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Surveillance of *Mycobacterium caprae* infection in a wild boar (*Sus scrofa*) population in south-western Hungary

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ABSTRACT

Bovine tuberculosis (bTB) is a re-emerging infectious disease in Europe, which causes a classical One Health problem in certain regions of the Continent. European experiences related to the wild boar's role in the epidemiology of bTB suggest that this species can be a maintenance host of the disease. In south-western Hungary, *Mycobacterium caprae* infection is known to be endemic in connection with south European bTB infected wild boar populations. Our goal was to carry out surveillance among wild boars inside this region to determine the prevalence and possible risk of the disease. In the study area (9600 ha) three large-scale cattle farms existed and over the previous 10 years bTB outbreaks were confirmed on each. Between 2008 and 2013 we sectioned 791 hunter-harvested wild boars on a hunting ground during evisceration. Of the 267 bacteriologically examined specimens, 36 (13.5%) proved to be infected by *M. caprae*. In the field we found 233 carcasses with suspect tuberculosis lesions (TBL). TBLs were generally found in the submandibular lymph nodes; while only two carcasses (n = 2; 0.25%) were found with TBL exclusively outside that region. These lesions could not be inspected without incision; on the other hand, generalization appeared to be very rare (n = 2; 0.25%) in the study area. These findings suggest that visual-only game meat inspection is insufficient to find the primary complex of bTB infection in wild boars. Although these localized small lesions are most unlikely to create any notable hazard for game meat consumers, nevertheless this lack in game meat inspection makes the surveillance system incomplete. Our study confirmed that wild boars play a maintenance role as a bTB host

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in this part of Hungary. However, development of an effective management strategy against bTB needs further investigations by a multidisciplinary research group.

Key words: bovine tuberculosis, *Mycobacterium caprae*, one health, submandibular lymph node, surveillance, wild boar

Introduction

In recent decades, natural reservoirs have become unavoidable in studying the epidemiology of bovine tuberculosis (bTB), because the test-and-slaughter method failed to achieve and maintain freedom from the disease in the presence of a wildlife reservoir. The recognition that emerging infectious diseases should cause complex human-animal-environmental health problems led to the One Health concept. This method is based on a holistic approach, as the human population, livestock, and the natural environment are considered as a comprehensive whole (ZINSSTAG et al., 2012).

Bovine TB is a zoonotic disease which can be maintained by natural reservoirs, therefore methods of ecology and population biology should be useful in the epidemiological investigation of the disease. Thereby bTB is a characteristic One Health issue. Special ecological factors determine the maintenance or spill-over roles of a host. These factors might be: population density, social behaviour, the feeding ecology of the host specie, or other characteristics of the local ecosystem (RHYAN and SPRAKER, 2010; MARTIN et al., 2011). Former studies have considered the badger to be an important reservoir species exclusively in the British Isles (GALLAGHER and CLIFTON-HADLEY, 2000; SOBRINO et al., 2008), whereas on the Continent, the species which may play a significant role in the epidemiology of bTB is the wild boar (NARANJO et al., 2008). *Mycobacterium caprae* has proven to be a major cause of bTB in central European livestock and wildlife (PRODINGER et al., 2005). In the last decade, several investigations have confirmed that wild boars can maintain bTB infection, even in the absence of another susceptible species (NARANJO et al., 2008).

Populations of wild boars expand all over Europe (MASSEI and GENOV, 2004; MARTIN et al., 2011). The causes of this increase are the special adaptability of the species (MASSOLO and MAZZONI, 2006) and artificial population development because of hunting interest (MERLI and MERIGGI, 2006). Intensive hunting treatment causes permanent perturbation, artificial feeding causes temporal overabundance, and social and dietetic stress for the animals (VICENTE et al., 2006; GORTÁZAR et al., 2011). These facts suggest that wild boar may become a maintenance host on any site where there is intensive boar hunting management.

A characteristic feature of wild boars' feeding ecology is scavenging, especially in winter; therefore wild boars may come into contact with infected materials (GORTÁZAR et al., 2008). Individuals of wild boar populations may be found in the closest vicinity to human settlements, hence they bring infection close to domestic animals (MASSEI and GENOV, 2004; LEMEL et al., 2003).

Continuous surveying of a potential reservoir species, such as the wild boar, is definitely important to determine the risk from the natural environment for bTB infection of cattle farms and game meat consumers. Although bTB is not a considerable health risk for game meat consumers (SANTOS et al., 2010), it is important to detect even early lesions in order to assess bTB prevalence in wildlife and the epidemiological risk caused by natural environment.

Earlier European studies' results show that visible suspect tuberculous lesions exist in the submandibular lymph node (smLN) of bTB infected wild boars very frequently (MARTÍN-HERNANDO et al., 2007; DE GARINE-WICHATITSKY et al., 2010). Based on these findings, we hypothesized that necropsy screening of these organs is able to provide information about bTB epidemics.

In this study, we inspected hunter-harvested wild boars in south-western Hungary, which is a bTB high risk area. Our goals were to calculate the prevalence of TBL and bTB in the studied population, and in so doing we attempted to determine the epidemiological role of wild boars in this part of Hungary. Our further aim was to promote the development of a management strategy against bTB in wildlife.

Materials and methods

Study area. Our study was implemented inside and in the close surroundings of the Zselic Landscape Protection Area, a part of the south Transdanubian Region of Hungary, from 2008 to 2013 (Fig. 1). In the study area (9600 ha), there were three large-scale cattle farms; and over the previous 10 years bTB outbreaks had been confirmed on each. Identical *M. caprae* strains had been previously isolated from all the infected cattle, red deer and wild boar individuals (personal communication by Szilárd Jánosi). This part of Hungary is a very important and well-known big-game hunting area, which is managed intensively.

Sample collection. Based on the strong site fidelity of wild boars, we attempted to select the hunting days in a certain season that represented a formerly not examined part of the area. Daily hunting bags consisted of 5-45 carcasses, and all these were included in the investigation except inadequate specimens, where the shooting had damaged the target organs. During the winter hunts, there was no selection for a specific age, size or health status; therefore the hunters' differing skills guaranteed randomness.

In the course of evisceration in the field, we carried out the post-mortem examination of 791 hunter-harvested wild boar carcasses. We inspected the tonsils, submandibular, retropharyngeal, tracheobronchial, mediastinal, hepatic, mesenteric and caecal lymph nodes for visible lesions, cutting specimens up into 3 mm slices, and we examined the cut surfaces. We also inspected the lungs, liver, spleen, kidneys and guts by palpation, and then we sectioned every suspicious deviation. We inspected the peritoneum and the pleura by observation and sectioned every suspicious granuloma or abscess.

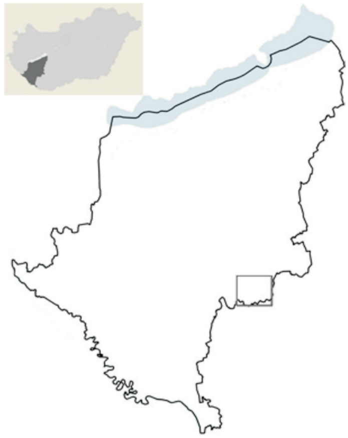

Fig. 1. Localisation of the study area in Hungary (small square)

Every purulent, caseous, caseo-calcareous abscess or calcification in any organ, and any sign of tuberculous pleuritis or peritonitis were qualified as TBL.

In each hunting season, we randomly chose one or two hunting days when the specimens (the tonsils, submandibular, retropharyngeal, tracheo-bronchial, mediastinal, hepatic, mesenterial and caecal lymph nodes and organs with lesions) from the whole hunting bag were sent to the laboratory; therefore 267 smLN samples came through bacteriological investigation.

Laboratory investigation. Samples were dissected and homogenized in a Stomacher (MiniMix 100WCC, Interscience, Arpents, France) with 10 mL sterile saline solution, decontaminated in 5% oxalic acid solution for 15 minutes; and centrifuged at 3000*g* for 10 minutes. The sediment was re-suspended in 2 mL sterile PBS and inoculated into Middlebrook broth, Herrold's, Lowenstein-Jensen and Lowenstein-Jensen slants, supplemented with pyruvate. Slants were incubated for at least 8 weeks at 37 ^{o}C, and checked for contamination and mycobacterial growth weekly, while the Middlebrook broth was checked by Ziehl-Neelsen (Z-N) staining every month.

All isolates were tested in a multiplex amplification system described by WILTON and COUSINS (1992) that can identify the genus *Mycobacterium* and then distinguish between *Mycobacterium tuberculosis* complex (MTC), *M. avium* complex (MAC) and *M. intracellulare* organisms.

MTC isolates were further tested with GenoType MTBC kit (Hain Lifesciences, Nehren, Germany) according to the manufacturers' instructions, which permits the genetic differentiation of *M. africanum*, *M. bovis* BCG, *M. bovis*, *M. caprae*, *M. microti* and *M. tuberculosis/"M. canettii"* strains on the basis of gyrase B gene polymorphisms.

Statistics. In order to determine whether the laboratory results are representative of the whole population, we compared the TBL prevalence rates of all the specimens with the one examined in the laboratory by Pearson's Chi-squared test using R-statistics software version 3.1.2.

Results

In the field study 233 (29.5%) of the 791 investigated carcasses proved to be positive on post-mortem examination (Table 1). Among the 267 carcasses submitted to the laboratory 88 (33.0%) showed suspect bTB lesions; while 36 (13.5%) proved to be infected by *M. caprae* (Table 2). No other *Mycobacterium tuberculosis* complex bacterium species were identified.

In most cases (n = 222; 28.1%), lesions were restricted to the submandibular lymph node, while generalized tuberculosis was extremely rare (n = 2; 0.25%).

Table 1. Post-mortem findings of wild boars examined in the field

<table>
<tr><td rowspan="6">Post-mortem examination in the field (n = 791)</td><td rowspan="5">Positives by post-mortem examination (n = 233)</td><td rowspan="3">Visible lesions in the submandibular lymph node (n = 231)</td><td>Only in submandibular (n = 222)</td></tr>
<tr><td>In submandibular and chest cavity (n = 7)</td></tr>
<tr><td>Generalized bTB (n = 2)</td></tr>
<tr><td rowspan="2">No visible lesion in the submandibular ln. (n = 2)</td><td>Lesions in a testicle and the hepatic lymph node. (n = 1)</td></tr>
<tr><td>Lesions in the caecal lymph node (n = 1)</td></tr>
<tr><td colspan="3">Negatives by post-mortem examination (n = 558)</td></tr>
</table>

Table 2. Laboratory findings

Number of wild boars examined in the laboratory	Number of negatives by post-mortem examination	Number of positives by post-mortem examination	*M. caprae* infection confirmed by culture
267	179 (67%)	88 (33.0%)	36 (13.5%)

The difference between the apparent prevalence of all the examined samples and laboratory examined ones was not significant using Pearson's Chi-squared test (P-value = 0.25), hence the two groups of samples showed statistical homogeneity.

Discussion

Analyzing other people's findings about the role of wild boar in *M. caprae* originated bTB epidemics, we hypothesized that wild boar can be a good indicator of bTB's environmental epidemiology in continental Europe. For this reason, we examined wild boar hunting bags in an intensively managed hunting area, in the close vicinity of *M. caprae* infected cattle herds.

Similarly to the results of VICENTE et al. (2006), we found that a considerable proportion of wild boars living in the infected area had TBL. The pattern of lesions we found in wild boars slightly differed from the results of ZANELLA et al. (2008) but was consistent with other studies (MARTÍN-HERNANDO et al., 2007; GORTÁZAR et al., 2008; SANTOS et al., 2010). Our study showed that the organs that had visible lesions most frequently were smLNs, whereas the exclusive occurrence of TBL outside these lymph nodes was extraordinary.

The severity of the pathological progress we found was lower on average than that found by others (MARTÍN-HERNANDO et al., 2007; SANTOS et al., 2010), since in our study generalized bTB cases were markedly rare (<1%). This phenomenon is presumably explainable by the different wildlife management systems, there is probably lower population density and less frequent drive hunting in this region of Hungary than on the Iberian hunting grounds. The studied area yields abundant feeding (mature oak and beech and agricultural lands) but insufficient water sources (temporary surface waters), which causes temporary aggregation of dense wildlife around waterholes in summer droughts. On the other hand, human activity is sparse because of abandonment of the countryside, and causes merely mild perturbation. Thus, migration is slight and the population may be relatively balanced. These factors should affect the immune response ability of wild boars and the number of infective contacts inside the population.

The experiences that lesions were principally localized to the smLN, the negligible number of examined animals with lesions exclusively outside that, and all the generalized bTB diseased carcasses with TBL in the smLN, suggests that necropsy of this organ alone provides valuable data about TBL prevalence in wild boar populations.

Nevertheless, investigation of the smLN is impossible without an incision as visual-only game meat inspection rules prescribe (HILL et al., 2014). Early lesions are very unlikely to be detected by visual inspection or palpation. Although, bTB might not cause a considerable health risk for game meat consumers, this weakness of game meat inspection shows the insufficiency of wildlife health surveillance. This infringes the One Health concept, because the chance to detect the initial stages of an endemic disease may be lost to the animal health service.

In our study, the prevalence of bTB infection proved to be 13.5% by culture of randomly selected wild boars but severe lesions and even generalized cases were very rare. Nevertheless, the outbreaks on cattle farms in the study area suggest that this epidemiological situation needs management. Experiences in Spain contradict our results, whereas in Mediterranean regions a higher prevalence and more severe lesions in wild boars cause outbreaks in livestock (MARTÍN-HERNANDO et al., 2007; SANTOS et al., 2010).

Since no such a systematic surveillance had ever been undertaken before in Hungary our results cannot be compared within the country. Nevertheless, it is suspected that wild boar may act as a maintenance host at a relatively low rate of infection. However, our study could not determine management strategies against bTB in wild boars. Whereas the population density in the study area is considered high, culling appears evident but the negative experience with badger culling strongly indicates the need to be cautious (GALLAGHER and CLIFTON-HADLEY, 2000). Before any active intervention, a prudent investigation is needed in the fields of ecology, population biology, and veterinary epidemiology. This needs a multidisciplinary research group with professionals of the above-mentioned specializations because emerging infectious diseases, which are maintained by natural reservoirs, require a special approach. The One Health concept provides an opportunity for veterinary epidemiologists to apply the knowledge of several professions.

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Conflict of interest

Authors declare that no financial or personal conflict of interest exists.

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SAŽETAK

Goveđa tuberkuloza je re-emergentna zarazna bolest u Europi koja dovodi do klasičnih poteškoća u pristupu „jedno zdravlje“ na određenim područjima. Europska iskustva o ulozi divlje svinje u epidemiologiji goveđe tuberkuloze govore da ta vrsta može biti rezervoar uzročnika. Poznato je da je zaraza vrstom *Mycobacterium caprae* endemska u jugozapadnoj Mađarskoj i da je povezana s populacijom divljih svinja južne Europe zaraženih uzročnikom goveđe tuberkuloze. Cilj je ovoga rada bio istražiti prevalenciju i moguće rizike od infekcije divljih svinja na tom području. Na istraživanom području (9600 ha) postoje tri velike goveđe farme na kojima je u prethodnih 10 godina potvrđena pojava tuberkuloze. Između 2008. i 2013. bila je eviscerirana 791 divlja svinja ustrijeljena u lovištima toga područja. Od 267 bakteriološki pretraženih, *M. caprae* dokazan je u 36 (13,5%) uzoraka. Na terenu su pronađene 233 lešine sa sumnjom na tuberkulozne promjene. One su općenito primijećene na submandibularnim limfnim čvorovima dok su izvan njih dokazane promjene samo u dvije lešine (n=2; 0,25%). Te lezije ne bi se mogle uočiti samo inspekcijom bez zarezivanja. Generalizacija je utrđena vrlo rijetko (n=2; 0,25%). Ti nalazi upućuju na zaključak da je samo inspekcija mesa divljači nedovoljna da bi se ustanovio primarni tuberkulozni kompleks u divljih svinja. Iako te lokalizirane male promjene vjerojatno ne mogu značajno naškoditi konzumentima mesa divljači, ipak ovaj nalaz govori da je samo inspekcija divljeg mesa nedostatna. Ovo istraživanje potvrđuje da divlje svinje imaju glavnu ulogu u održavanju goveđe tuberkuloze na istraživanom području Mađarske. Usprkos razvitku učinkovite strategije za kontrolu goveđe tuberkuloze potrebna su daljnja multidisciplinarna istraživanja.

Ključne riječi: goveđa tuberkuloza, *Mycobacterium caprae*, „jedno zdravlje“, submandibularni limfni čvor, nadzor, divlja svinja

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