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Serological survey for antibodies against *Mycobacterium avium* complex in hunting dogs in East-Central Portugal

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Abstract: The *Mycobacterium avium* complex (MAC) causes infections and diseases in humans, birds, livestock, and wildlife, among others. In dogs, MAC infection is not frequent; however, due to its potential zoonotic characteristics, mainly in immunocompromised patients, the study of MAC epidemiology is necessary. The aim of this work was to perform a study to detect the presence of antibodies against MAC in serum samples of 53 hunting dogs in the Idanha-a-Nova municipality of East-Central Portugal. A total of 23 dogs were seropositive by commercial ELISA test (43.4%, 95% CI: 30.1%–56.7%). Factors such as age, breed, and sex were not significant. The results of the current study indicate that further investigations about the diagnosis and epidemiological surveillance of the MAC in dogs must be carried out to highlight its significance in animal and public health.

Key words: ELISA, *Mycobacterium avium* complex, seropositive, hunting dogs

The *Mycobacterium avium* complex (MAC) belongs to the family of nontuberculous mycobacteria and it is a major health problem worldwide in humans and animals (1,2).

The MAC causes disseminated disease in patients. It causes avian tuberculosis as well as disseminated infections in immunocompromised patients and lymphadenitis in humans and other mammals (3).

MAC infection in dogs is not frequent (4–6) and it diagnosis is difficult because of its nonspecific presentation (7). However, a few reports indicated that dogs infected by the MAC present with lymphadenopathy, hematologic abnormalities, and cutaneous granulomas (8–10). The role of dogs in MAC epidemiology is currently unknown due to the scarce information available, although it could represent a potential risk for zoonotic transmission for immunocompromised patients (11). The aim of this work was to perform a study to detect the presence of antibodies against the MAC in hunting dogs in Portugal to improve the knowledge about its epidemiological role in wildlife.

During the hunting season of 2013 a total of 53 healthy dogs were blood-sampled by veterinarians for MAC detection in the municipalities of Idanha-a-Nova (39°55′11″N, 7°14′12″W) and Penamacor (40°10′8″N, 7°10′14″W), both belonging to the Castelo Branco district of East-Central Portugal. All dogs' owners were previously informed about the objective of the study.

Blood samples were transported within 8 h to the laboratory using isothermal portable containers. Serum samples were then obtained by centrifugation at 2500 rpm for 15 min and stored at –20 °C until use.

For statistical analysis, data such as age (>18 or ≤18 months-old), sex (male/female), breed (Podengo breed/crossbreed), body condition (good/poor), and intended use (hunting/pet and hunting) were collected.

Serum samples were tested for immunoglobulin G (IgG) antibodies (anti-multispecies IgG-HRP conjugate-concentrated, 10×) against MAC using commercial ELISA according to the manufacturer's instructions (ID Screen - *Mycobacterium avium* indirect multispecies, ID Vet, Grabels, France).

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The specific ELISA test is used to measure serum antibodies against the MAC using an absorption step to remove nonspecific antibodies. On each 96-well plate, serum samples were tested in single wells. The negative and positive control samples, provided by the manufacturer, were run in duplicate (first 4 wells). Results were expressed with the mean sample-to-positive ratio (S:P ratio) = [OD_{450} of sample – OD_{450} of negative control]/[OD_{450} of positive control – OD_{450} of negative control]. According to the manufacturer's instructions, readings were considered negative or positive when the reading was ≤40% or ≥50% of the positive control, respectively. Readings of >40% and <50% were considered doubtful. Doubtful results were run in duplicate wells with the same protocol. Differences in seropositivity among demographic categories in dogs were compared with a chi-square test ($\alpha < 0.05$) for statistical significance. Confidence limits for the proportions were established by exact binomial test with 95% confidence intervals (CIs).

Of the 53 dogs studied (Table), 23 (43.4%, 95% CI: 30.1%–56.7%) were positive for MAC by ELISA. Positive results were observed mainly in males (65.2%), dogs over 18 months old (95.7%), those belonging to Portuguese breeds (69.6%), and those with good body condition (87.0%). Moreover, positive results were higher in dogs exclusively used for hunting (82.6%). However, none of the demographic characteristics influenced the results ($P > 0.05$).

The MAC can cause infection in both humans and animals (7,12). In dogs, MAC infection has been recently described by PCR (5,13). However, data on the epidemiological impact and diagnosis techniques of this complex are still scarce. To the best of the authors' knowledge, this is the first report about MAC diagnostics in dogs by ELISA. Hunting activities represent important economic income in the geographical area under study. Since the geographical area under study presented a high prevalence of tuberculosis in game animals (14), the epidemiological studies published (14,15) suggested that wild animals could play an important risk factor for *Mycobacterium* spp. infection not just for animal health but also for public health. With the aim to improve tuberculosis surveillance and subsequent control in game, the National Veterinary Authority developed a specific program (16) to guarantee the public health, mainly focused on hunters.

The role of dogs in the epidemiology of the MAC is complex and unknown. The high number of positive animals among adults, males, and autochthonous hunting breeds could be associated with the higher preference for dogs with these characteristics by hunters and also by the widespread exposure to the etiological agent during hunting activities. Although the dogs surveyed did not present symptoms of MAC infection, some clinical signs such as anorexia, fever, diarrhea, lymphadenopathy, or lameness have been described depending on the dissemination degree and/or organ involvement (4,5,17,18). Previous studies also indicated that breeds such as miniature schnauzers and basset hounds are more predisposed to mycobacterial infection (5,10,17,19), although no differences were observed among Portuguese hunting breeds and crossbreeds. Moreover, immunocompromised animals are thought to be at greatest risk (5).

Table. Demographical characterization and seropositivity.

		Number of dogs tested (n)	Seropositivity (%)	95% CI (%)
Sex	Male	28	65.2	52.4–78.0
	Female	25	34.8	21.9–47.6
Age	>18 months old	47	95.7	90.2–100
	≤18 months old	6	4.3	0–9.8
Body condition	Good	48	87.0	77.9–96.1
	Poor	5	13.0	3.9–22.1
Breed	Podengo breed	33	69.6	57.2–81.9
	Crossbreed	20	30.4	18.0–42.8
Intended use	Hunting	44	82.6	72.4–92.8
	Pet and hunting	9	17.4	7.2–27.6
Total		53	43.4	30.1–56.7

The pathogenesis of mycobacterial infection in dogs is not well understood. As the high prevalence of tuberculosis in humans is mainly associated with immunocompromised status, a mechanism involving immunosuppression has therefore been proposed to occur in dogs (10), but there is no information available to prove this.

The possible role of hunting dogs in the epidemiology of the MAC in game needs to be ascertained. The source of infection is unknown, but dogs can be infected by contact with a contaminated environment (7) or by consumption of infected game meat (20). Since the significance of serological MAC diagnosis is limited by the dog's exposure to the etiological agent, further investigations about MAC infection by other techniques such as PCR could improve the knowledge about the epidemiology of the MAC in dogs as well as its relationship with other sources of mycobacteriosis in game and/or livestock. Moreover, close contact with owners could be considered as a risk factor for zoonotic transmission.

The zoonotic transmission route is still unclear because there is no information available about the excretion of the bacteria by dogs. Since dogs presented an innate resistance to *Mycobacterium* spp. (21), it is probable that excretion could be associated with cases of severe intestinal involvement such as enteritis or diarrhea since other mycobacteria such as *M. paratuberculosis* have been isolated from feces of infected sheep (22). As a consequence, there may be an increased risk of zoonotic infection in cases where dogs with intestinal clinical signs are in close contact with owners (23), and special attention should be paid to immunocompromised patients.

In conclusion, the MAC is an ubiquitous agent with wide host ranges. The immunological diagnosis of MAC in hunting dogs in an area with a high prevalence of mycobacteriosis indicates a potential relationship of MAC epidemiology with the wildlife–environment interface. Since the MAC is classified as a zoonotic agent, with a special interest in immunocompromised patients, the results of the current study indicate that further investigations about MAC diagnosis and epidemiological surveillance in dogs must be carried out to highlight their significance in game and public health.

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