

**University of Veterinary Medicine
Doctoral School**

Investigation on a sylvatic reservoir of bovine tuberculosis

Brief Summary of Doctoral Thesis

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Introduction and aims of the study

The cattle population of Hungary has been free from bovine tuberculosis (bTB) since 1980; though the European Union approved this status only in 2014. In Europe, twelve member states have got officially bTB free status; notwithstanding the mild increase of re-infection can be recognized all over the continent. In the background, the hardly manageable natural reservoir is suspected. In Hungary, two endemic areas are well-known. One of those is the Dunazug Mountains; while the other is the South Transdanubia with Zselic as a core area.

Despite the confirmed presence of bTB in wildlife, official surveillance system on natural reservoir does not run in either of the EU member states.

The primary goal of my study was to reveal, which species play a relevant role in the epidemiology of bTB within the South Transdanubian Region. My further aim was to create a simple method to investigate the emergence, maintenance and spread of bTB in field conditions.

The third goal of my work was to confirm that the application of ecological methods during an investigation on an emerging infectious disease is more useful than simple diagnostic investigation of reservoir species and statistical evaluation of prevalence data. *One World – One Health – One Medicine* (briefly *One Health*) approach that amalgamates skills of both epidemiology and ecology serves additional information. This new, multidisciplinary method helps to understand the progression of a local endemic.

Materials and methods

My work was carried out within the South Transdanubian region of Hungary, on five hunting premises between 2008 and 2017. It was based on post-mortem examination on harvested wild ungulates and bacteriological investigation of collected specimens. Bacteriological diagnostic procedures were accomplished by the Bacteriological Department of Veterinary Diagnostic Directorate, National Food-chain Safety Office, Budapest.

This doctoral thesis is based on six separate studies performed on five hunting premises of Somogy County. These premises are located in Zselic, Inner Somogy, Outer Somogy, Drava Plain and east Zala Hills. Out of the five studied area, Zselic was confirmed to be infected with bTB previously, as *Mycobacterium caprae* could be detected in both pastured cattle herds and wild ungulates. The other areas proved to be only sporadically infected. The common characteristic of all areas is intensively managed dense wild ungulate populations.

Particular areas can be distinguished by geographical features, though neither natural nor artificial barrier impedes migration of wildlife between the areas. This unbound migration of wild species is characteristic of the whole South Transdanubia, which is bordered by the River Drava in the south, by the River Danube in the east, by Lake Balaton and highway M7 in the north, while in the west, the landscape continues without any barrier in Zala Hills and Mura Plain.

Ecological, climatic, forestry and hunting management data of particular areas were partly provided by the manager of the areas and partly collected from Regional Forestry Plans available on official website of NÉBIH, the National Food-chain Safety Office (<http://portal.nebih.gov.hu/-/erdotervezes>).

In the first study, we were searching for those species, which have dense populations within the investigated ecosystem, are susceptible for bTB infection, are able to maintain the infection for a longer time and their lifestyle is suitable to spill the infection over toward other species, such as pastured cattle herds. Based on data provided by local hunting managers and literature review, wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*), red fox (*Vulpes vulpes*) and golden jackal (*Canis aureus*) were chosen for further investigation.

During hunting events, we carried out a post-mortem examination on all harvested individuals as far as possible, with the aim of determining the prevalence of tuberculosis-like lesions. After this, we collected specimens for bacteriological investigations. The submandibular, retropharyngeal, bronchial, mediastinal, hepatic, mesenteric and caecal lymph nodes and lesioned organs, if any, were submitted to Bacteriological Department of Veterinary Diagnostic Directorate, National Food-chain Safety Office, Budapest.

In the laboratory, specimens were processed by both aerobic and anaerobic and *mycobacteria* laboratory methods. All isolates were determined by classic morphological and biochemical methodology and stained by ZN staining. Bacteria belonging to the *Mycobacterium* genus were identified from ZN positive specimens. Discrimination of *Mycobacterium tuberculosis* complex (MTC), *Mycobacterium avium* complex (MAC) and other, non-avium, non-tuberculous (NANT) bacteria was carried out by molecular diagnostic methods.

Results of post-mortem and bacteriological investigations were summarized; then real prevalence was calculated by Sterne exact statistical method. This was necessary to determine, which species can reach enough high prevalence for surveying bTB by either post-mortem or bacteriological methodology.

The aim of the second study was to determine the efficacy of visual-only game meat inspection (VOI). For this reason, we compare the results of normal VOI and a detailed post-mortem examination. This study was carried out by examination of wild boar carcasses. For the first step, we accomplish a regular VOI; and then the whole body was necropsied, with special regard to the slicing of regional lymph nodes. Bacteriological background of tuberculosis-like lesions was confirmed by laboratory investigation.

During the third study, we attempted to develop a simple diagnostic method, which is suitable to apply among field conditions. We based our hypothesis on the observation that submandibular lymph nodes of wild boars are the most prevalent entry of *mycobacteria*, and bTB can be isolated from this organ most frequently. We hypothesised that a surveillance system, which based on solely the post-mortem examination of the wild boars' submandibular lymph node, can provide useful data on a natural reservoir of bTB within a certain ecosystem.

During social hunting events, we carried out necropsy investigation on each wild boar carcass. The finding of the submandibular lymph node was recorded separately. Specimens (as described at first study) were submitted to the bacteriological laboratory from both positive and negative carcasses.

On all study sites, we determined the apparent prevalence of both post-mortem and bacteriological positive findings; and then we calculated the real prevalence of bTB by Sterne exact method. Correlation between the annual prevalence of post-mortem and bacteriological findings was calculated by non-parametric Spearman method.

Comparing our surveillance method to the bacteriological investigation as the gold standard, the sensitivity and specificity were evaluated by Win Episcope 2.0 software.

The goal of our fourth study was to determine the epidemiological role of wild boar within a bTB hot-spot, based on the comparison of a natural free-range population with a dense stock of enclosures.

We hypothesised that if wild boar was a maintenance host of bTB, a denser population should show a higher prevalence of bTB; which correlates with the prevalence of tuberculosis-like lesions in wild boars' submandibular lymph node. On the other hand, if wild boars got the infection from an external source, the increase of population density would not result in a higher prevalence of neither bTB nor tuberculosis-like lesions in the submandibular lymph nodes.

We determined the population prevalence of tuberculosis-like lesions in wild boars' submandibular lymph node annually, in both free-range and enclosures. Annual prevalence of the two habitats was compared by Chi-squared test with R Statistics software version 3.2.1.

During the fifth study, the correlation of population density and bTB prevalence was investigated in the red deer population of the Zselic bTB hot-spot. This study was also based on the hypothesis we conceived in the fourth study.

Within the core area of the bTB epidemic, we compared the population of a red deer farm and the free-range area. Considering the fact that in cervid species, the frequently concerned organ cannot be detected as an indicator of bTB infection; therefore the determination of prevalence was based on post-mortem examination of the whole body and laboratory investigation of specimens.

After we confirmed that infection spread was density dependent, we determined the difference between the epidemiological risk of natural and farm populations. Prevalence of free-range and enclosed population was compared by Chi-squared test with R Statistics software version 3.2.1. For determination of population attributable fraction (PAF), we used Package 'epiR' in R-statistics software version 3.2.1.

During the sixth study, we analysed the epidemiological risk of different ecosystem elements. Our hypothesis was based on the previously confirmed fact that prevalence of tuberculosis-like lesions in wild boars' submandibular lymph nodes correlates with bTB prevalence in the concerned population. Therefore, we hypothesized that the risk of bTB is parallel with the prevalence of tuberculosis-like lesions in submandibular lymph nodes. On all five study sites, we carried out a post-mortem examination of submandibular lymph nodes of wild boars harvested on companion hunting events. On a hunting day, all carcasses were examined to keep the randomisation effect of this type of hunting regimen. Prevalence of tuberculosis-like lesions was determined separately by each site.

After analysing biotic and abiotic environmental factors of the different sites, we chose those ones, which might influence maintenance and spread of bTB. The hunting management data were assigned by the manager of the hunting premises. Moreover, data on forestry management, agricultural activity, land cover, climate, and geographical features

were collected from Regional Forestry Plans published online on the official website of the National Food-chain Safety Office (<http://portal.nebih.gov.hu/-/erdotervezes>).

Parasitic infection of wild boars as a special biotic factor was acquired from our own field observation and laboratory investigation. These parasitic investigations were carried out to confirm the occurrence of ecological interference in the studied ecosystems. Those parasite species were in focus, which might affect the course of bTB in the infected host. Based on the literature, we hypothesized that *Metastrongylus* spp. and *Macracanthorhynchus hirudinaceus* might impair the hosts' immune system and the course of bTB might worsen.

During parasitological investigations, prevalence and mean intensity were determined at a 95% confidence interval. From the five study sites, we examined 51 wild boar lungs to reveal the features of lungworm infection and the identity of worm species. For evaluation of the damage caused by *M. hirudinaceus*, we collected 618 randomized small intestine specimens from the study sites. The specimens were examined in the field to find granulomas characteristic to *M. hirudinaceus* infection, while 26 specimens were submitted to laboratory investigation. In the laboratory, we cut the intestine lengthwise, and then count the worm individuals. Prevalence and mean intensity of infection were determined by counting results. For analysis of wild boars' parasitic infection, we used Quantitative Parasitology 3.0 software.

We analysed the ecological tolerance of both *Metastrongylus* spp. and *M. hirudinaceus* species to exclude the bias in correlation with bTB infection caused by an external confounding factor. Considering that both parasites need an intermediate host to complete their biological cycle, we had to investigate the environmental requirements of these intermediate host species. In the case of *Metastrongylus* spp., the earthworms, while for *M. hirudinaceus*, the *Scarabidae* beetles play this role in the parasite life cycle. The environmental demands of these intermediate host species cited in literature were compared to environmental factors characteristic for the study sites. These data were collected from the Regional Forestry Plans published online on website <http://portal.nebih.gov.hu/-/erdotervezes>.

Prevalence and environmental data characteristic for the separate study sites were summarized in an MS Excel 2010 table. These data were processed by R Statistics 3.2.1 software. The correlation between the elements was determined by Spearman rank correlation test. And then we prepared a correlation matrix.

Results

Of the studied mammal species, the red fox did not show any visible lesion of bTB, despite among the studied nine individuals, we found 3 with bTB infection confirmed by bacteriological investigation.

In cervid species (red deer, N=25; fallow deer, N=23), all bTB infected specimens showed visible lesions, though at low prevalence of infection (red: 12.0%; fallow: 8.7%). In golden jackal, notwithstanding by very small sample size (N=3), bTB infection could not be confirmed. In wild boar (N=175), by both post-mortem and bacteriological examination considerable infection rate was possible; with a prevalence of 63.4% and 21.7%, respectively. Tuberculosis-like lesions were detected in the submandibular lymph node of this species most frequently. In this stage of our study, we could not find any bTB infected individual without lesion in the submandibular lymph node. In the case of other species, we could not detect such a regular pattern.

By visual-only game meat inspection (VOI), in 394 carcasses, we found two lesions, while further 129 carcasses contained lesions, which were detected only by detailed post-mortem investigation. Of the two VOI detected lesions, one was a generalised case, while the other was an abscess on the surface of the lung.

Forty-four lesioned submandibular lymph node specimens were submitted to the National Reference Laboratory, where 13 specimens were confirmed to be bacteriologically positive as *M. caprae* was detected in those. Only one carcass (0.3%; CI 95% 0.0-1.5%) was found, in which different regional lymph node contained small lesions detectable only by systematic slicing.

For evaluation of the efficacy of surveillance based on post-mortem examination of the wild boars' submandibular lymph nodes, we investigated 833 wild boars, harvested by company hunting. The core area of the bTB endemic proved to be the Zselic hills, where the prevalence of tuberculosis-like lesions in the wild boars' submandibular lymph nodes is 39.3%. On that part of Inner-Somogy, neighbouring to the Zselic hills, the prevalence proved to be 29.9%, while on the other sites this value was under 25%.

Comparing the annual prevalence of visible lesions in submandibular lymph nodes and confirmed bTB infection, we could ascertain the strong correlation of the two values ($r=0.91$, $P=0.03$). Calculation by Win Episcope 2.0 software revealed that sensitivity and specificity of the surveillance based on post-mortem examination of wild boars' submandibular lymph node proved to be 89.7% and 72.9%, respectively.

During the study on determination of wild boars' epidemiological role, we could collect samples in the free-range in every year, while in the enclosures seven hunting events were held, five in A, two in B enclosure. Based on data of seven hunting seasons, we calculated

apparent prevalence for the enclosures and for the free-range area as 24.0% and 27.7%, respectively. In six carcasses, we detected a generalised form of the disease, of which four were from enclosures, while two from the free-range area.

Though the apparent prevalence seemed to fluctuate, a difference was confirmed only between the “best” and the “worst” year of enclosure A ($P=0.002$). On the other hand, there was not any statistically confirmed fluctuation in enclosure B ($P=0.08$) and in the free-range area ($P=0.11$).

If the two enclosures were compared separately to the free-range population, enclosure A was different from both the free-range area ($P=0.002$) and enclosure B ($P=0.001$). Between the free-range area and enclosure B, the difference could not be confirmed statistically ($P=0.13$).

During the study on evaluation of the epidemiological role of red deer, we examined 284 deer carcasses, of which 127 were harvested in the free-range area, while 157 in an enclosure. As the causative agent of bTB, *M. caprae* was detected in both habitats.

Except for one carcass, all *M. caprae* strains ($N=17$) were isolated from visible lesions. In the enclosure, there were six carcasses; while in the free-range area, there were eleven ones with visible lesions but without any *Mycobacterium* infection in the background.

We could not find any regular pattern of lesions inside the body, as visible signs could be detected in retropharyngeal, mesenteric, mediastinal, submandibular lymph nodes and the lungs, and in one case, we could diagnose a generalised form of the disease.

Comparing free-range and enclosure population of red deer, we could reveal that the farm population was infected with bTB at a higher rate. The population attributable fraction for bTB, which characterise the epidemiological risk of farm circumstances proved to be 73.7% (CI 95% 4.7–92.7%).

During the study for determination the epidemiological risk of certain ecosystem elements, we carried out the post-mortem examination of 1987 submandibular lymph node specimens. Then we calculated the prevalence of lesions per sites. These prevalence data were compared with ecosystem elements having epidemiological relevance by literature review. Analysing connections between data, we experienced that relative relief, the area of beech forest microclimatic effect, mean intensity of *Metastrongylus* infection in wild boar, the area of croplands for games and the altitude above sea level have got strong positive correlation with prevalence of bTB lesions. Areal size of game damage and the size of the jackal bag showed a strong negative correlation with post-mortem prevalence of tuberculosis-like lesions. The power of test proved to be $P=0.08$, $P=0.09$, and $P=0.13$ for relative relief, beech forest microclimatic effect and both game damage and jackal bag size. Based on these data, we could determine that real correlation was possible in the case of these elements.

Discussion

During our long-term study, between 2008 and 2017, our goal was to reveal, which surveillance method would be suitable enough to study bovine tuberculosis in a natural reservoir. Our secondary aim was to determine the epidemiological risk for pastured livestock caused by the disease.

Based on previous literature data, we examined wild ungulates, such as wild boar and red deer, in the core area of bTB epidemic, the Zselic hills; and four, sporadically infected areas of Somogy County. Then, we collected data on other wild species of the studied areas with the aim to choose those ones, which answer the requirements of a real reservoir species, in other words, maintenance host.

The six mammal species that chosen previously were wild boar, red deer, fallow deer, roe deer, red fox, and golden jackal. Of the six ones, wild boar and red deer proved to be the most promising, which are suitable target species for survey bTB in an ecosystem.

In the case of red deer, all bacteriological positive findings were detected from a tuberculosis-like lesion. Based on this experience, we presumed that a detailed post-mortem examination of the whole body could be a very useful surveillance tool in this species. After field investigation, tuberculosis-like lesions should be submitted to the laboratory for confirmation bacteriological testing.

In the case of wild boar, most of the tuberculosis-like lesions had not bTB infection in the background. This experience agrees with others' findings; as in the background of lesions, non-tuberculous *Mycobacteria* or other bacteria from *Actinomycetales* genus, such as *Rhodococcus equi* or *Trueperella pyogenes* were detected.

During the examination of wild boars, the submandibular lymph node was concerned most frequently by tuberculosis-like lesions. In the primary phase of our study, we did not find any case, when another organ was lesioned but not the submandibular lymph node.

For this reason, we presumed that a surveillance system based on the post-mortem examination of wild boars' submandibular lymph nodes might provide useful data on bTB spread within an ecosystem. In cervid species, such a regular pattern was not observed. Moreover, in most of the cases, a detailed post-mortem investigation could detect a lesion concerned a single lymph node or a very small part of the lung tissue.

We investigated the potential of game meat inspection as a tool for surveillance bTB in wildlife. Because of very diverse lesion pattern in cervid species, we consider these species as less concerned in game meat inspection based surveys.

In the case of wild boar, we carried out a normal, visual-only game meat inspection in the field. Our investigation confirmed that this type of game meat inspection can detect only

1.5% of present tuberculosis-like lesions. This finding calls attention to the unsuitability of regular game meat inspection for monitoring emerging infectious diseases in ecosystems.

Recent European game meat inspection trend is visual-only inspection, which omits both palpations and cuts from the decision process. Previous food-chain risk analyses support the hypothesis that data acquired by palpation or cut cannot reduce the risk in such a rate as those activities can increase the risk of contamination.

Notwithstanding, it is worth noticing that in Europe, thousands of wild boar carcasses enter the game meat processing plants, where a post-mortem investigation based survey can be carried out with low cost, in a reasonable time with results representing even very large regions.

By application of experiences gained in other European bTB infected areas, and those we collected previously; we examined the potential of the surveillance method based on the post-mortem examination of wild boars' submandibular lymph node. The aim of our investigation was to reveal whether simple necropsy of this organ is suitable for tracking bTB in an ecosystem. By investigation of 833 wild boar carcasses, we confirmed that comparing with the bacteriological investigation as a gold standard our method had got a sensitivity of 89.7% and specificity of 72.9%. Based on this finding we can conclude that post-mortem investigation of wild boars' submandibular lymph node on its own is not suitable to determine the animal health statement of a certain carcass.

Notwithstanding, it was obvious that the annual prevalence of post-mortem lesions strongly correlates with the annual prevalence of bTB infections. For this reason, regular post-mortem examination of wild boars' submandibular lymph nodes is a very useful field surveillance method for tracking bTB epidemic in an ecosystem.

Based on this finding, we assume that with our surveillance method, a large number of wild boar carcasses can be examined in the game meat processing plants; and suspect hot-spots can be determined. Lesioned specimens can be submitted to the bacteriological laboratory with a sample size required for detection of 10% prevalence with a confidence level of 95%. By this method, high-risk areas can be detected in some simple steps.

Though bTB infection could be detected at a considerable rate, the prevalence of generalised cases was not more than 1% even within the core area. This observation contradicted other European researchers' findings. For this reason, we assume that the local wild boar population should play a different role in bTB spread than previously studied west European populations.

For clarification the infection spreading role of South Transdanubian wild boar population, we determined the prevalence of lesions in the submandibular lymph node in two types of habitats (free-range and enclosures) within the core area. The population of the enclosures was captured from the adjacent areas from year to year, therefore enclosed

population can be considered as a randomized sample from the free-range area. For this reason, we hypothesised that this captured population portion represents the free-range epidemiological situation. Inside the enclosure, population density is tenfold comparing to the free-range, therefore the theoretic possibility of infection spread increases as the animals enter the enclosure. Based on these facts, it was expected that infection began to spread rapidly within the captured population and reached a very high prevalence in a very short time.

Unlike, our investigations cannot confirm the above-mentioned hypothesis. Annual prevalence in enclosures was lower, though not significantly than that of the neighbouring free-range areas. Density-dependent spread of bTB infection in the local wild boar population cannot be supported.

In this case, it is very probable that wild boar is not a maintenance host of bTB within the South Transdanubian region; moreover, it gets the infection from an external source, another reservoir species.

Though, the density of the South Transdanubian wild boar population is recognizable comparing to other European populations; the prevalence of both infection and generalised cases are much lower than of those. In the background, the different quality of habitats should be supposed. The South Transdanubian population is still very far from the limits of carrying capacity of its habitat. Probably, this is why bTB cannot play an effective role in either population limitation or individual survival within the studied area.

Similar studies on red deer population of the core area led to a very different result. Infection prevalence of the farmed population was significantly higher than of free-range one. The population attributable fraction of captivity for bTB infection proved to be 73.7%. This value expresses the rate by which the infection prevalence would decrease, if the risk factor, namely captivity disappeared.

Captivity as a risk factor is very complex, containing a lot of different elements. An artificially dense population can cause social stress and increase the number of infective contacts between individuals, and enhance accumulation of infective agents in the environment. Studies in human populations and other mammal species confirmed that overcrowding, undernourishment and the consequent immune deficiency play the main role in the epidemiology of MTC caused diseases.

Captive red deer is not a real domestic animal; therefore it is not adapted entirely to the artificial circumstances. In captivity, these animals are fed with feeds developed for domestic livestock. These feeds are behind of wild ungulates in protein and microelement content. For this reason, artificial nutrition can provide enough energy but in the background, qualitative starvation develops. This affects disadvantageously the immune system of the animals, which leads to diseases.

The most obvious difference between the free-range and an enclosure is population density. Analysing literature, we presumed that exact epidemiological role of density cannot be clarified in the lack of detailed investigation of the concerned farm.

A further risk factor could be the less effective bTB surveillance system applied on the farm. In cervid species, an annual skin test cannot provide enough information on the real infection status of the herd. Only output-based surveillance systems, which are able to find even only one infected animal in any sized herd, are suitable for eradication and maintenance of free status.

Summarizing wild boar and red deer data; it is very probable that within the studied population, wild boars can contribute to the spread of bTB infection with a very low rate. On the other hand, red deer seemed to be a real reservoir of the disease, as in its population the spread of infection was supposed to be density dependent.

Though our study for clarification the role of red deer posed a lot of questions, it highlights the epidemiological risk of deer farms. The studied farm, in the lack of an effective surveillance system, should be considered as rather a great risk for adjacent natural wildlife and neighbouring livestock farms. Notwithstanding, our experience that bTB infection was isolated exclusively from visible lesions calls the attention to a surveillance possibility. Regular post-mortem examination of harvested animals can provide useful epidemiological information on bTB among farmed animals.

The exact risk definition of captivity needs further studies. By analysing several infected and bTB free herds with different technologies, it would be possible to determine those real risk factors, which should be eliminated to abolish infection accumulating role of deer farms. The investigation of such very complex, semi-intensive systems is inconceivable without a holistic approach, which includes a detailed analysis of the ecosystem where these farms run.

For this reason, One World – One Health – One Medicine (briefly One Health) is a very suitable new method. One Health does not examine epidemics as separate events but with its connections and ecological background.

For understanding the South Transdanubian bTB endemic, we applied this complex approach. During our analysis, we considered the prevalence of visible lesions in wild boars' submandibular lymph node as a variable, which expresses most effectively the infection rate of a certain study site.

On five sites (namely Zselic, Inner-Somogy, Outer-Somogy, Drava Plain, and east Zala), we compared numerical ecological features to the above-mentioned dependent variable, which characterizes the rate of infection. Based on literature data, those ecosystem elements were chosen, which might contribute to maintenance and spread of infection.

The population size of susceptible ungulate species and those elements which enhance population increase (such as mature, mast producing forests, arable lands and pastures, and surface water sources), were those variables supposed to be in direct correlation with the dependent variable of infection indicator.

On the other hand, the presence of predators and parasite infection were supposed to decrease the number of seriously diseased animals through ecological interference. With this phenomenon, predators and parasites can contribute to the health of the concerned ecosystem. For this reason, these factors were expected to be in a reverse correlation with the indicator of infection.

Factors that enhance the survival of *mycobacteria* in the environment (such as humid, cool microclimate, water carrying capacity of the soil, viz clay content) were supposed to increase the infection rate in wild boars.

As a result of our analysis, the relative relief, and the beech forest cover as an indicator of humid, cool microclimate were those factors, which showed a direct correlation with the indicator of infection at 90% confidence interval. Among independent variable reversely correlated with infection, the annual hunting bag of golden jackal proved to have a real correlation with the dependent variable.

Other variable showed an expected correlation with infection, though these correlations were not significant statistically. The only exception was the mean intensity of *Metastrongylus* spp., which correlates directly with the prevalence of tuberculosis-like lesions. An ancillary parasitological investigation was carried out within the core area of the endemic highlighted that the pathological role of lungworms in this ecosystem is neglectable. For this reason, ecological interference between the micro and the macroparasite (namely *mycobacteria* and lungworms, respectively) cannot manifest.

Notwithstanding, it can be observed that the mean intensity of metastrongylosis directly correlated not only with bTB infection but also with humid forest climate and clay content of the soil. In the background, a common tolerance range of *mycobacteria* and earthworms, intermediate hosts of lungworms are supposed to exist. Based on our results, it is possible that metastrongylosis cannot affect the course of tuberculosis considerably, in spite of its extremely high (96.1%) prevalence in wild boar population. The apparent correlation between the two variables is indirect through the common ecological demand of the two (micro and macro) parasites.

In the case of *M. hirudinaceus*, the hypothesis of apparent correlation also emerges. Though our results supported the presence of ecological interference, an in-host competition of the parasites is questionable. Ecological tolerance range of *M. hirudinaceus* is determined by environmental demands of its intermediate hosts, viz *Scarabidae*, especially cockchafer (*Melolontha* spp.).

The main prerequisite for successful production of intermediate hosts is quickly warming, easily drying, loose textured soil. This type of soil can be found in Inner-Somogy and the Drava Plain, which are the main habitats of golden jackal.

Our investigations could not reveal the role of golden jackal in the spread of bTB. Notwithstanding, a very similar mesopredator, the coyote is confirmed to be able to get the infection without transmission toward new hosts. In Hungarian ecosystems, golden jackal, in spite of its intermediate size, plays the role of the apex predator. For this reason, it is one of the most important regulator factors for wild ungulates, besides humans and stray dogs. Moreover, this small canid is the most important scavenging species in its habitats.

Environmental epidemiological studies confirm that within diverse ecosystems, where predators' guild presents, the dilution effect of biodiversity can manifest. Dilution effect is the phenomenon when predators decrease the number of infected individuals by removing the weakest members of a reservoir population. This phenomenon can prevail, if the predator species cannot develop advanced forms of the disease, therefore the pathogen reaches a dead-end and cannot spread towards.

Our results support the hypothesis that the presence of golden jackal is advantageous for the health status of the local ungulate population. In our recent study, we did not observe those factors that contribute to this phenomenon. Our assumption is that golden jackal can affect the spread of bTB by removing dead animals, hunting offals, and weakest individuals from the host populations, and also by impeding migration of host populations.

The main shortcoming of this environmental epidemiological study was that the five study sites can be regarded as samples, practically. Therefore the sample size is five, in spite of investigation on a very large amount of wild boar carcasses. For this reason, only those variables showed significant correlation with bTB prevalence, which is very different by certain sites.

Within the South Transdanubian endemic region, Zselic as core area needs regular monitoring. In my doctoral thesis, the main ecological factors for maintenance and spread of bTB infection were analysed by the methodology of One Health approach. By this work, I succeeded in finding ecological connections, which can be the starting points of further multidisciplinary research; and those can take us closer to successful management of wildlife tuberculosis.

Experiences gained during literature reviewing, fieldwork and data analysis supported our previous hypothesis that amalgamating methods of different disciplines enhances the efficacy of epidemiological work. Therefore it is suggested to apply One Health approach to investigate emerging infectious diseases.

New scientific results

1. Within South Transdanubian endemic area, wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*), and red fox (*Vulpes vulpes*) were confirmed to be infected with *Mycobacterium caprae*.
2. The visual-only game meat inspection was determined not to be a proper method for detection of bTB infection as only 1.5% of lesions were found by it.
3. We confirmed that in the wild boar, tuberculosis-like lesions were detected most frequently (99.9%) in the submandibular lymph node. The surveillance, which is based on the post-mortem examination of this organ had got a sensitivity of 89.7% and specificity of 72.9%.
4. *Mycobacterium caprae* transmission in wild boar proved to be frequency dependent.
5. In red deer, *Mycobacterium caprae* was isolated from visible lesions in 99.3%.
6. *Mycobacterium caprae* transmission in red deer proved to be density dependent; and in the lack of effective surveillance, deer farms could cause a relevant epidemiological risk for their environment.
7. By application of *One Health* approach, we ascertained that sub-montaneous climate in sylvatic habitat could enhance, while the presence of predators could impede the spread of the disease within natural ecosystems.

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