Dvorska L, Bartl J, Svastova P, Du Maine R & Rychlik I, Standardization of restriction fragment length polymorphism analysis for *Mycobacterium avium* subspecies *paratuberculosis*, *J Clin Microbiol*, 38 (1999) 155.

15 de Juan L, Álvarez J, Romero B, Bezos J, Castellanos E, Aranaz, A, Mateos A & Dominguez L, Comparison of four different culture media for isolation and growth of type II and type I/III *Mycobacterium avium* subsp. *paratuberculosis* strains isolated from cattle and goats, *Appl Environ Microbiol*, 72 (2006) 5927.

16 Collins DM, Cavaignac S & de Lisle GW, Use of four DNA insertion sequences to characterize strains of the *Mycobacterium avium* complex isolated from animals, *Mol Cell Probes,* 11 (1997) 373.

17 Whittington R J, Marsh I B & Whitlock R H, Typing of IS*1311*, polymorphisms confirms that bison (*Bison bison*) with paratuberculosis in Montana are infected with strain of *Mycobacterium avium* subspecies *paratuberculosis* distinct from that occurring in cattle and other domestic livestock. *Mole Cell Probes*, 15 (2001) 139.

18 Sevilla I, Singh S V, Garrido J M, Aduriz G, Rodriguez S, Geijo M V, Whittington R J, Saunders V, Whitlock R H & Juste R A, Molecular typing of *Mycobacterium avium* subspecies *paratuberculosis* strains from different hosts and regions. *Sci and Tech Rev,* (OIE), 24 (2005) 1061.

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

20 Yadav D, Singh S V, Singh A V, Sevilla I, Juste R A, Singh P K & Sohal J S, Pathogenic 'Bison-type' *Mycobacterium avium* subspecies p*aratuberculosis* genotype characterized from riverine buffalo (*Bubalus bubalis*) in North India. *Comp Immunol Microbiol Infect Dis,* 31 (2008) 373.

21 Singh S V, Sohal J S, Singh P K & Singh A V, Genotype profiles of *Mycobacterium avium* subspecies paratuberculosis isolates recovered from animals, commercial milk, and human beings in North India, *Int J Infect Dis*, (2009) doi:10.1016/j.ijid.2008.11.022 (In press).

22 Sonawane G G, Tripathi B N & Stevenson K, Molecular characterization of *Mycobacterium avium* subsp. *paratuberculosis* isolates from different host and geographic origins. *Inc Proc. 10th Int Coll Paratuberculosis*, (Minneapolis USA), 2009, 33.

23 Sevilla I, Garrido J M, Geijo M & Juste R A, Pulsed-field gel electrophoresis profile homogeneity of *Mycobacterium avium* subsp. *paratuberculosis* isolates from cattle and heterogeneity of those from sheep and goats, *BMC Microbiol*, 7 (2007) 18.

24 Bull T J, Hermon-Taylor J, Pavlik I, El-Zaatari F & Tizard M, Characterization of IS*900* loci in *Mycobacterium avium* subsp. *paratuberculosis* and development of multiplex PCR typing. *Microbiology*, 146 (2000) 2185.

25 Amonsin A, Li L L, Zhang Q, Bannantine J P, Motiwala A S, Sreevatsan S & Kapur V, Multilocus short sequence repeat

sequencing approach for differentiating among *Mycobacterium avium* subsp. *paratuberculosis* strains, *J Clin Microbiol,* 42 (2004) 1694.

26 Ghadiali A H, Strother M, Naser S A, Manning E J B & Sreevatsan S, *Mycobacterium avium* subsp. *paratuberculosis* strains isolated from Crohn's disease patients and animal species exhibit similar polymorphic locus patterns, *J Clin Microbiol,* 42 (2004) 5345.

27 Motiwala A S, Strother M, Amonsin A, Byrum B, Naser S A, Stabel J R, *et al.*, Molecular epidemiology of *Mycobacterium avium* subsp. *paratuberculosis*: evidence for limited strain diversity, strain sharing, and identification of unique targets for diagnosis, *J Clin Microbiol*, 41 (2003) 2015.

28 Motiwala AS, Amonsin A, Strother M, Manning E J B, Kapur V & Sreevatsan S, Molecular epidemiology of *Mycobacterium avium* subsp. *paratuberculosis* isolates recovered from wild animal species, *J Clin Microbiol*, 42 (2004) 1703.

29 Harris N B & Barletta R G, *Mycobacterium avium* subsp. *paratuberculosis* in veterinary medicine, *Clin Microbiol Rev*, 14 (2001) 489.

30 Overduin P, Schouls L, Roholl P, van der Zanden A, Mahmmod N, Herrewegh A van Soolingen D, Use of multilocus variable-number tandem-repeat analysis for typing *Mycobacterium avium* subsp. *Paratuberculosis*, *J Clin Microbiol*, 42(2004) 5022.

31 Biet F, Thibault V, Grayon M, Overduin P, Boschiroli M L, Hubbans C, Guilloteau L A & Supply P, Development of VNTR typing of *M. avium* subsp. *paratuberculosis*: comparisons of results with those obtained by IS900 RFLP. edited by E J B Manning and S S Nielsen, *Proc 8th Int Coll Paratuberculosis*, (Denmark), 2005, 366.

32 Marsh I & Whittington R J, Genomic diversity in *Mycobacterium avium*: Single nucleotide polymorphisms between the S and C strains of *M. avium* subsp. *paratuberculosis* and with *M. a. avium*, *Mol Cell Probes*, 21 (2007) 66.

33 Castellanos E, Aranaz A, Romero B, de Juan L, Alvarez J, Bezos J, Rodríguez S, Stevenson K, Mateos A & Domínguez L, Polymorphisms in *gyrA* and *gyrB* genes among *Mycobacterium avium* subsp. *paratuberculosis* Type I, II, and III Isolates. *J Clin Microbiol*, 45 (2007) 3439.

34 Whitlock H R & Buergelt C, Preclinical and clinical manifestations of paratuberculosis (including pathology), *Vet Clin N Am Anim Pract*, 12 (1996) 345.

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

36 Aduriz J J, Juste R A & Cortabarria N, Lack of mycobactin dependence of mycobacteria isolated on Middlebrook 7H11 from clinical cases of ovine *paratubeculosis*, *Vet Micobiol*, 45 (1995) 211.

37 Nielsen S S, Kolmos B & Christoffersen A B, Comparison of contamination and growth of *Mycobacterium avium* subsp. *paratuberculosis* on two different media, *J Appl Microbiol* 96 (2004) 149.

38 de Juan L, Álvarez J, Romero B, Bezos J, Castellanos E, Aranaz A, Mateos A G & Dominguez L, Comparison of four different culture method for isolation and growth of Type II and Type I/III *Mycobacterium avium* subsp. *paratuberculosis* strains isolated from cattle and goats, *Appl Environ Microbiol,* 72 (2006) 5927.

39 Whitlock R H, West S R, Layton B, Ellingson J, Stabel J, Rossiter C, *et al.*, Paratuberculosis in bison: A comparison of PCR, culture, serology and histopathology. in, E J B Manning and M T Collins, edited *Proc 6th Int Coll Paratuberculosis*, (1999) 424.

40 Merkal R S, Lyle P A S & Whipple D L, Decontamination media, and culture methods for *Mycobacterium paratuberculosis*. *Proc Annu Meet US Anim Health Assoc, Nashville*, Tennesse, 86 (1982) 519.

41 Whitlock R H, West S R, Layton B, Ellingson J, Stabel J, Rossiter C *et al.*, Paratuberculosis in bison: A comparison of PCR, culture, serology and histopathology, in *Proc 6th Int Coll Paratuberculosis*, edited by E J B Manning & M T Collins, (1999) 424.

42 Chiodini R J, Van Kruiningen H J, Thayer W R, Merkal R S & Coutu J A, Possible role of mycobacteria in inflammatory bowel disease. An unclassified *Mycobacterium* species isolated from patients with Crohn's disease, *Dig Dis Sci,* 29 (1984) 1073.

43 Armstrong, M C, Johne's disease of sheep in the South Island of New Zealand, *N Z Vet J,* 4 (1956) 56.

44 Seaman J T & Thompson D R, Johne's disease in sheep, *Aust Vet J,* 61 (1984) 227.

45 Collins D M, Gabric D M & de Lisle G W, Identification of two groups of *Mycobacterium paratuberculosis* strains by restriction endonuclease analysis and DNA hybridization, *J Clin Microbiol,* 28 (1990) 1591.

46 Ris D R, Hamel K L & Ayling A L, Can sheep become infected by grazing pasture contaminated by cattle with Johne's disease? *N Z Vet J,* 35 (1987) 137.

47 Pavlik I, Bejckova L, Pavlas M, Rozsypalova Z & Koskova S, Characterization by restriction endonuclease analysis and DNA hybridization using IS*900* of bovine, ovine, caprine and human dependent strains of *Mycobacterium paratuberculosis* isolated in various localities, *Vet Microbiol,* 45 (1995) 311.

48 Motiwala A S, Strother M, Theus N E, Stich R W, Byrum B, Shulaw W P, Kapur V & Sreevatsan S, Rapid detection and typing of strains of *Mycobacterium avium* subsp. *paratuberculosis* from broth cultures, *J Clin Microbiol,* 43 (2005) 2111.

49 Corn J L, Manning E J B, Sreevatsan S & Fischer J R, Isolation of *Mycobacterium avium* subsp. *paratuberculosis* from free-ranging birds and mammals on livestock premises, *Appl Environ Microbiol,* 71 (2005) 6963.

50 Singh P K, Singh S V, Singh A V & Sohal J S, Variability in susceptibility of different Indian goat breeds with respect to natural and experimental infection of *Mycobacterium avium* subspecies *paratuberculosis, Indian J Small Rumin Res*, 15 (2009) 35.

51 Clarke C J, The pathology and pathogenesis of paratuberculosis in ruminants and other species. *J Comp Pathol*, 116(1997) 217.

52 Manning E J B & Collins M T, *Mycobacterium avium* subsp. *paratuberculosis*: Pathogen, pathogenesis and diagnosis. *Sci & Tech Rev (OIE),* 20 (2001) 133.

53 Collins M T, Spahr U & Murphy P M, Ecological characteristics of *M. paratuberculosis, Bull Int Dairy Fed,* 362 (2001) 32.

54 Wu C W, Glasner J, Collins M, Naser S & Talaat T M, Whole-genome plasticity among *Mycobacterium avium* subspecies: Insights from comparative genomic hybridizations, *J Bacteriol,* 188 (2006) 711.

55 Marri P R, Bannantine J P, Paustian M L & Golding G B, Lateral gene transfer in *Mycobacterium avium* subspecies *paratuberculosis*. *Can J Microbiol,* 52 (2006) 560.

56 Whittington R J, Marsh I B & Reddacliff L A, Survival of *Mycobacterium avium* subsp. *paratuberculosis* in dam water and sediment, *Appl Environ Microbiol,* 71 (2005) 304.

57 Matte-Tailliez O, Brochier C, Forterre P & Philippe H, Archaeal phylogeny based on ribosomal proteins, *Mole Biol Evol,*19 (2002) 631.

58 Stratmann J, Strommenger B, Goethe R, Dohmann K, Gerlach G F, Stevenson K, Li L L, Zhang Q, Kapur V & Bull T J, A 38-kilobase pathogenicity island specific for *Mycobacterium avium* subsp. *paratuberculosis* encodes cell surface proteins expressed in the host, *Infect Immun,* 72 (2004) 1265.

59 Carniel E, The Yersinia high-pathogenicity island: An iron-uptake island, *Microb Infect,* 3 (2001) 561.

60 Bannatine J P, Zhang Q, Li L L & Kapur V, Genomic homogeneity between *Mycobaterium avium* subspecies *avium* and of *Mycobacterium avium* subspecies *paratuberculosis* belies their divergent growth rate, *BMC Microbiol,* 3 (2003) 10.

61 Marsh I B, Bannantine J P, Paustian M L, Tizard M L, Kapur V & Whittington R J, Genomic comparison of *Mycobacterium avium* subsp. *paratuberculosis* sheep and cattle strains by microarray hybridization, *J Bacteriol,* 188 (2006) 2290.

62 Marsh I & Whittington R J, Deletion of an *mmpL* gene and multiple associated genes from the genome of the S strain of *Mycobacterium avium* subsp. *paratuberculosis* identified by representational difference analysis and *in silico* analysis, *Mole Cell Probes,* 19 (2005) 371.

63 Cox J S, Chen B, McNeil M & Jacobs Jr W R, Complex lipid determines tissue-specific replication of *Mycobacterium tuberculosis* in mice, *Nature,* 402 (1999) 79.

64 Recht J, Martinez A, Torello S & Kolter R, Genetic analysis of sliding motility in *Mycobacterium smegmatis, J Bacteriol,* 182 (2000) 4348.

65 Tekaia F, Gordon S V, Garnier T, Brosch R, Barrell B G & Cole S T, Analysis of the proteome of *Mycobacterium tuberculosis in silico, Tuber Lung Dis,* 79 (1999) 329.

66 Weiss D J & Souza C D, Modulation of mononuclear phagocyte function by *Mycobacterium avium* subsp. *paratuberculosis*, *Vet Pathol*, 45 (2008) 829.

67 Sohal J S, Singh S V, Tyagi P, Subodh S, Narayanswamy K, Singh P K & Singh A V, Immunology of mycobacterial infections: With special reference to *Mycobacterium avium* subspecies *paratuberculosis*, *Immunobiology,* 213 (2008) 585.