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Post mortem examination of submandibular lymph node in wild boars (Sus scrofa) as a beneficial part of bovine tuberculosis surveillance systems

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ABSTRACT

In Europe the wild boar proved to be the most important reservoir of bovine tuberculosis (bTB) with visible lesions regularly in the submandibular lymph nodes. Based on these facts authors hypothesized that submandibular lymph node of the wild boar can be a good target for screening of bTB endemics. Samples were collected during evisceration of 833 carcasses between 2010 and 2014. The post-mortem examination of submandibular lymph nodes were compared with culture result of the whole lymph node set of each animal. Sensitivity and specificity proved to be 89.7% and 72.9% respectively, which suggests that surveillance based on post-mortem examination of the submandibular lymph node of wild boars could be an effective tool in disease management.
(Keywords: cost-effectiveness; reservoir; screening; wildlife)

INTRODUCTION

The main impediment of the struggle against bovine tuberculosis (bTB) is the existence of wildlife reservoir (*Fitzgerald and Kaneene,* 2012; *Naranjo et al.,* 2008). In Europe bTB is caused by two zoonotic agents *M. bovis* and *M. caprae* (*Aranaz et al.,* 2003; *Varga and Tekes*, 2007). It is confirmed that without an appropriate surveillance system disease management can't be successful (*Hadorn and Stärk,* 2008). In the course of surveillance planning it is essential to select the most eligible target species. In this point of view scavenger species, that act as bio-accumulators of infectious agents, can come into question (*VerCauteren et al.,* 2008).

Within certain regions of Europe one of the most important bTB reservoir species is the wild boar (*Fitzgerald and Kaneene,* 2012; *Naranjo et al.,* 2008); however, elsewhere wild boars and feral pigs proved to be spill-over hosts and good sentinels of the disease (*Nugent et al.,* 2002). These species are omnivorous and very frequently consume carrions. By scavenging, wild boars can get infections; hereby bTB as well. The most frequently affected organs are the submandibular lymph nodes (*Fitzgerald and Kaneene,* 2012; *Naranjo et al.,* 2008); moreover, severe disease proved to be rare in most wild boar habitats. Susceptibility, presence of visible lesions, but absence of notable mortality render suids adequate sentinels of the disease (*Nugent et al., 2002*). By these experiences we hypothesized that screening by post-mortem examination of submandibular lymph node can yield valuable data to estimate the true prevalence of bTB in wild boar populations.

MATERIAL AND METHOD

Our study was carried out on six separate sites of Somogy County, in Hungary 2010-2014. One of these sites is a bTB hot spot where four cattle farm outbreaks, caused by *Mycobacterium caprae*, were detected during the previous 10 years; while on the others *M. caprae* could be detected sporadically only in wildlife (*Jánosi et al.*, 2009).

Samples, which contained the submandibular, retropharyngeal, tracheobronchial, mediastinal, hepatic, mesenterial and caecal lymph nodes; moreover, every other organs with suspect bTB lesions were collected during drive hunting when boars were shot randomly without any special selection for age, gender or health status. We evaluated every purulent, caseous or calcification process as suspect bTB lesion. If this was detected in the submandibular lymph node we qualified the specimen as 'positive by post-mortem examination'.

The whole lymph node set was submitted to bacterial culture, irrespectively to the result of post-mortem examination. Samples of each carcass were pooled, homogenized and decontaminated in 5% oxalic acid solution for 15 min; then centrifuged at 3000 *g* for 10 min. The sediment was re-suspended in 2 mL sterile phosphate buffered saline and inoculated into Middlebrook broth and onto Herrold's, Lowenstein-Jensen and pyruvate supplemented Lowenstein-Jensen slants; which were incubated for at least 8 weeks at 37 ^{o}C and checked for contamination and mycobacterial growth weekly, while Middlebrook broth was checked by Ziehl-Neelsen (Z-N) staining every month. All isolates were stained by Z-N and tested in a multiplex amplification system described by *Wilton and Cousins* (1992).

Mycobacterium Tuberculosis Complex (MTC) isolates were further tested with GenoType MTBC kit (Hain Lifesciences, Nehren, Germany) according to the manufacturer's 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. In all cases when *M. caprae* bacteria were isolated we qualified the individual as 'bTB positive'.

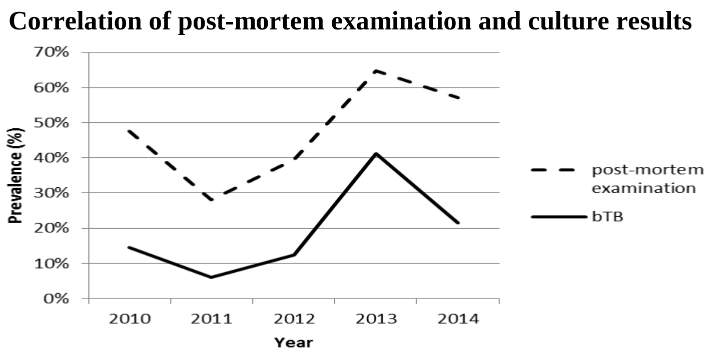
The post-mortem examination of the submandibular lymph nodes was compared with culture result of the whole lymph node set by Win Episcope 2.0 (*Thrusfield et al.,* 2001); while true prevalence of *M. caprae* infection was calculated by Sterne's exact method (*Lang and Reiczigel,* 2014; *Reiczigel,* 2003). The correlation between the prevalence of positives by post-mortem examination and culture was determined by analysing annual data collected on the bTB hot spot; where both positive and negative samples were available in an adequate count. For calculation of correlation we used R-statistics software version 3.2.1.

RESULTS AND DISCUSSION

The investigation of 833 wild boar carcasses resulted a strong correlation (r=0.91, P=0.03) between the prevalence of visible lesions of submandibular lymph node and bTB infection *(Figure 1).*

Sensitivity (89.7%; CI95%: 81.8–97.5%) and specificity (72.9%; CI95%: 69.8–76.0%) of this surveillance method make it eligible to be a screening component of a surveillance system (*Table 1*).

Figure 1



Prevalence of positives by post-mortem examination: rate of hosts with suspected tuberculous lesion in the submandibular lymph node; bTB prevalence: rate of hosts from which *Mycobacterium caprae* was isolated

Table 1

Detection of suspect tuberculous lesions and *Mycobacterium caprae* i n lymph node samples of wild boars

Origins of samples	*Number of wild boars sampled*	*Number of positives by post-mortem examination (% positive)*	*Number of false positive samples*[a]	*Number of false negative samples*[b]	*True prevalence of M. caprae infection (CI 95%)* [c]
Zselic [d]	422	166 (39.3%)	116	5	10.0 – 16.6%
Kelet-Zalai Dombvidék [e]	115	28 (24.4%)	28	0	0.0 – 3.3%
Külső-Somogy [e]	89	22 (24.7%)	22	1	0.1 – 6.0%
Nyugat-Belső-Somogy [e]	78	12 (15.4%)	12	0	0.0 – 4.8%
Kelet-Belső-Somogy [e]	94	28 (29.8%)	26	0	0.4 – 7.3%
Drávasík [e]	35	6 (17.1%)	6	0	0.0 – 9.6%
Total	833	262 (31.5%)	210	6	6.2 – 8.7%

[a]Suspected tuberculous visible lesion in the submandibular lymph node without detection of *Mycobacterium caprae* in the lymph node set
[b]*M. caprae* was isolated from the host without suspected tuberculous visible lesion in the submandibular lymph node
[c]Calculated from apparent prevalence statistically; CI = confidence interval
[d]Endemic area
[e]Sporadically infected areas

Our experiences about the pathology of bTB in wild boars differ from Iberian studies in severity (*Naranjo et al.*, 2008); as during our study we detected only 1–3 generalized cases annually; with the most lesions in submandibular lymph nodes. Nevertheless, agreement in the involvement of submandibular lymph nodes suggests that our experiences can be applied in any type of wild boar habitats as well.

In Europe wild boar populations are in expansion and thousands of wild boars are hunter-harvested and processed by game meat industry. Carcasses enter the game meat processing plants without viscera but with the head; accordingly with submandibular lymph nodes. These plants should be adequate places for large-scale screening of wild boars by post-mortem examination. Each wild boar carcass must go through meat inspection; thus our method doesn't need additional human resource because game meat inspectors can carry out this screening.

CONCLUSIONS

Although visible lesions cannot determine a specific pathogen unequivocally; we assume that cost-effectiveness compensates the relative inaccuracy. Our experiences suggest that in endemics true prevalence of bTB infection in wild boars can be estimated by post-mortem examination of submandibular lymph nodes; while in merely sporadically infected areas it can be applied to pre-select bacteriology specimens improving the cost-effectiveness of the surveillance system.

Our study highlights the potential zoonotic risk of natural environment; and confirms the great need of regular bTB surveillance inside abundant game populations.

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