

NONTUBERCULOUS MYCOBACTERIA AND THEIR DIVERSITY IN THE FIELD OF VETERINARY INFECTIOUS DISEASES

MICOBACTERIILE NETUBERCULOASE ȘI DIVERSITATEA IMPLICAȚIILOR ACESTORA ÎN SFERA PATOLOGIEI INFECȚIOASE VETERINARE

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ABSTRACT | REZUMAT

Nontuberculous mycobacteria (NTM) constitute the most diverse and comprehensive group, within the *Mycobacterium* genus. They are inhabitants of various environmental niches, being isolated from water, both in urban networks and in natural waters, soil or vegetation. Although they are characterized as having a moderate level of pathogenicity, an increase in the incidence of infections caused by them is registered, both in humans and animals. At the same time with the advance achieved in the molecular diagnosis, that gradually completed and replaced the time-consuming identification methods based on biochemical characters, an increase in the number of NTM species was recorded, currently over 160 being included in this group. The present synthesis highlights the importance that these species have for the infectious pathology of animals.

Keywords: *Mycobacterium avium* complex, nontuberculous mycobacteria, paratuberculosis

Micobacteriile netuberculoase (MNT) constituie grupul cel mai cuprinzător și divers din cadrul genului *Mycobacterium*. Acestea populează numeroase nișe din mediul înconjurător, fiind izolate din apă, atât cea din rețelele urbane cât și apele naturale, sol sau vegetație. Deși sunt caracterizate ca având un nivel de patogenitate moderat, se remarcă o creștere a incidenței infecțiilor determinate de acestea, atât la oameni cât și la animale. Concomitent cu avansul înregistrat în diagnosticul molecular care a completat și înlocuit treptat metodele de identificare în baza caracterelor biochimice, consumatoare de timp, s-a înregistrat o creștere a numărului de specii de MNT, actualmente peste 160 fiind încadrate în acest grup. Prezenta sinteză reliefează importanța pe care aceste specii o prezintă pentru patologia infecțioasă a animalelor.

Cuvinte cheie: complexul *Mycobacterium avium*, micobacterii netuberculoase, paratuberculoza

The comprehensive group of nontuberculous mycobacteria (NTM) brings together more than 160 members of the genus *Mycobacterium*, but the species included in the *Mycobacterium tuberculosis* complex MT BC, *Mycobacterium leprae* (*M. leprae*) and other "host-restricted" species, that are not linked to environmental sources (50). Even though other mycobacterial species were described not long after Robert Koch's important discovery of the tuberculous bacilli, their pathogenic potential for humans was later recognized.

NTM have been described in the literature under various names, such as "atypical", "environmental", "mycobacteria other than tuberculosis (MOTT)", but the most currently widespread name is "nontuberculous" or "NTM", also used by the American Thoracic Society (ATS), in their 2007 statement (19).

The raised awareness on the topic of NTM, especia-

lly in the human medicine, has been heightened as a result of their opportunistic association with the HIV patient's pathology but also due to the increased frequency of NTM infection reports. An important contribution in this matter was brought about by the current techniques used for the isolation and identification of mycobacteria, the use of automated culture systems, but more importantly, the use of modern molecular techniques in the current diagnostic procedures.

Nontuberculous mycobacterial infections may affect a broad range of species, both wild and domestic mammals, birds, fish, reptiles and humans. For immunocompromised people, infections due these bacterial agents represent an important cause of morbidity and mortality in developed countries (22). Following tuberculosis and leprosy, the third most frequent mycobacterial disease, common in Africa, but which evolves in other parts of the world, is considered to be "Buruli" or "Bairnsdale ulcer", caused by *Mycobacterium ulcerans* (*M. ulcerans*) (19).

NTMs have been isolated not only from animal orga-

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nisms, but also from the environment, where they can be found in numerous niches, such as: organic matter from soils, natural waters and urban water systems, amoebae, aerosols and dust, moss and vegetation from marshy areas, other plants or biofilms, oils. They may exhibit an increased resistance to the disinfectants used in water treatments, such as chlorination, and some species have the ability to survive and multiply in protozoa, aspect related to intracellular resistance in macrophages, both in humans and animals.

From a veterinary perspective, infections due to NTM have gradually gained importance, at the same time with the decreasing of bovine tuberculosis incidence, mainly in developed countries, due to the intense surveillance of the implemented tuberculosis control programs. The importance of NTM derives from two main aspects: firstly, from the direct impact that these species may have on animal health due to their potential to cause significant infections, therefore leading to considerable economic losses, and secondly, from the indirect impact derived from their interference with the tuberculosis screening programs in cattle (8). These species are also incriminated in reducing the efficacy of the BCG vaccination.

Some NTM species are important especially due to their role as etiological agents of animal infections. *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the etiologic agent of paratuberculosis (Johne's disease), a chronic granulomatous enteritis with a wide global distribution, acknowledged since the end of the 19th century, that affects many species of domestic and wild ruminants: cattle, sheep, goats, camelids, buffaloes, deer (9). MAP was also isolated from other species, such as wild boars, bears, rabbits, foxes, rats, monkeys, and also from human Crohn's disease patients. *M. avium* subsp. *avium* (MAA) is the causative agent of avian tuberculosis, which affects exotic, wild and domestic birds, being also isolated from cattle, pigs or horses (11). *M. avium* subsp. *hominissuis* (MAH) is an opportunistic environmental mycobacterium, belonging to the *Mycobacterium avium* complex (MAC), that can cause infections in pigs leading to significant economic losses. Usually it affects the digestive system's lymph nodes, determining granulomatous lesions most often observed during slaughterhouse inspection. Other organs, such as the liver, lungs or kidneys may also be affected (21).

The growing number of mycobacterial species included in this group and their relevance emphasise the relevance of understanding the relationships between the species and their implications in veterinary

and human pathology. We aimed at enhancing the main aspects regarding the importance and the implications of the species belonging to this heterogeneous group, focusing on their relevance for veterinary pathology.

NTM RESEARCH CONTEXT IN ROMANIA

In our country, NTM studies are mainly centralized on the veterinary clinical field and on MAC members, in particular MAP. The histopathological investigations carried out by Paul (35) on the clinical aspects and the prognosis of paratuberculosis in ruminants contributed to the understanding of this infectious disease.

Important recent contributions regarding paratuberculosis seroprevalence screening in domestic ruminants were brought by Savuța et al. (41, 42), Petriceanu (37) and Papastergiou (34). The implementation of molecular methods (PCR) in paratuberculosis diagnosis was conducted for the first time in Romania by Savuța (41).

Macovei (26) carried out epidemiological studies on cattle, sheep and goats, from various counties in the north-eastern region of the country, reporting a MAP seroprevalence of 19.54% and 32.35% in sheep, respectively in goats, and 6.25% for the tested cattle. Confirmation of mycobacterial infections, not only in ruminants but also in backyard poultry, was performed by bacterioscopic, histopathological, cultural examination and by identification of the specific insertion elements: IS1245 and IS901 for MAA and IS900 for MAP. Furthermore, characterization of the isolated strains was performed by Mycobacterial interspersed repetitive unit-variable number tandem repeats (MIRU-VNTR). Further studies on mycobacterial infection in hens were conducted by Bostănaru (10), using similar molecular identification procedures.

Investigations on the molecular diversity of NTM strains, identified in animal and human samples, were conducted by Anton et al (2019). Bacteriological, histopathological and molecular (real time PCR) diagnostic procedures were used for mycobacterial infection confirmation in ruminants and hens and MAC members were further characterized by MIRU-VNTR (2, 3).

Wildlife can play an important role in maintaining and spreading the MAC nontuberculous mycobacteria. In this context, *Mycobacterium avium* (*M. avium*) seroprevalence research in wildlife species, foxes and wild boars, was conducted in Romania evaluating the existence of a potential reservoir. The results obtained in this preliminary investigation suggest that, in the

evaluated region (counties from the north-eastern region of Romania), wild boars and foxes didn't contribute significantly to the dissemination and maintenance of NTM (2).

The data regarding the environmental NTM, reported in Romania, is scarce, mainly focusing on the isolation of NTM from the hospital environment. In an attempt to establish effective measures to prevent nosocomial infections in paediatric and oncological units, 21 strains of NTM were isolated from 500 samples of tap water and dust (4.2% positive samples), between 1994 and 1997 (49), the most common recovered species being *Mycobacterium marinum* (*M. marinum*). Stavri et al. (2005) demonstrate the usefulness of the ELISA test for the serodiagnosis of human infections due to environmental mycobacteria, using a panel of glycolipid antigens for MAC, *Mycobacterium kansasii* (*M. kansasii*), *Mycobacterium xenopi* (*M. xenopi*), *Mycobacterium scrofulaceum* (*M. scrofulaceum*) and *Mycobacterium gordonae* (*M. gordonae*) on 195 sera, the results of the cultural examination for MAC correlating with the results of the serological analysis (47).

The incidence of NTM in human respiratory specimens from the Bacteriology Laboratory of the Clinical Hospital of Pneumology in Iași was evaluated (10), based on their differentiation from MTBC members by the rapid immunochromatographic test, highlighting the efficiency and rapidity of the test in establishing the diagnosis and NTM discrimination from *M. tuberculosis*.

Further studies on NTM respiratory isolates from human patients, from the abovementioned institution, were conducted between May 2015 and March 2017. Amongst the isolates, those belonging to MAC: *Mycobacterium avium* (MAH and MAA) and *Mycobacterium intracellulare* (*M. intracellulare*) are the slow growing species most commonly encountered and *Mycobacterium peregrinum* (*M. peregrinum*) and *Mycobacterium chelonae* (*M. chelonae*), the most frequent isolated rapidly growing species (2,3).

The rapid immunochromatographic test was also used by Stavri et al., emphasizing the usefulness of this test in the current diagnosis of mycobacterioses, thus facilitating the rapid start of treatment (46).

IMPORTANCE OF NTM SPECIES INVOLVED IN ANIMAL INFECTIONS

Sometimes overlooked, NTMs may lead to bovine TB misdiagnosis, at both individual and herd level, aspect that is not to be neglected, considering the zoonotic character, economic impact and restrictions co-

related with this infectious disease. The interference with the bovine TB surveillance and control programs is very important, since NTM may induce false positive and false negative responses at the intradermal testing in cattle and may also influence the IFN- γ detection. This aspect is related to the antigenic features, shared between the *Mycobacterium* genus members.

The routes of NTM contamination are digestive, respiratory (via aerosols and dust) and cutaneous (14). NTM species are commonly isolated from bovine respiratory tissues, collected at abattoir inspection during TB diagnostic procedures, but digestive tissue might be more suitable for NTM detection (8).

Pre-exposure of the animal organisms to NTM may induce modulation of the immune response to BCG vaccination, having either a false negative or false positive effect, depending on the encountered NTM species. Cross-reactivity to mycobacterial antigens is an important issue to be considered, especially for bovine TB surveillance and control programs (20). Some NTM species, like *M. kansasii*, *M. marinum*, *Mycobacterium szulgai*, or non-pathogenic *Mycobacterium flavescens* and *Mycobacterium gastrii* harbour genes for important CE (ESAT-6/CFP-10) protein homologues (52). These peptides are diagnostic markers in TB infection in humans and animals and the genes encoding them are located in the RD1- Region of difference 1. In this matter, cross-reactivity for *M. kansasii* and *Mycobacterium bovis* in cattle was observed by Vordermeier et al. (52), suggesting that in case of previous exposure to NTM, a weaker response from the host immune system to MTBC species an BCG may be observed. In turn, the results of the studies conducted by Thom et al. (48) suggest that pre-exposure to *M. avium* may have a beneficial effect on the host response to BCG.

The "original antigenic sin" (OAS) is a phenomenon encountered in mycobacterial infection: an inefficient immune response due to cross-reaction to immunogenic antigens, common to NTM and BCG. Thus, standard testing procedures, intradermal skin and IFN γ tests, may be misinterpreted as false negative, especially in NTM prevalent regions (20). Aspects regarding the capacity of NTM persistence within the host, growth speed and also common antigens homology were correlated with the variation in the BCG vaccination response. It was suggested that the elimination of a mycobacteria is quicker in the case of fast-growing species.

In mycobacterial context, the OAS can be explained by the common antigens encountered in *Mycobacterium* species. According to Jenkins et al., in case of pre-exposure of an animal organism to an environmental

NTM, a "mild, non-inflammatory response" "and generation of specific T cells" may be present, and if the animal organism comes across a pathogenic mycobacterium, a cross-response is reactivated by the epitopes of the common antigens, recognized from the previous NTM exposure, as well as a T cells recall (20).

Even though the use of the comparative intradermal tuberculin test, as in the current procedure in our country, eliminates un-specific reactors, the shared mycobacterial antigens contained by the PPD may induce cross-responses, thus positive results may appear for both avian and bovine tuberculin. In general, NTM sensitization is a temporary state, but species like MAP may induce a "chronic sensitization" state for the animal, leading to false positive results at skin testing (8) and in the IFN γ test (5). Other species had been correlated with unspecific intradermal responses such as: *Mycobacterium fortuitum* (*M. fortuitum*) (31), *Mycobacterium terrae* (*M. terrae*) complex members, in particular *Mycobacterium nonchromogenicum* (7), *M. kansasii*, *M. chelonae* or *Mycobacterium phlei*. Presence of NTMs was also reported in healthy non-reactive cattle, with absent characteristic lesions (17).

Aside from the implication for diagnostic procedures, an important aspect regarding this large group is the ability to cause infections in both animal and human. Due to the slow development of mycobacterial infection, most often these are observed in animals with a longer life, like dairy cattle, breeding pigs and pets (14). Members of the MAC are of particular interest to veterinarians, especially the *M. avium* subspecies. Apart from paratuberculosis, their involvement in other types of infections, such as mastitis, testitis, pneumonia or systemic infection in cattle, has been reported. Currently, this group comprises the following species: *M. avium* with the following subspecies: *M. avium* subsp. *avium*, *M. avium* subsp. *hominissuis*, *M. avium* subsp. *silvaticum* (MAS) and *M. avium* subsp. *paratuberculosis*, *M. intracellulare*, *Mycobacterium chimaera* (*M. chimaera*), *Mycobacterium arosiense*, *Mycobacterium yongonense* (*M. yongonense*), *Mycobacterium timonense*, *Mycobacterium paraintracellulare*, *Mycobacterium colombiense*, *Mycobacterium marseillense*, *Mycobacterium vulneris*, *Mycobacterium bouchodurhonense* and *Mycobacterium lepraemurium* (51). Novel sequencing technique has led to the assignment of *M. chimaera*, *M. yongonense* and *M. intracellulare* as a subspecies of *M. intracellulare* (50).

MAA (serotypes 1, 2 and 3) is the most common cause of avian tuberculosis, an infectious disease with global spread. Numerous wild and domestic bird spe-

cies are receptive and clinical manifestations are observed in adult birds, hens and roosters of two years (14). In mammals, in case of MAA infection, lesions are usually observed in mesenteric and retropharyngeal lymph nodes, with granulomatous or purulent aspect (11). It was isolated from wild boars presenting mesenteric and submaxillary lymphadenitis (25) and from domestic pigs with granulomatous and caseous lesions in spleen, liver and mesenteric lymph nodes (36).

Due to its negative impact from the animal health and economic points of view, MAP is a considerable important pathogen. Paratuberculosis (or "Johne's disease") is a chronic granulomatous enteritis present in regions with important ruminant livestock across Europe, North America or Australia. Premature culling, decreased milk production, body weight loss, increased infertility percentage, higher mortality rate, prevention and control strategies' costs are key aspects that enhance the importance of this disease (8).

Paratuberculosis control programs are implemented in many European countries, given the high cattle herd prevalence estimations, of approximately 50% and a value of 20% for within herd prevalence (32). In France, a 63% flock level prevalence was reported for goats (30) and in central Italy, over 70% of the sheep flocks present a minimum of one seropositive animal (39).

The EFSA assessment on paratuberculosis (12) states that no information is available for Romania, Latvia and Malta, concerning MAP presence. The study conducted by Macovei, in the eastern region of our country reports a seroprevalence rate of 6.25% for cattle, 19.54% in sheep and 32.35% in goats and MIRU-VNTR characterization of MAP isolates was also performed (3, 26). Aside from cattle, goats and sheep, the paratuberculosis agent was isolated from a variety of animal species, both ruminants and non-ruminants like: wild boars, foxes, hares, rabbits, brown bears, badgers, skunks, armadillos, crows, rooks, wild ungulates, such as the red deer or bison (11), with variability in clinical manifestations and lesions. Both mild and severe intestinal and lymphoid associated tissues lesions were identified in rabbits. Digestive disease with paucibacillary granulomatous lesions were observed in foxes, weasels, rats, hedgehogs or otters (29). Similar to the paratuberculosis pathology in domestic ruminants, diarrhoea and weight loss associated with lesions of necrosis and mineralization in the ileal lymph nodes were reported in deer (38).

MAH is an environmental mycobacterial species, with opportunistic pathogenic capacity for birds and mammals and the MAC species with the greatest geno-

mic heterogeneity (50). As the name suggests, it was isolated from pigs, usually from the head, cervical and mesenteric lymph nodes, with or without macroscopic lesions (28), and humans with pulmonary infection, disseminated infection and children with cervical lymphadenitis. Most often, cases of pig infection are discovered due to the presence of the lesions in mesenteric lymph nodes and sometimes in the liver, kidneys or lungs, observed during carcasses and organs inspection in the abattoir (21). Though not common, unspecific symptomatology, like weight loss or reproductive disorders, may be observed, MAH isolation from aborted fetuses, uterus and genital swabs from breeding sows, sperm and pigs with wasting being reported (13). Furthermore, the isolation of this nontuberculous species has also been reported in dogs, pet parrots, horses and cattle (23, 24, 43).

MAS, typically known for its role as the etiological agent of tuberculosis in wood pigeon, is commonly considered to present pathogenicity for birds (wood pigeons, chickens). Ronai et al. reported the isolation from samples of deer, wild boars, red foxes, cattle and badgers (40).

NTMs are also correlated with mammary gland pathology, usually depicting mastitis or thelitis. Various species were recovered from bovines with nontuberculous-mastitis: *Mycobacterium smegmatis* (*M. smegmatis*), *M. fortuitum* (15), *Mycobacterium goodii*, *Mycobacterium lacticola* after intramammary administration of oily treatments (45).

Although rare, other slow growing nontuberculous species: *Mycobacterium malmoeense*, *Mycobacterium bohemicum*, *Mycobacterium palustre*, *Mycobacterium heckeshornense*, *Mycobacterium celatum* were recovered from mandibular lymph node samples of pigs (1). *M. fortuitum* was isolated from mesenteric lymph nodes and joint fluid (6). The lack of visible lesions in pigs at slaughter house inspection could be correlated with the short life of pigs.

Fish mycobacterioses are reported in various regions around the world, in wild as well as in farming fishes, with numerous species affected. The importance of these infections derives not only from the veterinary view, concerning the health of the fish population, but also from the one health concept, considering the risk for human skin infections when in contact with infected fish.

Gauthier and Rhodes provided a clear overview on fish mycobacterioses in their review (16). *M. marinum*, *M. fortuitum* and *M. chelonae* are the most common reported species involved in fish mycobacterioses, but

others like *Mycobacterium salmoniphilum*, *Mycobacterium shottsii*, *Mycobacterium pseudoshottsii*, *Mycobacterium montefiorensis*, *Mycobacterium haemophilum*, *Mycobacterium abscessus* (*M. abscessus*) or *M. peregrinum* were isolated from a variety of fish species. The fish present skin pigmentation modifications, scales and weight loss, behaviour changes or spinal deformities. Internal organs, livers, kidney or spleen are usually increased in size and present nodules. Granulomatous lesions are revealed through histopathological examination (16).

Mycobacterial infections may also affect companion animals. MAC complex members are involved in cases of regional lymphadenitis in cats, but also gastro-intestinal and pulmonary disseminated infections, and granulomatous lesions affecting bowels and mesenteric lymph nodes, livers and spleen, in dogs (18). Panniculitis, most often in obese cats, caused by rapidly growing NTM species like *M. fortuitum* and *M. smegmatis* and pneumonia due to *M. chelonae* or *M. fortuitum* in dogs were reported. Skin disorders determined by other mycobacterium species apart from the MTBC members comprise cutaneous infections due to atypical mycobacteria, canine leproid granuloma (CLG) or canine leprosy and feline leprosy.

CLG is common in Australia and Brazil, but it was also reported in the United States and Europe. It is characterized by nodular, self-limiting lesions, affecting the skin and subcutaneous tissues, especially in breeds with short hair. The causal factor remains uncharacterized, but it is phylogenetically close to *Mycobacterium simiae* (27). *Mycobacterium tilburgii*, *Mycobacterium simiae*, *Mycobacterium genavense* have been linked to the aetiology of this infection. Due to the localization of the lesions, the bacteria is thought to be acquired through biting arthropods.

The aetiology of feline leprosy involves more than one uncultivable mycobacterial species. The most common cause is *Mycobacterium lepraemurium*, a fastidious growing species belonging to MAC, that determines nodular lesions with ulcerative tendency in the infected cats, usually on the head and forelimbs, correlated with injuries after altercations with rodents. "*Mycobacterium lepraefelis*" determines regional or generalised nodular skin disease, with weak therapeutic outcome, and shares similar genomic traits with *M. leprae*, *Mycobacterium lepromatosis* and *Mycobacterium uberis* (33). "*Mycobacterium tarwinense*" is usually identified in cats presenting ocular and periocular nodular lesions, involving the skin and the subcutaneous tissue. Similar to the CLG *Mycobacterium* sp., is

closely related to *Mycobacterium simiae* and is not cultivable by traditional bacteriological procedures. "*Mycobacterium visibile*" is correlated with multisystemic granulomatous infection (4).

ASPECT CONCERNING PUBLIC HEALTH

Inhabitants of various natural and man-made environmental niches, NTM can cause opportunistic infections in both animal and people. Factors like the use of molecular techniques and automated culture systems in current diagnostic procedures and raising prevalence of immunosuppressive diseases contributed to the rising number of reports regarding NTM infection in humans. Broadly, clinical aspects encountered in human NTM infections comprise pulmonary infection, lymphadenitis, disseminated infection and skin infection. Person to person transmission generally does not happen and the primary source for these mycobacterial organisms is environmental, mainly soil and water. Members of the MAC, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. abscessus* are among the top species identified in human clinical specimens, but the prevalence of the isolates varies according to their geographical distribution (51).

Still under debate, the link between MAP and the aetiology of human Crohn's disease, an inflammatory bowel disease, arises from the clinical similarities with ruminant paratuberculosis and the identification of this mycobacterial species in samples from patients. Furthermore, Wynne et al. provided genomic based proof for zoonotic transmission (53).

In addition to Crohn's disease, MAP was associated with other disorders: Blau syndrome (granulomatous dermatitis, arthritis and uveitis), sarcoidosis (granulomatous multisystem disorder), autoimmune diabetes and thyroiditis and multiple sclerosis. For the latter three, a "molecular mimicry" mechanism between MAP and human proteins is involved, triggering an autoimmune reaction, a cross-response on the base of host and MAP epitope homology (44).

Furthermore, the particular structural characteristics of the outer-membrane of the *Mycobacterium* genus members determine their hydrophobicity and impermeability proprieties, facilitating their resistance to disinfectant substances, heavy metals and antibiotics, attachment to surfaces and aerosolization. These aspects underline the importance of NTM presence in engineered and natural environments, their adaptability to new conditions, given that repeated exposure constitutes a risk factor for humans.

FINAL REMARKS

Nowadays, access to advanced diagnostic techniques facilitates the identification and characterization of NTM, species heterogeneity, epidemiology, distribution, or susceptibility to treatment. All these aspects contribute to a better understanding of the potential hazard to animal and human health and facilitate the adoption of optimal control and prophylaxis measures. Furthermore, uniformity in the diagnostic procedure would facilitate the comparison of the isolated strains. As it was presented, NTM do not represent an important topic only due to their pathogenic potential, but also by reason of diagnostic procedures specificity, in case of tuberculous infection. Not all the species of this group are familiar for veterinary clinicians, hence the necessity of further studies regarding the species involved in mycobacterioses in animal populations on our country.

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