Preliminary Seroepidemiological Investigation Regarding *Mycobacterium Avium* Infection in Wild Boars and Foxes

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Abstract

The present study aimed at identifying *Mycobacterium avium* seroprevalence in wild boars (*Sus scrofa*) and red foxes (*Vulpes vulpes*) samples, from the Eastern region of Romania, by using an indirect ELISA assay. A total of 367 wild animal samples were collected from eight counties: 275 wild boars sera samples from Iasi (n=209), Botosani (n=11), Bacau (n=17), Galati (n=27) and Covasna (n=11) and 92 fox samples of thoracic fluid from Iasi (n=30), Suceava (n=20), Neamt (n=19), Vaslui (n=11) and Galati (n=12). Specific antibodies were not detected in wild boar serum samples tested. From the fox samples, one was positive (1.08%), originating from Galati County. Our results may be correlated with a reduced distribution of *Mycobacterium avium* subspecies in the environment and a low prevalence of infections caused by these bacteria in wild animals. The current assessment shows that foxes have a limited role in the epidemiology of nontuberculous mycobacterial infections.

Keywords: *Mycobacterium avium*, seroprevalence, wildlife

Introduction

Infections caused by nontuberculous mycobacteria (NTM) of the *Mycobacterium avium* Complex (MAC) were reported in various domestic or wild species, ruminant, omnivorous or carnivorous. MAC members, most notably *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and *Mycobacterium avium* subsp. *avium* (MAA), are the most commonly reported species, causing in some wildlife species similar lesion aspects to those encountered in domestic animals (Thorel *et al.*, 2001).

Evidence of natural infection with MAC members in wild animals is provided by studies

conducted in various countries as Scotland (Beard *et al.*, 2001), Spain (Boadella *et al.*, 2011), Portugal (Matos *et al.*, 2015) or Norway (Tryland *et al.*, 2004). The wild boars (*Sus scrofa*) are amongst the species with a large territorial distribution in Europe. NTM infection and dissemination can be possible in wild boars, considering the species adaptability to environmental changes and the ability to cross natural barriers, the omnivorous diet, the contact with many other wild and domestic animal species and the wide distribution (Boadella *et al.*, 2011). Numerous studies have highlighted the role of feral pigs in maintaining and spreading

mycobacteria from MAC and *Mycobacterium bovis* (Trcka *et al.*, 2008; Naranjo *et al.*, 2008, Richomme *et al.*, 2019). The lesions are rarely observed, more frequently in adult animals and are usually present in the lymph nodes. MAC infections in carnivores are most often localized at the digestive tract and occur as a result of ingestion of the bacilli during feeding, environmental contamination being unlikely. Small rodents, birds and rabbits included in fox diet are the most likely the source of infection.

Determining the prevalence of nontuberculous mycobacterial infections among wild animal populations provides significant additional information on the distribution and transmission of these infections. In the context of a common habitat, where there may be interactions between cattle and wild animals and taking into account the opportunistic infectious capacity of the NTM, their resistance in the environment and the possibility of developing a sensitisation to tuberculin condition in cattle, information regarding the epidemiology of NTM infection in wildlife is of valuable use in assessment of the tuberculosis control program efficiency. Furthermore, the evaluation of the health status of wild animal populations should not be neglected.

To our knowledge, no serological investigations have been carried out so far regarding infections with MAC members in wild animals, in Romania.

The purpose of this study was to conduct a seroprevalence study regarding the presence of anti-MAC antibodies in wild boars and wild foxes, from seven counties located in Eastern Romania and one in the central region of the country.

Materials and methods

In this study, 275 randomly collected wild boar sera samples from the following counties in Eastern Romania were tested: Iasi (n=209), Botosani (n=11), Bacau (n=17), Galati (n=27), along with eleven samples from Covasna County. Sampling was performed during the 2017-2018 hunting seasons, sera being stored at -20°C, until the time of testing. Age, gender and the origin hunting fund of wild boars from Iasi County are presented in Table 1. These data were not available for the wild boars in the other counties.

In addition, 92 samples of thoracic fluids from wild foxes (*Vulpes vulpes*) were tested. Fox samples were collected during 2015, after rabies vaccination campaign and previously tested for effectiveness of oral rabies vaccinations. The study included fox samples originated from five counties in N-E area of Romania (Figure 1).

For the identification of anti-*Mycobacterium avium* IgG antibodies in serum samples and thoracic fluids, an indirect ELISA commercial kit was used (ID Screen® *Mycobacterium avium* Indirect Multi-species, ID.vet, Innovate Diagnostics, France).

Figure 1. Geographic origin of the fox samples

Table 1. Data regarding the distribution and origin of the samples from Iași County

Hunting fund (HF)	Number of tested samples /funds	Gender		Age category		
		M	F	Yearling	Juvenile	Adult
1 Tatarusi	1		1			1
2 Motca	2		2			2
3 Miroslovesti	2	2				2
4 Halaucesti	1		1			1
5 Stolniceni Prajescu	4		4			4
6 Stroesti	1	1			1	
7 Harmanesti	1	1				1
8 Siretel	2	2				2
9 Bahlui	24	8	16		8	16
10 Catalina	3	2	1	2		1
17 Gropnita	1	1				1
22 Turia Perieni	23	11	12		6	17
23 Larga Jijia	2	1	1			2
24 Probota	3	2	1			3
25 Letcani	2	2				2
27 Victoria	8	4	4		1	7
28 Golaesti	3	2	1	1		2
29 Tutora	2	2				2
30 Prisacani	4	4				4
31 Strunga	3	3				3
32 Crivesti	5	3	2		4	1
33 Braiesti	6	2	4			6
35 Sinesti	4		4	4		
37 Gheorghitoaia	8	8			3	5
38 Horlesti	4	2	2	2		2
40 Mogosesti	7	2	5	1	2	4
41 Barnova	1	1				1
42 Poieni	14	4	10	1	3	10
43 Schitu Duca	4	2	2		2	2
44 Pietrosu	7	1	6		3	4
45 Pocreaca	2	1	1			2
46 Bradicesti	3		3		3	
47 Bunesti	2	1	1		1	1
48 Crasnita	14	11	3	1	2	11
49 Gorban	4	3	1		1	3
50 Raducaneni	7	5	2			7
51 Grajduri	16	11	5		3	13
52 Mironeasa	4	1	3		2	2
53 Tibana	1	1			1	
54 Tibănești	4	2	2		2	2

Sample testing was performed according to the manufacturer's specifications. The test presents a wide applicability, not only to detect the circulation of *Mycobacterium avium* subsp. *hominissuis* at herd level, in domestic pigs, but can also be used on serum and plasma samples from a variety of domestic (sheep, goats, cattle and pigs) and wild animals. According to the values provided by the manufacturer and preliminary studies (Lesceu and Pourquier, 2011; Eisenberg *et al.*, 2012), the used ELISA method presents a sensitivity ranging from 34.48% to 44% and a specificity of 100%.

The plates were read at 450nm wavelength, using the ELISA Sunrise™ Absorbance Reader (Tecan Group Ltd., Männedorf, Switzerland). The evaluation of the obtained results was performed according to the protocol formulas, samples being considered negative if the Cut-off was lower than 40% and positive if higher than 50%. Confidence intervals were calculated using the on-line platform VassarStats: Website for Statistical Computation (<http://vassarstats.net/>).

Results and discussions

Following the serological testing of the 275 wild boar sera, with the use of a commercial indirect ELISA kit, no anti-*Mycobacterium avium* antibodies were detected in evaluated serum samples. As such, no correlation could have been made with gender and age. Only one fox sample from Galati County was detected positive (1.08%, CI 95%: 0.1% - 5.9%) for *Mycobacterium avium* antibodies.

Amongst MAC members, MAP is the nontuberculous mycobacteria with the greatest negative impact on animal welfare. The importance of wildlife in paratuberculosis transmission is so far recognized (Stevenson *et al.*, 2009), the aetiological agent being isolated from different wild species, including *Suidae* -wild boars and *Canidae* -foxes (EFSA AHAW Panel, 2017).

Wild boars are known to be relevant reservoirs for *Mycobacterium bovis* in the Iberian Peninsula (Aranaz *et al.*, 2004, Boadella *et al.*, 2011), especially in Spain, where game-management practices are common (Naranjo *et al.*, 2008). Moreover, tuberculous mycobacteria were isolated from this species in other European countries also and depending on the epidemiological context, wild boars may act as spill-over hosts, maintenance

hosts or super-shedders for bovine tuberculosis (Richomme *et al.*, 2019).

Studies on MAC infection in wild boar populations are quite a few. The wild boar is an omnivore which, in the wild, occasionally feeds on small animals and birds, such as rabbits (*Lepus europeus*), pheasants (*Ithaginis* spp.), partridges (*Perdix perdix*) or young ruminants. The wild boar also feeds on remains of carcasses from dead wild animals or domestic animals improperly disposed of (Machackova *et al.*, 2003). Thus, mycobacterial infection may be transmitted through both respiratory (during digging) and digestive (ingestion of debris from contaminated pastures, offal from infected dead animals) routes (Machackova *et al.*, 2003).

In general, a low frequency of MAP is expected in wild boars, up to 5% according to the results reported by Alvarez in Spain or Machackova in various countries in central Europe (Alvarez *et al.*, 2005, Machackova *et al.*, 2003). Moreover, it is considered that in areas where other mycobacterial infections are prevalent, wild boars are not the most relevant marker for paratuberculosis (Carta *et al.*, 2013). Boadella *et al.* (2011) evaluating the presence for MAP antibodies (PPA 3) in wild boars' sera, using an ELISA method, suggested that wild boars cannot be considered sentinels for MAC infections. MAA infection in wild boars is very difficult to detect, however Lara *et al.* (2011) have succeeded in isolating the mycobacteria from mesenteric and submaxillary lymph nodes.

The lack of seroconversion revealed by this study could be explained by a real low level of exposure to MAC mycobacteria. Using the same method, Matos *et al.* (2015) identified a prevalence of 23.9% for the wild boar tested in Portugal. Moreover, our results can be correlated with a lower number of paratuberculosis outbreaks (n=27) identified between 2014-2018 in our country and reported in the World Animal Health Information System (WAHIS) portal of World Organisation for Animal Health (WOAH-OIE). Moreover, in the N-E region of the country, only one outbreak was reported in Iasi County (1 outbreak in 2015) and two in Botosani (two outbreaks in 2016).

Garrido *et al.* (2010) observed that in case of MAA experimental infection in wild boars, clinical signs are absent, and lesions occur in the head and mesenteric lymph nodes at an infective dose of 2.5×10^6 mycobacterial cells (Garrido *et*

al., 2010). These observations are reinforced by the lesions observed by other authors in natural infections (Machackova *et al.*, 2003). In addition, it was suggested that depending on the path of infection, respiratory (in close contact with shedders) or digestive tract (consumption of infected tissues), the development of the immune response is different and may be correlated with the mycobacterial infection manifesting potential (Garrido *et al.*, 2010).

The use of serological methods for MAC infections evaluation in wild mammals is not widely performed, making a difficult analysis of our results. Using the same testing procedure, Matos *et al.* (2015) identified a single positive fox sample out of 42 tested (2.4%). MAP was isolated or identified by PCR in foxes in Europe (Beard *et al.*, 2001, Florou *et al.*, 2008). In Portugal, infection with MAP is common among wild carnivores (Matos *et al.*, 2014).

Furthermore, in countries where the paratuberculosis prevalence is higher, evidence of infection is encountered in wild animal hosts. Our results may be correlated with a reduced distribution of MAC members in the environment and a low prevalence of infections caused by these bacterial agents in animals, either wild or domestic. A lack of involvement or a limited role of foxes in the epidemiology of nontuberculous mycobacterial infections is revealed from the current assessment. As in many grazing areas from the assessed counties cattle, sheep and goats are raised in extensive systems, the common interface between domestic and wildlife is created. Furthermore, as wild lagomorphs are linked to foxes and considered to pose the highest MAP transmission risk for livestock, of the non-ruminant hosts, a more accurate epidemiologic situation may be revealed by extending the number of fox samples and continue the evaluation in other species, such as rabbits.

Conclusions

To the extent of our knowledge, this is the first MAC seroprevalence study conducted in our country on wild animal species. Considering the fact that serosurveys on MAC infection in wildlife are not widely performed, and only few published results are available, the comparison of our results proved to be difficult. Though negative, the serologic response identified in this study opens

the research in the context of nontuberculous mycobacterial infections in wildlife, and further analysis is considered appropriate in order to observe whether in our country the mycobacterial infection shifted in wildlife direction and even if there is an underestimation in livestock.

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