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Review on microbial genetics and virulence factors of mycobacterium bovis

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Abstract
Bovine tuberculosis is a disease caused by Mycobacterium bovis that affects cattle and other species, including humans. Mycobacterium bovis resides mainly in macrophages, so bacilli survival within macrophages is related to virulence. The role of bacterial genetic variability in the infection's outcome remains uncertain. Until the early 1990s, when DNA fingerprints were introduced, it was believed that the M. tuberculosis complex was a group of highly genetically conserved bacteria, with limited phenotypic differences that influenced pathogenesis. However, epidemiological data suggest that differences in transmissibility and virulence between strains are related to their genotypes. Bovine tuberculosis is a chronic, debilitating infection that infects a wide range of hosts including domesticated and wild animals. Unlike M. tuberculosis, M. bovis has an unusually extensive host range including humans as recognized by the World Health Organization4 with a greater zoonotic potential in developing countries. M. bovis is also the progenitor of the Bacillus Calmette–Guérin (BCG), the only licensed tuberculosis vaccine and the gold standard for protection against childhood disseminated tuberculosis. Isolation and strain identification are important for disease control. However, little is known about virulence factors of the circulating strains in cattle populations. Therefore, the aim of this paper is to review the genetics and virulence factors of intracellular Mycobacterium bovis strains in pathogenicity and pathogenesis of the disease.

Keywords: Bovine, genetics, mycobacterium bovis, virulence

1. Introduction
Mycobacteria belongs to the Order *Actinomycetales*, Family *Mycobacteriaceae*. The genus Mycobacterium includes the Mycobacterium tuberculosis and *Mycobacterium avium complexes*, other pathogenic mycobacteria, and numerous species of saprophytic microorganisms present in soil and water. The Mycobacterium tuberculosis complex includes *M. tuberculosis, M. africanum, M. Canetti, M. bovis, M. pinnipedii, M. caprae* and *M. microti*. The *M. avium* complex includes *M. avium* subsp. avium, *M. avium* subsp. hominissuis, *M. avium* subsp. paratuberculosis and *M. intracellulare*. Some other mycobacteria of clinical signifi cance are *Mycobacterium chelonae*, *Mycobacterium fortuitum*, *Mycobacterium kansasii*, *Myco bacterium* leprae, *Mycobacterium marinum*, Mycobacterium ulcerans and *Mycobacterium scrofulaceum*.
Mycobacteria have a unique and complex cell wall structure comprising layers of protein, polysaccharide (Carbohydrate) and particularly lipid (fat). Slow growers Growth rates for mycobacteria are slow, with generation times ranging from 2 to more than 20 h. *M. bovis* has a broader host range and is able to infect multiple host species, mainly cattle and including humans, with variable populational persistence. Genome sequencing demonstrate that the M. bovis genome (4 345 492 bp for the virulent bovine isolate AF2122/97) is a down-sized version of the genome of M. tuberculosis (4 411 532 bp for the human isolate H37Rv), with more than 99.95% identity and no new genetic material as compared to M. tuberculosis. The virulence of M. bovis relates to its ability to survive and multiply in host macrophages. Specific toxins have not been identified, but rather virulence is a consequence of many pathogen factors working in concert to establish infection. Several alternative approaches have been attempted for the rapid and specific diagnosis of tuberculosis, but molecular methods, especially polymerase chain reaction (PCR) assays, are the most promising for diagnoses in live cattle and direct detection post mortem diagnosis in bovine tissue samples.

2. Literature review

2.1 Nature of mycobacteria

Mycobacteria are unusual amongst bacteria in their robustness, resilience and slow growth characteristics, and the chronic and insidious nature of the diseases that they cause. M. bovis is a facultative intracellular 'parasite', meaning that it can survive and thrive inside the host's macrophages (cells of the immune system that are meant to engulf and destroy the invading bacteria). It has many adaptations to intracellular life and may become quiescent (dormant) or divide very slowly, which enhances its survival. It has a tendency to become walled off in granulomas (small nodules of chronic inflammation) in the tissues (Hirsh *et al*., 2004) [13].

Mycobacteria have a unique and complex cell wall structure comprising layers of protein, polysaccharide (carbohydrate) and particularly lipid (fat). These components confer structural strength and great resistance, both to attack in the intracellular environment and enhance survival outside of the host (Gordon, 2019).

Their high cell wall lipid content excludes standard aniline dyes, so that once stained with special staining procedures, mycobacteria are resistant to decolorization even by acid alcohol. This property is termed acid fastness, so that mycobacteria are commonly referred to as acid-fast bacilli. In contrast, these microorganisms are not readily stained with the Gram method and are considered weakly gram-positive. On different generation times, mycobacteria can be divided into slow and rapid growers. Slow growers Growth rates for mycobacteria are slow, with generation times ranging from 2 to more than 20 h. Based require more than 7 days to form visible colonies on solid medium, whereas rapid growers form colonies within 7 days (Holt *et al*. 1994) [14].

2.2 A brief background on mycobacterium bovis genomics

To precisely interpret genotyping and WGS data, it is necessary to understand the genetic make-up of *M. bovis*. This pathogen is part of the *Mycobacterium tuberculosis* complex (MTBC), a bacterial group composed of 11 species or ecotypes with variable host tropism and virulence (Galagan, 2014) [7]. Mycobacterium tuberculosis is the leading etiological agent of TB in humans, while *M. bovis* has a broader host range and is able to infect multiple host species, mainly cattle and including humans, with variable populational persistence. MTBC genomes are highly similar, with >99.95% identity over homologous nucleotide sequences, including the ribosomal RNA genes, while horizontal gene transfer and large recombination events are considered absent These pathogens have solely A evolved through single nucleotide polymorphisms (SNPs), indels (small insertions and deletions), deletions of up to ≈26 Kb, insertion sequences (IS) and duplication of few paralogous gene families (Gagneux and Small, 2007) [6].

The average size of a virulent *M. bovis* genome is 4.3 Mb, containing approximately 4200 genes, including a single copy of each of the ribosomal RNA genes (5S, 16S, and 23S) and 45 tRNAs. As with other Actinobacteria it's (Goodfellow *et al*., 2012) [10] genome has a high GC content (≈65%), which implies the use of appropriate sequencing reagents for library preparation in WGS (Tyler *et al*., 2016) [20]. MTBC genomes, including *M. bovis*, have a substantial number of repetitive elements, constituting one of the main challenges for WGS data analyses. These include, but are not restricted to, mobile elements (e.g., insertion sequences-IS), proline-glutamate (PE) or proline-proline-glutamate (PPE) family genes, integrases, two phage sequences, a CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and the 13E12 repeat family genes. In particular, PE-PPE gene families account for approximately 10% of MTBC genomes and have been associated with TB pathogenesis (Delogu *et al*., 2017). Repetitive elements are difficult to handle in genomic studies because the majority of and most commonly used sequencing platforms generate short reads, usually ranging from 50 to 300 bp, which are often shorter than the repeats themselves (Torresen *et al*., 2019) [19]. Some of these repetitive regions are the basis for the traditional genotyping techniques developed over the years.

2.3 Mycobacterium bovis virulence factors

In recent years, remarkable progress has been made in understanding the basis of virulence and the pathogenesis of mycobacterial infections particularly through the application of whole genome sequencing and comparative genomic analysis.

Genome sequencing demonstrate that the M. bovis genome (4 345 492 bp for the virulent bovine isolate AF2122/97) is a down-sized version of the genome of M. tuberculosis (4 411 532 bp for the human isolate H37Rv), with more than 99.95% identity and no new genetic material as compared to M. tuberculosis. Thus, DNA deletions in M. bovis are the major contributors to these differences, which have been found to affect genes involved in transport, cell surface structures, and intermediary metabolism. These deletions may remove genes that are unnecessary for host adaptation and lead to a different and sometimes even wider host range. Point mutations also play a role in defining the phenotype, which is the case for M. bovis resistance to pyrazimamide. In addition, sequence variations have been found in genes coding for cell wall and secreted proteins, such as the PE_PGRS and PPE protein families. Another notable change is a mutational event in the M. bovis pyruvate kinase gene that renders M. bovis unable to use glycerol as a carbon source. (Garnier *et al*. 2003) [8].

2.4 Virulence gene regulation

Successful infection is dependent on the ability of mycobacteria to adapt to different conditions such as exposure to the resistance to reactive oxygen (ROI), ROI and resistace to nitrogen intermediates (RNI), hypoxia, low pH, nutrient starvation, and damage to the cell surface. Mycobacteria thus have several transcriptional regulators that control expression of distinct sets of genes. The best described of these are sigma factors and two-component systems. Two-component systems consist of a transmembrane sensor histidine kinase that reacts to various stimuli and subsequently activate its transcriptional regulator. Genome sequencing has revealed that conserved two-component systems are present in these species and may have similar functions. One two-component system that is important for virulence is PhoPR. An outbreak of M. bovis in humans was caused by a strain that elicited increased expression of PhoP due to insertion of IS 6110 in the promoter region (Soto *et al*. 2004). DosRS, or the dormancy regulon, is believed to be relevant for latent infections and is activated during hypoxia and by nitric

oxide. Other two-component systems such as PrrAB and SenX3-RegX3 also appear to play a role in virulence, and MprAB is important for persistent infections. The MprAB also regulates stress responses, including the sigma factors SigB and SigE (He et al. 2006) [12]. Sigma factors form a reversible complex with RNA polymerases that provide promoter recognition and thus affect gene transcription. The primary sigma factor SigA and the primary-like SigB are conserved in mycobacteria and allow transcription of housekeeping genes (Manganelli *et al*. 2004) [15]. SigA is essential for growth, whereas SigB may serve as a backup mechanism since the subdomains responsible for promoter recognition are almost identical to those of SigA. SigA may be involved in virulence since a mutant with an amino acid substitution led to attenuated growth of M. bovis in guinea pigs. Mycobacteria also have several sigma factors (11 in M. bovis and 17 in *M. avium* subsp. paratuberculosis) that are responsive to changes in the environment and belong to the group of extracellular function sigma factors. One of these is SigF, which in M. bovis BCG is induced after exposure to cold-shock, hypoxia and oxidative stress. The sigma factors are part of a tightly regulated network the complexity of which is illustrated by the presence of both anti-sigma factors and of anti-anti-sigma factors. Furthermore, sigma factors also regulate each other and as already mentioned, can interact with two-component systems (Sechi *et al*., 2007) [18].

2.5 Molecules of cell wall components in pathogenesis and virulence

2.5.1 Role of complex carbohydrates and lipids

The cell wall core of mycobacteria is composed of three covalently attached molecules: peptidoglycan, arabinogalactan and mycolic acid. Lipids and glycolipid complexes present in the cell wall of virulent and attenuated strains of mycobacteria have been extensively examined to understand their significance in granuloma formation (Ehrt and Schnappinger 2007) [3]. Mycobacteria differ in sulfur-containing glycolipids. Sulfolipids and sulfatides are present in M. tuberculosis but absent from M. bovis because the glycolipid sulfotransferase and arylsulphatase genes are disrupted in the latter. This difference may also contribute to determining the host range and tissue tropism of M. bovis. Another glycolipid, lipoarabinomannan (LAM), may contribute to arresting phagosome maturation. In addition, LAM is a powerful scavenger of reactive oxygen (ROI) and nitrogen (RNI) intermediates. Moreover, M. bovis contain mannosylated LAM (ManLAM), which provides a way for mycobacteria to enter phagocytes via mannose receptors (Schlesinger, 1993).

2.5.2 Role of proteins and lipoproteins

Mycobacterial proteins and protein complexes (i.e., lipoproteins) also play important and diverse roles in pathogenesis.

FAP-P, a protein located in the inner part of the cell envelope is used for attachment and internalization invasion of bacilli to bovine epithelial cells. Proteins encoded by the mce operons seem to play a role in the entry and survival of mycobacteria within phagocytic cells and invasion of epithelial cells. (Gioffre *et al*., 2005) [9].

The secreted proteins play an important role in the development of cell-mediated immunity. These proteins also possess enzymatic activity and catalyze mycolyltransfer reactions involved in the final stages of mycobacterial cell wall assembly. They possess a redundant system of SODs. The iron manganese-dependent SOD (Sod A) is secreted and seems the more critical enzyme for resistance against ROI. Another membrane-associated copper-zinc-dependent SOD (SodC) may play an additional role in protecting tubercle bacilli against the oxidative burst of activated macrophages. Protection against RNI is provided by two alkyl hydroperoxidases denominated AhpC and AhpD (Piddington *et al*. 2001) [16].

2.6 Process of disease development and intracellular life

The pathogenesis of M. bovis is complex and largely attributed to its ability to evade or manipulate the host's immune response. These strategies facilitate the establishment of the primary infection, its persistence, quiescence and reactivation under favourable conditions. The organism deploys multiple remarkable biochemical, immunological, and genetic strategies to gain access to host macrophages and then resist the usual bacterial killing mechanisms, reprogramming them and thriving in this hostile environment (Vitale *et al*., 1998).

Of particular note and importance is the tolerance of mycobacteria to acidic conditions. This is needed because the host acidifies the phagosomes, small envelopes within the macrophages containing the engulfed bacteria intended to kill them. Mycobacteria have evolved to resist this, which has consequences for the ability of M. bovis to survive in other acidic environments, such as silage. The host's response of granuloma formation (so called 'walling off of infection') in its attempt to limit bacterial dissemination, also serves to provide a secure niche protected from the immune response. This reduced exposure to the host's immune cells also means that a diagnostically detectable antibody response, which is common for other bacterial and in viral diseases, is often delayed for a considerable period. Therefore, tests that measure cell-mediated immune responses, such as the tuberculin skin and interferon-gamma tests are more suitable for the diagnosis of TB in animals and man (Cambier *et al*., 2003).

As an obligate aerobe requiring oxygen for survival, M. bovis survives the low-oxygen environment within the granuloma by entering a state of non-replicating quiescence in which it can survive for months or years until conditions become more favourable, perhaps when host immunity wanes through stress or intercurrent disease. The quiescent state is brought about following the increased storage of nutrients, reduction in metabolic pathways and changes in the structure of the cell wall, particularly in its lipid component. These attributes also confer on M. bovis the ability to survive harsh conditions in the environment for considerable periods, maintaining its ability to infect susceptible hosts (Schlesinger 1993).

2.7 Pathogenesis and Pathogenicity

The virulence of M. bovis relates to its ability to survive and multiply in host macrophages. Specific toxins have not been identified, but rather virulence is a consequence of many pathogen factors working in concert to establish infection. Following entry to the host via the respiratory tract, mycobacteria are engulfed by macrophages and by dendritic cells. Mycobacteria engulfed by dendritic cells travel to the draining lymph nodes. The initial response to infection is non-specific and triggered by the foreign body effect of

waxes and lipids in the mycobacterial cell wall. Survival within the phagosome of macrophages is promoted by interference with phagosome-lysosome fusion, probably through retarding maturation of the phagosome and failure of lysosomal digestion. Bacilli released from dead macrophages are engulfed by surrounding viable phagocytes. Migration of macrophages containing viable mycobacteria can disseminate infection. The complex lipid and waxy composition of the mycobacterial cell wall contributes not only to virulence but also, in association with M. bovis proteins, to the immunogenicity on which the development of the host responses and the lesions depends. Before activation, macrophages permit the survival and replication of mycobacteria. Infected macrophages accumulate in the alveolus as the primary site of infection and secrete a range of cytokines which recruit lymphocytes to the lung, thus aiding granuloma formation and containment of the organisms. The cytokines TNF-α and IFN-γ are essential in the development of resistance to mycobacteria and their production is stimulated through both the innate and adaptive immune responses. These cytokines activate infected macrophages and enhance their ability to destroy contained mycobacteria. Given that activated dendritic cells are unable to kill engulfed mycobacteria but limit their replication, these cells may act as a reservoir for mycobacteria, in particular within the lymph nodes (Hope and Villarreal-Ramos, 2008). With the development of cell-mediated immunity some weeks after infection, macrophage recruitment accelerates under the influence of cytokines produced by T lymphocytes sensitized to M. bovis cellular constituents. Both CD4 + and CD8 + T cells are necessary for immunity to mycobacteria although studies in cattle have shown that CD8 + T cells may also play a role in the immunopathology of M. bovis infection. The gradual accumulation of macrophages around the developing lesion and the formation of a central necrotic core result in a tubercle or granuloma, the typical host response to M. bovis infection. The architecture of the granuloma is important as it facilitates close interaction between the constituent macrophages, dendritic cells and T cells and containment or destruction of the pathogen. In addition, the layered structure helps to physically separate the necrotic core from the surrounding tissue. Granulomas may be histologically visible as early as 3 weeks after experimental infection of cattle with high doses of M. bovis . Frequently, only a small number of cattle within a herd show positive reactions to the tuberculin test and develop pathological lesions. It is likely that these animals represent one end of the spectrum of responses to exposure to M. bovis. Other animals in the herd may clear infection without becoming sensitized to the tuberculin test. Animals which test positive but in which no lesions can be detected may be latently infected or may have cleared the infection but remain sensitized to tuberculi (Harris, 2006) [11].

2.8 Immune system evasion

Unlike other pathogens, MTB infects and resides within immune cells, this bacterium has the ability to live within the dynamic and heterogeneous environment of macrophage phagosome. Here, the bacilli use a plethora of strategies to evade the microbicidal mechanisms of macrophage, including: phagosome-lysosome fusion, recruitment of hydrolytic lysosomal enzymes, production of reactive oxygen/nitrogen species, antigen presentation and apoptosis. Disruption of those functions in turn disrupts the adaptive immune response. Phagocytosis is an active process that depends on the interaction with various surface receptors expressed on the macrophage such as complement receptor type 3 (CR3), FCγ receptors and lectin receptors and it can be opsonic or non-opsonic. However, non-opsonic phagocytosis of MTB results in higher intracellular survival, although it is difficult to assess if the engagement of specific receptor determines the course of infection. MTB uses PDIM lipids to evade detection by TLRs, thereby preventing mycobacteria (Cywes *et al*., 1997) [2]. L delivery into microbicide macrophages expressing iNOS (Cambier *et al*., 2003). Moreover, MTB actively blocks the phagosome maturation by their cell wall components or through the secretion of various macromolecules that interferes with this process, which enables bacterial survival in a non-acidified intracellular compartment.

Bovine Tuberculosis (TB) is an infectious disease of cattle. It is caused by the bacterium Mycobacterium bovis (M. bovis) which can also infect and cause disease in many other mammals including humans, deer, goats, pigs, cats, dogs and badgers. In cattle, it is mainly a respiratory disease but clinical signs are rare. TB in humans can be caused by both Mycobacterium bovis and the human form, Mycobacterium tuberculosis (Fratti *et al*., 2003) [5].

2.9 Granulomatous lesion development

Aerosol exposure to acid-fast bacilli generally leads to involvement of pulmonary lymph nodes and lungs, while animals exposed by ingestion of contaminated food and water usually develop primary foci in lymph nodes of the head and other lymph tissues associated with the gastrointestinal tract (Thoen 1988). The mucociliary clearance in the upper respiratory passages provides defense against infection by inhalation of mycobacteria. However, microorganisms on small particles such as dust and water droplets that do not impinge against the mucociliary layer can pass through terminal bronchioles, thus gaining access to alveolar spaces. The estimated size of terminal endings of bronchioles is about 20 m as compared to 1-4 m for an acid fast bacillus. Mycobacteria multiply within macrophages and after 10-14 days, CMI responses develop and host macrophages acquire an increased capacity to kill the intracellular bacilli. The CMI responses are mediated by lymphocytes, which release lymphokines IFN) that attract, immobilize and activate additional blood-borne mononuclear cells at the site where virulent mycobacteria or their products exist. The cellular hypersensitivity that develops contributes to cell death and tissue destruction (caseous necrosis). In some instances, liquefaction and cavity formation occur due to enzymatic action on proteins and lipids. Rupture of these cavities into the bronchi allows aerosol spread of bacilli. Activated macrophages migrate to blind endings of lymphatic vessels and course to one or more of the thoracic lymph nodes, either bronchial or mediastinal. Ultrastructural studies show that engorged macrophages enlarge and develop marked increases in the number of lysosomes, Golgi complexes, and vesicles. Lymph nodes are more commonly infected than other tissues because fluids in an animal eventually pass through the nodes where the meshwork of trabeculae entraps the organisms. The enlargement and presence of macrophages in impenetrable passageways between reticular cell fibers of the lymph node provide an environment for mycobacterial

growth and development of the granulomatous lesion in the node. Occasionally, some phagocytized mycobacteria remain in the lung, and both lung and thoracic nodes are affected. Primary lesions often become localized in a node(s), which may become large and firm. Fibrous connective tissue development probably contributes to localization of the granulomatous lesions. Granuloma formation is an attempt by the host to localize the infection and to allow inflammatory and immune mechanisms to destroy the bacilli. A few lesions may appear to be regressing and becoming encapsulated by well-organized connective tissue; such lesions may contain viable bacilli. Typically, the microscopic appearance of a granuloma (a tubercle) is focal and has some caseous necrosis in a central area encircled by a zone of epithelioid cells, lymphocytes, and some granulocytes. Mineralization may be present in necrotic centers; in more advanced lesions, several foci of mineralization may coalesce. The zone near the necrotic area often contains multinucleated giant cells that contain several nuclei, often in a horseshoe or ring shape near the cytoplasmic border. An outer boundary of fibrous connective tissue is usually present between the lesions and normal tissue. Occasionally, fibrous tissue is not apparent and the lesion assumes a more diffuse appearance. Lesions of lymph nodes associated with the gastrointestinal tract, as with *Mycobacterium avium* in cattle and swine, suggest infection by ingestion. The medial retropharyngeal lymph nodes are a frequent site of M. bovis infection and are the most commonly infected site in the head. These nodes receive afferent lymph vessels from the floor of the mouth and adjacent parts. Other lymph nodes of the head (Mandibular, parotid and lateral retropharyngeal) are occasionally involved. The liver is only infrequently involved; hepatic nodes have afferent lymph vessels from the liver, duodenum, and abomasal lymph nodes are commonly involved. The greater part of the blood supply to the liver is derived from the portal vein, which drains the blood and lymph from the intestine; therefore, mycobacteria can pass directly to a hepatic node from the intestine, or through the portal vein to the liver only or subsequently into hepatic nodes. Occasionally, only mesenteric lymph nodes are infected. Localized tubercles have not been reported in the mucous membrane of the small intestine; mycobacteria are apparently able to diffuse into lymphatics of the lamina propria and be transported by phagocytes via the lymphatic vessels to mesenteric lymph nodes. In 72 Chapter 6some animals, lesions may develop in superficial cervical lymph nodes. Superficial iliac and popliteal lymph node lesions are seen only infrequently.

2.10 Diagnosis and controls of mycobacterium bovis

There is a growing perception that no single method is sufficient for detecting all cattle infected with BTB (Salfinger *et al*., 1994) [17]; therefore, a multidisciplinary approach must be employed, based on currently available methods. Some of the diagnostic methods and combinations of methods that are regularly used for diagnosing BTB are shown in Figure 1. Bovine tuberculosis infection in cattle is usually diagnosed in the live animal. The diagnosis is based on delayed hypersensitivity reactions (intradermal tuberculin tests), a method that may lack both sensitivity and specificity. However, a definitive diagnosis is still established by isolation and identification of the etiological agent (*M. bovis*) from clinical samples, using a combination of traditional culture and biochemical methods, which is considered the "gold standard". These methods are slow, cumbersome, unreliable, and time-consuming (it may take more than 4 weeks to grow the microorganism, and an additional 2 weeks to identify it). Several alternative approaches have been attempted for the rapid and specific diagnosis of tuberculosis, but molecular methods, especially polymerase chain reaction (PCR) assays, are the most promising for diagnoses in live cattle and direct detection post mortem diagnosis in bovine tissue samples (Figueiredo *et al*., 2010) [4]. A tentative diagnosis of bovine tuberculosis can be made following the macroscopic detection at necropsy of typical lesions. Histo-pathological examination of the lesion may increase the confidence of the diagnosis but bacteriological isolation of Mycobacterium bovis from the lesion is the only way to make a definitive diagnosis. The sensitivity of gross post mortem examination is affected by the method employed and the anatomical sites examined (Vitale *et al*., 1998).

To determine the significance of cattle that give a positive reaction in diagnostic tests but do not have visible lesions (NVL), a bacteriological examination is necessary. NVLs may be due to early infection, poor necropsy technique or infection with mycobacteria other than M. bovis. M. bovis was found to survive best in frozen tissue and the tissue preservative, sodium tetraborate, was found to have adverse effects on viability. It was found desirable to use two different culture media for the primary isolation of M. bovis; agar media for rapid growth and egg media for control of contamination.

BTB is typically controlled using isolation or quarantine of infected herds, test-and-slaughter policy, and pasteurization of milk (10) and other control measures, such as culling, vaccination and their combination, are also used (Zanini *et al*., 1998) [21].

3. Conclusions

The pathogenic Mycobacterium bovis strains differed in their capacity to modulate the M1-type activation phenotype induced by IFN-γ. In contrast to the mycobacterial strains demonstrating moderate ability to grow intracellularly which enhanced classical activation of Macrophages by INF-γ, the slow growing strain of Mycobacterium bovine induced an atypical, mixed M1/M2 phenotype, leading to inhibition of Macrophage bactericidal activity. Functional diversity of Mycobacterium bovis strains circulating in animal population, highlighting novel strategies of intracellular adaptation of the pathogenic mycobacteria. Elucidating the functional significance of diversity of virulence-associated properties of Mycobacterium bovis is important for understanding the diverse outcomes of infection and mechanisms of pathogenesis of bovine tuberculosis.

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