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Epidemiology and Zoonotic Significance of Non-Tuberculosis Mycobacteria in Ethiopia

Mishamo Lechamo[1], Tariku Geinoro[2*]
[1]Livestock and Fishery Office, Doyogena Woreda, Southern Region, Ethiopia
E-mail: *mishamolechamo@gmail.com*
[2]Veterinary Drug and Feed Administration and Control Authority, Southern Branch Office, Hawassa, Ethiopia
E-mail: *geinorotariku@ymail.com*

Abstract

Non-tuberculosis Mycobacteria (NTM) are groups of Mycobacteria species which are not a member of the Mycobacterium tuberculosis complex and also known as environmental mycobacteria, or typical mycobacteria. They are capable infecting the cervical lymph nodes, skin, soft tissues and pulmonary (especially lung) of both humans and animals. Pulmonary NTM disease is most commonly caused by *Mycobacterium avium-intracellulare* and *M. kansasii;* and occasionally by *M. xenopi, M. fortuitum*, or M. chelonae**.** Mycobacterium avium complex can causes disease in poultry and cattle, characterized by wasting manifested noticeable atrophy of muscles, malabsorption, diarrhea, unilateral lameness, pulmonary infections, fibrocavity disease and fibronodular disease. In Ethiopia, non-tuberculosis mycobacteria infection in cattle, birds, pigs, camel and goat have been reported in different parts of the country. The pathogens that are transmitted between the environment, wildlife, livestock and humans represent major challenges for the protection of human and domestic animal health, the economic sustainability of agriculture, and the conservation of wildlife. The risk factors for distribution and pathogenesis of NTM are broadly categorized as environmental factors, host factors, pathogen factors. Even though the zoonotic significance of NTM is not well established in Ethiopia, isolation of NTM from livestock of the country combined with high prevalence of HIV/AIDS in human population indicates the potential exposure of humans for infection by NTM resulting in opportunistic disease in immune-compromised patients. Therefore, increased understanding about the epidemiology of the non tuberculosis mycobacterial disease is important concept in the control and prevention of the disease.

Keywords: Epidemiology, Ethiopia, Non-tuberculosis Mycobacterium, Zoonotic Significance.

1. Introduction

Tuberculosis is a chronic, granulomatous, debilitating infectious disease caused by acid-fast bacilli of the genus *Mycobacterium* [1]. Tuberculosis occasionally, assumes an acute and rapidly progressive course. The disease affects all species of vertebrate and before control measures were adopted, it was a major disease of a man and domestic animals [2].

Non-tuberculosis Mycobacterium (NTM) is the group of the *Mycobacteria* species which are not a member of the *Mycobacterium tuberculosis* complex (MTC) and also known as environmental mycobacteria, or typical mycobacteria or MOTT (mycobacteria other than tuberculosis). However; NTM are capable of causing mycobacterial infection or disease especially when the individual is predisposed to these species under the influence of certain risk factors [3].

The range of infection in human caused by the NTM is broad and includes pulmonary and cervical lymphadenitis caused by *Mycobacterium avium* complex (MAC), *M. kansasii* and *M. intracellulare*, skin infections caused by *M. marinum* and *M. haemophium* and nosocomial infections brought about by *M. chelonae* and *M. fortuitum* species [4].

In animal, NTM caused by infections [5] whose clinical manifestations different from those of classical tuberculosis infection; their veterinary importance lies instead in a sensitization to the tuberculin test. NTM have been known to cause disease characterized by the formation of tuberculoses-like lesions in the lymph nodes of different species of domestic animals including pigs, cattle, camel, and small ruminants [5, 6, 7]. In some cases, they may result non visible tuberculoses lesions and thus the lesions may not be detected during routine veterinary meat inspection, which makes them difficult to diagnose at postmortem examination. Currently, there are more than 140 identified species of NTM [8].

There is an important zoonotic aspect of these disease caused by infection of *Mycobacterium avium* complex (MAC) and other NTM in human particularly in immune-compromised individual such as AIDS patients. It likely that infections in human and animals in specific environment might originate from the environment where NTM are abundant. In addition, human through consumption of unpasteurized animal products from infected animal can be exposed to the infection with NTM. In most developing countries like Ethiopia, whose livestock resources are mainly managed under the traditional and poor management systems that favors for transmission of NTM infection in animals coupled with together with the HIV/AIDS pandemic in human increased the potential zoonotic importance of NTM in human population [9].

Therefore; the objective of this paper is: To review the epidemiology, zoonotic significance and control options of the non-tuberculosis mycobacteria species in Ethiopia.

2. General Biology of Mycobacterium species

Morphologically, Mycobacterium species are thin-rod-shaped, of varying lengths (0.2-0.6 x 1.0-10.um), acid-fast bacilli with high lipid content in their cell wall, which has been the focus of much attention. Sometimes they occur as a branching filamentous form but these may easily fragmented. Mycobacteria are non motile, nonspore forming, aerobic. Although, cytochemically gram positive, they do not take up dyes of Gram stain, because, the cell wall is rich in lipids, mycolic acids forming the bulk part of cell wall. They are characteristically acid-fast, as once the cells have taken up a dye; they do not easily decolorized, even by acidic alcohol [10].

2.1. Taxonomy

In 1959, Botanist Ernest Runyon put these human disease associated with bacteria into four groups. According to the Runyon classification system these disease are based on rates of growth and pigment production (Table 1) [8].

Table 1: Non-tuberculosis mycobacteria classified based on their rate of growth and pigment production

Group Names	Species	Pigment production	Rate of growth
Photochromogenic	*M. kansasii*	Yellow colonies	Only slow growing and requires seven days and above
	M. marinum	After exposure to light	
	M. simiae		
	M. asaticum		
Scotochromogenic	*M. scrofulceum*	Yellow or orange colonies the absence of light	Slow growing
	M. szulgai		
	M. gordonae		
Non-photochromogenic	*M. avium*	No pigment or light production with exposure to light	Slow growing
	M. intracellulare		
	M. xenopi		
	M. malmonese		
	M. ulcerans		
	M. haemophium		
	M. genavense		
	M. terrae		
Rapid growers	*M. chelonae*		Rapid-growing in that there is visible growth
	M. abscess		
	M. fortuitum		
	M. peregrinum		Require less than seven days.
	M. smegamatics		
	M. flavescens		

Source: [11]

2.3. Morphology

Generally, Mycobacterium species in living tissue appears as rod-shaped that can assume different shapes where as in the culture *M. avium* is cocoid shape.

While mentioning the morphology, it is worth to rise up some points about the chemical structures of the cell wall (Table 2) that is responsible for their resistance humoral defense mechanisms and to disinfectant [12].

Table 2: Cell wall components of mycobacterium and their function

Cell wall components	Functions
Mycolic acid and their esters	Make up the bulk of the cell wall lipids for acid-fastness
	Properties of retaining carbol-fuchsin after application of the decolorizer, acid-alcohol
Glycolipids	For toxicity, granulomatous response, and enhanced survival of phagocytosed Mycobacterium
	Colonial growth of virulent Mycobacterium
Surface mycoids	Resistance to water soluble enzymes, antibiotics, disinfectants
	Ensures bacterial survival with in macrophages
Wax-D and various proteins	Activates macrophages
	Adjuvant activity
	Stimulates, particularly ecll-mediated immune response and induce granuloma formation
Sulfo-lipids and phospholipids	Prevents the respiratory burst
	phagolysosomal fusion

Source: [11]

2.3. Staining and Biochemical characteristics

When they are poorly stained by aniline, they are gram-positive [12]. But otherwise, MAC and other non-tuberculosis mycobacteria stain readily Ziehl-Neelson Method; other methods like kinyon modification of Ziehl-Neelson Method. There are to be no significant biochemical distinction between *M. avium* serovar 1,2 and 3 (avian tubercle bacilli) known to be virulent for chicken and serovar 4-20 which are known to be virulent for other domestic mammals [13].

2.4. Growth requirement

The optimal temperature for growth of MAC is $40^{0c,}$ except the *M. avium ssp*. Paratuberculosis, whose optimal temperature for growth is $38\text{-}39^{0c}$ [12] while MAC and related mycobacteria grow at $25\text{-}45^{0c}$, with optimal temperature being near the top of that range. *M. avium* complex grow poorly or not all ordinary laboratory media because they are strict aerobes. Therefore, they grow best on complex media (i.e. containing serum, potato and egg) and *M. avium ssp*. Paratuberculosis requires mycobactin supplement for its growth [1].

2.5. Pathogenesis

The most common clinical manifestation of NTM disease is lung disease, but lymphatic, skin/soft tissue, fibro-cavity disease, fibro-nodular disease and disseminated disease are also important. Pulmonary disease caused by NTM [8].

Pulmonary infections: - the pathogenesis of lung disease due to NTM is poorly understood. Pulmonary infection with NTM is poorly acquired by inhalation, most likely aerosols from natural surface water or from domestic and institutional hot water systems and through ingestion with subsequent aspiration from the stomach [14].

Host response to MAC infection: - the observation that pulmonary *Mycobacterium avium* complex (MAC) disease is relatively rare, despite the high prevalence of skin test reactivity to *M. avium* complex (MAC) in young adulthood suggests that the host immune response is highly effective at containing or eliminating the infecting microbes. Both age and preexisting lung disease or lung injury are major risk factors for active NTM lung disease. Pulmonary NTM disease is most frequent in older adults. In general terms, MAC is taken up by macrophages and survives and proliferates within vacuoles in their cytoplasm as an intracellular pathogen [15].

Lymphocytes ultimately interact with the infected macrophages to induce intracellular destruction of the mycobacterium or to destroy the infected macrophages itself. T. helper lymphocytes are the basis of acquired (antigen-specific) immunity, while natural killer (NK) cells are key components of the innate immune response to these pathogens. This interaction between macrophages, lymphocyte, and microbe is the focal point in the pathogenesis and immunology of MAC infection that leads to granuloma formation, successful control of the infection, or clinical disease [16].

Fibrocavity disease: - lung disease due to MAC has been recently differentiated into two distinct forms, fibrocavitary and fibronodular. In the more familiar fibrocavity form, upper lobe disease occurs most commonly in elderly male smokers with chronic pulmonary symptoms due to underlying lung disease. Symptoms and radiographic changes may be difficult to differentiate from the underlying disease, but MAC is often easily recovered from the sputum of these patients [16].

Fibronodular disease: - the fibro nodular and bronchiectatic form of MAC lung disease appears most frequently in nonsmoking women over the age of 50 who do not have underlying lung disease. Body morphotype is a possible predisposition to this second form of disease. Some patients with fibronodular NTM lung disease appear to have similar clinical characteristics and body types, including scoliosis, pectus excavatum; mitral valve prolapsed, and joint hyper mobility [17].

Disseminated disease in HIV-infected patients: - NTM infection in patients with AIDS primarily presents as disseminated disease in association with very low CD4 lymphocyte counts (usually below 100/microL) in developed countries. Exposure has been documented from potable hot water [18]. Disseminated NTM disease occurred in 5.5 percent of AIDS cases reported to the Centers for Disease Control and Prevention from 1981 to 1987, 96 percent of which were due to MAC [19].

Virulence: - inhibit phagolysomal fusion, cause disruption of the phagosomes, interfere with release of hydrolytic enzymes from the attached lysosome, and inactivate lysosome enzymes release into the cytoplasmic vacuole, even survive within the fused phagolysosomes some by the help of the fatty acid and mycoids [20].

Lesion: - the capacity of the MAC to produce progressive disease may be associated with certain complex lipid components of the cell wall, such as cord factor, sulfur-containing Glycolipids (sulfides) or strongly acidic lipids. Delayed type of hypersensitivity (DTH) develops following exposure to Mycobacteria; once activated, macrophages demonstrate an increased capacity to kill Mycobacteria. Activated macrophages that lack sufficient sub cellular microbicidal components to kill virulent tubercle bacilli are destroyed by the intracellular growth of the organisms and a lesion develops [21]. At postmortem, which die after prolonged illness, are in poor to emaciated condition. There are no body-fat, the pectoral muscles are atrophied, and the keel bones prominent and occasionally deformed. Tuberculoses nodules can be seen in liver, spleen, intestine, bone marrow, lung, mesentery, heart, kidney and ovary. In other domestic animals, such as pig and cattle, exposure to MAC is common but it takes chronic nature in most conditions and involves lymph nodes of head and cervical regions and also mesentery [20].

3. Epidemiology of Non-tuberculosis Mycobacterium

NTM are widely distributed in the environment, particularly in wet soil, marshland, streams, and rivers. Different species of NTM prefer different types of environment. Human disease is believed to be acquired from environmental exposures, and unlike tuberculosis and leprosy, there has been no evidence of human-to-human transmission of NTM, hence the alternative label "environmental bacteria'' [8]. Carcasses of infected birds are the sources of dissemination of avian tubercle bacilli. Ulcerative swine intestinal lesions also serve as a source of infection (for other animals and birds. Cattle infected with MAC can serve as source of infection [20, 22].

3.1 Risk factors

3.1.1 Environmental risk factor

Housing can predispose to the NTM infections, as does intensive keeping; so that the disease is common and serious where these forms of husbandry are practiced. The closer the animals packed together, the greater the chance to acquire NTM infection. In addition, confinement also increases acquiring of the NTM infection. For instance, the necessity of keeping birds in close confinement during winter provides favorable conditions of spreads of disease [23].

3.1.2 Host risk factor

Zebu breeds are thought to be more resistant to MAC infections that the European breeds and the effects on local animals are much less severe but under intensive management conditions, the morbidity rate reaches above 50%. Young animals are the most vulnerable to infection of NTM species such as *M. avium ssp paratuberculosis* but older animals can be infected only with very large inocula [1]. Infection with other species of *M. avium* complex appears to be less prevalent in young because the younger are more resistant to infection with these groups of *M. avium* complex. However, in older birds, the MAC has a greater opportunity to cause the disease longer periods of exposure [2]; this condition is also true in pigs [24].

3.1.3 Pathogens risk factors

It has been suggested that glycolipids, peptidolipids that accumulate on the periphery of the cell envelop of pathogenic NTM contribute to the pathogenecity of the organisms [25]. It is more resistant to most of anti mycobacterial, drugs and grows optimally at 40^0c. *M. avium*, which is the causative agent of the avian tuberculosis, has a tremendous ability to persist in the environment for quite a longer period of time. This makes it a good candidate to pose a serious hazard to the environment and to other domestic animals [22]. This also true for *M. avium ssp paratuberculosis* [1]. These characteristics of the *M. avium* complicate the prevention and control measures directed against the disease [13].

3.2 Distribution of NTM

3.2.1. Distribution of NTM in the world

NTM diseases have been seen in most industrialized countries, where incidence rates vary from 1.0 to 1.8 cases per 100,000 persons. Recent studies, including one done in Ontario, Canada, suggest that incidence of NTM is much higher. Pulmonary NTM is estimated by some experts in the field to be at least ten times more common than TB in the U.S., with at least 150,000 cases per year. The most NTM disease cases involve the species MAC, *M. abscessus*, *M. fortuitum* and

M. kansaii. The *M. abscessus* is being seen with increasing frequency and is particularly difficult to treat [8]. Mayo Clinic researchers found a three-fold increasing incidence of cutaneous NTM infection from years 1980 to 2009 in a population-based study of residents of Olmsted Country, Minnesota. The most common species were *M. marinum*, according for 45% of cases and *M. chelonae* and *M. abscessus*, together accounting for 32% of patients. *M. chelonae* infection outbreaks, as a consequence of tattooing with infected ink, have been reported in the United Kingdom and the United States [19].

M. avium complex in chicken is worldwide in distribution but occurs most frequently in the North Temperate Zone. The highest incidence of the infection in the USA occurs in the flocks of the North Central States while in Western and Southern State is low. The explanation for this low incidence is not entirely obvious, but there are several contributing factor such as climate, flock, management and duration of infection. There is also high prevalence of MAC infection in homosexual AIDS patients in USA [26]; the disease has also been reported in Canada, European, countries like Czech Republic; Australia and some Latin America countries, but the incidence of the MAC infection varies from country to countries and /or from countries to and/or continents to continents [20].

On Africa conditions, the disease has been reported as low incidence by South Africa in its poultry flock while Kenya has reported the disease in its lesser flamingoes [27]. In Tanzania 17 strains of NTM were isolated. Among these, *M. terrae* were the most common which is isolated from wildlife in Tanzania. This report suggests that *M. terrae* may be a ubiquitous NTM across Africa [28].

3.2.2. Distribution of NTM in Ethiopia

In Ethiopia, non-tuberculosis mycobacterium infection in cattle, camel and goat have been reported in different parts of the country [6, 7, 29]. These studies mainly based on molecular characterization of isolates from tuberculous-like lesions obtained from abattoir examination. In Ethiopia, MAC infection in chicken has been confirmed but the distribution and prevalence of MAC at a national level is unknown.

A study done by [22] on prevalence of MAC in indigenous backyard managed chicknen showed a statistically significant difference between agro-climatic zones of Central Ethiopia. According to this study, relatively higher temperature of low and mild altitude favors the survival of the organisms better than the coldest temperature altitude. For example, high (Debre Berhan, located at 2780m altitude; mean annual temperature range between 6.3 and 18.8^{o}c), mid (Sebeta, located at 2240m altitude; mean annual temperature range between 15 and 21^{0}c), and low (Nazareth, located at 1300m altitude; mean annual temperature range between 15 and 28^{o}c [22].

3.3. Prevalence in human

HIV- infected patients were at especially high risk of disease due to NTM. The majority of disease in this population (95%) is due to *M. avium*. Disseminated *M. avium* infection is the most common bacterial infection in patients with AIDS, occurring in 20 to 40% of all patients. Disease in these patients is highly correlated with sever immunosuppression, with the average CD4 cell count at the time of dissemination in the 25 to 30 ranges. Patients with, 100 CD4 cells, not receiving prophylaxis, develop disseminated *M. avium* at the rate of approximately 20% per year. The overall incidence of *M. avium* as an initial diagnosis has increased among AIDS patients while other complications of AIDS, such as pneumocystis pneumonia, have increased. Disseminated *M. avium* occurs in similar rates in all geographic regions and various HIV risk groups. Localized pulmonary disease in AIDS due to *M. avium* occurs in less than 5% of patients [30].

Table 3: Non-tuberculosis complex mycobacteria (NTM) identified by 16S rDNA locus sequencing results

Bacterial species	Sources
Mycobacterium terrae complex	1 goat
Mycobacterium arupense	1 goat
Corynebacterium pseudotuberculosis	1 goat
Mycobacterium fortuitum	2 cattle
Mycobacterium flavescens	1 human sputum

Source: [28]

The isolation of NTM from camelus having tuberculosis-compatible nodules with granulomatous and caseous lesions in lymph nodes and lung indicate the importance of these species in camels of Ethiopia. Research carried out on camels slaughtered at Metehara and Akaki abattoirs isolated 18 NTM out of the 21 isolates (85%) obtained from tuberculoses lesions on different tissues of camel [7]. Similarly, a study carried out on camels of the East Ethiopia isolated 65% NTM from the total culture isolates [31].

Table 4: Non-tuberculosis mycobacteria isolated from cattle in Ethiopia

Species	Woldiya	Gimbi	Butajira	Jinka
M. ocapulcensis		1		
M. colombiense			1	
M. engbaekii		1		
M.fortuitum	2			1
M. gordonae		3		2
M. intracellulare			2	
M. monacense		1		
M. mucogenicum				1
M. nonchromogenicum	1		7	
M. peregrinum		1	1	
M. voccae			1	

Sources: [6]

4. Zoonotic Significance

In immunocompetent patients, the infections caused by MAC agents are principally pulmonary. In children a recent study has shown that the most predominant species in cervical lymphadenitis caused by non-tuberculosis mycobacteria was *M. scrofulaceum* (60%) followed by the MAC agents (40%) among the members of MAC, *M. avium subspp avium* predominate (87 to 98%) in AIDS patients and induce disseminated mycobacteremia rather than bacteria restricted to the lungs as for immunocompetent patients [32].

Among the several opportunistic pathogens affecting patients infected with human immunodeficiency virus (HIV), members of NTM, mainly *M. avium subspp avium*, are the causes of significant problems in these immune suppressed HIV/AIDS patients accounting for 95% of the cases [33]. Disseminated M. avium occurs in similar rates in all geographic regions and various HIV risk groups. Localized pulmonary disease in AIDS due to *M. avium* occurs in less than 5% of patients [30].

Potable water is considered as the primary source of NTM infection in humans [34] and has been shown to be a source of *M. avium subspp avium* infection in virus inoculated Simian immunodeficiency macaques. Food has also been to be a possible source and route of transmission of *M. avium subspp avium*.

In Ethiopia, the zoonotic significance of NTM is not well established. However, isolation of NTM from livestock of the country combined with high prevalence of HIV/AIDS in human population indicates the potential exposure of humans for infection by NTM resulting in opportunistic disease in immunocompromised patients. A study done by [28] in pastoralist of Borena zone isolated *M. flavescens* from sputum of human pulmonary tuberculosis patient and this confirms the role of NTM in pastoralist areas of the country [29].

Livestock in pastoralist regions is major source of food and income, in addition, possession of livestock provides a measure of social status in the pastoral communities. In pastoralist communities of Ethiopia habit of consumption of raw animal product particularly milk is common and the pastoralists have also close physical contact with their animals.

5. Control Options

Control and eradication programmes of non-uberculosis disease or other mycobacteria pathogens could be extremely complicated by the existence of the strong involvement of wildlife and environment reservoirs. Therefore, the epidemiological knowledge of these pathogens in their natural reservoirs is an important factor to be taken into account for the success of control programmes. For assessing epidemiological studies of mycobacteria in wildlife the environment improved diagnostic tools are needed [35]. The spread of the distribution of diseases caused by NTM, the high incidence in some areas, importance of the poultry and swine industries and increasing isolation of the these agents in AIDS and other immune-compromised patients makes control and eradication of NTM [21]. The main goal is to reduce or prevent the excretion of bacilli from wildlife by breaking the chain of infection from feral to domestic animals [35].

5.1. Other Control Options

5.1.1. Test and Removal

Tuberculin test is a considered as practical value in testing and removal of the reactive animals like infected chickens, which helps to the elimination of the many foci. The test is capable to detect many infected chickens, fowl and swine before the disease reaches a severe or chronic state, it repeated tests are made and reactors removed, dissemination of the bacteria to the environment may be reduce [21]. So regular tuberculin testing provides a monitoring system. Recently, a whole blood rapidly agglutination test has been developed for the detection of the tuberculosis in poultry and preliminary trials indicates that it is more useful supplementary test to the wattle tuberculin test [12].

5.2.2. Sanitation

Special consideration should be applied to poultry since they are the main source of the infection to other domestic animals and disseminators to the environment, so through cleaning and disinfecting of buildings and other premises with cresylic or phenol compounds should be used at (2-5) percent of concentration to kill the tuberculin [13].

6. Conclusion and Recommendation

In Ethiopia, in recent years a number of studies indicated the wide spread distribution of NTM in livestock and isolation of NTM species in pulmonary TB patients from pastoralist area showed the zoonotic importance of these organisms in our country. In addition, the high prevalence of HIV infection in Ethiopia indicates the existence of important risk factor for human NTM infection.

Based on the above conclusion, the following recommendations are forwarded:

- Further research and detailed investigations are needed about zoonotic significance of the bacteria in Ethiopia.
- Public education should be practiced to increase awareness of the community on the potential risk of contamination of food and water by NTM organisms and education of the community to practice of pasteurization of food of animal origin to reduce risk of infection.
- Great attention should be given by medical professionals on the importance of the NTM disease especially MAC as public hazards since NTM are one of the opportunistic diseases in immunocompromised HIV/AIDS patients.

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