Impact of piglet oral vaccination against tuberculosis in endemic free-ranging wild boar populations

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Abstract

In Mediterranean woodlands of Iberia, the Eurasian wild boar (*Sus scrofa*) is considered as the main wild reservoir of the *Mycobacterium tuberculosis* complex (MTC), and a key risk for cattle tuberculosis (TB) breakdowns. In this context, wild boar vaccination might represent a valuable tool for TB control. We tested two vaccines in natural and managed sites: heat-inactivated *M. bovis* (IV) and BCG, each one deployed during four consecutive summers using selective piglet feeders. Piglets from natural sites had lower bait uptake rates (50 to 74%) than those from managed sites (89 to 92%). Piglet TB lesion prevalence increased by 6% in the Control and Managed BCG sites, and decreased 36% in the Natural IV site. In the Natural BCG site the control year sample size was too low for comparisons. In the managed IV site, piglet TBCL prevalence remained 0% throughout the study period. At the population scale, TB lesion prevalence increased in the Control sites (5.6%), while a significant decline occurred in the Managed IV site (34.4%). No changes were recorded in the remaining sites. We conclude that IV can become part of integrated TB control schemes, although its performance is context dependent and requires tailored field protocols.

Keywords: tuberculosis control, field vaccination, *Mycobacterium bovis*, BCG, heat-inactivated *Mycobacterium bovis*, wild boar.

INTRODUCTION

Vaccination is one of the most effective tools to prevent, control and eradicate infectious diseases (Rappuoli et al. 2001, McVey et al. 2010). In free-ranging wildlife, the technical and logistical difficulties and the cost of vaccination limit its application to diseases that have significant public health, economical or conservation impact (Cross et al. 2007). The turning point of wildlife vaccination was the control of fox (*Vulpes vulpes*) rabies in Europe (Freuling et al. 2013).

This success prompted the investigation of field vaccination strategies to control other relevant diseases in wildlife, including animal tuberculosis (TB). This chronic infection is caused by *Mycobacterium bovis* (*M. bovis*), Myc*obacterium caprae* (*M. caprae*) and other members of the *Mycobacterium tuberculosis* complex (MTC). It is a zoonosis, although nowadays the number of human cases is low in industrialized countries (Langer and LoBue 2014). Hence, the impact of animal TB is mainly socio-economical and derives from the eradication campaign costs, associated movement restrictions, and indirect losses to the livestock industry (Zingsstag et al. 2006), as well as regionally to the hunting industry (Barasona et al. 2016). Moreover, animal TB causes conservation concerns, e.g. in the endangered Iberian lynx, *Lynx pardinus* (López et al. 2014).

Most TB-control efforts focus on cattle as the main target host (Reviriego Gordejo and Vermeersch 2006). However, TB is a paradigmatic example of multi-host infection and its eradication remains unlikely without targeting all relevant hosts (Gortázar et al. 2015). In Mediterranean woodland habitats of the Iberian Peninsula, the MTC host network is complex and includes several relevant domestic and wild host species (Aranaz et al. 2004, Gortazar and Boadella 2014). In this region, the native Eurasian wild boar (*Sus scrofa*) is considered as the main wild reservoir for MTC (Naranjo et al. 2008). This suid is also regarded as a key risk for cattle TB breakdowns (La Hue et al. 2016, Hardstaff et al. 2014), mostly through indirect contacts (Kukielka et al. 2013, Cowie et al. 2015).

In consequence, wild boar are becoming an additional target for TB control. There is evidence suggesting that interventions on wild boar such as biosafety measures reducing wildlife– cattle contacts (Barasona et al. 2013), or culling (Boadella et al. 2012), manage to reduce TB prevalence in sympatric ruminants such as cattle and red deer (*Cervus elaphus*). In this context, wild boar vaccination might represent a valuable additional tool for TB control in Mediterranean Iberia.

Proof of principle of TB disease reduction by vaccination with the life attenuated BCG has been demonstrated for several wild reservoirs in controlled experiments (Buddle et al. 2006, Lesellier et al. 2006, Nol et al. 2008). Further field experiments have been conducted in brush-tailed possums (*Trichosurus vulpecula*; Corner et al. 2002, Tompkins et al. 2009 and Nugent et al. 2016) and Eurasian badgers (*Meles meles*; Chambers et al. 2011 and Gormley et al. 2017) with promising results regarding protection (Table 1).

Two vaccine candidates administered by the oral route have been tested in laboratory trials in the wild boar model: BCG (Ballesteros et al. 2009a, Garrido et al. 2011, Gortázar et al. 2014) and heat-inactivated *M. bovis* (IV) (Garrido et al. 2011, Beltrán-Beck et al. 2014a). Both vaccines decrease disease severity, reducing lesion and culture scores, when compared to unvaccinated controls. Vaccine safety and species-specific delivery at field has been assessed in additional trials (Beltrán-Beck et al. 2014b).

Both vaccines are prophylactic and non-sterilizing. Thus, their protective effect is expected to reduce the severity of the disease and subsequent transmission, rather than preventing infection. The vaccination target are 2-6 month old wild boar since they have a higher chance of being uninfected (Ballesteros et al. 2009b). The vaccines are formulated for oral delivery, as oral administration via baits is the most practical means for wildlife vaccination at large scales (Cross et al. 2007). This coupled with complementary tools such as species-specific baits (Ballesteros et al. 2009c) marked with chemical compounds (Ballesteros et al. 2011) and selective baiting stations (Ballesteros et al. 2009b) enables a targeted vaccine delivery and accurate bait uptake assessment.

An injectable version of the IV vaccine successfully reduced TB lesion prevalence in a wild boar farm (66% reduction; Díez-Delgado et al. 2016). However, extensive field trials are needed to assess vaccine performance under realistic oral delivery conditions in free ranging populations. Therefore, in 2012 a large-scale (ca. 460 km^2), four-year wild boar oral vaccination experiment was implemented in a high prevalence area of Montes de Toledo, Spain.

Our aims were, first, assessing vaccine impact measured as changes in TB prevalence at the wild boar population scale based on pathology and culture; second, assessing a key operational aspect of oral vaccination (bait uptake) under field conditions; and third, modelling field vaccination in order to gather additional insights regarding (i) the influence of different levels of vaccination success on disease prevalence and the

influence of continued vaccination (25 years) and eventual cessation on (ii) population density and on (iii) disease prevalence in two scenarios representative of the situations encountered at field.

MATERIALS AND METHODS

Animal use

The experiment was conducted under a research license (828493/2011) issued by D.G. Agricultura y Ganadería, Junta de Castilla - La Mancha. Post-mortem inspection and sampling was performed on hunter-harvested wild boar. No animal was culled because of the experiment.

Study area

The study was conducted in Montes de Toledo, a mountain chain located in Central Spain (39 º 25 'to 39 ° 16'N, 4 ° 05' to 4 ° 23 'W). This region has a Mediterranean wood and scrubland habitat dominated by evergreen oaks (*Quercus* sp). The climate is typical Mediterranean, with mild to cold winters, hot summers and rainfall mostly limited to spring and autumn.

The study area is composed by an array of private owned hunting estates, communal lands and protected natural areas representing a gradient of wildlife management. Land use changes have favoured the upsurge of a commercial hunting industry economically relevant for the area, which main big game species are red deer and wild boar, leading to high ungulate densities (Vicente et al. 2013).

In this TB endemic area wild boar TB-compatible lesion (TBCL) prevalence ranges from 52% to 70% and shows an increasing trend in time (Vicente et al. 2013). In contrast, lower (12%) and relatively stable TCBL prevalence has been described for red deer (Vicente et al. 2013).

A total of 19 sites were selected for TB monitoring. Out of them, 2 private owned hunting estates and a natural park were devoted to vaccination (96 km^2) and the remaining sites were used as control (n=15, ca. 360 km^2).

BCG was deployed in one of the private owned estates (Managed BCG) and IV in the other one (Managed IV). The natural park was divided in two areas separated by a topographical barrier (a flat area of 4 km with less vegetation, crossed by the main road that separates the north and south mountain chains); BCG was deployed in the north

area (Natural BCG) while IV was deployed in the south (Natural IV; Figure 1). Further site characterization is given in Supplementary Material 1.

Vaccination program

Vaccines

The live attenuated BCG vaccine derives from *M. bovis* Danish (CCUG strain 27863) and was prepared as described elsewhere (Ballesteros et al. 2009a, Garrido et al. 2011, Gortázar et al. 2014). Vaccine doses of 0.15 ml of a suspension containing 5.2 to 7.6 $x10^5$ c.f.u. were placed into sterile airtight polypropylene 0.2 ml vials (VWR®, Radnor, Pensilvania, USA). BCG was freshly prepared for each cycle and stored at 4ºC until deployment in the field (24 to 72 hours).

The IV vaccine derives from a heat-inactivated field isolate obtained from a naturally infected wild boar (Neiker1403, spoligotype SB0339) and is prepared as described in Garrido et al. (2011). Each IV vial contains a dose of approximately $6x10^7$c.f.u. in 0.2 ml of PBS.

Vaccine delivery

(i) Baits

BCG and IV vaccine vials are deployed in baits specific for wild boar piglets (Ballesteros et al. 2009c). These baits have demonstrated their stability, safety and effectiveness in reaching the target species and age class in the field (Ballesteros et al. 2011). A chemical marker, iophenoxic acid (IPA; PR EuroCHEM Ltd., Cork, Ireland), is added to the baits (as described in Ballesteros et al. 2011) to determine the proportion of wild boar piglets consuming baits (bait uptake). Two IPA derivatives, each one associated to a vaccine type, were employed. Propil-IPA was associated with BCG baits and ethyl-IPA to IV baits.

(ii) Selective piglet feeders and spatial distribution

Baits were placed at selective piglet feeders (Ballesteros et al. 2009a). Treatment surfaces were divided into a 2 km^2 grid by means of GIS analysis (QGIS version 1.8.0 Lisboa). Piglet feeders were distributed by couples per grid. These two piglets feeders were separated approximately 100 meters to avoid monopolization by any dominant family group. They were placed nearby a permanent waterhole (to ensure passing by) in a spot where they received

afternoon shadow (to avoid thermic pressure over vaccines). Managed sites (BCG and IV) had 10 couples of piglet feeders each and natural sites 14 couples each (total piglet feeders =96). A detailed map of piglet-feeder distribution is provided in Supplementary Material 1.

Vaccination schedule

Vaccination took place in summer to target the main peak of 2-6 month old wild boar once they are into solid food consumption and to take advantage of the limited natural food resources available in Mediterranean habitats in this season. Maize was pre-baited 2-5 times a week for 8 weeks prior to vaccine deployment and sham baits (without vaccine or marker) were placed as well to habituate wild boar piglets to baits. Pre-baiting helps getting wild boar used to visit piglet feeders and limits uptake by non-target species (Kaden et al. 2000, Ballesteros et al. 2011).

The vaccination campaign included 3 cycles that consisted of three nights each. Two cycles were held consecutively on early summer (end of June-July) and one in late summer (end of August-September).

Twenty baits per piglet feeder were deployed each day at dusk leading to a total of 17.280 vaccine baits per year (180 baits/km^2 and year). Non-consumed baits were retrieved the next morning and fresh vaccine baits were newly placed each day (maximum exposure to environmental temperatures was 12 hours).

Vaccine impact assessment

Hunter-harvested wild boar (n=1140) were sampled during the regular hunting season (October to February) from 2011-12 to 2015-16. Samples obtained prior to vaccination (hunting season 2011-12, from now on control year) serve as pre-intervention background providing baseline data on infection and disease. A representative sample stratified by age and sex of the hunted animals was randomly selected at each hunting event. Each specimen was subjected to collection of biometrical data, sex and age determination, blood collection from the cavernous sinus (Arenas-Montes et al. 2013) and a general inspection of the whole carcass. Age was assessed based on tooth eruption patterns (Saenz de Buruaga et al. 1991), establishing three categories: wild boar less than 12 months old were classified as piglets (n= 245), those between 12 and 24 months as yearlings (n= 305), and those over 2 years as adults (n= 590).

Organ samples taken in the field include the mandibular lymph nodes (LNs), tonsils, the

lung with the tracheobronchial LNs and mediastinal LN, the spleen, and the mesenteric LNs (Martín-Hernando et al. 2007). Detailed inspection by serial sectioning in the laboratory allowed recording TBCL presence and lesion scoring. A simple lesion scoring method was developed to inform on lesion severity (Díez-Delgado et al. 2014). Briefly, the lesion score is based on lesion size (0 if no lesion is present, 1 for lesions <1 cm and 2 for larger sized lesions) and inspection of the routine target organs (considering each lung lobe separately and excluding the tonsil). Hence, the total lesion score of an individual ranges from 0 to 26. Individuals with lesion score >0 are defined as TBCL positive. Processed tissues were stored at -20ºC. Mandibular LN and tonsil pool plus a thoracic LN pool were cultured following the procedures described in Garrido et al. (2011). All isolates were spoligotyped (Kamerbeek et al. 1997). Wild boar with mycobacterial growth confirmed by spoligotyping as *M. bovis* or *M.caprae* were defined as culture positive.

Bait uptake assessment

Bait uptake is determined by the presence of chemical marker in serum. The IPA derivatives analysis was done following the extraction method and LC/ESI-MS analysis described in Ballesteros et al. (2010). The detection time of marker in serum after bait ingestion lasts for up to 18 months (Ballesteros et al. 2010). Thus, marker presence is used to estimate bait uptake in the vaccination campaign previous to the hunting season by individual wild boar (discriminating if older wild boar consumed bait as piglets is not possible).

Statistics

Descriptive analysis, predictors, and logistic regression

Changes in temporal trends were analysed by means of a Chi square test or Fisher exact test (two tailed) when required. Data on study area rainfall was obtained from National Agency of Meteorology, Station 4184. The cumulative annual rainfall was calculated from September to August to match sampling years rather than natural years.

For wild boar population monitoring, relative wild boar abundance estimates based on a dropping frequency index (FBII; Acevedo et al., 2007) were obtained yearly for the vaccinated sites (n=4) and the majority of control sites (n=11).

In order to assess vaccine impact (defined as the combined probability of bait uptake and protection) for each site as compared to control sites while controlling for known

disease drivers, a logistic regression model was fit using lesion presence as dependent variable.

Independent variables introduced in the model where known drivers of TB (Vicente et al. 2013): age, rainfall (cm), relative wild boar abundance (FBII), years (1 to 4); and initial TB prevalence (proportion) to account for the situation prior to intervention. All analyses and data visualization were undertaken in the R statistical package (R Development Core Team, 2015) using the packages: ggplot 2 (Wickham 2009), reshape2 (Wickham 2007). Significance was fixed at $p < 0.05$. The 95% confidence intervals (CI) were calculated by bootstrapping.

Modelling

To answer questions that could not be tested in this field trial and gain insight into the mechanisms that govern the dynamics of vaccinating against TB in wild boar, a mathematical model representing the key processes in this system was developed. The model reflects a single geographical estate containing a homogeneously mixed population with parameters that are representative of the field trial sites. Two different scenarios representing our vaccination sites were modelled: (a) site with medium initial prevalence where piglets have a low chance of infection prior to vaccination (default disease transmission rate and no pseudo-vertical transmission); and (b) site with higher initial prevalence and greater rates of transmission combined with a greater proportion of piglets infected prior to vaccine delivery (double transmission rate and 100% pseudo-vertical transmission). Three situations were addressed: (i) the influence of different levels of vaccination success (effective immunization) on disease prevalence, (ii) the influence of continued vaccination (25 years) and eventual cessation on population density, and (iii) on disease prevalence. The model framework, parameterization and interpretation are explained in Supplementary Material 2.

RESULTS

Bait uptake

The proportion of individual wild boar with chemical marker presence in serum by site and age class is displayed in Figure 2. Piglets from Natural sites had lower uptake rates (50 to 74%) than those from managed sites (89 to 92%). The chemical marker was detected as well in older wild boar (42-59%).

The topographical barrier separating different vaccine treatments in the Natural sites was not fully effective: consumption of both vaccine types (positive for both markers) was detected in 22-39% of vaccinated wild boar from the Natural sites.

Vaccine impact

Figure 3 presents the observed temporal trend of TBCL prevalence in piglets and at the population scale. The agreement between TBCL and culture had a kappa value of 0.68 (raw data on TBCL, lesion score and culture are listed in Supplementary Material 3).

Piglets showed a high initial infection pressure as the mean initial TBCL prevalence was 50% for the treatment sites and 53% for control sites. However, the initial piglet TBCL prevalence was highly variable and ranged from 0% to 100% among sites. At the end of the experiment, piglet TBCL prevalence had increased by 6% in both, the Control and Managed BCG sites, and decreased 36% in the Natural IV site. In the Natural BCG site the control year sample size was too low for comparisons. In the managed IV site, piglet TBCL prevalence remained 0% throughout the study period.

At the population scale, the TBCL prevalence evolution during this five-year period increased steadily but not significantly in the Control sites (5.6% increase, $X^2 = 0.922$, 1 d.f., $p > 0.05$). Regarding the treated sites, a significant decline occurred in the Managed IV site (34.4% reduction since control year; $X^2 = 7.665$, 1 d.f., $p < 0.01$). In this site, vaccination appeared to prevent infection and reduce disease severity (see Supplementary Material 3). No significant changes were recorded in the remaining sites ($p > 0.05$). The inter-annual variability in TBCL prevalence was high, particularly in the Natural sites (Figure 3).

Table 2 displays the results of the logistic regression model. Vaccination had a significant effect when IV was used in the Managed site ($p < 0.001$). However, the effect of vaccination was negligible for the sites in which BCG was deployed ($p > 0.05$) as well as for the Natural IV site ($p > 0.05$). Other significant variables explaining TBCL presence in our model were increasing age, low rainfall and initial prevalence.

Modelling

Two scenarios representing our vaccination sites were investigated: (a) similar to Managed sites (medium initial prevalence where piglets have a low chance of infection prior to vaccination) ; and (b) similar to Natural sites (high initial prevalence and greater

rates of transmission combined with a greater proportion of piglets infected prior to vaccine delivery).

Effects of vaccination success on disease prevalence

Figure 4 a(i) & b(i) shows that as the proportion of successfully vaccinated piglets (those that receive vaccine and are receptive to immunization thus resulting effectively immunized) increases, TB prevalence decreases (30% and 20% decrease, respectively, when vaccination success is 100%). This decrease in total prevalence is driven by less generalized infections and is greatest when piglets have a lower risk of infection prior to vaccination (a(i)).

Effects of continued vaccination (25 years) and eventual cessation on population density and disease prevalence

Figure 4 a(ii) & b(ii) shows the epidemiological dynamics regarding population density for a 25 year vaccination program when a 75% vaccine success level its assumed. It indicates that there is an initial reduction in the level of infected and generalized individuals, which lowers disease transmission and reduces mortality as less individuals progress to the generalized class in which disease induced mortality is substantial. The drop in the force of infection following vaccination drives an increase in total population density. Therefore, the reduction in disease prevalence shown in Figure 4 (a(iii) & b(iii)) is a consequence of an increase in total population density rather than a decrease in the density of infected and generalized individuals.

Population increase is driven by an increased pool of susceptibles which, in turn, can support a greater density of infected and generalized individuals. This explains why vaccination did not eradicate the disease in the model. The results for population density and disease prevalence on both scenarios are qualitatively similar but vaccine impact is less marked when pseudo-vertical and intense transmission take place.

Moreover, model results highlight how observations from the early years of a vaccination program may not give a clear picture of the effectiveness of a long-term vaccination strategy, since in the initial stages the benefits of vaccination are not compensated by an increase in total population density.

The model results also indicate that when the vaccination program is stopped there is an initial increase in disease prevalence and density of infected and generalized wild boar before levels return to those prior to vaccination. This is a consequence of the elevated population density resulting from vaccination and of the temporary nature of vaccine-derived immunity (see also Supplementary Material 2).

DISCUSSION

Our results confirmed the feasibility of bait deployment targeting wild boar piglets in Mediterranean habitats. Under optimal conditions of 90% piglet bait uptake and moderate (50%) initial infection prevalence, IV appeared to prevent infection and reduce disease severity, lowering TB prevalence at the population scale by 34% after four years. This result is particularly relevant in a context of increasing prevalence in the control sites. By contrast, no significant effects were found at a lower IV bait uptake rate (74%) and high natural challenge or in the sites in which BCG was deployed.

A key aspect of vaccination is to target enough individuals. In free-ranging populations this goal is difficult to achieve and assess. Commonly, bait uptake by piglets is a limiting factor in oral vaccination via baits (Kaden et al. 2000) but this trial was able to reach more than 70% of this age class in three of four sites. This is relevant regarding the potential of vaccination for controlling other diseases, e.g. classical swine fever, in case of eventual emergence in Mediterranean regions. In this study, higher uptake was achieved in populations used to being fed and to human presence, i.e. managed hunting estates. Management, a risk factor for TB (Vicente et al. 2013), can thus be helpful in vaccine delivery while naïve populations might take longer to get used to new food sources (Delahay 2003). Nevertheless, in highly prevalent populations, aggregation at piglet feeders could offset vaccination benefits through increased transmission (Sorensen et al. 2014).
Despite using piglet feeders, a proportion of older age individuals (42-59%) gained access to baits. The effect of vaccination in adults is unknown. We speculate it could act as a protective revaccination prolonging immunity of the individuals (as long as they are uninfected) but it will decrease bait availability for piglets.

The need of assessing new tools, such as vaccination, to manage TB effectively in wild boar is evident from the baseline situation of Mediterranean populations. In this region, monitoring has shown a 13% increase in wild boar TB between 2000 and 2012 (Vicente et al. 2013). In the control sites, wild boar TB prevalence was already high prior to intervention (mean 63% in 2012) and grew 5.6% during the five-year study period.

The impact of BCG deployment was not significant, as prevalence remained stable. BCG is known to confer variable protection in humans and cattle (Fine et al. 1995, Buddle et al. 2013) and field trials where BCG failed to confer any protection have been reported (e.g. Baily et al. 1980 and Beggren 1981). Field trials deploying BCG in other wildlife hosts demonstrated protective effect over vaccinated individuals but effects at the population level were less evident (Table 1). While we can ensure BCG viability through the study (Beltrán-Beck et al. 2014b), we cannot rule out interference due to non-tuberculous mycobacteria priming, genetic differences, nutritional status, co-infections etc. (Fine et al. 1995 and Buddle et al. 2013).

Regarding IV, a significant 34% reduction in TBCL prevalence was observed in one of the sites but vaccine impact was negligible in the other. This suggests that the effect of vaccination is context dependent. Vaccine performance can be affected by initial prevalence, pre-existing infection, population and disease dynamics (Gormley et al. 2011) and vary over time (Halloran et al. 1997) or in space (Kaden et al. 2000). Potential explanations for the different vaccine impact in these two settings are: heterogeneous exposure to MTC, different vaccination success achieved and inter-population mixing.

Regarding the first explanation, exposure heterogeneity (in terms of infective dose and number of reinfections), would to some extent explain the results obtained as vaccines are believed to protect better against a light challenge (Clemens et al. 2011). Since experimental challenge is thought to be more severe than the one occurring under natural circumstances, it has been proposed that vaccine efficacy will be greater under field conditions. Studies in Table 1 were conducted in settings where the initial TB prevalence ranged from 5 to 35%. In our study, the Managed IV site was characterized by a moderate initial prevalence (50%), no generalization (lesions restricted to mandibular LNs) and low infection pressure for piglets, whereas the Natural IV site was characterized by high initial prevalence (77%), high proportion of generalized individuals (36%) and high proportion of diseased piglets (86%). Although in field trials the challenge dose is unknown, in the latter setting challenge might have been intense enough to resemble the one in laboratory trials where all individuals develop disease despite getting the vaccine. The results obtained by modeling are along these lines and suggest that increased transmission intensity and proportion of already infected piglets reduces the impact attainable through vaccination (Figure 4).

The bait uptake achieved (second explanation) in both IV sites was not significantly different (92 and 74%) but the proportion of successfully vaccinated individuals (those that receive vaccine and are receptive to immunization), might have been. The existence of already infected piglets by the time of vaccination (in which despite vaccine consumption no protection is expected) and the consumption of both vaccines (with possible non-protective outcomes; Diez-Delgado et al. 2014) can decrease the proportion of effectively immunized individuals.
Third, permeable fences in Natural IV allow inter-population mixing (immigration/ emigration). These movements complicate the assessment of vaccine efficacy (dilution effect) and may act as a source of infection. Thus, we expect that closed and well-delimited (wild boar proof fenced) populations will benefit most from vaccination.

Predictive modeling suggests that vaccination is useful to control TB in wild boar but not enough to achieve eradication as a stand-alone tool. Moreover, we are considering the wild boar system in isolation, which is not realistic in Mediterranean settings, where several other wild and domestic hosts are likely contributing to MTC maintenance. Such complex settings would benefit from an integrated control scheme combining several tools, including wild boar vaccination, and targeting several hosts.
The impact generated at population scale by vaccination reaches its maximum ca. 5 years after starting the campaign. This is roughly the timeframe of this field experiment. During the vaccination campaign, a considerable pool of susceptibles builds up so once vaccination ceases; disease prevalence is expected to recover quickly. At the same time, the increase in susceptibles drives an increment of the overall population density. This implies that increased hunting or increased population control is required in order to balance the consequences of vaccination on population dynamics.

CONCLUSIONS

Our efforts to mimic a realistic bait deployment in free-ranging wild boar populations provide practical insights into the logistics of oral vaccination in Mediterranean ecosystems. Oral IV can contribute to TB control in its main Iberian reservoir, the wild boar. However, this study showed that IV performance is context dependent. This implies that successful vaccination will depend on tailored field protocols. We suggest that IV deployment should be regarded as just one tool among several others, and that

successful control schemes will require integrated strategies including all key maintenance hosts.

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Figure captions

Figure 1. Study area. Location of study area, Montes de Toledo, central Spain. Sites involved in the field trial. Three types of sites can be differentiated: control sites where no treatment is employed (light grey), sites vaccinated with BCG (blue) and sites vaccinated with heat-inactivated M.bovis vaccine (IV; yellow).

Figure. 2. - Bait uptake. Proportion of wild boar individuals positive to chemical marker detection in serum by site and age class. Bars are the percentage of individuals positive to detection of chemical marker, light grey bar represent single chemical marker detection and dark grey presence of both markers. Error bars are bootstrap 95% confidence intervals (CI). Red dashed line stands for the minimum theoretical 70% uptake threshold to achieve an effective intervention (Anderson et al. 2013).

Figure 3.- Temporal trend of TBCL prevalence by site of the total population and the piglet age class. The solid line represents total population and the dashed line the piglet age class. In the treatment sites control trend for total population (solid line) and piglets (dashed line) appears in light grey to provide background information. Error bars are bootstrap 95% CI.

Figure 4.- Results for the wild boar TB. (a) represents a site with medium disease prevalence in which piglets have a low chance of infection prior to vaccination (default disease transmission rate and no pseudo-vertical transmission) (b) represents a site with higher initial prevalence and greater rates of transmission combined with a greater proportion of piglets infected prior to vaccine delivery (double transmission rate and 100% pseudo-vertical transmission). Row (i) shows disease prevalence against proportional vaccination success, vp, with results determined at the stable endemic steady state when the specified level of vaccination is included; (ii) shows changes in population density against time (years) for a vaccination level of 75%, ($vp = 0.75$); and (iii) shows changes in disease prevalence against time (years) for a vaccination level of 75%, ($vp = 0.75$). In the figures N (blue) is the total population density; I (magenta) the total density of infected but not generalized; G (red) the total density of generalized; S (green) is the total density of susceptibles; and PV (cyan) is the total density of vaccinated piglets. Also, *ptot* (black) is the proportion of the total population infected

with TB (*ptot* = (I+G)/N); *pinf* (magenta) is the prevalence of infected but not generalized (*pinf* = I/N); and *pgen* (red) is the prevalence of generalized infection (*pgen* = G/N).

Table 1.- Summary of the results and characteristics of vaccination field trials against TB conducted under natural exposure conditions in wild species.

Species	Vaccine			Vaccine assessment		Reference
	Type	Dose	Route (delivery)	Endpoint	Efficacy	
Possum	BCG	10^6 cfu	Intranasal and conjuctival (trapping)	Clinical signs, lesion presence and cultured tissues	69%*	Corner et al. 2002
Possum	BCG	10^7 cfu	Oral (trapping)	Transition probability from susceptible to infected (estimated by modelling)	95%*	Tompkins et al. 2009
Possum	BCG	10^8 cfu	Oral (baiting)	Lesion presence and cultured tissues	81%	Nugent et al. 2016
Badger	BCG	10^6 cfu	Intramuscular (trapping)	Stat Pack serology INFg Culture	74%* 20% 27%	Chambers et al. 2011
Badger	BCG	10^8 cfu	Oral (trapping)	Stat Pack serology (hazard rate ratios)	36%-84%*	Gormley et al. 2017

* $p<0.05$

Table 2.- Results of the logistic regression model of TBCL presence. Estimates (B), estimate associated standard error (SE) and p-value. Reference values for the variables age and site are piglets and control respectively.

Predictor		B	(SE)	
(Intercept)		0.314	(0.472)	
Age	Subadults	0.993	(0.230)	***
	Adults	1.376	(0.214)	***
Rainfall		-1.574	(0.532)	**
FBII		-0.391	(0.363)	
Site	Managed BCG[a]	-0.613	(0.417)	
	Managed IV	-1.604	(0.295)	***
	Natural BCG	0.422	(0.356)	
	Natural IV	0.484	(0.250)	
Initial prevalence		0.839	(0.401)	*
Year		-0.074	(0.078)	

[a] Only results of three vaccination years available
*** $p<0.001$ ** $p<0.01$ * $p<0.05$

Supplementary Information for the TB Model

1.- Using mathematical modelling to examine the dynamics of the wild boar TB vaccination system

The model reflects a single geographical managed estate containing a homogeneously mixed population covering a 3x3 km^2 area. The population density of wild boar is separated into different age classes to capture distinct disease and biological characteristics for piglets (aged 0-1 year) P, yearlings (aged 1-2 years) Y, and adults (aged 2 years+) A. Further, the age-classes are split into susceptible, infected and generalised (super-shedder) classes (subscripts S, I, G, respectively) to reflect the disease status of the population. The population dynamics of the wild boar TB system are represented by the following set of non-linear differential equations (which is an extension of classical disease modelling frameworks (see Anderson and May 1979 [1]; Keeling and Rohani 2008 [2])) and a schematic representation is shown in Figure S1:

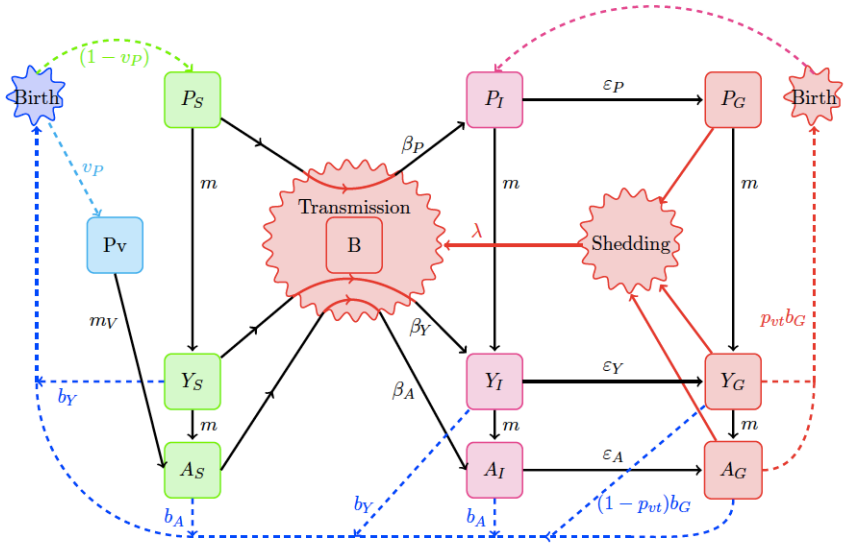


Figure S1: A schematic representation of the wild boar TB vaccination model represented by Equations 1. The model represents the density of piglets P, yearlings Y, and adults A with age-classes split into susceptible, infected and generalised (super-shedder) classes (subscripts S, I, G respectively). The class Pv represents vaccinated piglets and B represents the density of free-living TB particles. The parameters are detailed in Section 2.

$$\frac{dP_S}{dt} = (1-v_P)(b_Y(Y_S+Y_I)+b_A(A_S+A_I)+(1-p_{vt})b_G(Y_G+A_G))(1-qN) - mP_S - d_P P_S - \beta_P P_S B \quad (1a)$$

$$\frac{dP_I}{dt} = p_{vt} b_G(Y_G+A_G)(1-qN) + \beta_P P_S B - mP_I - d_P P_I - \varepsilon_P P_I \quad (1b)$$

$$\frac{dP_G}{dt} = \varepsilon_P P_I - mP_G - \alpha P_G - d_P P_G \quad (1c)$$

$$\frac{dP_V}{dt} = v_P(b_Y(Y_S+Y_I)+b_A(A_S+A_I)+(1-p_{vt})b_G(Y_G+A_G))(1-qN) - m_V P_V - d_P P_V \quad (1d)$$

$$\frac{dY_S}{dt} = mP_S - mY_S - d_Y Y_S - \beta_Y Y_S B \quad (1e)$$

$$\frac{dY_I}{dt} = \beta_Y Y_S B + mP_I - mY_I - d_Y Y_I - \varepsilon_Y Y_I \quad (1f)$$

$$\frac{dY_G}{dt} = \varepsilon_Y Y_I + mP_G - mY_G - \alpha Y_G - d_Y Y_G \quad (1g)$$

$$\frac{dA_S}{dt} = mY_S + m_V P_V - d_A A_S - \beta_A A_S B \quad (1h)$$

$$\frac{dA_I}{dt} = \beta_A A_S B + mY_I - d_A A_I - \varepsilon_A A_I \quad (1i)$$

$$\frac{dA_G}{dt} = \varepsilon_A A_I + mY_G - \alpha A_G - d_A A_G \quad (1j)$$

$$\frac{dB}{dt} = \lambda(P_G + Y_G + A_G) - \mu B \quad (1k)$$

Here, N represents the total wild boar population. Susceptible and infected yearlings and adults give birth to susceptible piglets at rates b_Y and b_A respectively. Generalised yearlings and adults give birth to piglets at rate b_G with a proportion p_{vt} assumed infected (through pseudo-vertical transmission from sow to offspring) and the remainder, $(1 - p_{vt})$, assumed susceptible. In this study we assume that $b_Y = b_A = b_G$. The total population is regulated through a crowding parameter, q, that acts to stabilise the total population to a carrying capacity, N = K, in the absence of disease. Maturity from piglets to yearlings and yearlings to adults occurs at rate m and piglets, yearlings and adults may die of natural causes at rates d_P, d_Y, d_A respectively. Here we assume $d_P = d_Y = d_A$.

The prime driver for infection in the wild boar TB system is through environmental contact with free-living TB particles, with density B. We assume that free-living particles are shed from generalised wild boar at rate λ and decay at rate μ. Susceptible may become infected through contact with free-living TB particles with transmission coefficients β_P, β_Y and β_A and infected can progress to the generalised class at rates ε_P, ε_Y and ε_A for the different age classes respectively. We assume that individuals in the generalised class suffer additional disease induced mortality at rate α. We assume piglets and yearlings are more susceptible to TB infection than adults and so set $\beta_P = \beta_Y$, which we assume to be three times greater than transmission for yearlings to be the same, $\beta_P = \beta_Y$, and three times the rate for adults, $\beta_A = 3\beta_Y$.

Similarly we set the rate of progression to generalised infection for piglets and yearlings to be the same, $\varepsilon_P = \varepsilon_Y$, and three times the rate for adults, $\varepsilon_A = 1/3\ \varepsilon_Y$ .In this way we have set the model so that the yearling class is the same as the piglet class in terms of disease characteristics, but the yearling class is the same as the adult class in terms of reproductive processes.

We represent vaccination in the model by assuming a proportion, v_P, of susceptible births enter the immune piglet class P_V. The vaccinated piglets lose their immunity at rate m_V maturing into the susceptible adult class. This implicitly assumes that when immunity is lost individuals have reached maturity and are able to reproduce but also have a reduced susceptibility to infection. Note, our vaccination coefficient combines the effects of both coverage and efficacy by representing the proportion of successful vaccinations. In the model the vaccination process is represented as a continuous process whereas in the field vaccination is applied to piglets aged 2-6 months. Therefore, there is a chance of infection prior to vaccination and we approximate this with the inclusion of pseudo-vertical transmission from generalised individuals.

2.- TB vaccination model parameters

We use empirical data to set the model parameters and where information is not available we set values to approximate the observed prevalence, representative of the wild boar TB system in Central Spain. The parameters are as follows:

$b_Y = b_A = b_G = \log(4)$ The population birth rate in a disease-free population when resources are unlimited. This constant rate means that for each reproductive member of the population, 3 piglets will be born, averaged over the population over a year. (This has been derived by assuming that there is a 50% sex ratio and that each female produces an average of 6 offspring per year when resources are not limited.)

K = 500 The carrying capacity for the total population in the target area in the absence of disease.

$q = \frac{1}{K}\left(1 - \frac{d_A(d_P+m)(d_Y+m)}{m(b_A m + b_Y d_A)}\right)$ This parameter limits the total population to the carrying capacity K in the populated disease-free steady state, and is derived from steady-state analysis of the model without infection.

$m = 1$ The rate that piglets mature to yearlings and yearlings mature to adults. These rates assume that it takes on average 1 year to enter the next age class.

$d_P = d_Y = d_A = 1/7$ The natural death rate of all classes which implies an average life expectancy of 7 years.

$\beta_P = \beta_Y = c\beta\beta_A = 20/K$ The infection rates are fitted to give prevalence levels observed in the wild

boar TB system in central Spain. We assume that $c_\beta = 3$ and so disease transmission to piglets and yearlings is three times that of the adult rate under the assumption that transmission is higher for piglets and yearlings than it is for adults.

$\varepsilon_P = \varepsilon_Y = 2$ The rate that infected piglets and yearlings become generalised. This assumes that it takes on average 6 months for an infected piglet or yearling to progress to the generalized class.

$\varepsilon_A = 2/3$ This is the rate that infectious adults become generalised. This assumes that it takes on average 18 months for an infected adult to progress to the generalised class.

$\alpha = 1$ This is the additional disease induced death rate of the generalised class and assumes that on average individuals spend 1 year in the generalised class before death.

$\lambda = 1$ The rate of shedding of infectious particles by generalised classes. We normalise this value to 1. This is valid as we have explored a range of values for β_P, β_Y and β_A which scale with the size of λ and the density of free-particles, B.

$\mu = 6$ This is the decay rate for free-living particles, indicating that they have an average life expectancy of 2 months.

p_{vt} The proportion of generalised births that result in pseudo-vertical transmission. In this study we assume $p_{vt} = 0$ or 1.

v_P The proportion of susceptible births successfully vaccinated. We explore the full range of possible values of v_P in this study.

$m_V = 1$ The rate that vaccinated piglets mature into the susceptible adult class. This assumes that when immunity is lost individuals are able to reproduce but also have the same reduced susceptibility to infection as adults.

3.- TB vaccination model results

In the results that follow we will refer to the total density of susceptibles as S where $S = P_S+Y_S+A_S$; the total density of infected but not generalised as I where $I = P_I + Y_I + A_I$; and the total density of generalised as G where $G = P_G + Y_G + A_G$. The total population density, N, can therefore be defined as $N = S+P_V+I+G$, which is at steady state, $N = P_S+Y_S+A_S = K$, in the absence of disease. All the densities are expressed in terms of population per geographical area. We define the total prevalence, $p_{tot} = (I+G)/N$, as the proportion of the total population infected with TB; the prevalence of infected but not generalised $p_{inf} = I/N$; and the prevalence of generalised $p_{gen} = G/N$; such that $p_{tot} = p_{inf} + p_{gen}$. We used MATLAB to obtain numerical results for the model as the proportion of successfully vaccinated piglets v_P is varied. We use the default parameter set detailed in Section 2 under conditions of 0 or 100% pseudo-vertical transmission, $p_{vt} = 0$ or 1. We consider results for both the default transmission coefficient, which results in a medium disease prevalence at steady state, and twice the default transmission value to reflect a greater risk of TB infection resulting in higher disease prevalence at the

endemic steady state. We run the model until it has reached a stable endemic steady state then include vaccination for a period of 25 years to achieve a stable vaccinated steady state. We examine how vaccination affects the disease prevalence statistics p_{tot}, p_{inf} and p_{gen} and the epidemiological dynamics.

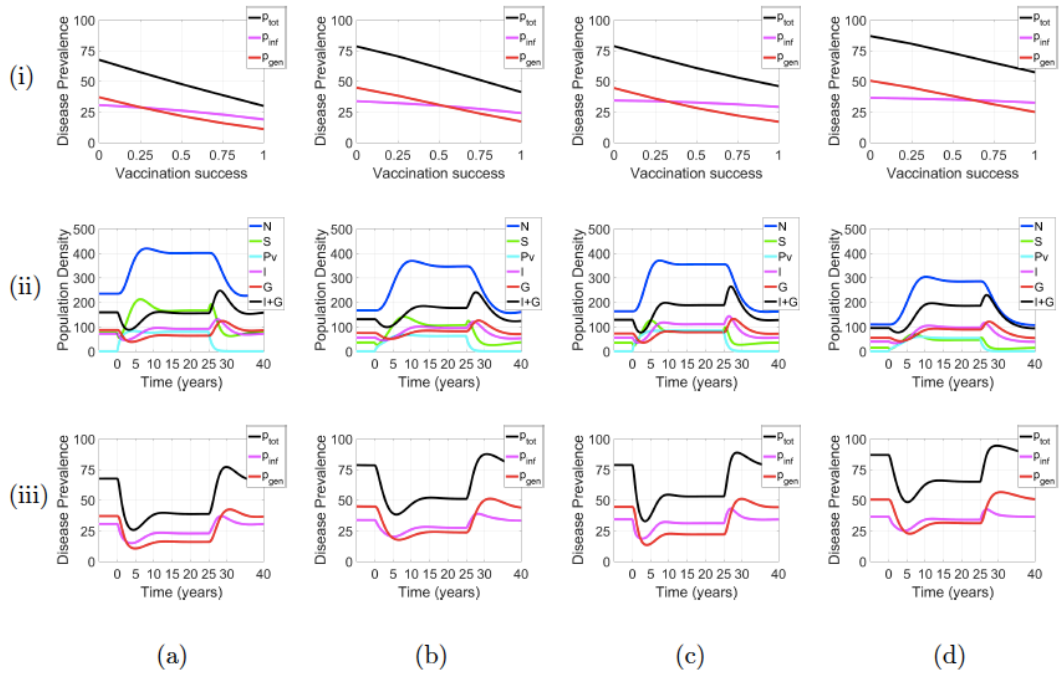
We examine results for the model described by Equations 1a-1k in different combinations of disease transmissions rates and pseudo-vertical transmission.
Figure S2 (a) shows results for the default parameter set from Section 2 and 0% pseudo-vertical transmission; Figure S2 (b) shows results for default parameters with 100% pseudo-vertical transmission; Figure S2 (c) shows results for twice the default rate of disease transmission and 0% pseudo-vertical transmission; and Figure S2 (d) shows results for twice the default rate of disease transmission with 100% pseudo-vertical transmission. Note, Figure S2 (a) and (d) also appear in the main text, denoted as Figure 4.- (a) & (b) respectively.
Figure S2 (i) shows the change in disease prevalence for different levels of vaccination success (see also Table S1). Figures S2 (ii) and S2 (iii) show the epidemiological dynamics and changes in disease prevalence over time when we assume a 75% level of vaccination success.

Table S1: Table showing changes in p_{tot}, p_{inf} and p_{gen} for different levels of vaccination when pseudo-vertical transmission is 0% or 100%. Other details are as in Figure S2.

v_P	Default Transmission						Doubled Transmission					
	$p_{vt} = 0\%$			$p_{vt} = 100\%$			$p_{vt} = 0\%$			$p_{vt} = 100\%$		
	p_{tot}	p_{inf}	p_{gen}	p_{tot}	p_{inf}	p_{gen}	p_{tot}	p_{inf}	p_{gen}	p_{tot}	p_{inf}	p_{gen}
0	68	31	37	79	34	45	79	34	44	87	36	50
0.25	57	29	29	70	32	38	70	34	36	81	36	45
0.5	48	26	22	61	30	31	61	33	28	73	35	38
0.75	39	23	16	51	27	24	53	31	22	65	34	31
1	30	19	11	41	24	17	46	29	17	57	33	25
	(a)			(b)			(c)			(d)		

Figure S2: *Using default parameter values described in Section 2 with (a) 0% pseudo-vertical transmission; (b) 100% pseudo-vertical transmission; (c) 0% pseudo-vertical transmission with disease transmission twice the default value; and (d) 100% pseudo-vertical transmission with disease transmission twice the default value. Row (i) shows disease prevalence against proportional vaccination success, vp, with results determined at the stable endemic steady state*

when the specified level of vaccination is included; (ii) shows changes in population density against for a vaccination level of 75%, (v_p = 0:75); and (iii) shows changes in disease prevalence against for a vaccination level of 75%, (v_p = 0:75). Here p_{tot} (black) is the proportion of the total population infected with TB; p_{inf} (magenta) the prevalence of infected but not generalised; p_{gen} (red) the prevalence of generalised; N(blue) is the total population density, I(magenta) the total density of infected but not generalised; G(red) the total density of generalised; S(green) is the total density of susceptibles; and PV (cyan) is the total density of vaccinated piglets.



References

[1] Anderson, R.M. and May, R.M. (1979). Population Biology of Infectious Diseases 1. Nature 280 361-367.

[2] Keeling, M.J. and Rohani, P. (2008). Modeling infectious diseases in humans and animals. Princeton University Press.

Supplementary Table 1.- Characterization of study sites.

Site id.	Status	Type of vaccine	Surface (km^2)	Type of site	Mean FBII	Fencing (permeability)	Supplementary feeding	Initial TBCL (%)
1	Control	None	8	Private hunting site	0.1	Yes (wild boar permeable)	Yes	53
2	Control	None	9	Private hunting site	n.a.	Yes (wild boar permeable)	No	60
3	Control	None	103	National Park	0.3	Yes (wild boar permeable)	No	67
4	Control	None	27	Comunal land	n.a.	No	No	50
5[a]	Vaccinated	BCG	19	Private hunting site	0.4	Yes (wild boar proof)	Yes	53
6	Control	None	30	Comunal land	0.1	No	No	36
7	Control	None	14	Private hunting site	0.5	Yes (wild boar proof)	Yes	92
8	Control	None	22	Private hunting site		Yes (wild boar proof)	Yes	82
9	Control	None	8	Private hunting site	n.a.	No	No	64
10	Control	None	22	Comunal land	0.1	No	No	40
11[b]	Vaccinated	BCG	27	Natural Park (leased hunting land)	0.2	Yes (wild boar permeable)	No	100
12[c]	Vaccinated	IV	29	Natural Park (leased hunting land)	0.2	Yes (wild boar permeable)	No	77
13	Control	None	9	Private hunting site	1.2	Yes (wild boar proof)	Yes	56
14	Control	None	26	Private hunting site	0.2	Yes (wild boar proof)	Yes	87
15	Control	None	20	Private hunting site	0.4	Yes (wild boar proof)	Yes	81
16	Control	None	21	Comunal land	0.3	No	No	33
17	Control	None	23	Private hunting site	0.3	Yes (wild boar proof)	Yes	88
18	Control	None	19	Private hunting site	0.3	Yes (wild boar proof)	Yes	79
19[d]	Vaccinated	IV	21	Private hunting site	0.5	Yes (wild boar proof)	Yes	50

[a]Managed_BCG [b]Natural_BCG [c]Natural_IV [d]Managed_IV

Supplementary Figure 1.- Distribution of piglet feeders in vaccination sites: (a) Managed (BCG and IV) and (b) Natural (BCG and IV).

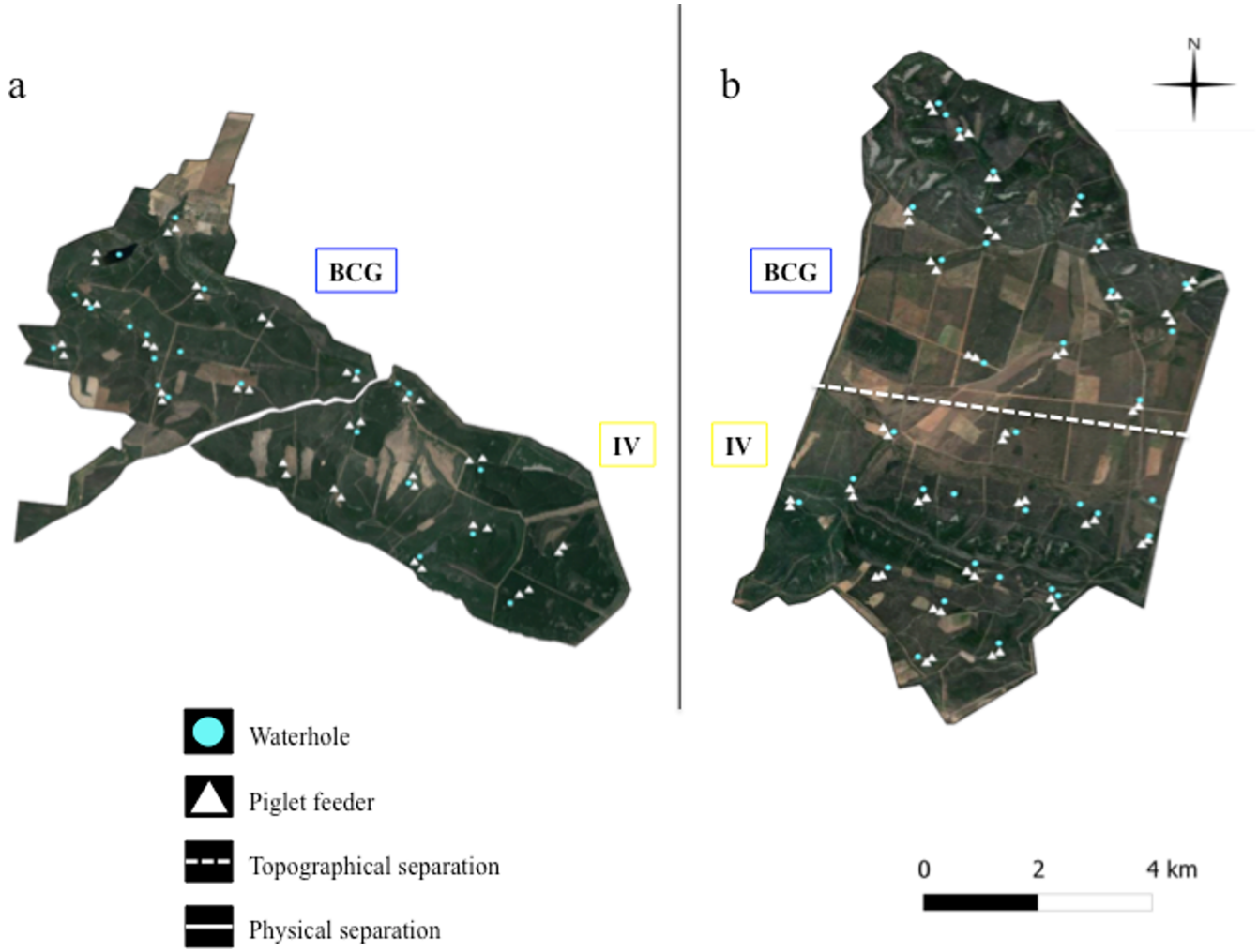


Table 1.- Raw data on presence of tuberculosis compatible lesions (TBCL, disease presence) of the total population and piglet age class by site and year. Sample size (n), absolute number of positives (+) and prevalence with associated 95%CI.

	Control year			Vaccination year 1			Vaccination year 2			Vaccination year 3			Vaccination year 4		
TBCL	n	+	Prevalence (95%CI)	n	+	Prevalence (95%CI)	n	+	Prevalence (95%CI)	n	+	Prevalence (95%CI)	n	+	Prevalence (95%CI)
Control															
Total population	160	101	63.13 (56.25-70)	155	104	67.10 (59.38-74.84)	102	74	72.55 (63.73-80.39)	161	111	68.94 (61.49-75.79)	112	77	68.75 (59.82-76.81)
Piglets	34	18	52.94 (34.94-70.59)	36	18	50 (33.33-66.66)	16	14	87.5 (68.75-100)	31	16	51.61 (35.48-67.74)	22	13	59.10 (40.90-77.27)
Managed BCG															
Total population	17	9	52.94 (29.41-76.47)	13	7	53.85 (30.58-84.62)				16	8	50 (25.00-75.00)			
Piglets	7	1	14.29 (0-42.86)	5	3	60 (20-100)				5	1	20 (0-60)			
Managed IV															
Total population	24	12	50 (29.71-70.83)	27	12	44.44 (25.93-62.96)	19	6	31.58 (10.52-52.63)				32	5	15.63 (3.13-28.13)
Piglets	1	0	0	2	0	0 (0-0)	6	0	0 (0-0)				4	0	0 (0-0)
Natural BCG															
Total population	11	11	100 (100-100)	35	33	94.29 (85.71-100)	14	9	64.29 (35.71-85.71)	33	23	69.70 (51.51-84.85)	26	25	96.15 (88.46-100)
Piglets	1	1	100				2	1	50 (0-100)	6	3	50 (16.67-83.33)	6	5	83.33 (50-100)
Natural IV															
Total population	22	17	77.27 (59.09-95.45)	24	21	87.50 (74.89-100)	41	25	60.98 (46.34-75.61)	49	44	89.80 (81.63-97.96)	47	35	74.47 (61.70-87.23)
Piglets	7	6	85.71 (57.14-100)	2	0	0 (0-0)	11	3	27.27 (0-54.54)	9	7	77.78 (44.44-100)	8	4	50 (12.50-85.50)

Table x.- Raw data on culture positivity (infection)of the total population and piglet age class by site and year. Sample size (n), absolute number of positives (+) and prevalence with associated 95%CI.

TB CULTURE	Control year			Vaccination year 1			Vaccination year 2			Vaccination year 3			Vaccination year 4		
	n	+	Prevalence (95%CI)	n	+	Prevalence (95%CI)	n	+	Prevalence (95%CI)	n	+	Prevalence (95%CI)	n	+	Prevalence (95%CI)
Control															
Total population	43	25	58.14 (44.19-72.09)	84	30	35.71 (26.19-45.24)	46	26	56.52 (41.30-69.57)	152	130	85.53 (79.61-90.79)	110	56	50.90 (40.91-60.02)
Piglets	15	11	73.33 (53.33-93.33)	36	12	33.33 (19.44-50.00)	16	14	87.5 (68.75-100)	30	24	80 (66.58-93.33)	22	10	45.45 (22.73-68.18)
Managed BCG															
Total population	12	6	50 ()	12	8	66.67 (41.67-91.67)				16	13	81.25 (62.50-100)			
Piglets	7	1	14.29 (0-42.86)	5	3	60 (20-100)				5	3	60 (20-100)			
Managed IV															
Total population	12	5	41.67 ()	27	4	14.81 (3.70-29.63)	18	6	33.33 (11.11-55.56)				32	5	15.63 (3.13-28.13)
Piglets	1	0	0	2	0	0 (0-0)	6	0	0 (0-0)				4	0	0 (0-0)
Natural BCG															
Total population	7	5	71.43 (42.86-100)	35	25	71.43 (57.14-85.71)	14	11	78.57 (57.14-100)	33	26	78.79 (63.64-90.91)	26	23	88.46 (76.92-100)
Piglets	1	0	0	1	0	0	2	1	50 (0-100)	6	4	66.67 (33.33-100)	6	5	83.33 (50-100)
Natural IV															
Total population	16	13	81.25 (62.34-100)	24	18	75 (58.33-91.67)	41	25	60.97 (46.34-75.61)	49	46	93.88 (85.71-100)	47	35	74.47 (61.70-85.11)
Piglets	4	4	100 (100-100)	2	0	0 (0-0)	11	2	18.18 (0-45.45)	9	7	77.78 (55.56-100)	8	7	87.5 (62.50-100)

Table 3.- Raw data on lesion score (disease severity) of the total population and piglet age class by site and year. Sample size (n), absolute number of positives (+), absolute number of generalized individuals OUT OF THE POSITIVES(G; more than one anatomical region affected, anatomical regions considered are head, thorax and abdominal cavity) and mean score (Mean).

TB LESION SCORE	Control year			Vaccination year 1			Vaccination year 2			Vaccination year 3			Vaccination year 4		
	n	+ (G)	Mean	n	+ (G)	Mean	n	+ (G)	Mean	n	+ (G)	Mean	n	+ (G)	Mean
Control															
Total population	160	101 (44)	2.81	155	104 (54)	4.30	102	74 (41)	4.59	161	111 (53)	4.27	112	77 (36)	4.71
Piglets	34	18 (3)	1.62	36	18 (6)	3.53	16	14 (9)	6.50	31	16 (10)	5.32	22	13 (5)	3.23
Managed BCG															
Total population	17	9 (3)	1.76	13	7 (7)	4.62				16	8 (1)	1.38			
Piglets	7	1(1)	0.57	5	3 (3)	8.20				5	1(0)	0.80			
Managed IV															
Total population	24	12 (0)	1.08	27	12 (4)	2.30	19	6 (3)	1.79				32	5 (1)	0.560
Piglets	1	0(0)	0	2	0 (0)	0	6	0	0				4	0	0
Natural BCG															
Total population	11	11 (4)	5	35	33 (25)	6.43	14	9 (5)	7.29	33	23 (17)	6.73	26	25 (15)	7.156
Piglets	1	1	2				2	1(0)	2	6	3 (1)	2	6	5 (3)	7
Natural IV															
Total population	22	17 (8)	4.64	24	21 (16)	5.67	41	25 (12)	3.68	49	44 (20)	6.27	47	35 (17)	4.43
Piglets	7	6 (2)	3	2	0 (0)	0	11	3 (2)	2.27	9	7 (4)	7.89	8	4 (2)	4.75