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## Short Communication

Towards harmonised procedures in wildlife epidemiological investigations: A serosurvey of infection with *Mycobacterium bovis* and closely related agents in wild boar (*Sus scrofa*) in SwitzerlandOlivia Beerli <sup>a</sup>, Sohvi Blatter <sup>a</sup>, Mariana Boadella <sup>b</sup>, Janne Schöning <sup>a</sup>, Sarah Schmitt <sup>c</sup>, Marie-Pierre Ryser-Degiorgis <sup>a,\*</sup><sup>a</sup> Centre for Fish and Wildlife Health (FIWI), Vetsuisse Faculty, University of Bern, Postfach 8466, Bern CH-3006, Switzerland<sup>b</sup> SaBio IREC (CSIC-UCLM-JCCM), Ronda de Toledo s/n, Ciudad Real 13071, Spain<sup>c</sup> Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 270, Zurich 8057, Switzerland

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## ABSTRACT

Bovine tuberculosis (bTB) is a (re-)emerging disease in European countries, including Switzerland. This study assesses the seroprevalence of infection with *Mycobacterium bovis* and closely related agents in wild boar (*Sus scrofa*) in Switzerland, because wild boar are potential maintenance hosts of these pathogens. The study employs harmonised laboratory methods to facilitate comparison with the situation in other countries. Eighteen out of 743 blood samples tested seropositive (2.4%, CI: 1.5–3.9%) by ELISA, and the results for 61 animals previously assessed using culture and PCR indicated that this serological test was not 100% specific for *M. bovis*, cross-reacting with *M. microti*. Nevertheless, serology appears to be an appropriate test methodology in the harmonisation of wild boar testing throughout Europe. In accordance with previous findings, the low seroprevalence found in wild boar suggests wildlife is an unlikely source of the *M. bovis* infections recently detected in cattle in Switzerland. This finding contrasts with the epidemiological situation pertaining in southern Spain.

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Tuberculosis (TB) is a chronic disease caused by mycobacteria of the *Mycobacterium tuberculosis* complex (MTBC). *Mycobacterium bovis* and *M. caprae* cause bovine TB (bTB) but have a large host range among domestic and wild animals and occasionally infect humans (SANCO, 2013; Schöning et al., 2013). Because wildlife reservoirs play a crucial role in the epidemiology of bTB in farm animals, assessment of the infection status of both wild and domestic hosts is essential to prevent or control disease spread (Boadella et al., 2011). In continental Europe, the wild boar (*Sus scrofa*) is a common host of *M. bovis* but, depending on geographical region, its epidemiological role varies from one of maintenance to spillover host. Nevertheless, clear comparison of the prevalence of infection in wild boar populations between regions is difficult as the methods used to determine this parameter and animal population densities vary greatly (Schöning et al., 2013). Harmonisation of investigation procedures across regions with different disease patterns is a necessary pre-requisite to the identification of risk factors for disease occurrence (Patz et al., 2004).

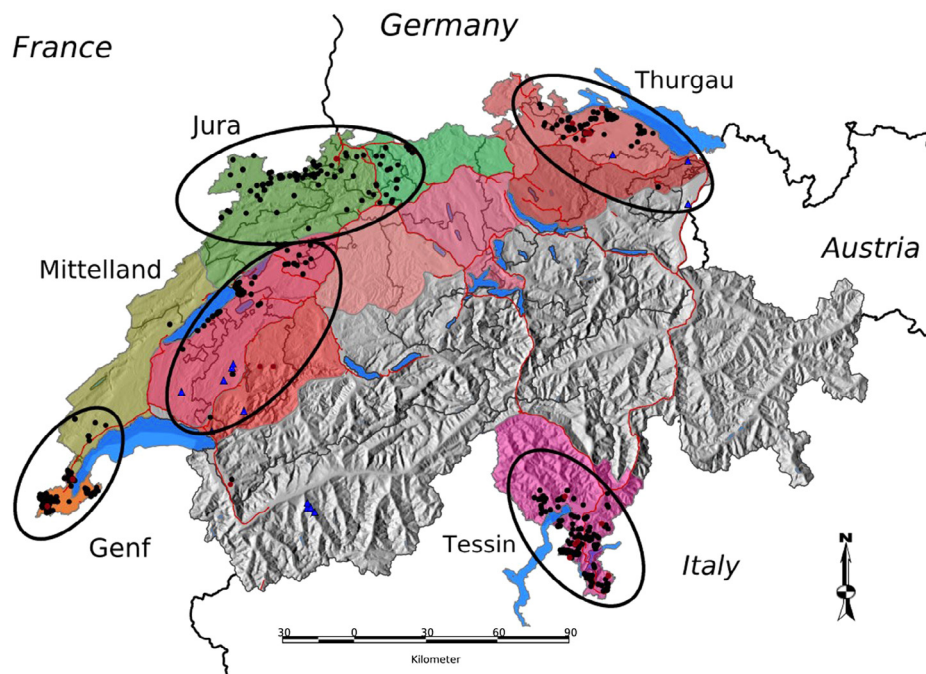
In Europe, bTB is (re-)emerging in both cattle and wildlife populations, including in the countries surrounding Switzerland which

has been officially bTB free since 1960 (Schiller et al., 2010; Schöning et al., 2013). Scanning surveillance of wildlife for several decades, and a recent cross-sectional study of wild boar and red deer (*Cervus elaphus elaphus*) from at-risk areas have not detected infection with either *M. bovis* or *M. caprae* (Schöning et al., 2013). However, in the latter study, 6/165 wild boar (3.6%, 95% CI, 1.4–7.8%) tested by culture and PCR were found to be infected with MTBC mycobacteria. While two animals were infected with *M. microti*, mycobacteria could not be further identified in four animals (Schöning et al., 2013). Furthermore in 2013, bTB was detected on several cattle farms in Switzerland. Outbreaks in the eastern part of the country were due to infection with *M. caprae* and were related to cattle movements from Austria (Federal Food Safety and Veterinary Office, 2014) where *M. caprae* infection is widespread in free-ranging red deer and occasionally found in cattle (Schoepf et al., 2012). In contrast, cases in western Switzerland were due to *M. bovis* infection and were traced back to a single farm, although the primary source of infection has not yet been identified (Federal Food Safety and Veterinary Office, 2014).

The aims of this study were to provide a more comprehensive picture of the scale of infection with *M. bovis* and possibly other closely related agents such as *M. caprae* in wild boar in Switzerland, and to promote a harmonised approach to such investigations in order to facilitate the comparison of data between European

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**Fig. 1.** Map of Switzerland with main lakes (pale blue), large roads (red lines), and sampling units (black circles). Blue triangles show the recent cases of bovine tuberculosis in cattle herds. Dots show the geographical origin of the sampled wild boar (black, seronegative; red, seropositive). The unit Jura is located in the bioregion Jura (shades of green). The units Geneva (Genf), Mittelland and Thurgau belong to the bioregion of the Swiss Plateau (shades of pink), adjacent to the Jura. The unit Tessin is separated from the Plateau by the Alps and corresponds to the canton of Tessin (purple) and to the bioregion South.

countries. Although the use of serology to detect infection with MTBC organisms is not considered particularly effective (de Lisle et al., 2002), it is the method of choice for population surveys of wild boar (Boadella et al., 2011). Serology can be rapidly performed and is an inexpensive methodology that is suitable for large-scale testing.

Blood samples collected from 743 wild boar hunted in Switzerland (41,285 km<sup>2</sup>) between 2008 and 2013 were available for analysis. Five sampling units were defined based on wild boar occurrence (Wu et al., 2011) and abundance, bioregion, and on anthropogenic and natural barriers (Fig. 1). Blood was sampled from the thoracic cavity: directly from the heart or by puncturing the cavernous sinus (Arenas-Montes et al., 2013). Samples were centrifuged at 1500 g and serum aliquots stored at -20 °C until analysed. Animals were categorised into three age groups, based on weight and coat colour (Wu et al., 2011): juveniles (<12 months), immature adults (12–24 months), and adults (>24 months).

Samples were tested using a bovine purified protein derivative (bPPD)-based indirect in-house ELISA evaluated for wild boar (79.2% sensitivity, 100% specificity for *M. bovis*; Boadella et al., 2011). Samples from MTBC culture-negative wild boar originating from bTB-free areas in Spain were used as negative controls. Pooled positive sera from *M. bovis*-positive wild boar were used as positive controls. Prevalence estimates, 95% CI calculations and two-tailed Fisher's exact-tests were carried out using NCSS 2007 statistical software<sup>1</sup> with a significance level set at  $P < 0.05$ .

Of the tested wild boar 18/743 were found seropositive, corresponding to an estimated seroprevalence of 2.4% (95% CI: 1.4–3.8%). There were no significant differences between sex or age categories. The prevalence was significantly higher in the north-east of the country (unit Thurgau, close to the border with Germany), than in the units Jura ( $P = 0.013$ ) or Geneva ( $P = 0.013$ ; Fig. 1, Table 1) adjacent to the border with France. Distances between seropositive wild boar and the closest

cattle farm with cases of bTB ranged from 5.6 to 23.9 km (average, 15.1 km). Sixty-one samples from animals from Geneva ( $n = 25$ ), Thurgau ( $n = 29$ ) and Tessin ( $n = 7$ ) had previously been tested by PCR and culture. Four of these wild boar were positive for MTBC on both tests, and in two of this sub-group, the mycobacteria detected by PCR were further identified as *M. microti* by spoligotyping (Schöning et al., 2013). Three of these four positive samples were seropositive by bPPD ELISA including the two positive for *M. microti*. All PCR negative samples were also negative by ELISA.

This serosurvey confirms the low prevalence of infections with MTBC mycobacteria in wild boar in Switzerland. ELISA screening resulted in a lower estimated prevalence compared to culture and PCR (2.4% vs. 3.6%) and, by investigating a larger sample size, we obtained a narrower confidence interval (1.5–3.9% vs. 1.4–7.8%). Furthermore, comparison of serological and culture/PCR results showed that at least a proportion of the seropositive reactions were due to *M. microti* infection. This finding indicates that the applied ELISA is not 100% specific for *M. bovis* infection but cross-reacts with other MTBC mycobacteria.

Considering the prevalence of infection identified in wildlife in Switzerland in this and a previous (Schöning et al., 2013) study, it seems unlikely that wildlife acts as a reservoir of *M. bovis* or *M. caprae*

**Table 1**

Blood samples of free-ranging wild boar (*Sus scrofa*) from Switzerland collected between 2008 and 2013 and tested for antibodies to mycobacteria of the *Mycobacterium tuberculosis* complex by ELISA. The number of samples and test results are given for the five selected study areas.

Study area	Positive/Tested	Prevalence (%)	95% Confidence interval
Geneva	2/192	1.0	0.1–3.7
Mittelland	3/138	2.1	0.4–6.2
Jura	1/142	0.7	0.0–3.8
Thurgau	6/88	6.8	2.5–14.2
Tessin	7/172	3.5	1.3–7.4

<sup>1</sup> See: <http://www.ncss.com>.

and is the source of the recently detected cases of bTB in cattle in Switzerland. The detection of several seropositive wild boar scattered throughout the sampled areas indicates that infection with MTBC mycobacteria occasionally occur, although at least a proportion are likely due to *M. microti*. Nevertheless, some of the seropositive wild boar may have been infected with *M. bovis* or *M. caprae*, either as a result of spill-over from cattle (some wild boar were found relatively close to infected cattle farms) or following contact with infected wildlife from neighbouring countries. In both scenarios the risk of future reservoir development needs to be considered. Currently, coordinated effort by animal health and wildlife management in Switzerland is improving targeted surveillance for bTB in cattle and wildlife in at-risk areas and implementing infection prevention strategies for wildlife and livestock.

In this study we harmonised our methodology with that of previous surveys of wild boar in Spain and France (García-Bocanegra et al., 2012; Muños-Mendoza et al., 2013; Richomme et al., 2013). Apparent overall seroprevalence in Switzerland was significantly lower than that in Southern Spain (54.6%, CI 45.6–63.4%,  $P = 0.000$ ), and France (7.8%, CI 6.7–9.1%,  $P = 0.000$ ), but did not differ from the seroprevalence reported from the Atlantic region of Spain (2.1%, CI 1.8–3.6). Comparisons with data from other countries such as Italy (Bollo et al., 2000) are more difficult as both study design and diagnostic methods differ. Given that blood samples are easier to collect than tissue samples, and that serology is less expensive and more rapidly carried out than other diagnostic methods, this methodology is the most appropriate in estimating the prevalence of infection with MTBC mycobacteria in wild boar on a national scale. However, because serological testing does not identify specific mycobacteria, the accuracy of serosurveys of *M. bovis/caprae* is limited in regions where other members of the MTBC occur. Nevertheless, serology is a valuable surveillance tool which can be complemented with more precise investigative methods where clusters of cases are identified or where regional increases in prevalence occur. A particular benefit of serology is that it can contribute to the harmonisation of diagnostic protocols across different regions/countries, given that it can be deployed in testing large numbers of animals. Such an approach may facilitate the identification of the risk factors contributing to the occurrence and maintenance of bTB.

### Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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