MINI-REVIEW

The puzzle of the evolutionary natural history of tuberculosis

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Abstract

Several pieces of the puzzle of the natural history of tuberculosis are assembled in this review to illustrate the potential reservoirs and sources of the *Mycobacterium tuberculosis* complex (MTBC) mycobacteria, their transmission to animals and humans, and their fate in populations, in a co-evolutionary perspective. Millennia-old companions of mammalian and human populations, MTBC are detected in the soil, in which they infect and survive within vegetative amoebae and cysts, except for *Mycobacterium canettii*. Never detected in the sphere of plants, they are transmissible by transcutaneous, digestive and respiratory routes and cause an infection of the lymphatic system with secondary dissemination in most tissues, in which they determine a specific and non-pathognomonic granulomatous inflammatory reaction; in which MTBC survives in dormant form irrespective of MTBC species and mammalian species; indicating that the current epidemiology in mammalian populations is essentially governed by the probabilities of contact between mammalian species and MTBC species. Individual variabilities in clinical expression of tuberculosis are related to MTBC species, strain and inoculum; host genetic factors; acquired modulations of the inflammatory response; and probably human microbiota. This review of the literature suggests an evolutionary natural history of telluric environmental mycobacteria, satellites of unicellular eukaryotes, transmissible to mammals via the digestive and then respiratory tracts, in which they determine a fatal contagious infection that is primarily lymphatic and a quiescence-mimicking encysted form. This review opens perspectives for microbiological and translational medical research.

Keywords: Microbiota, *Mycobacterium*, *Mycobacterium bovis*, *Mycobacterium tuberculosis*, natural history, sources, transmission, tuberculosis
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Introduction

Tuberculosis is one of the leading infectious causes of death worldwide, and it is estimated that approximately one-quarter of the world's population is latently infected with *Mycobacterium tuberculosis* [1,2]. This fatal infectious disease is caused by 13 species of closely related mycobacteria forming the *Mycobacterium tuberculosis* complex (MTBC) [3]. Because of their genomic proximity, the exact taxonomic status of these species is debated, and some authors consider the species of the MTBC as ecotypic variants of *M. tuberculosis* [4]. However, these species or ecotypes have genomic, genetic and phenotypic differences that may explain differences in reservoirs and modes of transmission. *Mycobacterium tuberculosis* does not cause tuberculosis only in humans, being described in a variety of animals, mainly mammals [5].

The outcome for persons infected with *M. tuberculosis* is highly variable, ranging from total elimination of the pathogen by the inflammatory and immune system, to long-term asymptomatic transport during so-called dormant tuberculosis, to symptomatic tuberculosis, and finally death [6]. The role of the microbiota in modulating the expression of *M. tuberculosis* infection is emerging, and the continued transmission of *M. tuberculosis* is the main factor in maintaining the high incidence of this disease [7].

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Mycobacteria of the MTBC

Of the 13 species or ecotypes currently described in MTBC, only nine have been associated with human tuberculosis, including the biliary bacillus Calmette–Guérin (BCG) vaccine [8,9]. MTBCs have 72 highly conserved genomic sequences (>99.9% nucleotide identity) but 73 different phenotypes. It has been suggested that MTBC evolved from a common ancestor via 74 successive DNA deletions/insertions, giving them their differences in pathogenicity [10]. The genomic evolution of these mycobacteria is achieved by a progressive decrease in the coding capacity of their genome, which is exclusively chromosomal, with loss of genomic fragments called deletion regions and invasion of the genome by insertion sequences [11]. *Mycobacterium canettii* is the closest species to the common ancestor of MTBC, having a genome of 4.48 ± 0.05 Mb [11] coding for unique functions such as the Trans cobalamin gene. *Mycobacterium canettii* is responsible for non-contagious tuberculosis [12] and has a geographical distribution limited to the Horn of Africa. Microbiologically, *M. canettii* has a 17-hour doubling time that is one-third shorter than that of *M. tuberculosis* (25 hours) and produces smooth colonies, unlike *M. tuberculosis*, which has a rough morphotype [12]. *Mycobacterium tuberculosis* has a worldwide distribution in six major phylogenetic lineages (lineages 1, 2, 3, 4, 7 and the recently described lineage 8) [13] that are unevenly distributed across the world. L1 and L2 lineages predominate in East and Southeast Asia, while L1 and L3 lineages predominate in the Indian subcontinent, L3 and L4 lineages predominate in Central Asia and Russia, and the L4 lineage predominates in Europe, the Americas, North Africa and the Middle East [14]. It is in sub-Saharan Africa that we find the greatest variety of lineages, because in addition to the L1, L2, L3 and L4 lineages, two new lineages have recently been identified, the L7 lineage in Ethiopia and the L8 lineage in Rwanda and Uganda [13–15]. *Mycobacterium africanum* has two lineages (L5 and L6) distributed exclusively in West Africa and responsible for tuberculosis that is indistinguishable from that caused by *M. tuberculosis* [16–18]. The virulence of MTBC is variable and differs within strains of the same species, for example: modern lineages of *M. tuberculosis* (L2–L4) are more virulent and responsible for most tuberculosis cases in the world [10]. Specifically, the Beijing (L2) strains are the most virulent MTBC for humans with a predisposition to develop further resistance to anti-tuberculosis drugs and have a high capacity for propagation, illustrated by the fact that these strains have been described in cattle and dogs [5,10,19]. This virulence of Beijing strains is correlated with deletions in the *pks15/1* gene and in the RD207 region [20]. In contrast, *Mycobacterium bovis* BCG is the least virulent strain among MTBC, its attenuation follows the loss of the RD1 region in its genome [21].

Human–environment interfaces: entrance doors and microbiota

Since the work of Villemin and the introduction of *M. tuberculosis* culture by Robert Koch, many scientists have tried to determine how tuberculosis is transmitted to humans [7,22]. A large part of the studies on tuberculosis transmission were carried out at the end of the nineteenth/early twentieth century. These studies focused on culture to demonstrate viability, and inoculation of animals to confirm the pathology [7]. Several theories existed at the time, and Calmette and Guérin reported in 1905 the intestinal origin of pulmonary tuberculosis [23]. Other works reported the culture of *M. tuberculosis* in the environment [7]. It should be considered that, at that time, knowledge of the diversity of mycobacteria (tuberculous and non-tuberculous) was more limited, so that several studies were carried out with environmental mycobacteria, or with MTBC different from *M. tuberculosis* [7]. An important part of our knowledge about tuberculosis transmission comes from the investigation of cases acquired during care. It has been well recognized since the 1950s that the respiratory tract is the main route of transmission of *M. tuberculosis*; however, this does not exclude other routes of transmission [7] (Fig. 1).

Respiratory route

Innovative studies by Riley and Wells in the 1950s and 1960s showed that exposure of guinea pigs to aerosol droplets from patients with pulmonary tuberculosis resulted in substantial tuberculin conversion rates in the guinea pigs [24,25]; and this work has recently been replicated with consistent results [7]. These studies have clarified the basic principles of airborne transmission of pulmonary tuberculosis, which is now widely accepted as the major route of transmission [7,26]. Cases of transmission of *M. tuberculosis* by aerosolized *M. tuberculosis* have been reported during autopsy [7] and surgical procedures of incision and irrigation of tuberculous abscesses [7]. Respiratory transmission also includes *M. bovis* tuberculosis, not only in persons exposed to aerosols from animals infected with *M. bovis* [27,28], but also by human-to-human transmission of *M. bovis* [28–30].

In mice, infection with *M. tuberculosis* aerosols leads to a loss of intestinal microbiota diversity 6 days after infection [31]. These rapid changes in the microbiota are attributed to the host immune response, with all mice showing a recovery of microbial diversity within a few days [31]. The role of the

respiratory microbiota in the development of tuberculosis remains uncertain. In addition, the data show that there is a unique microbial diversity between individuals with tuberculosis and healthy controls, but the results remain controversial [32]. Importantly for the diagnosis of tuberculosis by laboratory culture, the protocols include a step of chemical decontamination (sodium hydroxide) of the sputum to remove fast-growing microorganisms to facilitate the growth of mycobacteria [33]. This decontamination may have contributed to the non-observance by culture of certain bacterial communities that could coexist with *M. tuberculosis* in the lungs and respiratory tract, opening prospects for clinical microbiology work.

Digestive route

Historically, the digestive tract has been the primary route for BCG vaccine administration, illustrating the possibility of systemic passage of MTBC after ingestion [34,35]. This route of administration was abandoned in most countries following the 'Lübeck Disaster', and in 1976 Brazil was the last country to abandon this route, because of poor response to skin testing and for economic and operational reasons [36,37]. Currently, the possibility of a digestive gateway for MTBC is neglected despite published evidence, illustrated by the 'Lübeck Disaster' in Germany in 1929–1933 [38]. During this episode, oral administration to 251 neonates of BCG vaccine that was accidentally contaminated with *M. tuberculosis* caused tuberculosis in 228 children, all of whom developed lymph node involvement, while a pulmonary form was reported in 30 children; 71 (28.3%) died of tuberculosis [38]. This accident shed light on the transmissibility of *M. tuberculosis* through the digestive tract, and in this accident the form of tuberculosis caused by the *M. tuberculosis* was similar to the forms of tuberculosis caused by *M. bovis* in humans, most commonly affecting the lymph nodes after entry of the pathogen through the digestive tract [38]. For *M. canettii*, current clinical and experimental data [12,39] suggest the existence of an unknown environmental reservoir, and digestive transmission via food with local replication in the oropharynx and cervical lymph nodes and increased dissemination in the respiratory and digestive tracts [12]. Digestive transmission of tuberculosis is known particularly for zoonotic tuberculosis caused mainly by *M. bovis* [27]. Zoonotic tuberculosis is primarily a foodborne disease that follows consumption of unpasteurized milk or milk products [27]. Meat from animals with tuberculosis is not recognized as a vehicle for transmission of *M. bovis*, at least when cooked, and *M. bovis* is rarely found in muscle [27]. WHO has estimated that in 2018 zoonotic tuberculosis with *M. bovis* caused 143 000 new cases and 12 300 deaths [2].

Abdominal tuberculosis represents 12% of extrapulmonary tuberculosis cases and 1%–3% of total tuberculosis cases [40]; 15%–25% of abdominal tuberculosis cases have concomitant pulmonary tuberculosis [41]. Modes of MTBC infection in cases of abdominal tuberculosis include swallowing infected sputum, ingestion of infected food, lymphatic spread from an extra-abdominal focus, and by contiguous spread from urogenital organs [40,42].

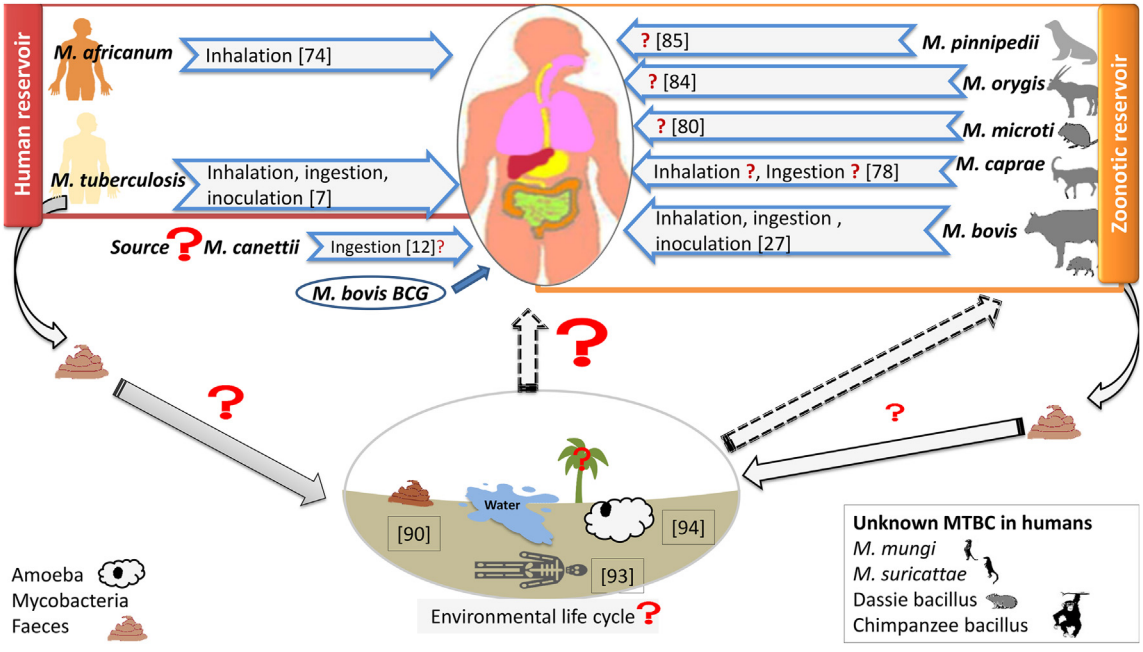

FIG. 1. Reservoirs of the *Mycobacterium tuberculosis* complex and routes of transmission to humans.

Abdominal tuberculosis can be classified into four forms: luminal (ileocaecal), peritoneal, nodal and visceral involving solid intra-abdominal organs [40].

Several series of cases of abdominal tuberculosis have been reported, in some studies this localization is mainly attributed to *M. bovis* then to *M. tuberculosis* [43–45].

Alteration of the intestinal microbiota after antibiotic administration has been reported in mouse models to cause susceptibility to tuberculosis. Mice with microbial dysbiosis showed a significant increase in *M. tuberculosis* in the lungs and spread to the spleen and liver [46]; similar results to those observed at the induction of dysbiosis by *Helicobacter hepaticus* infection [47].

Tuberculosis of inoculation

Cutaneous inoculation is also a rare route of transmission, reported in some accidental cases among health-care workers [48,49], or in patients following corticosteroid injections or skin trauma [50] and tattooing [51]. This route of inoculation results in primary cutaneous tuberculosis. On the other hand, facial cutaneous tuberculosis, peri-ocular cutaneous tuberculosis, has been described and the authors hypothesized that this unusual presentation could be due to minor trauma followed by inoculation [52]. Annobil *et al.* described primary tuberculosis of the penis with bilateral hypertrophy of the inguinal lymph nodes in a 4-month-old baby circumcised at 6 weeks of age; possibly related to the barber-operator having moistened the razor with sputum before sharpening it [53]. Also, an anecdotal report of sexual transmission of *M. tuberculosis* has been described, involving penile cutaneous tuberculosis followed by endometrial tuberculosis in the patient's partner; the two strains of *M. tuberculosis* were identical by molecular typing [54]. In rare cases, tuberculosis can be contracted after solid organ transplantation from an infected organ donor, and because of immunosuppression, transplant recipients frequently develop extrapulmonary or disseminated TB; however, tuberculosis after solid organ transplantation is most often caused by primary infection or reactivation of a latent infection [55].

Fate of *M. tuberculosis* in humans

Once inhaled, *M. tuberculosis* travels from the trachea to the lungs where it is phagocytosed by alveolar macrophages in which it is internalized in the phagosomes and then in the phagolysosomes [56,57]. However, *M. tuberculosis* can block the acidification and maturation of phagosomes to survive in the host alveolar macrophages [58,59]. Macrophages and other immune cells aggregate to form the granuloma [60], in which mycobacteria are both intracellular within macrophages and extracellular [61]. This granuloma is formed mainly by macrophages infected and uninfected with *M. tuberculosis* bacilli surrounded by immune cells including granulocytes, dendritic cells, natural killer cells and T and B lymphocytes [60] (Fig. 3). Under these conditions, both innate and adaptive immune defences are involved in the control of *M. tuberculosis* [62], limiting their replication and leading to a state of equilibrium between the host organism and the pathogen. In the granuloma, *M. tuberculosis* bacilli are exposed to a variety of stress conditions, in particular hypoxia, which promotes the dormancy of the mycobacteria [62]. This dormant state in *M. tuberculosis* results in an ability to persist in host tissues without replication for months or even years, without causing tuberculosis disease, and resulting in chronic asymptomatic infection in up to 90% of infected persons—known as latent tuberculosis infection [6,63–65]. On the other hand, 5% of infected persons will develop active tuberculosis [66], whereas others will be competent to eliminate the pathogen. Dormant mycobacteria may reside in old granulomatous lung lesions (in the macrophage and/or caseum), pulmonary lymph nodes [67], or adipose tissue, which is described as a large reservoir housing dormant mycobacteria and preserving them from antimicrobial agents and the host immune system [68]. In 5%–15% of latently infected persons, *M. tuberculosis* can reactivate, leading to active tuberculosis [68]. Indeed, when host immunity is compromised and environmental conditions around *M. tuberculosis* become conducive to its reactivation, these bacilli accelerate replication, leading to necrosis of infected macrophages and release of intracellular mycobacteria, which could infect new cells and spread to other tissues [69,70]. In addition to their ability to reactivate, the important role of dormancy in the natural history of tuberculosis lies in the fact that dormant mycobacteria are potentially infectious. Indeed, this has been demonstrated not only experimentally [69,70] but also clinically, where it has been found that dormant mycobacteria can be even more infectious than metabolically active mycobacteria in the expectorant of patients with pulmonary tuberculosis [71].

Tuberculosis in the animate environment: animals

Data from the literature indicate that except for *M. bovis* BCG, which is a vaccine strain, and *M. canettii*, all other MTBC (11/13 species) can infect one or more animal species, including humans (Fig. 2). MTBC species are genetically closely related (>99.9% nucleotide identity) and some authors classify them into strains that are adapted to the human host, such as *M. tuberculosis* and *M. africanum*, and those that have the potential to spread and transmit in a wide variety of wild and

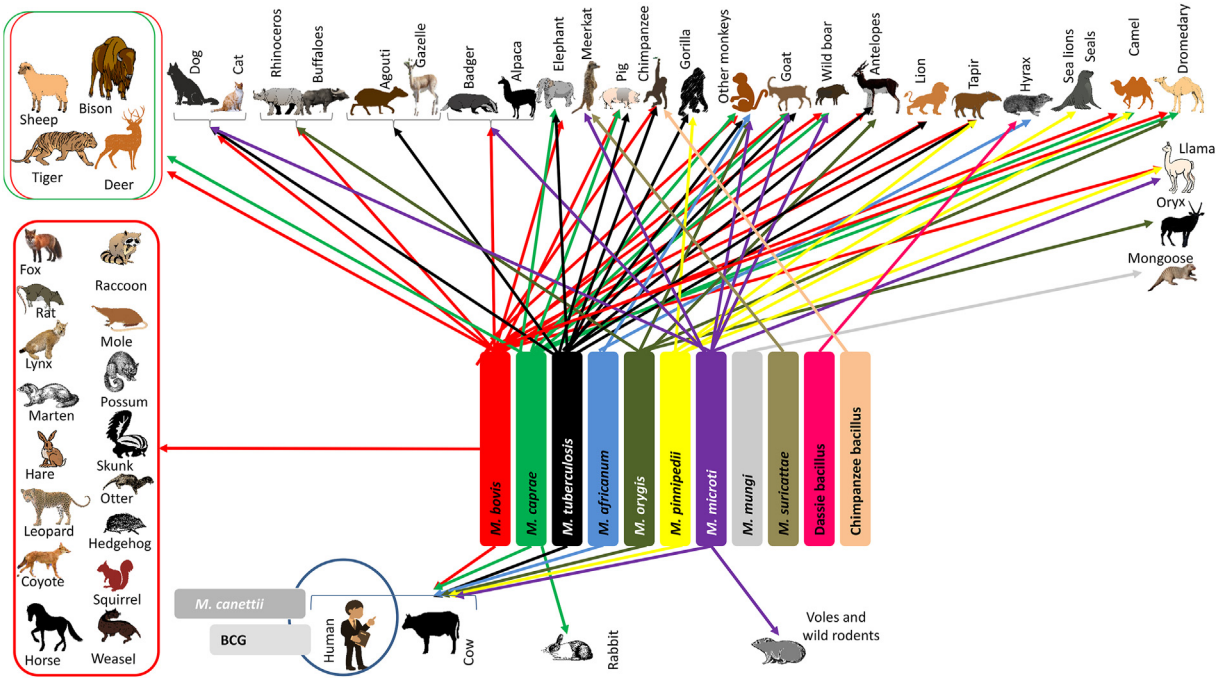

FIG. 2. Species distribution of *Mycobacterium tuberculosis* complex in mammals; based on references [8,23,26,28,29,32,33,37,38,85].

domestic animal hosts [3]. However, it should be noted that the species barrier is not strict and in rare situations *M. tuberculosis* can infect domestic animals, or wild animals in captivity. More than 15 species of animals can be infected with *M. tuberculosis*, including parrots [5,72,73]; similarly, *M. africanum* has been sporadically isolated from African monkeys with active tuberculosis and from cows [18,74]. Five MTBC have already been reported as zoonoses, causing tuberculosis in animals and transmissible to humans, including *M. bovis* [27,75–77], *Mycobacterium caprae* [78,79], *Mycobacterium microti* [80–82], *Mycobacterium orygis* [83,84] and *Mycobacterium pinnipedii* [85,86]. However, the main agent of tuberculosis in animals is *M. bovis*, which can infect a wide range of mammals and has a worldwide distribution in cows [75,87].

Other MTBC species identified in animals have never been reported in humans, including *Mycobacterium mungi*, *Mycobacterium suricattae*, Dassie bacillus and Chimpanzee bacillus [8,9,86,88]. These data suggest that there is no specificity between MTBC species and infected mammalian species, and that the current distribution of MTBC species among mammals results from the probability of contact.

Tuberculosis in the inanimate environment

Current data in the literature depict a scenario in which MTBC were initially environmental mycobacteria that evolved from unicellular eukaryotic (amoeba) opportunistic pathogens to mammalian opportunistic pathogens and then to contagious pathogens in humans (Fig. 1). Some studies have shown the persistence of *M. tuberculosis* and *M. bovis* in soil experimentally inoculated in the laboratory under controlled temperature and humidity conditions for a period of 12 months [89]. This experimental observation was followed by field observations in Tehran, Iran, where 1% of the soil samples and 10% of the water samples were found to have grown *M. tuberculosis*, which was re-cultivated 9 months after sampling; and whose genotypes determined by spoligotyping corresponded in part to those of tuberculosis patients diagnosed in Tehran [90]. Recent work has reviewed all the experimental and field observation data to confirm the possibility of prolonged storage of MTBC in soil [7]. Patients with pulmonary tuberculosis pass *M. tuberculosis* in the stool, which is an alternative to sputum for the diagnosis of tuberculosis by culture [91] and by molecular biology [92]. Individuals infected with *M. tuberculosis* could contaminate the environment [90]. In addition, there has been a reported case of transmission of *M. tuberculosis* to an embalmer from the corpse of a patient who died of pulmonary tuberculosis [93], illustrating that the corpses of tuberculosis patients could be a source of soil infection [5].

Some experimental observations indicate the survival of MTBC within the vegetative forms of free amoebae of the genus *Acanthamoeba* [94]. It has been shown experimentally that the five free amoebae *Acanthamoeba polyphaga*, *Acanthamoeba*

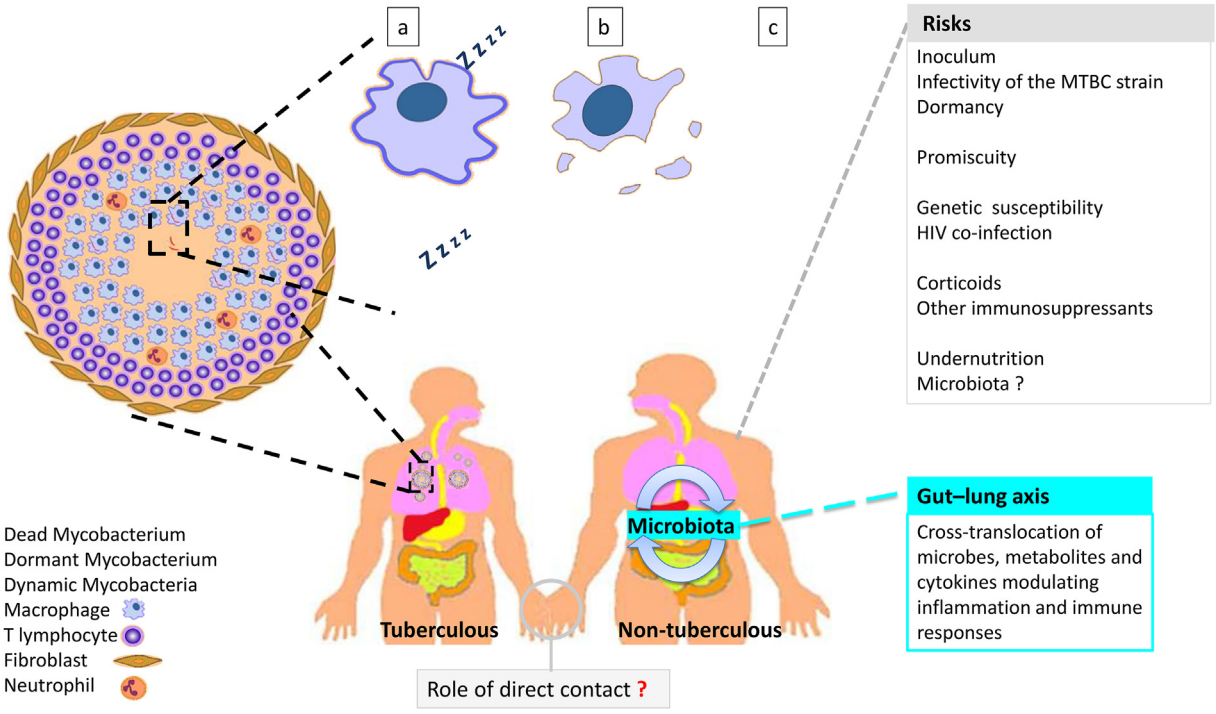

FIG. 3. Risks predisposing to the acquisition of tuberculosis and the life cycle of *Mycobacterium tuberculosis* in the granuloma. (a) Dormant *M. tuberculosis* bacilli persist in macrophages and caseum for extended periods of time. (b) When environmental conditions become conducive to reactivation, mycobacteria actively replicate leading to disruption of the integrity of the granuloma and spread to other tissues. (c) Under the action of antibiotic treatment and the immune system, initially replicating mycobacteria will be eliminated while a proportion may become dormant and persist.

castellanii, *Acanthamoeba lenticulata*, *Vermamoeba vermiformis* and *Dictyostellium discoideum* can be infected by *M. bovis*; it is encysted by each of these five amoebae; it persists for at least 60 days within the cysts; and that experimental inhalation of vegetative amoebae and cysts infected with *M. bovis* causes pulmonary tuberculosis in BALB/c mice [95]. This intra-amoebic life was the occasion for genetic exchanges between the host amoeba and the mycobacteria [96]. Interestingly, only *M. canettii* is not cyst-positive at the time of amoeba cyst formation, unlike *M. tuberculosis*, which can probably survive for extended periods of time within the cyst, as it has been shown that *Acanthamoeba* amoeba cysts can survive for 50 decades [97]. The mechanism of *M. canettii*'s early exit is not known, even though this MTBC codes for an active cellulase that could cleave the cellulose wall of the developing cyst [94]. Also, the modality of survival of MTBC inside the amoeba cyst is unknown even though they could be dormant mycobacteria [98]. All these observations suggest the possibility of an environmental cycle independent of the usual hosts, the possibility of which is not documented. However, the role of soil as a source of contamination of certain mammals does not seem unreasonable (Fig. 1).

Conclusions and perspectives

This review of the literature from different geographical and thematic sources describe scenarios of reservoirs, sources, modes of transmission and fates of MTBC in animal and human populations that are broader than those usually reported. Indeed, tuberculosis can be understood as an infection by telluric bacteria initially pathogenic to unicellular eukaryotes, which progressively acquired the capacity to infect pluricellular eukaryotes and then to be directly transmissible from host to host conferring the contagious character currently observed in human populations. The observation of pulmonary tuberculosis alone obscures the knowledge of these possible scenarios, diminishing vigilance on current and past alternative modalities for prehistoric populations.

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Conflict of interest

None declared.

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