Chapter 4

Mycobacterium avium Complex in Domestic and Wild Animals

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1. Introduction

Mycobacteria from the *Mycobacterium avium* complex (MAC) cause a variety of diseases including tuberculosis-like disease in humans and birds, disseminated infections in AIDS patients and otherwise immunocompromised patients, lymphadenitis in humans and mammals and paratuberculosis in ruminants. *M. avium* subsp. *paratuberculosis* (*Map*) is the etiologic agent of Johne´s disease in cattle and it has been identified in human patients with Crohn's disease. The MAC comprises slow growing mycobacteria that are ubiquitous in the environment (soil and water), and have a wide source range, causing disease in various domestic and wild mammals and birds [1].

The aim of this study was to discuss the classification and biology, epidemiology, clinical signs, pathology, diagnostic techniques, and public health concerns in *Mycobacterium avium* complex in domestic and wild animals.

2. Classification and biology of *Mycobacterium avium* complex

The phylum Actinobacteria is large and very complex; it contains one class (Actinobacteria), five subclasses, six orders, 14 suborders, and 40 families. The orders, suborders, and families are defined based on 16S rRNA sequences and distinctive signature nucleotides. The suborder Corynebacterineae contains seven families with several well-known genera. Three of the most important genera are *Corynebacterium*, *Mycobacterium*, and *Nocardia* [2].

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The species of *Mycobacterium*, sole genus of the family Mycobacteriaceae, is composed of a group of high genomic C+G content (~61 to 71%), facultative intracellular, Gram-positive microorganisms comprising more than 130 established and validated species and subspecies [3], with surprisingly diverse phenotypes related to growth rate, metabolic activity, colony appearance, environmental distribution, and pathogenic potential for eukaryotic hosts [4]. Although most of these species are saprophytic, important human and animal pathogens have been identified. Pathogenic members are usually characterized by their slow growth in culture, with generation times of 12 to 24 h, and must be incubated for 2 to 40 days after inoculation of a solidified complex medium to form a visible colony, whereas nonpathogenic members grow considerably faster [5]. Mycobacteria are acid-fast bacilli, acidophilic, small, slightly curved or straight rods that sometimes branch or form filaments. Mycobacterial filaments differ from those of actinomycetes in readily fragmenting into rods and coccoid bodies when distributed. They are aerobic, immobile, non-sporulated and catalase positive bacteria. Their cell wall is lipid-rich and contain waxes with 60 to 90 carbon mycolic acids, which are complex fatty acids with a hydroxyl group on the β-carbon and an aliphatic chain attached to the α-carbon. The presence of mycolic acids and other lipids, in high concentration outside the peptidoglycan, makes mycobacteria acid-fast dye resistant (basic fuchsin cannot be removed from the cell by acid alcohol treatment), as well as resistant to immune system defense mechanisms and disinfectants [2,6].

2.1. *Mycobacterium avium* complex (MAC)

Bacteria from the *Mycobacterium avium* complex (MAC) differ in virulence and ecology, and are the most frequently isolated non-tuberculous mycobacteria [7]. *Mycobacterium* members of MAC have the capacity to survive and multiply under a wide range of environmental conditions, including low pH, extreme temperatures, chlorine or ozone treatment and low oxygen level. Thus, plus their ability to utilize many substances as nutrients, enables them to grow successfully in many biotopes [1]. The environmental sources responsible for MAC infection in different populations, the specific routes of infection and transmission, the potential for latent infection and reactivation of disease are not yet well defined [4,8]. Ingestion of environmental organisms followed by invasion through the gastrointestinal tract has been suggested as the main route of infection because the organisms are frequently isolated from stools of different animals. There is also an important positive correlation between the presence of MAC in respiratory samples and the subsequent development of disseminated disease [9]. Traditionally, MAC includes two species, *Mycobacterium avium* and *Mycobacterium intracellulare* [3]. Recently, advances in molecular taxonomy have fuelled identification of novel species within the MAC, including the *Mycobacterium chimaera* incorporating sequevar MAC-A organisms isolated from humans with pulmonary cavitations, pulmonary abscess, chronic obstructive pulmonary disease and bronchiectasis [10]; the *Mycobacterium colombiense* incorporating sequevar MAC-X organisms isolated from the blood and sputum of HIV infected patients in Colombia [11], and from diseased lymph nodes in children [12,13]; the *Mycobacterium arosiense,* recently described in an immunocompromised child with disseminated osteomyelitic lesions [14]; the *Mycobacterium vulneris* [15], *Mycobacterium marseillense, Mycobacterium timonense* and *Mycobacterium bouchedurhonense* isolated from patients

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with pulmonary disease. On the basis of genotypic, phenotypic and growth characteristics, biochemical tests and historical reasons, multiple subspecies of *Mycobacterium avium* are recognized. These include the subsp. *avium*, subsp. *paratuberculosis*, subsp. *hominissuis* and subsp. *silvaticum* [3]. All four *Mycobacterium avium* subspecies and *Mycobacterium intracellulare* are capable of infecting a diverse range of host and possess a high degree of genetic similarity [17]. Contemporary methods for MAC identification, e.g., high performance liquid chromatography (HPLC) of cell wall mycolic acids and genetic probes based on rRNA targets, e.g. AccuProbe, cannot discriminate among *Mycobacterium avium* subspecies. Given the differences in pathogenicity among *Mycobacterium avium* subspecies and the implications regarding the infection source, a practical and accurate method of simply identifying *Mycobacterium avium* subspecies is needed [18].

2.2. *Mycobacterium avium* subsp. *avium*

Before establishing the *Mycobacterium avium* subsp. *avium (Maa)* designation, this bacterium was simply referred to as *Mycobacterium avium* and has long been recognized as a primary pathogen causing avian tuberculosis in wild and domestic birds as well as in a variety of fowl, game birds and water-fowl. The most common route of infection for susceptible animals is the alimentary tract. Respiratory tract is also suggested as a potential source of infection [19].

2.3. *Mycobacterium avium* subsp. *paratuberculosis*

Mycobacterium avium subsp. *paratuberculosis (Map)* is the etiologic agent of Johne’s disease or paratuberculosis, a chronic granulomatous enteritis of ruminant livestock and wildlife, with worldwide distribution having a significant impact on the world economy [5]. For veterinary medicine, *Map* is the MAC member of greatest importance, and is capable of infecting and causing disease in a wide array of animal species, including nonhuman primates, without the need for co-existent immunosuppressive infections [18]. *Map* is one of the slowest growing mycobacterial species, hence primary isolation from specimen, requires prolonged culture incubation and can take several months. Unlike most other *Mycobacterium avium* subspecies, isolation of *Map* requires the addition of the siderophore mycobactin to culture media [20]. From phenotypic analysis, the *Map* group has been subdivided into two main types, bovine and ovine, that vary in hosts, diseases caused, and growth phenotypes [21]. Genotypically, these findings were based primarily on comparisons of the integration *loci* of the IS*900* insertion sequence (IS) and used polymorphisms in IS*1311* to separate sheep and cattle isolates into separate populations [22].

2.4. *Mycobacterium avium* subsp. *hominissuis*

MAC isolates of genotypes IS*901*- and IS*1245*+ and serotypes 4 to 6, 8 to 11 and 21 are less virulent for birds and are designated *M. avium* subsp. *hominissuis* (*Mah*). *Mah* was proposed to distinguish organisms found in humans and pigs from those isolated from birds [3]. Those are genomically diverse, the more diverse group of strains, low-virulence, opportunistic pathogens for both animals and humans [18]. Considered ubiquitous in the environment (the most likely source of infection for humans), *Mah* can cause serious systemic

infection in immunocompromised patients, such as those infected with HIV. Additionally, this opportunistic pathogen can cause cervical lymphadenitis in children with cystic fibrosis, and lung infections in patients with underlying lung disease [23]. Domestic water distribution systems have been reported as possible sources of *Mah* infections in hospitals, family houses, and commercial places [24]. In animals, *Mah* is found as a cause of lymphadenitis of the head and mesenteric lymph nodes of swine documented at slaughter [18], and can also lead to systemic infection of parenchymatous organs [23]. *Mah* were recovered from affected lymph nodes of red deer from Austria [25].

2.5. *Mycobacterium avium* subsp. *silvaticum*

Mycobacterium avium subsp. *silvaticum* applies to the previously named wood pigeon bacillus, an acid-fast organism causing tuberculosis-like lesions in these wood pigeons. The inability to grow on egg media, the stimulation of growth by pyruvate and at pH 5.5 and their mycobactin dependency upon primary isolation, gradually losing this phenotype upon subculture, have been described as characteristics of *Mycobacterium avium* subsp. *silvaticum* [17].

2.6. *Mycobacterium intracellulare*

Mycobacterium intracellulare, initially named *Nocardia intracellularis,* is an environmental organism and opportunistic pathogen, isolated from a variety of animal hosts and environmental sources. *Mycobacterium intracellulare* is a closely related pathogen of birds with a lower prevalence [26]. In general, it has been subject to less study than *Mycobacterium avium*, as the latter is more prevalent in clinical and environmental samples, has a wider apparent host range, and contributes almost exclusively to disseminated MAC disease in human immunodeficiency virus patients [3]. The type strain of *Mycobacterium intracellulare* (ATCC 13950) was isolated from a human, specifically responsible for enlarged lymph nodes in children, who died from disseminated disease [3], and progressive pulmonary disease in elderly women [27]. *Mycobacterium intracellulare* appears to have a distinct ecological niche, more prevalent in biofilms and at significantly higher CFU numbers than *Mycobacterium avium* [28].

3. Clinical signs and morphology in domestic and wild species

All ruminant species, captive or free-ranging, are susceptible to disease and death due to MAC infection [29], and a wide diversity of non-ruminant species can become infected with mycobateria belonging to MAC, especially with *Map* and *Maa*. Paratuberculosis has been described in cattle, small ruminants, deer, and in South American camelids (llamas and alpacas) [30,31]. This chronic disease is one of the most serious affecting dairy cattle worldwide showing symptoms of an insidious intestinal pathology responsible for significant economic losses [5]. The close relationship between wild, captive and domestic ruminants and other species like birds is, nowadays, clinically relevant as the wild population could act as reservoir for this agent [32].

In cattle this disease is scored in four stages according to its evolution and symptoms, two of them evolving sub-clinically. Stage I, or silent infection, is the most observed in young animals, without significant clinical signs and only in *post mortem* evaluation it is possible to identify the agent by culture or histopathology analysis [33].

Stage II remains a subclinical disease, being observed in adult animals. It may be detected by alterations in immunological serological and/or cellular parameters. Intermittently, fecal culture and histopathology analysis of these animals could be positive to *Map* [33].

In stage III the clinical signs can be observed, occurring after several years of incubation. The initial clinical signs are subtle with gradual weight loss despite normal appetite, intermittent diarrhea along several weeks, drop in milk production and roughness of hair coat. These symptoms are included in the differential diagnosis of multiple diseases, so it is often misdiagnosed [34]. Usually, animals in this stage are positive upon ELISA and other serological tests, as for histopathological analysis of lesions, which are common in the terminal ileum [35] (Figure 2A).

The last stage of the disease (stage IV) comprises animals that rapidly progress from the stage III with rapid condition deteriorated. They became increasingly lethargic, weak and emaciated and present intermandibular edema due to hypoproteinemia. In this stage, the culture of the agent, molecular biology techniques of PCR, ELISA, serology and histopathology (Figure 1), all are positive for the majority of animals tested. The gastrointestinal tract is the preferential local to sample in order to isolate the agent, but in some conditions it can even be present in extraintestinal lesions, with the liver and lymph nodes being the most common sites [33].

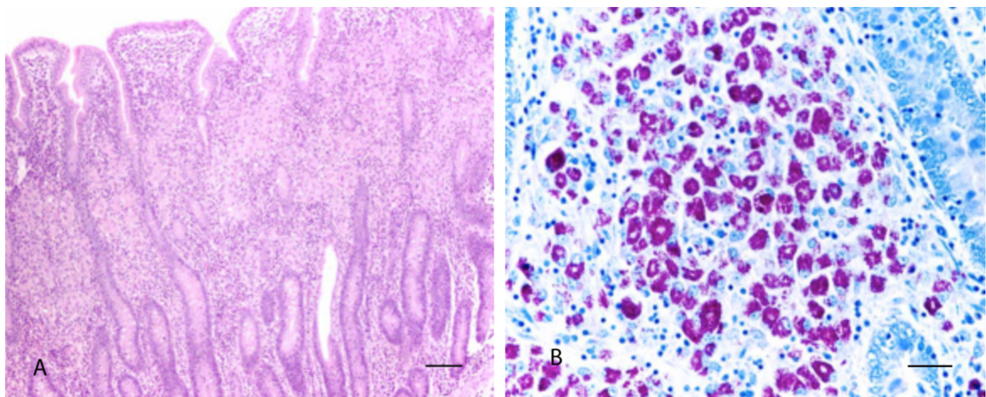

Figure 1. Morphological aspects of paratuberculosis lesions in the ileum of sheep. **A**- Thickening of the mucosa by an inflammatory infiltrate composed by epithelioid cells, macrophages and some multinucleated giant cells. (H&E stain, Bar=50µm) **B**- Acid-acohol resistant bacilli within the macrophages and epithelioid cells that infiltrate the mucosa. Notice the abundant number of mycobacteria that are visible at moderate amplification (Ziehl-Neelsen, Bar=30µm)

3.1. Clinical signs and lesions in wild species

Mycobacteria belonging to the MAC can affect a wide-range of wild animals, but little has been published on the clinical signs, which are rarely perceived or not documented. When present, the occurrence of clinical signs and lesions is highly variable in timing but often similar to those of their domesticated counterparts. The vast majority of reports on MAC species affecting wildlife mention the *Map* and the *Maa* as the mycobacteria most commonly isolated in these animals.

3.1.2. Wild ruminants

The lesions observed in wild species of sheep and goats are identical to those of their domestic counterparts, while in the South American camelids the lesional pattern is similar to that of cattle. However in llamas and alpacas, in contrast to what is generally described in cattle, lymph node necrosis and mineralization, along with multiorganic dissemination, have also been reported [30,31]. As in the previously mentioned species, the most significant MAC species capable of causing clinical disease in free-living, captive and farmed deer are *Map* and *Maa*. Although *Mah* has been also isolated from lesions in deer [25,26] and *Mycobacterium intracellulare* was also found in deer species but they are not so common and it's infection is usually subclinical. Despite the occurrence of paratuberculosis in adults, outbreaks of the disease frequently occur in young deer of 8-15 months of age, contrary to the clinical disease in sheep and cattle which usually affects adults of 3-5 years of age [36]. Clinical signs of paratuberculosis in deer are similar to those described in sheep and cattle, with diarrhea and loss of weight and body condition [37]. Accordingly, the intestinal lesions of paratuberculosis in deer primarily affect the jejunum and ileum, and are identical to the typical lesions observed in sheep and goats [30]; yet, necrosis and mineralization in lymph nodes draining the gastrointestinal tract, especially those draining the ileum and ileocecal valve, are a common feature (Figure 2B). The lymph nodes are often enlarged, and a range of changes from yellow watery areas to caseous necrosis is observed on cut surfaces. The histologic changes in these lesions are very similar to those caused by *Mycobacterium bovis* and other members of the MAC genus [38,39]. Balseiro et al., have proposed an histopathological classification of lesions observed in natural occurring cases of paratuberculosis in free-ranging fallow deer (*Dama dama*), according to which the lesions would be graded into four categories: focal, multifocal, diffuse multibacillary, and diffuse intermediate (multibacillary-lymphocytic) lesions. Focal lesions are composed of small granulomas, mainly in the jejunal and ileal lymph nodes, whereas multifocal lesions consist in well-demarcated granulomas in the intestinal lymphoid tissue and also in the intestinal lamina propria. Diffuse multibacillary lesions are characterized by a severe granulomatous enteritis and lymphadenitis. Macrophages and numerous Langhan's multinucleatd giant cells (L-MGC) containing many mycobacteria are present, resulting in macroscopic changes in the normal gut morphology. These changes are found from the proximal jejunum to the ileocaecal valve, but lesions are always particularly severe in the distal jejunum. In the diffuse intermediate (multibacillary-lymphocytic) lesions, there is a prominence of lymphocytes, macrophages and L-MGC, with small numbers of mycobacteria [40]. In deer with clinical signs of paratuberculosis, disseminated granuloma-

tous lesions in the lung and liver can also be observed [38]. A recent report in free-ranging red deer (*Cervus elaphus*) supports the possibility of multiorganic dissemination of *Map* in deer, since the agent was isolated from kidneys with granulomatous lesions [41].

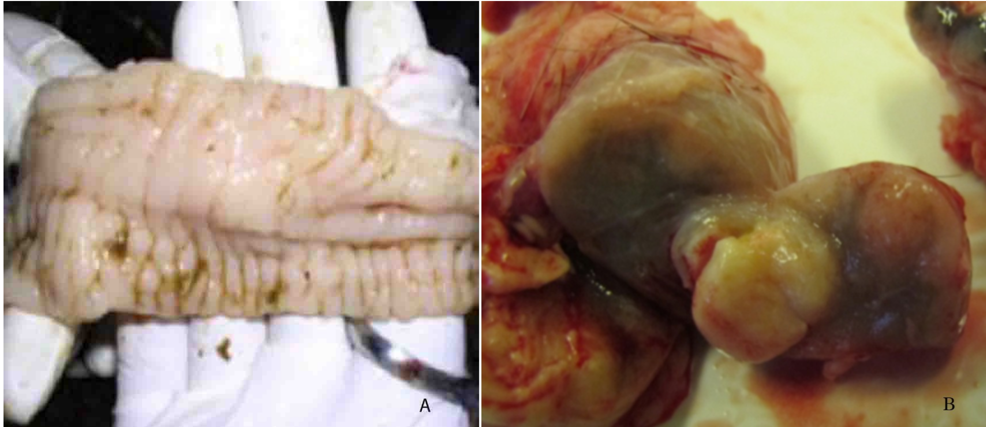

Figure 2. A – Paratuberculosis gross lesions in the ileum of sheep. Notice the increased thickness of the mucosa, with the characteristic folds and gyros B – Paratuberculosis lesions in the submandibularis lymph nodes of red deer (*Cervus elaphus*) with caseous necrosis.

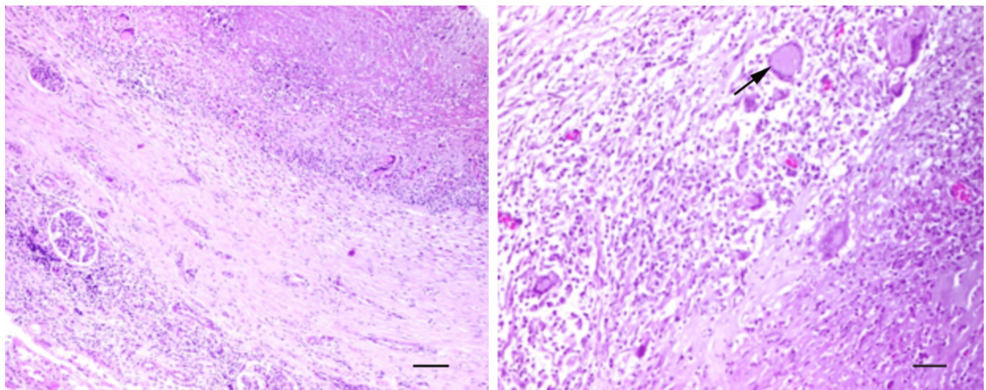

Figure 3. A - Morphological aspects of paratuberculosis lesions in the kidneys of deer (*Cervus elaphus*). A single granuloma with central caseous necrosis, surrounded by a thick fibrous capsule (H&E 100μm). **B** – Higher magnification of the previous image. Notice the presence of Langhan's MGC (Arrow. H&E 50μm).

In deer, the infection by *Maa* is self-limiting as in other mammalian species [36,42]. The lesions may be purulent, caseous, or granulomatous [26], and are mainly present in the retropharyngeal lymph nodes and lymph nodes draining the intestinal tract (mesenteric and ileocaecal), consistent with the feco-oral route of infection. The granulomatous le-

sions are grossly and histologically identical to the lesions caused by *Mycobacterium bovis* [42]. *Mah* lesions in deer are similar to those observed in animals with *Maa* infection, and although rare, both mycobacteria can cause systemic disease [26] with hematogenous spread to the liver and lungs to produce miliary lesions and a terminal septicemia [43]. Despite of these findings, *Map* and *Maa* infections can be present in apparently asymptomatic deer herds [44,45]. Furthermore, a study of wild Tule elks (*Cervus elaphus nannodes*) from California revealed no significant associations between MAC infection and microscopic lesions, such as presence of macrophages and/or multinucleate giant cells (MGC) in tissue sections [45] (Figure 3).

3.1.3. Non-ruminant species

Map has been isolated in a wide range of wild mammals, from rodents, badgers, racoons, nine-banded armadillos, opossums, northern short-tailed shrew, striped skunks [46-48], wild boars [32,49] and rhinoceros [50] to bears [51], but not all of them present the same susceptibility and develop clinical signs or lesions when infected. The lesions produced by *Map*, representative or not of paratuberculosis, as well as the clinical signs seen in non-ruminant wild animals are more subtle than those observed in wild-ruminant species. Monkeys (*Macaca arctoides* and *Papio sphinx*) have demonstrated susceptibility to *Map* infection and develop lesions that are confined to the intestine and abdominal lymph nodes, resembling the lesions of paratuberculosis in cattle and in humans Crohn’s disease [52,53]. When infected by *Map* lagomorphs produce an intestinal disease similar to paratuberculosis in ruminants, and severe infection can occur naturally, in which extensive granulomas with numerous giants’ cells carrying multiple acid-fast bacilli can be observed in the small intestine [54]. In foxes and stoats, *Map* affects the intestines and mesenteric lymph nodes with microscopic changes similar to those described in ruminants with subclinical paratuberculosis. The lesions are composed of single macrophage-like cells or discrete granulomas consisting of small numbers of macrophages, in the cortex and paracortex of the mesenteric lymph nodes. In the small intestine, only few numbers of intracellular acid-fast bacteria are present within the macrophages, and Langhan’s-type MGC, irregularly scattered in the granulomas, in all layers of affected intestine [46,54,55]. The typical pathology of paratuberculosis has also been noted in wood mice, weasels, badgers [46], and rats infected with the predominant ruminant strains [56]. When present, in brushtail possum and hedgehogs infected with *Map*, the lesions observed are in the grastrointestinal tract [57]. A recent report revealed that wild Eurasian otters (*Lutra lutra* L.) could be infected with *Map*. In that study, no gross lesions were found, but the retropharyngeal and mesenteric lymph nodes presented disrupted architecture, lymphoid depletion and diffuse inflammatory infiltrate composed mainly of macrophages and, to a lesser extent, neutrophils. *Map* was identified by direct PCR in several organs, including the intestine and lymph nodes [58]. Reports in which *Map* was isolated from Eurasian wild boars (*Sus scrofa*) revealed that the infection can occur with or without lesions, the later being the most frequent. If present, lesions generally consist of granulomatous enteritis and mesenteric lymphadenitis [32,49]. In mesenteric lymph nodes, lymphadenitis with multifocal lesions ranging from less than 1 cm to large areas

of more than 1 cm in diameter, of either necrotic or necrotic calcified granulomas are observed (Figure 4). The presence of lymphocytes and caseous necrosis are the most common findings in these lesions [59].

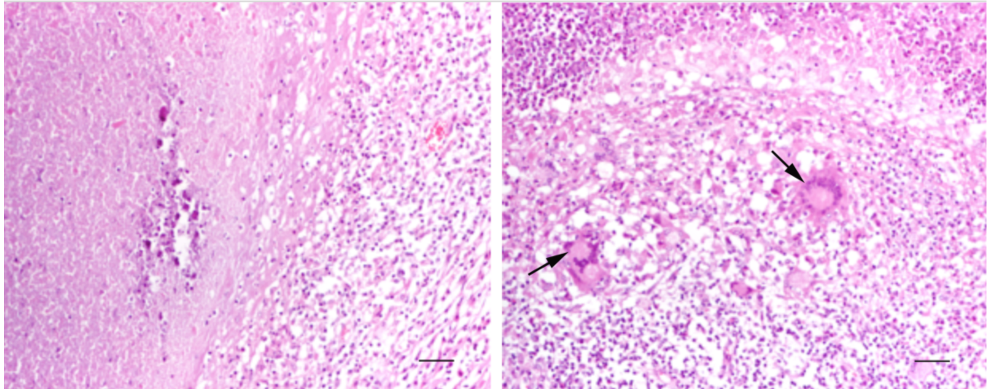

Figure 4. Morphological aspects of paratuberculosis lesions in wild boar (*Sus scrofa*). Presence of of multiple granulomas in the mesenteric lymph nodes (H&E 50µm). **A** – Notice the caseous necrosis surrounded by inflammatory cells predominantly macrophages and some fibroblasts. **B** – Higher magnification of the former image. Note the Langhan's MGC (Arrows. H&E 50µm).

Despite *Maa* being widely reported in wild boar, data on clinical infection or mortality are scarce [49]. Apparently, natural infection with this mycobateria causes barely detectable clinical signs or lesions [49]. However, there are reports of *Maa* isolated from free-ranging Eurasian wild boars with tuberculous lesions in intestinal lymph nodes [60], and the experimental infection with high doses of *Maa* results in gross and histopathological lesions of tuberculosis in tracheobronchial and mandibular lymph nodes. All visible lesions are less than 10 mm in diameter and consist of typical tuberculous granulomas with a central caseous necrosis, variably mineralized, surrounded by macrophages, lymphocytes, neutrophils, eosinophils and occasional MGC surrounded by fibrous tissue. Acid-fast bacilli are rarely detected in the necrotic debris of these lesions [61]. Another study also showed that in wild boars with mesenteric and submaxillar lymphadenitis, *Mycobacterium avium* subspecies type 1 and *M. avium* subspecies type 2 were the most frequently isolated mycobacteria [62].

Regarding *Mah* in wild boar, recent reports suggest that this animal species may act as a reservoir for these mycobacteria, since it was detected in lymph nodes without gross lesions or microscopic lesions [60,63].

3.2. Bird species

Bird species, either domestic or free-living can be infected with MAC mycobacteria, but they are more susceptible to *Maa*, the causative agent of avian tuberculosis [19]. Infection by *M. avium* subsp. *paratuberculosis* has been also documented in birds, with and without clinical and pathological findings [46,57].

Maa belonging to serotypes 1, 2, 3, and 6 is the most common agent of avian tuberculosis, but other species belonging to MAC, such as *M. intracellulare*, are also sporadic causes of disease [19]. Tuberculosis affects a wide-range of bird species, and it has been documented in waterfowl, galliformes, columbiformes, passerines, psittacines, raptors, and ratites [64-67]. According to their susceptibility to the disease, it has been proposed the classification of bird species into four groups, from highly susceptible to highly resistant: (1) domestic fowl, sparrows, pheasants, and partridges; (2) guinea fowl and domestic turkeys; (3) domestic goose and duck; (4) domestic pigeon [19]. The clinical signs of avian tuberculosis are well established but are not pathognomonic, and are different according to the phase of infection and the predominant form of the disease in the bird species, which in turn is related to the species susceptibility to the agent. Avian tuberculosis usually presents three stages or phases: latency, lesion development, and period of cachexia [68,69]. In most reports on the initial phase of avian tuberculosis the absence of clinical signs is a common feature. On the contrary, in advanced cases, progressive weight loss, depression, white diarrhea with soiled feathers, increased thirst, respiratory distress, fatigue, and decreased egg production may be observed [68]. Generally, avian tuberculosis is an intestinal and hepatic disease that can disseminate to other organs including the lungs, air sacs, spleen, bone marrow, and skin [69]. This is considered as the classical form of infection, and it's marked by the presence of tubercles or granulomas in multiple organs. Lesions in the intestinal tract characterize a second form of infection, and a third type of infection, especially reported finches, canaries, and psittacines occurs without the development of tuberculous lesions [19,68,69]. The clinical signs of the intestinal form of tuberculosis are characterized by chronic wasting disease. In severe cases and as disease progresses, feathers are often dull or ruffled and comb, wattle, and earlobes are paler, thinner and dry. Birds become lethargic and emaciated with marked atrophy of breast muscles, displaying the typical "knife edged" keel. In extreme cases, the body fat disappears, and the bird's face looks smaller than normal. The body temperature of the affected birds remains normal, even in severe cases [68,69]. Sudden death may also occur, as well as dyspnea, granulomatous ocular lesions [70] and skin lesions, which are less frequent [68,69]. Sudden death may be the result of massive hemorrhage secondary to liver or spleen rupture. In such cases, the birds may exhibit good body condition but frequently show advanced lesions of tuberculosis. Lameness can be the result of bone dissemination, in particular to the bone marrow of long bones. Joints can also be affected, and as consequence, some birds may adapt a sitting position or even show paralysis. There are also reports of neurological signs, due to the involvement of vertebral or central nervous system [68]. The lesions of avian tuberculosis are mainly composed by epithelioid cells containing large numbers of bacterium, that may either diffusely infiltrate the organ or form discrete tubercles or granulomas [68]. In the classical and intestinal forms of the disease, studded greyish-white to greyish-yellow nodules are frequently observed. The nodules, which appear as tumour-like masses, bulge from the serosal surface of the intestine and can be palpable. These nodules may suffer ulceration, and the caseous material within may be discharge into the intestinal lumen leading to the excretion of mycobacteria in droppings. During dissemination, typical caseous lesions, without calcification, are always found in the liver and spleen, with considerable enlargement of the organs. Nodules are firm but can be incised easily

since mineralization is rare in avian tuberculosis. Due to this, spleen takes irregular and "knobbly" appearance. With the disease progression, tubercular nodules in the bone marrow, ovaries and testes, are often seen. Pulmonary lesions, which are a striking feature of tuberculosis in other species, are rarely observed in birds [68,69]. Pulmonary avian tuberculosis is not common, but it has been reported occasionally in pigeons and water-fowl [19,65,69]. In the cachexia stage of avian tuberculosis, massive tubercles with large numbers of bacilli are observed [68,69].

In accordance to the clinical signs and lesional patterns of tuberculosis in domestic species, captive, exotic and wild birds, including raptors, generally develop the disseminated form of avian tuberculosis, involving the digestive tract, liver and spleen [66,71,72]. However, some studies reveal that exotic bird species may have lesions in the liver and spleen without intestinal involvement. These lesions are typical granulomas with a caseous or coagulative necrotic centre and MGC. Acid-fast bacilli are numerous in the central zone of the tubercle [69].

Infection of birds by *Map* has been reported, but it often occurs without clinical signs or lesions, despite the typical lesions of paratuberculosis being described in jackdaws (*Corvus monedula*), rooks (*Corvus frugilegus*), and crows (*Corvus corone*) [46,57]. A study also reported the occurrence of diarrhea, respiratory signs, hepatomegaly and splenomegaly in a diamond sparrow (*Emblema guttata*) with liver granuloma lesions, predominantly composed of lymphocytes and mononuclear cells [73].

4. Diagnostic techniques

The diagnosis of Mac is based on the clinical signs, post mortem gross lesions, and by demonstrating the presence of acid-fast bacilli using Ziehl-Neelsen staining. This is normally sufficient to establish the diagnosis [68,69,74,75]. *Ante mortem* diagnosis is based on clinical signs, leukograme, serology, culture and also acid-fast stain or biopsy samples of fluids or organs [76-80]. Radiography and ultrasonography are also useful in the medical evaluation of birds [79].

4.1. Isolation and identification

The golden standard test for mycobacterium diagnosis is the microbiological culture. Tissue culture seems to be slightly more sensitive than faecal culture and it allows the infection to be detected in some animals that had no specific lesions. The culture of bacteria requires weeks or months of incubation before colony growth occurs. This means that a significant amount of time is needed before a diagnosis can be made. It is also difficult to isolate bacteria in culture due to intermittent shedding and a low number of bacilli in faeces and tissues [81]. *M. genavense* is extremely difficult to isolate by culture [82,83]. *M. avium* complex grows best in media containing eggs or egg yolk and the incubation temperature should be set at 37ºC-40ºC. Culture can be performed in Dorset´s, Herrold´s egg yolk medium, Middlebrook 7H10 and 7H11 or Coletsos medium supplemented with 1% sodium piruvate [68,74,75]. For

the isolation of *Map* or *M. silvaticum* addition of mycobactin is required in all media. *Map* is the slowest growing of the culturable mycobacteria [84]. Cultures should be incubated for at least 8 weeks. Typically, *M. avium* produces smooth colonies within 2-4 weeks and rough variants can occur [74]. Culture of *Map* from faeces or tissues of other animals such as sheep and goats is less successful due to the "S" strains that usually infect these animals [84]. The best organs to use for culture are usually liver and spleen but bone marrow can be used if carcass is decomposed, as it could be less contaminated [74]. Non-sterile specimens need to be processed with detergent alkali or acid to eliminate rapidly growing microorganisms before culture decontamination in order to remove faster growing microbial species. Incubation with various decontamination agents such as 0.6-0.75% hexadecylpryridinium chloride (HPC) or NaOH for 3 hours to overnight, have been used. It is important that decontamination does not remove too many viable mycobacterium cells [85,86]. Other methods like sedimentation and centrifugation can be employed if small numbers of mycobacteria are expected in the sample [85,87]. Shorter incubation times can be achieved using automated broth based systems, like liquid culture BACTEC system MGIT 960 [84]. These systems have been reported to be highly sensitive for culture [88]. For *M. genavense* the use of BACTEC system with no additives and pH 6.0 is recommended [89,90]. Middlebrook 7H11 with pH 6.0 supplemented with blood and charcoal is also recommended to promote growth of *M. genavense* [91]. In human AIDS patients, laboratory diagnosis of MAC infection is usually made by the BACTEC blood culture [92]. Conventional biochemical tests for species identification are lengthy and fail to distinguish between *M. avium* and *M. intracelullare* [74]. Identification of isolates by phenotypic characteristics of majority of clinically relevant mycobacteria can be based on growth rates, colony pigmentation and biochemical tests such as niacin production, nitrate reduction, tween 80 hydrolysis arylsulphatase, urease, tellurite reduction, thiophen-2-carboxylic acid hydrazide sensitivity catalase (qualitative and quantitative) growth on MacConkey and sodium chloride tolerance [88,93].

Classification of MAC organisms has been made by seroagglutination [19,74]. Seroagglutination is based on sugar residue specificity of surface glycopeptidolipids, and allows classification of MAC organisms into 28 serovars: 1 to 6, 8 to 11 and 21 are currently ascribed as *M. avium*, while serovars 7, 12 to 20 and 25 to *M. intracellulare*. However, no consensus was achieved on the other serovars [74]. MAC colonies can also be identified using high performance liquid chromatography (HPLC) for detecting mycolic acid [19]. HPLC and the use of monoclonal antibodies to major serovars in ELISA also facilitate typing of mycobacteria [19,75].

4.2. Immunological methods

The enzyme linked immunosorbent assay (ELISA) has been used for detecting antimycobacterial antibodies in the serum of ruminants [94]. However, serological assays for detecting *Mycobacterium* are problematic. The sensitivity of ELISA is dependent on the stage of the disease with a higher sensitivity of the test in case of higher bacterial load. The test can detect the most severe infections in multibacillar lesions but shows lower sensitivity in animals with paucibacillar lesions [84]. One important disadvantage is the inability to distinguish be-

tween different mycobacterial infections probably due to close antigenic relationship between *Maa* and *Map* [95].

Tuberculin test is the most widely used method in domestic fowl and the only for which an international standard for the reagent exists. Birds are tested by intradermal inoculation in the wattle with 0.05 ml or 0.1 ml of tuberculin (avian purified protein derivate – PPD) [74]. A positive reaction is identified as a hot and oedematous swelling at the site or by the presence of a small firm nodule of approximately 5 mm in diameter after 48 hours [19]. The tuberculin test and the haemagglutination test (stained antigen) are the immunological methods most frequently used for export testing [74]. In the stained antigen test an antigen stained with 1% malachite green is used for the rapid blood plate agglutination test [96].The diagnosis of *Mycobacterial* infections in live wild animals remains a challenge [95]. The comparative cervical tuberculin (CCT) skin test has been applied in wild animals such as in cervids [97], but the test presents two major limitations. It has been proved that CCT cannot detect some stages of infection [98], and wild ruminants must be captured twice increasing the stress and the risk of accidents for the animals and for handlers [95]. Alternative probes like the detection of interferon-γ (IFN- γ) assay and ELISA, which employ blood and serum respectively, could be an alternative in wild animals [95]. Advantages of ELISA and IFN-γ assay is that they enable testing without handling the animals twice and allow repeated testing, which are important advantages in case of wild ruminants [99]. ELISA detects humoral immune response whereas the CCT and IFN-γ assay detect cellular immune response [95]. The dominant response to mycobacterial infections in ruminants is cell-mediated. However, a recent study demonstrated that the IFN-γ assay may be of limited usefulness in some species of cervids [100].

4.3. Genetic methods

4.3.1. The contribution of molecular biology to MAC research

During the past several years, many molecular methods have been developed for direct detection, species identification, and drug susceptibility testing of mycobacteria. These methods can potentially reduce the diagnostic time from weeks to days with a higher sensibility. Molecular biology methods offer new opportunities to differentiate, identify and type bacterial species and strains. These methods use the variability of nucleic sequences of genes such as 16S rDNA, beta subunit RNA-ase (rpoB), gyrase (gyrB), rDNA internal transcribed spacer among other genes. Some of the methods available to differentiate and identify species of mycobacteria at the DNA sequence level are PCR, PCR-REA, sequencing analysis, spoligotyping and DNA fingerprinting. These methods have been applied to both the “universal” part of the genome and to specific mycobacterial genes.

Isolation of mycobacterial DNA can be done from living mycobacteria, not only from mycobacterial isolates but also directly from body fluids (sputum, bronchoalveolar lavages, and bronchial and tracheal aspirates, semen, milk, blood, cerebrospinal fluid), from tissues and from faeces and can be done using dead mycobacterial cells, namely from formalin-fixed

and paraffin-embedded tissues and from forensic and archaeological samples [101]. One of the challenges with molecular detection of *Map* is to get the genomic DNA out of the bacteria, which is protected by its thick and waxy cell wall. One method commonly used is the mechanical disruption by the use of bead beating, a general term for using small beads mixed with the sample, usually in the presence of a proteolytic enzyme and lysis buffer, to break tissues or tough cell walls and spores by forceful shaking in a cell disrupter, or "bead beater". It is one of several suggested methods to lyse *Map* [102-105]. Others include homogenized (grinded) sample under liquid nitrogen in a mortar and pestle, combinations of enzymatic treatment, freeze-thaw/boiling and kits for plant DNA purification or for animal tissues DNA purification [103,105-109]. Commercially available kits, developed for DNA isolation from different matrices, are commonly used for the rapid isolation and detection of *Map* in milk.

4.3.2. Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is an in vitro method for the amplification of DNA that was introduced in 1985 [110]. With the performance of a previous reverse transcription step, PCR can also be applied to RNA [111]. Reverse transcription PCR is a modification of this method used when the initial template is RNA rather than DNA, the reverse transcriptase enzyme first converts the RNA target into a complementary DNA copy (cDNA), that can be used to amplify the much higher numbers of copies of messenger or ribosomal RNA than the number of DNA copies present in bacteria, and it may detect specific expression of certain genes. Some modifications to single PCR were done to improve results and were used for MAC species detection, the multiplex PCR, the assay that include several primer pairs specific to different DNA targets to allow amplification and detection of several pathogens at the same time, and nested PCR, the product from one PCR reaction serves as template in a second reaction with fresh reagents, thus diluting any PCR inhibiting substances and increasing the sensitivity. As example differentiation of *M. tuberculosis* complex, *M. avium* and other non-tuberculous mycobacteria (NTM) has been done by using hybridization probes [112]. Targeting the 16S rRNA gene, 3 different probes, specific for mycobacteria, *M. tuberculosis* complex and *M. avium,* were constructed and the thermal melting temperature (Tm) was different for *M. tuberculosis*, *M. kansasii*, *M. avium, M. intracellulare* and *M. marinum* allowing the differentiation. TaqMan PCR assay targeting 65 kD heat shock protein gene has been used for the detection of *M. genavense* and *M. avium* complex species in avian tissue samples [83].

Specific probes are available for the identification of *Mycobacterium avium* and *Mycobacterium intracellulare*. Amplification of the DT1 and DT6 fragments was considered equally sensitive for species identification [113]. Recently, numerous isolates suspected of be *Mycobacterium intracellulare* were reclassified as *Mycobacterium chimaera* sp. nov., as part of the MAC [114]. Strains of MAC can be identified by serological procedures, on the basis of differences in the C-mycoside glycopeptidolipids. To date, using 16S rRNA probes, 28 MAC serotypes have been identified from which the serotypes 1–6, 8–11, and 21 belong

to *Maa*. Serovars 7, 12–20, and 25 have been ascribed to *Mycobacterium intracellulare*. Serovar-1 is the most common organism isolated from birds and from human. Serotypes 1 and 2 are most commonly isolated from domestic birds, and serovar 3 is recovered sporadically from wild birds. Serotypes 1, 2, and 3 are considered virulent for chickens. Serotypes 1, 4, and 8 have been reported to predominate among isolates from AIDS patients [19].

Other approach to the differentiation of MAC strains was obtained with the description of repetitive insertion sequence IS*900* in *Map* strains and IS*901* or IS*902* in *M. avium* subsp. *silvaticum* strains [115]. Examination of serotyped strains revealed IS*901* only in strains of serotypes 1, 2, and 3 [19].

The discovery of insertion sequences in mycobacterial genomes, e.g. IS*900* in *Map* [116], IS*901* [117], IS*1245* [118], IS*1311* [119] in the MAC strains was a major breakthrough in the study of mycobacterial infections. When characterized and used in the proper context, the species-specific IS (insertion sequences) elements can be useful classification tools to distinguish subsets of the MAC [18,120]. However, there are two problems described that can question their utility for this purpose. First, a number of IS elements have been uncovered in strains considered to be MAC organisms, but without adequate strain characterization, it is difficult to judge which organisms harbour such elements. Second, IS elements are by nature mobile elements, so there is a risk that similar elements are found in unrelated bacteria because of mobility to or from MAC organisms [3].

IS*900* was the first IS characterized within the *Mycobacterium* genus [116,121] and is the most widely used target sequence for detection of *Map* and presently considered specific for this agent. The *Map* genome is reported to have 15 to 20 copies of the insertion element, and the sequenced strain K-10 has 17 copies [122]. Cousins et al. recommended that restriction digestion should be used to confirm the profile of the IS of the amplified product [123]. However, Englund et al. identified a *Mycobacterium* sp. with an IS*900* like sequence in which the restriction sites after amplification with the original primers were identical to the restriction sites in amplified DNA of *Map* [124]. Therefore, restriction endonucleases analysis did not solve the problem of false positives. Englund et al. recommended that a positive IS*900* PCR should be confirmed by subsequent sequencing or by a PCR assay targeting another gene in *Map* [124]. In a study performed by Vansnick et al. two sets of newly developed PCR primers based on the insertion sequence IS*900* and the unique sequence f57 were developed and the combination of the two PCR assays has proven to be useful for the identification of *Map* [125]. *Map* genome has revealed the presence of 17 IS*900*, 7 IS*1311*, and 3 IS*1245* insertion elements. The IS*900* element seems unique to *Map* and has been widely used as a diagnostic tool to detect *Map* in clinical samples from both animals and humans [5].

The specific DNA sequence IS*900* was also used as the target for *Map* detection in Nested PCR. In 2002, IS*900*-nested PCR was used to determine the specificity and sensitivity of a commercial ELISA test [126]. However, the nested PCR method is now being replaced by Real-Time PCR [127].

RFLP analysis of the IS*900* element has been used a molecular tool to type *Map* isolates and allowed the division of *Map* into different groups, associated to different host species [3]. The IS*900* element is by far the most widely used target for the molecular detection of *Map* and has been used in the form of direct PCR [108,125], *in situ* PCR [128] nested PCR [126,129,130], and real-time PCR [131-133]. Sequencing of the amplified product for IS*900* is therefore necessary to confirm that the amplicon is truly IS*900*.

Additional gene loci specific for *Map* have been identified and suggested for use in diagnostics: ISMav2 [134], f57 [135], IS*Map*02 [136], and other *Map* specific coding sequences [137].

To identify the methods which are best suited for diagnostics, eight single-round and five nested PCR systems including twelve different primer pairs based on IS*900* (9x), 7 ISMav2 (1x), f57 (1x), and locus 255 (1x) sequences were compared by Möbius et al., which concluded that stringent selection of IS*900*-specific primers ensures that IS*900* remains a favourite target sequence for amplification of *Map* specific loci [138]. A PCR system targeting two different *Map* specific regions would have a still higher specificity. The following six single round PCR-systems were recommended by Möbius et al. [138]: IS*900* based PCRs of Englund et al. [139], and Doran et al. [140], the f57 based PCR assay [125], and the locus 255 based PCR assay [137]. However, despite their advantages, such as a hundredfold enhancement of sensitivity, nested PCR assays bear a high risk of contamination and crossing over and, therefore, cannot be recommended as a reliable method for routine diagnosis of *Map*.

The insertion sequence IS901 was discovered by Kunze et al. and shows around 60% sequence homology to IS*900* [117]. The stability of IS*901* in strains isolated primarily from clinical material from birds, domestic animals and from the environment is used for the rapid identification of IS*901*+ strains using the PCR method. Screening across a larger panel of isolates revealed that most isolates from birds and some animals contained the element, whereas isolates obtained from AIDS patients or the environment did not. Furthermore, it was found that most bird isolates had similar IS*901* patterns [3].

IS*1311* was first reported as a GenBank entry in 1994 (U16276) and was subsequently used for RFLP analyses [119,141]. This element is present in all members of the *M. avium* subspecies, including *Maa*, *Mah*, and *Map* [142], and is not present in *M. intracellulare* [22,141]. The element itself has 85% sequence identity to IS*1245*. With the wide range of *M. avium* hosting for this element, it is possible that IS*1311* represents an “older” IS element which may have been present prior to subspecies divergence [3]. RFLP analysis of IS*1311* and the use of IS*1311* PCR-REA also revealed distinct pattern types, corresponding to different genotype species strains of *M. avium* subsp. *paratuberculosis* [22,142].

The IS*1245* was first described in 1995, is present in up to 27 copies in *Mycobacterium avium* [118], was presented as having a more restricted range than IS*1311*, and was found to be stable during in vivo and in vitro passage, making it a popular target for restriction fragment length polymorphism strain typing. It is described as limited to the subspecies of *M. avium*, i.e., *Maa* (that would include *Mah*), *Map* subsp. *paratuberculosis*, and *M. avium* subsp. *silvaticum*. By PCR analysis, this element was not found in *M. intracellulare* or in 17 other mycobacterium species [3]. Standardization of IS*1245* RFLP analysis was proposed in 1998 as a tool

for MAC molecular epidemiology [143]. Some *M. avium* isolates have been documented as being IS*1245* negative, but only a few such reports have presented further documentation of strain identity by a sequence-based method [144]. Beggs et al. found IS*1245* in strains of *M. intracellulare*, demonstrating that the element is present in other species of the *M. avium* complex [144]. In some reports, IS*1245*-negative isolates have been described that contain an hsp65 sequence identical to that of *M. avium* but that differs from *M. avium* in other taxonomic targets, such as the 16S rRNA gene and the ITS sequence [3].

Other identification methods of *M. avium* species or its subspecies are based on 16S rDNA [145], PCR-REA (Restriction Endonuclease Analysis) [146], sequence analysis of hsp65 [8] or a strategy based on large sequence polymorphisms [147]. Semret et al. evaluating the distribution of genomic polymorphisms across a panel of strains, verified that it was possible to assign unique genomic signatures to host-associated variants and based on these polymorphisms proposed a simple PCR-based strategy that can rapidly type *M. avium* isolates into these subgroups [147].

The sequence of the 16S rDNA gene is specific at the species level and is also a stable property of microorganisms. Wilton and Cousins described a method for the simultaneous identification of genus, species and strains of *Mycobacterium* sp. using conserved and variable sequences of the 16S rDNA gene [145]. Comparing the 16S rDNA sequences of mycobacterial pathogens, they found variable regions specific for individual species and used the information to develop a duplex amplification system, which makes it possible to identify the genus *Mycobacterium*, and the species *M. a. avium* and *M. intracellulare*. If combining the primers for 16S rDNA with primers specific to the gene that encodes the secretion protein MPB70 (specific for *Mycobacterium tuberculosis* complex) this system permits the detection and identification of clinically important mycobacteria in one single PCR. The disadvantage of this method is that it does not distinguish between *M. a. avium* and *M. a. paratuberculosis*.

Combining PCR amplification of the 16S rDNA gene and subsequent restriction analysis we have the PCR-REA (or PRA) method. Using the 16S rDNA gene primers according to Thierry et al. and the resulting PCR products, 1 300 bp in size digested with *Rsa I* it is possible to distinguish species of MAC (*M. a. avium* serotypes 1–3, 8–11 and 21, *M. a. paratuberculosis*) and *M. intracellulare* (serotypes 7, 12–20, 22–28) [148].

Standard (housekeeping) genes offer a higher level of sequence variation than do ribosomal genes but are nonetheless useful for taxonomic purposes due to the relative sequence conservation imposed to maintain function. In this category, the stress protein gene hsp65 is a preferred target for mycobacterial identification to the species level, having been routinely used in diagnostics since the development of rapid PCR-restriction enzyme analysis (PRA) methods. The *dnaJ* gene encodes a stress chaperone protein and is highly conserved among the bacterial genera [149]. Morakova et al. designed primers specific for the *dnaJ* gene in the *M. avium* species that allow amplification of the *dnaJ* gene in all isolates of all *M. avium* subspecies and the authors suggest using them as an internal standard in the multiplex PCR to control inhibition of the amplification, and consequently false negatives, because are highly specific for at least *M. avium* [108]. The same team designed a fast and specific PCR strategy for the detection and differentiation of *M. avium* subspecies for use in routine veterinary di-

agnosis [108]. They have developed a multiplex PCR based on IS*900*, IS*901*, IS*1245* and the *dnaJ* gene. This method allows the detection of *M. a. paratuberculosis*, *M. a. hominissuis* and *M. a. avium/M. a. silvaticum* in one PCR reaction (PCR multiplex) and theoretically enables the detection of mixed infections of *M. a. paratuberculosis* and *M. a. avium* or M. *a. paratuberculosis* and *M. a. hominissuis*. The sensitivity of this multiplex PCR is 103 CFU for each bacterial strain in one PCR reaction, which also enabled the use of this test directly for DNA isolated from the tissue of the heavily infected sheep.

Shin et al. designed a five-target multiplex PCR to discriminate MAC organisms isolated. This MAC multiplex was designed to amplify a 16S rRNA gene target common to all *Mycobacterium species*, a chromosomal target called DT1 that is unique to *Maa* serotypes 2 and 3, to *M. avium* subsp. *silvaticum*, and to *M. intracellulare*, and three insertion sequences, IS*900*, IS*901*, and IS*1311*. The results for the pattern of amplification allowed to determine whether isolates were mycobacteria, or members of MAC, and to classify them into one of the three major MAC subspecies, *Map*, *Maa*, *Mah* [18].

5. Public health concerns

Zoonotic aspects of mycobacteria transmitted by the environment and wildlife highlights a major health problem. MAC causes a variety of disorders including tuberculosis-like diseases in animals and in human immunocompetent or immunocompromised patients. Susceptibility to mycobacterial infections depends of risk factors since they are ubiquous in the soil and water [150]. Human exposure to mycobacterium present in wildlife and in nature can occur by a variety of routes. Humans are continuously exposed at a low level (50 to 5000 bacilli per day). Contact with water, municipal or natural are also important routes for mycobacteria infection. Birds are major excretors of the agent in their faeces and the bacteria can persist in the soil and in water for long [1].

Healthy humans have a low susceptibility to MAC infection and only a very small percentage of mycobacteria progress trough to infection, but in immunocompromised individuals infected with HIV or leukaemia patients, treated with steroid therapy, chemotherapy or other immunosuppressive medication, should be carefully considered regarding their possibility to come in contact with birds with mycobacterial infection [1,151]. Prior to the introduction of highly active antiretroviral treatment more than 40% of patients developed *M. avium* complex bacteriemia two years after the AIDS diagnosis [152] and a disseminated MAC infection was found in as much as 50% of autopsied AIDS patients [153,154]. This has predominantly been attributed to the impairment of the adaptative part of the immune systems in HIV-1 infected individuals due to the loss of CD4+ T cells, as the susceptibility to opportunistic infections including *M. avium* infection is correlated with a decline in this cell type [155]. MAC usually produces clinical disease only when CD4+ are very low (< 50 cells/ml), which is seen in 4 to 5 per cent of HIV infected patients [88]. A recent study showed that exposure of dendritic cells to HIV-1 promotes or facilitates the intracellular growth of *M. avium* [153]. Signs and symptoms associated with MAC disease in AIDS cases are persistent

high grade fever, high sweats, anaemia and weight loss in addition to nonspecific symptoms of malaise, anorexia, diarrhea, myalgia and occasional painful adenophaty [88]. Epidemiology of MAC complex in patients without HIV infection remains somewhat difficult to determine since the disease is relatively uncommon, it is not a reportable health event and environmental exposure varies greatly according to the geographic region [156]. In an epidemiological survey in USA from 2000 to 2003 performed in [156] in patients without HIV infection the rate of positive non-tuberculous cultures was 17.7 per 100,000. Surveys conducted in Europe estimated the rate of disease anywhere in the human body to be 0.8-3.1 per 100,000 [157,158]. In a recent study, Bodle et al. estimated the incidence of non-tuberculous mycobacteria in the respiratory tract disease in 2.0 per 100,000 and the disease in anywhere in the human body in 2.7 per 100,000 [156]. Another recently published study showed that the patient´s lung disease was likely acquired by inhalation of aerosols while showering [159]. *M. avium* may reach the lungs by aspiration because a considerable percentage of patients with non-tuberculous mycobacteria (NMT) disease have been found to experience gastroesophageal reflux disease [160,161].

Disease patterns of MAC are different in immunocompromised patients. In adults, infection is mainly pulmonary [1,151]. MAC is the most common of the nontuberculous mycobacteria found in apparently healthy children [162] and it's infection is characterized by a chronic granulomatous lymphadenopathy in the neck region that preferably is treated by excision of the affected lymph node [162,163]. The main hypothesis of infection is that oral contact with *M. avium*-infected water courses causes lymphadenitis in the head and neck region in children [162,164]. Among the members of MAC, *Maa* is predominant (87-98%) in AIDS patients and induces disseminated mycobacteriemia rather than restrict the bacteria to the lungs [1]. Since the advent of AIDS, HIV has become the major risk factor for MAC infection. In AIDS patients the main route of infection is the gastrointestinal tract and *M. avium* is naturally tolerant to the low pH that exists in the stomach [19,165]. Regarding therapeutics, *M. avium* is of special concern because drugs commonly used from treating tuberculosis in humans are not effective [166] and MAC strains are resistant to isoniazid, the most popular anti-tuberculosis drug [19,167]. Preexisting pulmonary conditions, patients with current illness or immunosuppressive medication are the most important risk factors for MAC infection amongst patients without HIV infection. MAC was also reported as the most common pathogen causing post transplant non-tuberculous mycobacteria disease [156]. Other factors are local traumas and surgical procedures injuries [168]. Chronic obstructive pulmonary disease, emphysema, pneumoconiosis, aspiration due to oesophageal disease, previous gastrectomy and chronic alcoholism are some of the conditions which have been linked to disease [88,154]. Disseminated MAC infection is more frequent in caucasians compared with Hispanic or African-americans individuals. However, there are no differences related with age [169,170]. Infection is more frequent in men than in women, particularly in homosexual and bisexual men, when compared to other HIV risk categories.

The zoonotic potential of *Map* has been debated for almost a century because of similarities between Johne´s disease in cattle and Crohn´s disease in humans. A quarter century later since *Map* was first proposed as an etiologic agent in Crohn´s disease based on the isolation

of the organism from several patients the association as a causative agent or in an incidental away remains unresolved. Milk and water are potential sources for acquiring *Map* infection [171]. However, only a few samples of milk, positive by PCR for the presence of *Map* have yielded positive results in culture, suggesting that *Map* remains undetectable probably because the low number of viable *Map* in samples [172]. Published reports indicate that *Map* may not be completely inactivated by pasteurization of milk [173]. Serological response to *Map* in humans is also not conclusive [172]. Traditional methods of detecting bacteria, culture and stain are largely ineffective in detecting *Map* in humans. Bacteria are very difficult to culture and *Map* is able to exist in a spheroplast (cell wall deficient form) in humans so it cannot be identified by Ziehl-Neelsen staining [174-176]. Polymerase chain reaction has identified *Map* in greater than 90% of biopsy specimens from Crohn´s patients [177] and viable *Map* was detected in peripheral blood and serum in a higher proportion of individuals with Crohn's disease [178,179]. However, culture of *Map* from human specimens is a controversial question since some authors pointed out the difficulty of culturing *Map* [180,181]. At this moment is not possible to know if *Map* is a primary etiological agent or secondary invader and further research is need to understand the possible links between this agent and Crohn disease [1].

There is a recent interest in *Map* as an immune trigger of several autoimmune diseases [182,183]. Environmental agents are postulated to trigger autism. Recently, a theory proposing a mechanism by which *Map* triggers autism through molecular mimicry to the heat shock protein HSP65, which stimulates antibodies that cross react with myelin basic protein, a common feature of autism [184]. Another recent study has associated the presence of *Map* with Blau syndrome, an autossomal dominant, and systemic inflammatory disease. The mutations of Blau syndrome are on the same gene on chromosome 16 (CARD 15) that confers susceptibility to Crohns´disease [185]. *Map* was also implicated in sarcoidosis [186,187], which is a multisystemic granulomatous disease with many features in common with mycobacterial infection, and that, like Crohn´s disease, can be mimicked by slow bacterial infections [188,189].

Map was also linked to ulcerative colitis, irritable bowel syndrome, autoimmune (type 1) diabetes, Hashimoto thyroiditis and multiple sclerosis [179,184]. Increasing evidence suggests a role for *Map* in autoimmune (type I) diabetes. It is postulated that this bacterium acts via molecular mimicry between its antigens (HSP65) and the pancreatic enzyme glutamic acid decarboxylase (GAD) [190-195].

6. Conclusion

MAC comprises slow growing mycobacteria that are ubiquitous in the environment (soil and water), and have a wide source range, causing disease in various domestic and wild mammals and birds. MAC can affect a wide-range of wild animals, but little has been published up to the moment on the clinical signs, which are rarely exhibited or not documented. When present, the occurrence of clinical signs and lesions is highly variable in timing, though often similar to those of their domesticated counterparts.

The evidence for the zoonotic potential should not be neglected particularly in immunocompromised patients, both humans and animals.

Recent reports, suggesting an association between MAC and autoimmune and other chronic human diseases, alert to the importance of developing new studies on MAC biology, molecular diagnosis and epidemiology.

Research to understand the impact of MAC in public health is needed as well as the determination of transmission routes between humans and wildlife, which requires interdisciplinary collaboration among medical, veterinary and other public health officials.

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