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Bovine Tuberculosis: A Review of Molecular Diagnostic Methods and Impact on Public Health

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Abstract Bovine tuberculosis (BTB) is a zoonotic infectious disease of cattle, other domesticated animals, and certain wildlife populations. It has been widely distributed throughout the world, and it has been a cause for great economic loss in animal production. In developed countries, the eradication programs have reduced or eliminated TB in cattle. Many factors contribute to the persistence of the disease, such as the limitations of diagnostic tests, larger herd sizes, increase in animal movements and trade, and limited options for control, such as restrictions on whole herd depopulation. The available advanced TB diagnostic techniques can detect and differentiate the causative mycobacterial species, and help an early confirmation of the diagnosis, which is useful to design appropriate control measures in the national BTB control programs. Molecular diagnostics, such as polymerase chain reaction, spoligotyping, restriction fragment length polymorphism (RFLP), variable number tandem repeats typing (VNTR), and polymorphism GC-rich repeat sequence (PGRS) are the techniques used for concurrent detection and typing of *Mycobacterium* species at the strain level. The molecular epidemiology is also being used to identify the source of contamination, to determine the risk factors of BTB transmissions, to investigate the drug resistance pattern, and to track the geographic distribution and spread of clones of *Mycobacteria* species. The molecular diagnosis is the tool to check whether active transmission or reactivation of BTB, hence, it is better to adopt these methods for the epidemiological survey of BTB. Since BTB is a major public health problem, at least a single reference laboratory should be available for molecular based diagnosis as part of its control.

Keywords: *bovine tuberculosis, molecular diagnostic techniques, molecular epidemiology, polymorphism GC-rich repeat sequence (PGRS), public health, restriction fragment length polymorphism (RFLP), variable number tandem repeats typing (VNTR), zoonosis*

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1. Introduction

Bovine tuberculosis (BTB) is a chronic bacterial disease characterized by progressive development of tubercles in any tissue/organ of the body and is important from economic as well as public health point of view [1,2,3,4]. It is predominantly caused by *Mycobacterium bovis* and is transmitted from infected animals to humans through close contact and ingestion of raw animal products [3,5,6]. It is reported that globally, 3.1% of human cases are attributed due to *M. bovis* [5].

It is an significant zoonotic disease transmitted from animals to humans and makes a significant economic impact due to high cost of eradication programs and has serious consequences for movements of animals and their products, biodiversity, and public health impact [2,7,8]. It has been recognized from 176 countries as one of the important bovine diseases causing great economic loss [9]. It is a highly infectious disease, which can affect most warm-blooded animals, including human beings [6]. *Mycobacterium bovis* are excreted in the exhaled air, in sputum, feces (from both intestinal lesions and swallowed sputum from pulmonary lesions), milk, urine, vaginal and uterine discharges, and discharges from open peripheral lymph nodes of infected animals [10].

World Health Organization classified BTB among seven neglected zoonotic diseases having potential to infect humans [11]. A study conducted between 1998-2005, over European badger (*Melesmeles*) and cattle populations showed close association between *M. bovis*

strain types isolated from cattle and associated badgers indicates intra-specific transmission [12]. In cattle, exposure to this organism can result in a chronic disease that jeopardizes animal welfare and productivity, and in some countries leads to significant economic losses by causing ill-health and mortality [13]. Moreover, human TB of animal origin caused by *M. bovis* is becoming increasingly evident in developing countries [14].

Knowledge of the genomic sequence of *M. bovis*has facilitated the development of high molecular typing techniques that allow greater insight into the epidemiology, evolution, and population structure of *M. bovis* [15]. The molecular diagnostic techniques can help to determine the source of infection and outbreaks, understand the relationship between different outbreaks, and identify wild animal reservoir of *M. bovis*. In addition, they can provide insight into the risk factors for BTB transmission by allowing identification of the dynamics of this disease [14]. Therefore, the objective of this manuscript is to present an overview on the public health significance, epidemiology, diagnosis, prevention, and control of BTB.

2. Review

2.1. Etiology and Characteristics

Bovine tuberculosis (BTB) is caused by *Mycobacterium bovis (M. bovis)* in the members of *Mycobacterium tuberculosis* complex (MTBC) [3,16,17]. *Mycobacterium bovis* is obligate aerobic, non-capsular, slow growing, non- motile, non-spore forming bacteria [6]. Its cell wall is rich in lipids which is responsible for acid fastness and hydrophobicity. Its waxy coat lipid layer, mycolic acid, contributes greatly to resistance of the bacteria towards many disinfectants, antibiotics and physical injuries. It may also reduce growth rate of some species by limiting the uptake of nutrients [18]. *Mycobacteria* have a distinctive cell wall structure and composition that differs from that of Gram-positive and Gram-negative bacteria. It contains peptidoglycan but has large amounts of high molecular weight lipids in the form of long chain length fatty acids (mycolic acids) attached to polysaccharides and proteins. This high lipid content gives the mycobacteria their acid-fast properties (retaining a stain on heating in acid), which allows them to be distinguished from other bacteria (e.g. positive Ziehl-Neelsens stain) [19].

Mycobacterium tuberculosis complex (MTBC) has seven approved members, and these are *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. microti*, *M. tuberculosis* subsp. canettiand *M. bovis* subsp. caprae. Four members of this group cause human TB i.e. *M. bovis*, *M.* tuberculosis, *M. africanum* and *M.* canetti [20,21]. Members of the MTBC are extremely similar genetically having at least 99.9% similarity on the nucleotide level and an identical 16S rRNA sequence [22]. Mycobacterium species grows on medium containing serum, potato and egg. The most commonly used media are Lowenstein-Jensen (LJ) that contains egg, glycerol, asparagine, mineral salt and malachite green and stone brinks medium. *M. bovis* grows more slowly than M. tuberculosis, which needs more than 8 weeks to appear on primary culture and the optimal growth temperature is 37°C [23].

2.2. Pathogenesis and Virulence Factors

The main aspects of the pathogenesis of TB in cattle are fairly settled, including the main route of infection, infective dose, and incubation period before infectivity [24]. Animals exposed by ingestion of contaminated feed or water often develop primary foci in lymph nodes associated with the intestinal tract, while aerosol exposure leads to the involvement of the lungs and associated lymph nodes. In case of respiratory infection, the mucociliary clearance in the upper respiratory passages may prevent infection in some exposed animals [25]. In the bronchi; the organism penetrates the mucosa and are trapped and phagocytized in the bronchial and mediastinal lymph nodes. In the lungs, the bacterial are phagocytized by alveolar macrophages. In case of oral infection, the organism presumably penetrates the buccal or intestinal mucosa and, via the lymphatics, reaches the phagocytes in the draining lymph node. The phagocytosis causes a localized inflammatory reaction and recruitment of mononuclear cells from neighboring blood vessels. The cellular response results in the accumulation of large number of phagocytes leading to the formation of the granuloma or the tubercle that characterizes the disease. The granuloma consists of infected macrophages surrounded by epithelioid cells, granulocytes, lymphocytes, and later, multinucleated giant cells [26].

Tubercle bacilli that are inhaled usually lodge in the alveolar spaces where they are ingested by macrophages. The tubercle bacillus enters the macrophage by binding to cell surface molecules of the phagocyte [27]. The ingestion of the tubercle bacillus by the phagocytes into the phagosome or intracytoplasmic vacuole protects the organism from the bactericidal components of serum. Following ingestion of the bacillus, lysosomes fuse with the phagosome to form a phagolysosome and it is there that the phagocytes attempt to destroy the bacillus. Virulent bacilli have the ability to escape destruction and survive inside a mononuclear macrophage by inhibiting phagosome fusion with preformed lysosomes, thereby limiting acidification of the phagolysosome [28]. It has been suggested that pathogenicity of *M. tuberculosis* complex is a multifactorial phenomenon requiring the participation of the cumulative effect of several glycolipid complexes, such as lipoarabinomannan that may interfere with phagosome maturation [29].

2.3. Clinical Signs and Immune Response

Animals infected with TB slowly develop symptoms and may, if not carefully observed by animal keepers, suffer unnecessarily. Depending on which organ or associated lymphnode is affected, animals can display a wide range of symptoms, such as coughing, dyspnea, gastrointestinal problems, bone deformation, and emaciation [6,30]. Few studies have been conducted to investigate loss of productivity due to BTB, but it is probable that there is a productivity impact in animals with BTB due to the nature of the disease and thus this is likely linked to economic losses. It has previously been suggested that reduced milk production, the food value of the carcass, and reproduction (or fertility) are factors that affects the animal productivity [31]. Bovine TB can be

difficult to diagnose based only on clinical signs, that some cattle with extensive military tubercular lesions may appear clinically normal [32].

An essential component of immunological response to BTB in cattle is the cell mediated immunity (CMI) and it is responsible for the defense from the infection and development of lesion [33,34]. In particular a key role is played by-lymphocytes (T-Cell), i.e. T-helper 1-CD4 T-cells that are responsible for the production of interferon (IFN-gamma) and when sensitized by contact with antigen, drive the so called delayed type hypersensitivity (DTH). DTH is responsible for localized inflammatory reaction also mediated by macrophages which typically occurs at least 48hr after of exposure to antigen [33]. On the other hand, humoral immune responses are supportive rather than essential and the specific role of B-cells remains controversial. Experimental infection of cattle with virulent strains of *M. bovis* showed that robust CMI response begins as early as 2-3 weeks after challenge whereas, humoral responses (IgM and IgG) appear in the more advanced stages of infection, starting 2-4 weeks later [34].

2.4. Public Health Significance

Human TB due to *M. bovis* is usually underestimated or under diagnosed because of no clinical, radiographical and histopathological differentiation of TB caused by *M. tuberculosis* and *M. bovis* [35]. *Mycobacterium bovis* is not the major cause of human TB but it can infect human beings too either by consuming raw milk, meat and their products from infected animals, or by inhaling infective droplets or direct exposure to infected animals [2,16,36]. Referred to as Butcher's Wart (analogous to Prosector's Wart, which is caused by *M. tuberculosis* and is an occupational risk associated with performing autopsies), this skin lesion can occur in persons handling infected meat. It is exceedingly rare and generally self-limiting. Since *M. bovis* is either enzootic or found sporadically in much of the developing world; there is clearly a risk of cow to human transmission by either ingestion or inhalation [5].

The prevalence of BTB transmitted human infections has considerable reduced in the developed countries. This is attributed to the pasteurization of milk and testing the cattle for the infection and practicing culling and slaughtering of infected animals [37]. This unfortunately is not the case in developing countries of the African region where people often consume unpasteurized milk. As a result, up to 15% of human TB cases in these geographical regions are attributed to BTB [16,39,40].

2.5. Epidemiology

2.5.1. Occurrence and Distribution

Bovine TB affects cattle throughout the globe and some countries have been able to reduce or limit the incidence of the disease through process of 'test and cull' of the cattle stock. Most of Europe and several Caribbean countries are virtually free of *M. bovis*. BTB is endemic to many developing countries particularly African countries [41]. It is pertinent to mention that mandatory pasteurization of milk combined with tuberculin testing and culling (slaughter) of infected cattle resulted in dramatic decline in the incidence of human TB due to *M. bovis* in developed nations of the world [37]. The largest decrease in regional BTB trends was observed in Oceania and Europe (by more than 45%) followed by Asia (38% of decrease); whereas, the decrease in BTB notification was slower in Africa (25% reduction) and 18% reduction in Americas over the 30-years period [42].

2.5.2. Host Range

Bovine TB is a chronic infectious disease, which affects a broad range of mammalian hosts including cattle, pigs, goats, sheep, badgers, possums, domestic cats, deer, camelids, omnivores, and wild carnivores [2,3,6,43,44,45]. Cattle, goats, and pigs are found to be most susceptible, while horses are showing a high natural resistance [46]. Moreover, a large number of wild animals like elephants, rhinoceroses, foxes, primates, opossums, bears, warthogs, and large cats are naturally infected with *M. bovis*. However, little is known about the susceptibility of birds to *M. bovis*, although they are generally thought to be resistant [10].

2.5.3. Source of Infection and Mode of Transmission

Cattle serve as the principal reservoir of *M. bovis* and human can be infected with *M. bovis* where cattle are reared for milk production [47]. The organisms are excreted in the exhaled air, nasal discharge, milk, urine, vaginal and uterine discharges and discharges from open peripheral lymph nodes. Animals with gross lesions that communicate with airways, skin or intestinal lumen are obvious disseminators of infection. In the early stages of the disease before any lesions are visible, cattle may also exert viable *Mycobacterium* in the nasal and tracheal mucus. In experimentally infected cattle, excretion of the organism commences about 90 days after infection [48]. The transmission of *M. bovis* can occur between animals, from animals to humans and vice versa and rarely, between humans. Close contact between animals during the intensive farming practice, water points, salt licks, market places, transports, auctions contributes to the effective spread of *M. bovis*. The ingestion of contaminated products, such as carcasses of prey, pastures, and water is considered as a secondary way to spread the disease in cattle, however, it is an important pathway in the introduction of infection in wildlife [49].

2.6. Diagnosis of Bovine Tuberculosis

2.6.1. Conventional Techniques

The laboratory diagnosis of bovine TB depends on the isolation of bacteria from clinical specimens collected from the lesions of infected/suspected animals using culture. Traditionally, the culture is performed on Lowenstein-Jensen's (LJ) medium, a solid medium consisting of whole hens' egg. Previous studies have recommended the use of 0.4% sodium pyruvate over glycerol in LJ medium which improves the growth of *M. bovis* [2,50].

Mycobacterium bovis can be demonstrated microscopically on direct smears from clinical samples or prepared tissue

materials. The acid fastness of *M. bovis* is normally demonstrated with the classic Ziehl-Neelsen's stain [51]. Positive if tubercular granuloma displaying central necrosis with or without mineralization surrounded by macrophages, lymphocytes; Inconclusive lesion characterized by irregular un-encapsulated clusters of epithelioid macrophages but no multinucleated giant cells and necrosis, consistent with an initial stage and negative features not consistent with tubercular granuloma [52].

2.6.2. Serological Tests

The skin tests are the international standard for ante-mortem diagnosis of bovine TB in cattle herds and individual animals. The main variants of the tuberculin test in use today are single intradermal tuberculin test (SITT), which is performed using only one bovine tuberculin purified protein derivatives (PPD) and single intradermal comparative tuberculin test (SICTT), which is performed using both bovine and avian tuberculin PPD [53,54].

Single intradermal comparative tuberculin test (SICTT) measures hypersensitivity to tuberculin (both for *M. bovis* and *M. avium*) injected into the neck of cattle. The results are read after 72 hours. It compares the responses of *M. bovis* and *M. avium*. It only assumes that infection with *M. bovis* promotes a larger response to *M. bovis* tuberculin than to *M. avium* tuberculin and that infection with other types of mycobacterium promote the reverse relationship. When the change in skin thickness is greater at the avian PPD injection site, the result is considered negative for BTB. Thus, if increased in the skin thickness at the injection site for the bovine PPD is greater than the increase in the 1+ skin thickness at the injection site at the avian PPD the animal is positive for bovine TB. A maximum volume of 0.2 ml containing a minimum of 2000 IU of bovine and avian PPDs should be injected at cervical site [55].

The disadvantage of the SITT is its lack of specificity and the number of No-Visible-Lesion reactors (NVLs) which occur and failure to detect cases of minimal sensitivity, in old cows and in cows which have recently calved. As well as in early infection, in some cattle in an unresponsive state, referred to as anergy which is developed due to antigen excess or immune suppression which in turn caused by non-specific factors such as malnutrition and stress [56]. Enzyme Linked Immunosorbent Assay *(*ELISA) appears to be the most suitable of the antibody detection tests and can be a complement, rather than an alternative, to tests based on cellular immunity. It may be helpful in anergic cattle and deer [57].

Lipoarabinomannan (LAM) is a glycolipoconjugate composed by an anchor mannosyl phosphate inositol, a polysaccharide backbone and diverse capping motifs species. Correct translocation of LAM in to the cell wall constitutes an important feature for the mycobacterial stability, the lack of *O*-manosilation was associated with increased production of LAM and increased release of LAM/LprG protein and consequently with a reduction in virulence of MTB *O*-manosilated deficient strain [58]. During mycobacterial disease LAM in a soluble form is released both from metabolically active and degrading bacterial cells. In active mycobacterial disease LAM occurs in serum and subsequently may be cleared through the kidneys and occur in urine in an antigenically intact form. Furthermore, as LAM is a carbohydrate antigen, and thus inherently heat-stable, LAM may be detectable by sensitive immunological techniques, even after heat treatment of urine samples [59]. Lipoarabinomannan (LAM) test is currently the fastest and most specific *in vitro* test by rapid detection of mycobacteria LAM in urine. This innovative and sandwich type rapid test is based on lateral flow immunochromatography. Detection of mycobacterial antigens in urine as a diagnostic test has several potential advantages compared with all currently used diagnostics. Urine samples are simple to collect, process, and store [60].

Bovine TB antibody rapid test utilizes recombinant *M. bovis* antigens to capture the antibody developed during infection. The captured IgG's are detected using colloidal gold conjugated detection antibody. Bovine TB antibody rapid test works on chromatographic immunoassay and has basic components of test strip which includes conjugate pad, which contains detection antibody, colloidal gold conjugated; a nitrocellulose membrane strip containing two lines T: recombinant *M. bovis* antigens and C: goat anti mouse [55].

2.6.3. Molecular Diagnosis

Molecular typing is a valuable tool in epidemiological investigations and for identification of potential sources of infection and has recently been recommended as a means of drawing conclusions as to whether and to what extent transmission from wild animals to cattle takes place [61]. Restriction fragment length polymorphism (RFLP) analysis with probes derived from the insertion element IS6110, the polymorphic GC-rich sequence (PGRS), and the direct repeat (DR) sequence has proved to be the most useful method of differentiating *M. bovis* strains. Spoligotyping and MIRU-VNTR are still by far the most used typing methods in molecular epidemiological studies of bovine and zoonotic TB. There is a growing research showing that mycobacterial interspersed repetitive unit variable number tandem repeat (MIRU-VNTR) is the most discriminatory technique to genotype *M. bovis* isolates [62].

2.6.3.1. Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) analysis was observed as a means to identify the strain and an especially useful tool for epidemiologic studies of BTB. RFLP based on the presence of the insertion sequence IS*6110* has been widely used as a genetic marker. The IS*6110* fingerprinting via RFLP has been standardized by using *Pvu*II as the restriction enzyme of choice to digest mycobacterial genomic DNA [63]. RFLP, as a molecular marker, is specific to a single clone/restriction enzyme combination and most RFLP markers are co-dominant (both alleles in heterozygous sample will be detected) and highly locus specific. It requires large amount of deoxyribonucleic acid (DNA) and restricted to the mycobacterial cultures which take around 20 to 40 days to obtain sufficient DNA and applied to strains of all mycobacterium species for which suitable probes have been identified. International consensus has been achieved

regarding the methodology of IS*6110* RFLP typing of *Mycobacterium tuberculosis* complex isolates and IS*1245* RFLP typing of *M. avium* strains. This is technically demanding, slow, and expensive and requires sophisticated analysis software for result analysis [64]. After electrophoresis of digested DNA on agarose gel, Southern blotting is carried out. Polymorphic banding patterns are revealed after hybridization by using a fragment of IS*6110* as a probe. This insertion sequence is present in up to 20 copies in *M. tuberculosis*, thus enabling the application of IS*6110*-RFLP as the gold standard genotyping technique for this organism [65]. In contrast, only 1-5 copies of IS*6110* are found in *M. bovis*, which limits the ability of this element to discriminate between different *M. bovis* strains [66].

The application of RFLP in the study of bovine TB is plagued by the fact that it requires the culture of tuberculous bacteria from clinical specimens. And it is a known fact that tuberculous bacteria are slow growers which may interfere with the results of RFLP based assays [67]. RFLP based identification systems are costly, laborious, technically demanding, and require advanced computer software for the analysis of results [68].

2.6.3.2. Polymorphic GC-Rich Repeat Sequence (PGRS)

Application of polymorphic GC-rich repeat sequence (PGRS) technique in the strain identification of *M. bovis* was noted to be similar and probably superior over the standardized IS6110 finger printing method. In both these techniques, purified DNA is necessary for southern blot hybridization and banding pattern analysis [69].

The PGRS-based RFLP probe was identified as the single most discriminatory type currently available for *M. bovis*strain typing. Such probes are present in 30 copies among members of *M. tuberculosis* complex that exhibits a high level of polymorphism between unrelated isolates. The interpretation of results by PGRS DNA method was noted to be difficult [70,71].

2.6.3.3. Polymerase Chain Reaction (PCR) Based Techniques

i. Multiplex Polymerase Chain Reaction (mPCR)

The multiplex PCR may be viable diagnostic method in endemic TB areas, where both of human and bovine TB co-occurs and differentiation between them is necessary for monitoring the spreads of *M. bovis* to human. Multiplex PCR as molecular technique differentiates MTBC from *M. avium*, *M. intracellularae*, and other mycobacterial species [72,73]. Heat killed acid fast bacilli (AFB) positive sample DNA was used as source of DNA template. The PCR targets the sequence of the genus *Mycobacterium*, within the16S rRNA gene (G1,G2) sequences, within the hyper variable region of 16S rRNA, that is known to be specific to *M. intracellularae* (MYCINT-F) and *M. avium* (MYCAV-R),and the MTB70 gene specific for MTBC (TB-A, TB-1B) [14,74]. PCR that is more sensitive than culture, a fact that may be attributable to the decontamination method before culture that may kill a high proportion of bacteria. Multiplex PCR is faster than culture-based detection, reducing diagnosis time from 120 days to approximately 2 days, even when automated culturing with broth medium is used [75].

ii. *Regions of Difference (RD) Deletion Typing*

Regions of difference (RD) are used to differentiate between species in the MTBC. It is a rapid, simple, and reliable PCR-based MTBC typing method that makes the use of MTBC chromosomal region-of-difference deletion loci. The phylogeny of MTBC showed recently that the strains found in animals belong to a single lineage which showed the deletion of the “Region of Difference” 9 (RD9) [76]. Indeed, *M. bovis* is the most recent strain in his lineage showing the deletion of RD4 [77]. Several specific primers are used to amplify specific loci which together formed a MTBCPCR typing panel. The final pattern of amplification products of all reactions, given by failure or success, segregates the tested strains from NTM isolates and by MTBC subspecies identity. The panel not only provides an advanced approach to determine the subspecies of MTBC isolates but also differentiate them from clinically important NTM species [78]. Molecular deletion typing had been found to be an important tool to differentiate *M. bovis* from the other strains of the MTBC. Pattern of presence or absence of these RDs would allow discrimination among MTBC strains [79].

iii. *Spoligotyping*

Spoligotyping is a useful PCR-based method to detect and type MTBC bacteria simultaneously. This method uses RLB (Reversed Line Blotting) and offers an alternativefor typical Southern blotting when rapid results are required. The method described based on DNA polymorphism present at one particular chromosomal locus, the direct repeat (DR) region, which is uniquely present in *Mycobacterium tuberculosis* complex bacteria [64,80].

The DR region in *M. bovis* BCG contains direct repeat sequences of 36 BP, which is interspersed by the non-repetitive DNA spacers of 35-41bp in length. Other MTBC strains contain one or more IS*6110* elements in DR-region. DNA extracts from clinical samples or lysed bacteria (from freezer or Lowenstein) can also serve as template. The clinical importance of spoligotyping is determined by its rapidity, both in detecting causative bacteria and in providing epidemiologic information on strain identities. It can also be useful for identification of outbreak and can facilitate contact tracing of TB. The specificity and sensitivity of this technique has been found to be 98% and 96%, respectively [81].

Spoligotyping also called spacer oligonucleotides typing is a novel method for simultaneous detection and typing of *Mycobacterium tuberculosis complex* bacteria, has been recently developed [67]. The direct repeat region in *M. bovis* BCG contains direct repeat sequences of 36 base pairs, which is interspersed by the non-repetitive DNA spacers of 35-41 base pairs in length. Other *Mycobacterium tuberculosis* complex strains contain one or more IS*6110* elements in direct repeat region [68].

iv. *Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeat (MIRU-VNTR) Typing*

This is a PCR based method that analyses multiple independent loci containing variable numbers of tandem repeats (VNTR) of different families of interspersed genetic elements collectively called *Mycobacterial* interspersed repetitive units (MIRU) [82]. In its original format, the PCR primers are run in each separate reaction

and the sizes of the products are analyzed by gel electrophoresis. Currently, the most widely used version of MIRU-VNTR analysis includes 12 tandem repeat loci. A set of 24 MIRU-VNTR loci were standardized to increase the discrimination power [83].

Mycobacterial interspersed repetitive units-variable number tandem repeat (MIRUVNTR) typing relies on PCR-amplification using primers specific for the flanking regions of the VNTRs and on the determination of the sizes of the amplicons, after electrophoretic migration. As the length of the repeat units is known, these sizes reflect the numbers of the amplified MIRU-VNTR copies [84]. The result is a numerical code, corresponding to the repeat number in each VNTR locus. Such numerical genotypes are intrinsically portable and are thus particularly convenient for both intra- and inter-laboratory comparative studies. Initial VNTR typing systems for MTBC strains made use of extremely limited sets of loci including exact tandem repeats (ETR), mycobacterial interspersed repetitive units (MIRUs) [73].

MIRU-VNTR 24-loci typing has been proposed for international standardization based on analysis of the clonal stability and evolutionary rates of MIRU-VNTR markers in the genetic lineage of tubercle bacilli collected worldwide. In addition, the method has been improved as high-speed automated genotyping system with the use of multiplex PCRs for the target MIRU-VNTR loci on a fluorescence-based DNA analyzer with computerized automation of the genotyping [83]. MIRU-VNTR genotyping has been used in a number of molecular epidemiologic studies, as well as to elucidate the phylogenetic relationships of clinical isolates. VNTR analysis has also been used to evaluate *M. bovis* transmission. In addition to their use for tracing TB transmission at the strain level, MIRU-VNTR markers can also provide useful predictions for classifying strains into genetic lineages [78].

2.7. Control and Prevention

Control programs of BTB have been primarily focused on the control of *M. bovis* in cattle [84,85]. The effective control and eradication of *M. bovis* depend on identifying and isolating potential sources of infection. Although test and slaughter policy are guaranteed to eradicate TB from domesticated animals, the policy has a negative economic impact [86].

Prevention of BTB mainly based on the herd hygiene and biosecurity practices, is aimed to reduce the exposition to pathogen; routine surveillance and slaughter surveillance [56]. The eradication of *M. bovis* from domestic herds can be achieved through whole herd depopulation (stamping-out). However, the emergence of financial and animal welfare constraints, as well as the emotional impact made by the farmers and opinion of public community made this strategy be indicated only on rare occasions and under specific circumstances [87].

3. Conclusion

Bovine TB is an infectious bacterial zoonotic disease transmitted from animals to humans and makes a significant economic impact due to high cost of eradication programs and has serious consequences for movements of animals and their products. In almost every country of the world, BTB is prevalent and causes loss of productivity. Molecular epidemiology of BTB is used to identify the source of contamination, to determine the risk factors BTB transmissions, to investigate drug resistance pattern and to track the geographic distribution and spread of clones of *Mycobacteria* species. Molecular methods used to differentiate between MTBC and NTM and also applicable for culture negative samples where strain identification is not possible by conventional approach. Due to the grave consequences of *M. bovis* infection on animal and human health, it is necessary to introduce rigorous control measures to reduce the risk of the disease in human and animal populations.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the manuscript.

Authors Contribution

All authors have contributed equally during the manuscript preparation.

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