Resumo: A tuberculose aviária é uma doença infeciosa de carácter crónico e desenvolvimento insidioso, que muitas vezes apresenta um desfecho fatal. Devido ao seu potencial zoonótico assume um importante papel na saúde pública. O diagnóstico em animais vivos mantém-se difícil de obter, destacando-se a biologia molecular como uma alternativa rápida e específica, comparativamente aos outros métodos diagnósticos disponíveis. O objetivo desta revisão foi compilar informação sobre os principais agentes etiológicos responsáveis por provocar a infecção em fauna selvagem, bem como os dados epidemiológicos conhecidos, de forma a prestar apoio a médicos veterinários, alunos, técnicos e investigadores da área.

Palavras-chave: tuberculose aviária; fauna selvagem; Mycobacterium; biologia molecular.

Summary: Avian tuberculosis is a chronic infectious disease with an insidious development, which often presents a deadly outcome. Because of its zoonotic potential, it takes on an important role to public health. The diagnosis in living animals is still hard to get, and molecular biology stands out as a quick and specific alternative, when compared to other available means of diagnosis. The aim of this revision was to compile information on the main etiologic agents responsible for causing the infection in wild animals, as well as the known epidemiologic data, so as to give support to veterinaries, students, technicians and researchers in the area.

Keywords: avian tuberculosis; wildlife; Mycobacterium; molecular biology.

Introduction

The mycobacteria are divided into groups according to some criteria: pathogenicity to animals or humans, growth rate and optimum temperatures, and effect of visible light on pigment production (for example, M. avium produce yellow pigment on the absence of light) (Inderlied et al., 1993).

The genus Mycobacterium includes pathogenic species for many animals: birds (M. avium), mammals (M. avium subsp. paratuberculosis, M. bovis, M. tuberculosis), fishes (M. marinum), frogs (M. fortuitum) and rodents (M. leprae) (Converse, 2007).

Tuberculosis, caused by M. tuberculosis (MTC), was considered the "typical" mycobacteriosis, thus making all other, except M. leprae, being described as "atypical" (Inderlied et al., 1993; Cangelosi et al., 2004).

Mycobacterium avium complex

Avian mycobacteriosis presents ubiquitous distribution, and is described in wild and captive birds all over the world (Converse, 2007). It's caused by Mycobacterium avium serotypes 1-3, less frequently by Mycobacterium genavense (Mijs et al., 2002) and rarely by M. intracellulare, M. fortuitum, M. tuberculosis, M. gordonae and M. nonchromogenicum (Kunze et al., 1992; Dvorska et al., 2004), M. genavense's detection has increased in captive birds, instead of M. avium (Pollock, 2006; Converse, 2007). Sporadically, other potentially pathogenic mycobacteria are found in bird tissues, e.g. M. celatum, M. simiae and M. chelonae (Moravkova et al., 2011).

Although the term mycobacteriosis can be applied to any infection of mycobacteria, the term avian tuberculosis is more used for the disease caused by M. avium or related agents, due to its typical tuberculous
lesions (Converse, 2007). The agent *M. tuberculosis* is occasionally found in birds, but its clinical signs are different (Kearns, 2003).

Avian tuberculosis is a chronic disease, with a long incubation period depending on the physical health of the bird and virulence of the strain of *M. avium avium* (Moravkova et al., 2011), that leads to anorexia, lethargy, emaciation and dyspnea; death can occur in a few months. The agent can persist in the environment and populations during years. It has been verified a high incidence of avian tuberculosis in captive populations, with high number of birds in a contaminated area (Converse, 2007).

Avian tuberculosis is still difficult to diagnose and control in living birds (Kearns, 2003). There are no reports of birds species that are resistant to all mycobacteria (Converse, 2007).

The agents that are included in *M. avium* complex (M AC) are opportunistic microorganisms, able to cause disease in humans and animals (Inderlied et al., 1993), and are subdivided in four subspecies: *M. avium* subsp. *avium*, *M. avium* subsp. *silvaticum*, *M. avium* subsp. *hominisuis* e *M. avium* subsp. *paratuberculosis* (Converse, 2007). *M. avium* is an important pathogen for humans and animals (Bonó et al., 1995). M AC’s microorganisms were responsible for an opportunistic infection in 70% of the people with Acquired Immune Deficiency Syndrome (AIDS) in developed countries before the appearance of modern anti-retroviral drugs (Cromie et al., 2000). *M. avium*’s serotypes 1, 2 and 3 are the most frequently isolated from animals; serotypes 4-8 are the most often involved in immuno compromised individuals (Kunze et al., 1992).

*Mycobacterium avium* subsp. *avium* affects mainly birds, despite being documented also in bovines and pigs (Moravkova et al., 2008). Most of birds’ infections are caused by serotypes 1-3 of *M. avium* (Cromie et al., 2000). *M. avium* subsp. *hominisuis* (serotypes 4-6, 8-11 and 21) is an opportunistic agent, mainly infectious to immunocompromised humans, pigs, cattle and cervids (Dvorska et al., 2004). *M. avium* subsp. *silvaticum* is documented only in wood pigeons (*Columba palumbus*). *M. avium* subsp. *paratuberculosis* is the causative agent of paratuberculosis (Johnne’s Disease), a chronic inflammation of gastrointestinal tract, that mainly affects ruminants (Moravkova et al., 2008; Castellanos et al., 2012), despite having a large spectrum of hosts (Motiiwala et al., 2004). Paratuberculosis is described in many wild species, such as the roe deer (*Capreolus capreolus*), the European rabbit (*Oryctolagus cuniculus*) and the red fox (*Vulpes vulpes*) (Greig et al., 1999; Carta et al., 2012). A recent study written by Carta et al. revealed that the red deer (*Cervus elaphus*) doesn’t contribute, as a reservoir, to maintain *M. avium* subsp. *paratuberculosis* on the Iberian Peninsula. On the other hand, that study refers the high prevalence of *M. avium* subsp. *paratuberculosis* in the fallow deer (*Dama dama*) in the same geographic area (Carta et al., 2012). In some predator carnivores, the same agent was isolated 6 times more than it was in preys (Castellanos et al., 2012). The association of paratuberculosis with the Crohn’s Disease in humans is still being studied (Greig et al., 1999; Motiiwala et al., 2004; Rodriguez-Lázaro et al., 2005; Castellanos et al., 2012).

Eventually, *M. avium* can cause mycobacteriosis in all bird species, but it affects mainly the waterfowl, Galliformes, Columbiformes, Passerines, Psittacines, ratites and raptors (Aranaz et al., 1997; Pollock, 2010).

**Epidemiology**

Mycobacteria are opportunistic microorganisms that live under various temperatures and pH conditions, in damp environments (Cromie et al., 2000; Converse, 2007). Avian tuberculosis is primarily transmitted by direct and indirect contact with infected birds by *M. avium* (Converse, 2007). Raptors can be infected by ingestion of contaminated preys (Pollock, 2006). Mycobacteria can also be dispersed as aerosols, in case of animals with respiratory signs. The environment can persist contaminated for a long period of time. Liver and intestinal granulomas continuously liberate bacillus to fecal material. *M. avium* can persist outside the animal host, producing mycobactin and acquiring iron, essential to his growth and survival in the environment (Converse, 2007).

The most important infection routes are the respiratory and gastrointestinal tracts (bronchial and intestinal mucosa, respectively) (Inderlied et al., 1993). There is evidence that *M. avium* can be transmitted mechanically, by arthropods (Converse, 2007). Some mycobacteria have been isolated from coleopterans (Fischer et al., 2004). In case of paratuberculosis, *M. avium* subsp. *paratuberculosis*, it is mainly transmitted by feco-oral route, through colostrum, milk or contaminated pasture, but it can also be transmitted by other routes, such as intravenous, intramammary and intrauterine (Castellanos et al., 2012).

Susceptibility to mycobacterial infections depends on different factors, both genetic and environmental (Aranaz et al., 1997; Converse, 2007). Apparently, *M. avium* subsp. *avium* infection makes the infection with concurrent less virulent mycobacterial species easier (Moravkova et al., 2011).

The agents of avian mycobacteriosis in pet birds are rarely identified, due to lack of specific findings at necropsy and difficulties in isolating mycobacterial species (Aranollá et al., 2009).

Avian tuberculosis is more frequently diagnosed in adult birds, between 3 and 10 years of age (Pollock, 2010). The diagnosis in birds with less than 2 years old it’s very rare (Solé et al., 2009).

Incidence and prevalence of avian tuberculosis remain unknown, due to the lack of specific clinical
Clinical signs

The most common clinical signs are: anorexia, progressive weight loss, weakness, feather damages, diarrhea, abdominal distension, lethargy and death. In case of pulmonary, ocular or bone involvement, dyspnea, blindness and lameness can occur (Converse, 2007). Granulomatous lesions are common in raptors, but rare in Anseriformes, Columbiformes, Coraciiformes, Passeriformes and Psittaciformes. The diffuse form of disease was mainly described in Coraciiformes (Alcedo spp.) and Passeriformes (Pollock, 2006).

Infections by M. avium and M. genavense produce very similar clinical signs in birds (Converse, 2007). In veterinary epidemiology, the virulent strains that induce avian tuberculosis are the most important ones (Pavlik et al., 2000).

Paratuberculosis, an intestinal granulomatous disease, is characterized by emaciation, at first with an increased appetite and after with anorexia, lethargy and chronic or intermittent diarrhea. A bdominal distension can occur due to hepatomegaly or small intestines enlargement. Musculoskeletal disease on avian tuberculosis is described with various incidences, being the carpometacarpal and elbow joints the most commonly involved. Skin above the affected places appears thickened and ulcerated. Respiratory form of disease results in dyspnea and exercise intolerance, due to pulmonary granulomas and/or air sacs compression, secondary to enlargement of the liver. Nodules within the infraorbital sinus, nares and syrinx are rare, despite being described. Skin disease, also rare, involves dermatitis, non pruritic thickening, xantomatosis and soft subcutaneous masses (Pollock, 2006).

In humans, M. avium infection results in one of the following presentations: 1) primary localized lymphadenitis, 2) pulmonary disease or 3) generalized disease, associated to immunosuppression (Aranaz et al., 1997). The serotypes 1, 4 and 8 are the most commonly responsible for the mycobacteriosis associated to AIDS (Aranaz et al., 1997).

Mammals’ immune response to M. avium includes leucocytes, such as macrophages and T lymphocytes, particularly citotoxic T-cells and natural killer cells, and citocines (TNF, IFN-γ, IL-2). Chikens’ response appears to be similar (Cromie et al., 2000).

Prevalence of mycobacteria in wild animals in Portugal

In Portugal, the epidemiology of M. bovis in wild boar (Sus scrofa) was described by Santos et al. (2009). In this study, in the total of mycobacterial isolates, 43% were M. bovis, 19.5% were M. avium and 36.6% were other mycobacteria. In the same study the isolation rates for M. bovis were 6%, 22%, and 46% in tuberculosis-infected areas.

In the Iberian Peninsula, paratuberculosis is uncommon in red deer (Cervus elaphus) and roe deer (Capreolus capreolus). However, in the same area a high M. capreolus infection prevalence among fallow deer (Dama dama) was detected (Carta et al., 2012).

As far as we know, prevalence of avian tuberculosis in wildlife is not well described in Portugal, yet.

Impact on wildlife populations

Tuberculosis is considered an emerging infectious disease among wildlife, and is already described in a wide number of wild species. Once established, it is extremely difficult to eradicate. The only successful case of eradication was in Australia, with the de-population of water buffalo (Bubalus bubalis), the maintenance host of the infectious agent, M. bovis (Santos et al., 2009). Tuberculosis is not a dangerous threat to most wild carnivore populations, although it could have a devastating effect in small populations, like the Iberian linx (Lynx pardinus) (Briones et al., 2000). Several wild mammal species are involved in the transmission and maintenance of M. bovis infection, such as Eurasian badgers (Meles meles) in Great Britain, white-tailed deer (Odocoileus virginianus) in the United States and wild boar (Sus scrofa) in Spain (Lyashchenko et al., 2008).

Avian tuberculosis occurs sporadically in wild birds. The prevalence of the disease may be underestimated, because many carcasses remain undetected in the wilderness or are removed by predators or hunters. Bacterial infections are diagnosed, sometimes, when epizootic episodes of other diseases happen, causing high mortality (such as avian cholera).

Avian tuberculosis (M. avium serovar 1) is described as the cause of high mortality in lesser flamingos (Phoenicopterus minor) (Kock et al., 1999). The potential of disease transmission increases in gregarious species; predators, whose diet is sick birds, are also susceptible to higher risk (Converse, 2007). It’s relatively rare in non-gregarious species, such as some raptors (Tell et al., 2004).

Free-ranging raptors assume an important role on mycobacteria dispersion (Soler et al., 2009). However, they are not, apparently, a relevant source of infection to captive birds (Pollock, 2006). Mycobacterium avium Complex (MAC) species also have been isolated.
in wild and domestic mammals (cattle and pigs) (Bar-tos et al., 2006). Prevalence of *M. tuberculosis* is de-scribed in wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*), potential reservoirs of bovine tuberculosis (Vicente et al., 2006). Bovine tuberculosis is reported in many wildlife species and countries (M artín-A ntece et al., 2005). The identification of reservoirs is crucial to the implementation of effective controlling mea-sures (Naranjo et al., 2008).

**Mycobacterial diseases in wild animals**

The most significant mycobacterial diseases of free-living, captive and farmed deer are bovine tuber-culosis (*M. bovis*), paratuberculosis (*M. avium* subsp. *paratuberculosis*) and avian tuberculosis (*M. avium* subsp. *avium*) (Mackintosh et al., 2004).

The reported prevalence, by some countries, of *M. bovis* infection in wild deer doesn’t reach the 5%, except in New Zealand, where the main suspect cause for such a high level of infection is the spread from other wildlife (Mackintosh et al., 2004). In Great Britain, the badger (*Meles meles*) is the main wildlife reservoir of bovine tuberculosis. The transmission between cattle, badgers and deer has been proved, but the direction of spread has not been disclosed. In Spain, the transmission between cattle, deer and wild boar is reported, and tuberculosis caused by *M. bovis* has been a major problem in collections of wildlife (Mackintosh et al., 2004).

The gross lesions of disease in deer tend to be simi-lar to those found in cattle (Table 1). However, in deer there are more abscesses containing liquid pus, rich in bacillus, fewer calcification and without fibrosis. The lymph nodes of the head are infected in almost half of the cases, with caseous necrotic lesions. Infections with *M. avium* subsp. *paratuberculosis* and *M. avium* subsp. *avium* cause identical lesions to those caused by *M. bovis* (Mackintosh et al., 2004).

**Diagnosis**

*Ante mortem* diagnosis is very difficult to obtain (Tell et al., 2004). Avian tuberculosis must be consid-ered as a differential diagnosis when adult raptors are found unhealthy, weak and in poor condition without any apparent reason. The probability increases if ce-lomic abnormalities are detected during physical ex-amination or diagnostic exams (Tell et al., 2004; Pol-lock, 2006). Granulomatous lesions in birds don’t tend to calcify, as they do in mammals, what results in a more difficult radiographic diagnosis (Pollock, 2006). Minimum database analysis can reveal variable results. However, a complete blood count might reveal a marked heterophilic leukocytosis, monocytosis, thrombocyto-sis and biochemistry might reveal elevated fibrinogen, as well as a polyclonal gammaglobulinopathy (Pollock, 2006). The most useful *ante mortem* diagnostic tests are radiography, ultrasonography, laparoscopy and biopsy or fine-needle aspiration with acid-fast staining. How-ever, definitive diagnosis requires mycobacterial culture or PCR test (Tell et al., 2004). Serologic tests Enzyme-Linked Immunoadsorbent Assay (ELISA), complement fixation and hemagglutination are available for only a little number of species (Pollock, 2006).

*Mycobacterium avium* isolation or its amplification in clinical samples doesn’t necessarily mean active in-fection, because *M. avium* is a common agent in the environment and might be present in cases of absent disease (Aranaz et al., 1997).

*Post mortem* diagnosis of avian tuberculosis is based on the presence of typical lesions and mycobacterial detection on blood or tissues. Caseous granulomas or other inflammatory lesions along the intestine, liver, spleen, lungs, bone marrow, gonads or kidneys can be found in post mortem examination (Cromie et al., 2000; Converse, 2007; Moravkova et al., 2011). A presumptive diagnosis is supported by microscopic findings of acid-fast bacillus, if at least 5x10⁴ mycobacteria/ml of material are present (Aranaz et al., 1997; Converse, 2007). Mycobacterial culture is difficult and delayed (might require a minimum of 4 weeks) (Converse, 2007). Isolation process includes 3 steps: enrichment, decontamination and prolonged incubation (Coelho et al., 2007). Some media can be used to MAC’s culture, but the most sensible method requires, at least, culture in 2 media, 1 solid and 1 liq-uid. M ultiple combination of culture media results in higher sensitivity (Inderlied et al., 1993; Aranaz et al., 1997). Several methods can be used to culture MAC from blood, bone marrow or other specimens (Inder-lied et al., 1993). Most mycobacterial strains can be cultivated on the Löwenstein-Jensen medium at 28°C and 37°C. Culture of MAP has good results on Middle-

### Table 1 – *M. intracellulare* and MAC microorganisms’ ability to produce macroscopic lesions in determinate species (adapted from Mackintosh et al., 2004).

<table>
<thead>
<tr>
<th>Animal species</th>
<th><em>M. avium</em> subsp. <em>avium</em></th>
<th><em>M. avium</em> subsp. <em>hominisissis</em></th>
<th><em>M. avium</em> subsp. <em>silvaticum</em></th>
<th><em>M. avium</em> subsp. <em>paratuberculosis</em></th>
<th><em>M. intracellulare</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Deer</td>
<td>yes</td>
<td>rarely</td>
<td>yes</td>
<td>yes</td>
<td>unknown</td>
</tr>
<tr>
<td>Cattle</td>
<td>yes</td>
<td>rarely</td>
<td>unknown</td>
<td>yes</td>
<td>unknown</td>
</tr>
<tr>
<td>Pigs</td>
<td>yes</td>
<td>yes</td>
<td>unknown</td>
<td>rare</td>
<td>probably</td>
</tr>
<tr>
<td>Humans</td>
<td>rarely</td>
<td>yes</td>
<td>unknown</td>
<td>rare</td>
<td>rarely</td>
</tr>
</tbody>
</table>
Table 2 – Serotypes and DNA insertion sequences of the agents of the Mycobacterium avium complex (MAC) and M. intracellularare
(adapted from Mackintosh et al., 2004).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>M. avium subsp. avium</th>
<th>M. avium subsp. hominisuis</th>
<th>M. avium subsp. silvaticum</th>
<th>M. avium subsp. paratuberculosis</th>
<th>M. intracellulare</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotypes</td>
<td>1, 2, 3</td>
<td>4-6, 8-11, 21</td>
<td>2, 3</td>
<td>-</td>
<td>7, 12-20, 23</td>
</tr>
<tr>
<td>IS900</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>IS901/IS902</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>IS1245</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>IS1311</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>

brook-Cohn 7H10 agar with OADC and mycobactin enrichment (Harmsen et al., 2003). The culture on a liquid medium can be done in a modified Middlebrook 7H9 liquid medium containing radioactively labelled palmitic acid (BACTEC TB System; Becton-Dickinson Diagnostic Instrument Systems, Sparks, Md.) (In-derlied et al., 1993).

Molecular diagnosis provides a helpful and quick alternative to biochemical test profiles of pure cultures (Harmsen et al., 2003). Polymerase Chain Reaction (PCR) based techniques need a small amount of sample to obtain results, detect a reduced number of microorganisms, give results in a short period of time and might distinguish mycobacterial species (Aranaz et al., 1997; Cangelosi et al., 2004; Coelho et al., 2007). Application of PCR to detect mycobacterial genome is valuable for laboratory confirmation of the infectious disease (Coelho et al., 2010). The gold standard test for mycobacteria detection is 16S rDNA gene (Mijs et al., 2002), but other targets have proven to be helpful, such as the internal transcribed spacer (ITS) region and the hsp65 gene (Harmsen et al., 2003). The main advantage of 16S rDNA gene analysis is that it can be applied to all bacteria, including uncultivable or dead bacteria (Harmsen et al., 2003).

For detection of MAC microorganisms, PCR searches for specific insertion sequences (IS900, IS901 and IS1245) (Moravkova et al., 2000). Pavlik et al. (2000) distinguished IS900 to M. a. paratuberculosis and IS901 or IS902 to M. a. silvaticum (Begg et al., 2000). IS1141 to M. intracellularare and IS1110 to M. avium are also described (Guerrero et al., 1995) (Table 2).

IS900 is a fragment repeated 15-20 times in all strains of M. a. subsp. paratuberculosis’ genome (Cousins et al., 1999; Coelho et al., 2007; Castellanos et al., 2012). PCR assays for detection of IS900 are highly sensitive to identify M. a. subsp. paratuberculosis (Kunze et al., 1992; Eriks et al., 1996; Whittington et al., 1998). However, to epidemiological studies, strains differentiation is useful, which can be obtained through Restriction Fragment Length Polymorphism (RFLP) (Whittington et al., 1998). IS901 is specific from M. avium serotypes 1, 2 and 3 isolates and there are 2-13 copies in the genome (Dvorska et al., 2004). The IS1245 was detected on isolates from bird samples, corresponding to M. avium subsp. avium (Mijs et al., 2002), and are present 6-20 copies in isolates from M. avium subsp. hominisuis (Dvorska et al., 2004).

Mycobacterium avium complex’ serotypes 7, 12-20 and 22-28 which don’t contain IS901 nor IS1245 were designated M. intracellularare (Dvorska et al., 2004).

Restriction fragment length polymorphism (RFLP) isn’t still currently used in epidemiological studies about M. avium infections. There are 2 insertion sequences, IS1245 and IS1311, who shares 83-85% of DNA identity, consistently present in M. avium and exhibit high RFLP diversity on human’s isolates (Picardeau and Vincent, 1996; Whittington et al., 1998).

Conclusion

The members of MAC are the most prevalent opportunistic pathogenic mycobacteria causing infection both in animals and humans. Mycobacterium bovis is the major responsible for tuberculosis in mammals. The culture of mycobacteria is difficult and fastidious, so other diagnosis strategies were developed. There are specific PCR protocols which are reliable, fast and cheap for the detection and differentiation of M. avium subspecies and M. bovis, from solid plate cultures and heavily infected tissues, for use in routine veterinary diagnosis. It also contributes to increase the amount of epidemiological studies, describing prevalence and incidence of mycobacterial infections in wild populations and identification of maintenance hosts.

The study of mycobacteria in wild animals contributes to diminish the role of these animals as vectors on the transmission to domestic animals and people.

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