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ORIGINAL ARTICLE

Shared use of mineral supplement in extensive farming and its potential for infection transmission at the wildlife-livestock interface

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Abstract

Recently, the survival of *Mycobacterium bovis* on livestock mineral blocks has been confirmed, but little is known about its implication in the transmission of animal tuberculosis (TB) under field conditions. The objective of this study was to describe the shared use of mineral supplements in four extensive beef cattle farms from a high TB prevalence area in South Central Spain, to identify the main factors explaining their use, and characterize its potential role for the transmission of *Mycobacterium tuberculosis* Complex (MTC). This is relevant to design control measures at the wildlife-livestock interface. Animal activity was monitored by camera-trapping at 12 mineral supplementation points during spring and fall. Additionally, swabs were periodically taken from the mineral substrates and analyzed by PCR searching for MTC DNA. Cattle, pig, goat, sheep, wild boar, and red deer were all recorded licking on mineral supplementation points. Livestock species were the main users and presented a diurnal use pattern. Wild ungulates presented a nocturnal-crepuscular use pattern, with scarce overlapping with livestock. Wild boar presence was positively related to cattle presence at mineral supplementation points, whereas red deer presence was higher in supplemental points closer to forested areas and in farms without hunting pressure. We recorded 266 indirect wildlife-livestock interactions (i.e., two consecutive visits that occurred within 78 h), all of them derived from 21 unique wildlife visits. All the analyzed swabs resulted negative to MTC DNA. Comparing to other environmental sources of MTC in our study area, mainly water ponds, this research evidenced that mineral blocks are less attractive to wildlife. However, the potential for interspecific transmission of MTC or other pathogens cannot be discarded. The risk for interaction at mineral supplementation points and further transmission can be prevented by implementing specific measures in the context of integral biosecurity plans at the wildlife-livestock interface, which are proposed.

Keywords Bovine tuberculosis · Interactions · Interspecific transmission · Mineral block · *Mycobacterium tuberculosis* Complex · Photo-trapping

Introduction

Animal tuberculosis (TB, also commonly referred to as bovine tuberculosis, caused by *Mycobacterium bovis* and other members of the *Mycobacterium tuberculosis* Complex, MTC) is shared by wildlife and livestock in different epidemiological contexts worldwide (Gortázar et al. 2015). The presence of the MTC at this interface is of economic, sanitary (including human health) and conservation concern (Krebs et al. 1998; Gortázar et al. 2010; Gormley and Corner 2018). The wide range of (domestic and wild) host species together with the combination with cultural and environmental factors leads to many different epidemiological scenarios with their own risks for transmission (Humblet et al. 2009; Fitzgerald and Kaneene 2013).

Previous studies highlighted the shared use of resources by wildlife and extensive livestock in a wide range of epidemiological settings all around the world. For instance, the cattle-badger (*Meles meles*) interface in UK, where indirect contact seems to be the main driver for TB transmission, occurring in cattle pastures, farm buildings, or latrines (Böhm et al. 2009; Drewe et al. 2013; Woodroffe et al. 2016, 2017; Silk et al. 2018), or the cattle-white-tailed

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(*Odocoileus virginianus*) deer in Michigan, USA, where indirect contact is usually caused by deer accessing to food storage sites (Berentsen et al. 2014; Lavelle et al. 2015, 2016; VerCauteren et al. 2018). In Spain, shared use of resources by wildlife and extensive livestock has been documented for cattle-badger, cattle-Eurasian wild boar (*Sus scrofa*), and red deer (*Cervus elaphus*), and these wild ungulates also with domestic pigs (Kukielka et al. 2013; Carrasco-García et al. 2016; Acevedo et al. 2019; Triguero-Ocaña et al. 2020). These species, along with goats and sheep, are part of the MTC maintenance host community in the Iberian Peninsula (Santos et al. 2020). In this complex epidemiological system, the transmission of MTC is mainly indirect (Cowie et al. 2016), and it has been attributed to shared pastures and water or feed contaminated with saliva, urine, or feces from infected animals (Santos et al. 2015; Barasona et al. 2017b, a). It is known that mycobacteria from MTC are relatively resistant to environmental factors and under appropriate conditions may persist in the environment for weeks or months, prolonging the likelihood of indirect transmission by ingestion (Fine et al. 2011). Recently, it has been reported that *M. bovis* can be isolated up to 78 h post inoculation in mineral blocks, depending on the composition of the block and the environmental conditions (Kaneene et al. 2017). Previous studies highlighted the shared use of mineral blocks and their potential role as aggregation points between wildlife and livestock in extensive farming systems (Payne et al. 2016). However, little is known about their potential for *M. bovis* and other members of the MTC transmission, which depends on both survival of mycobacteria and the specific use of the blocks by hosts.

In this context, the aim of this study was to describe and quantify the shared use of mineral supplements by wildlife and livestock during two seasons in beef cattle farms from a high TB prevalence area (South and Central Spain, SCS), and to assess the presence of MTC DNA on the blocks. Results should be relevant to the design of control measures to reduce risk of transmission of bacili of the MTC at the wildlife-livestock interface.

Materials and methods

The study was carried out in four beef cattle farms from Ciudad Real (Castilla-La Mancha) and Córdoba (Andalucía), two provinces from SCS, during two different seasons, spring (April to May) and fall (September to October) of 2016. The mineral supplements used can be divided in two types: (i) *natural salt rocks* with impurities and variable composition, mainly sodium chloride (NaCl) with mineral traces, and (ii) *artificial mineral blocks* composed of 38% sodium (Na), 1% calcium (Ca), and 0.6% magnesium (Mg). Three mineral supplementation points (MP) were selected for monitoring in each farm. The type and location of all MPs were those used by the farmers (Table 1).

Camera traps (Ltl-5310, Ltl ACORN® Futian, Shenzhen, China), one per MP, were attached to trees or wooden posts at 5 m from the mineral supplement to record the presence of animals. Camera traps were set to take 3 consecutive pictures after animal detection, with a 1 min interval between consecutive activations. Camera traps remained in the field a minimum of 14 days per season, resulting in 315 operative

Table 1 Characterization of the selected farms in terms of livestock census, mineral supplementation, land use, and game management. Game information includes hunting bag data (hunted animals/year) and trapping rates (TR, visits/camera·day) for both red deer and wild boar (see below)

		Farm 1	Farm 2	Farm 3	Farm 4
Census	Goat	80	0	0	6
	Pig	110	200	250	0
	Sheep	0	16	0	340
	Cattle	80	150	60	306
Mineral supplement	Composition	Artificial	Natural	Artificial	Natural
	Disposal	Hanging (1.2 m)	Iron grid (0 m)	Hanging (0.4 m)	Ground (0 m)
Land use	% *dehesa* (open oak woodland)	50%	61%	99%	11%
	% scrubland/woodland	50%	39%	1%	89%
	Total (ha)	300	560	181	728
	Use (livestock/hunting)	Both	Both	Livestock	Livestock
Hunting bag/year	Red deer (*n*)	30	0	3	0
	Wild boar (*n*)	20	20	3	0
Wildlife trail camera	Red deer (TR)	2.58	0.65	0	0.83
	Wild boar (TR)	0.27	0.83	0.36	1.05

camera days that were used for the statistical analysis (147 camera-days were discarded due to operating failures).

In order to measure wildlife activity independently to that at the MP, we set camera traps in two active wildlife trails per farm and season. Two cameras per farm were installed in natural and obvious wildlife trails up to 250 m from a MP and separated by an average of 1 km from each other to ensure spatial independence.

The pictures recorded by each camera trap in MP were visualized to determine the animal activity at "visit" level. A *visit* was defined as a consecutive series of pictures where a single animal or a group of them (belonging to the same species) were recorded in a given camera, and separated more than 15 min of the next series of the same species. The interval between visits (IBV, 15 min) was established following the procedure described in Kukielka et al. (2013). Briefly, IBV was assessed after a trial of 5, 15, 30, 45, and 60 min carried out with the data from three randomly selected CT. The smallest changes of number of visits per IBV at each camera appeared when selecting for 15 min IBV or more (i.e., defining IBV as 15 or 30 or 45 or 60 min resulted in similar number of visits). For each visit, we recorded date, time, visit duration (difference between the first and last picture of the series), the species involved (cattle, sheep, goat, pig, wild boar, or red deer), the maximum number of individuals in the group, and number of pictures in each visit where at least one individual is licking directly on the mineral supplement.

Due to grazing management conducted by farmers, not all the livestock species could access to MP at any time (they rotate over grazing plots). In order to address this condition, when assigning the camera traps with presence of a given species, we used only the days in which the given species could potentially be captured by camera traps (Potentially Camera-trap day, PCT day; data provided by the farmers). For wildlife species, we assume that all camera-trapping days were PCT days, since fences were permeable to wildlife.

To characterize animal activity at the MPs, we calculated different parameters for each species: (i) the daily presence rate (PR), calculated as the proportion of PCT days with presence of a given species; (ii) the daily visit rate (VR) per species, calculated as the number of visits per PCT day; (iii) the animal rate (AR), calculated as the sum of the maximum number of individuals of each species per visit and PCT day; (iv) the use index (UI), calculated as the time spent (in seconds) by each species in each visit per PCT day; and (v) the daily use pattern (DUP), assessed as the proportion of visits to MPs by hour of the day. Differences in the activity parameters among species and seasons were explored using non-parametric statistical tests.

Concerning the interactions between livestock and wildlife, a *direct interaction* was defined as a visit where two or more individuals of different species were captured in the same picture. Otherwise, an *indirect interaction* was defined as two consecutive visits that occurred within a specific Critical Time Window (CTW). We established a conservative CTW (78 h), based on risk of TB transmission, using the maximum survival time of *M. bovis* on a mineral substrate reported by Kaneene et al. (2017). Both types of interactions can be classified as *interspecific* or *intraspecific*, depending on if the subsequent (or simultaneous) visit is a different species or not, respectively. Additionally, indirect interactions were classified depending on the number of visits that occurred between the first visit and the visit that produced the interaction (interaction visit) within the CTW. For that purpose, we named as first-order interactions those in which the interaction visit first occurred after the first visit, second-order interactions those in which the interaction visit happened after a first-order interaction, and so on.

We followed the protocol for data exploration described by Zuur et al. (2010) in order to avoid type I or type II errors and potentially erroneous ecological conclusions. We tested if the animal activity parameters differed between species using non-parametric tests (Kruskal–Wallis and Mann–Whitney-Wilcoxon), since data was not normally distributed. Generalized lineal mixed models (Poisson distribution and log link function) were developed to identify the factors related with the activity of red deer and wild boar in MPs. We used as dependent variable the VR of a given species per camera trap and season, since this parameter reflects the use of mineral supplementation, and the potential for interaction and consequent transmission of pathogens. Regarding the explanatory variables, we used season (spring vs fall, categorical), land use (livestock/hunting), type of mineral supplement (natural on the ground or artificial hanging), the UI of each livestock species (seconds, as indicative of livestock presence), the distance to cover (distance in meters from each camera to the nearest forest/scrubland patch, as a measure of proximity to wildlife habitat), and the relative abundance of wildlife (VR; visits/PCT day in wildlife trails). Farm ID was included as a random effect in order to control its effect and avoid masking the effects of the rest of the variables associated to farm. We selected the most parsimonious model using the Akaike Information Criterion (Δ_i AIC >2; Burnham and Anderson 2004). Models were run separately for wild boar and red deer. Sequentially, we implemented hierarchical variance partitioning (Moustakas and Evans 2015) of the covariates of the most parsimonious model to account for the contribution of each explanatory variable to the total variance of Wild boar VR and Red deer VR. All statistical analyses were conducted using computing software R 3.5.1 (R Core Team 2019).

Additionally, samples were collected from the surface of the mineral substrate every 2–3 days with a sterile swab (Copan Diagnostics Inc., Murrieta, CA, USA), properly tagged and frozen at −18 °C until laboratory diagnostics were performed. Sixty swabs were selected and analyzed for the presence of MTC DNA. The sample selection was based on the previous presence of wildlife at the MP by camera-trapping in order assign any positivity to the use of the MP, and to maximize the probability of MTC detection. In the laboratory, the swabs were cleaned in buffered tampon and centrifuged. Manual DNA extraction was performed with FluoroLyse Kit (Hain Lifesciences, Nehren, Germany). MTC DNA amplification analyses and control elaboration were carried out following the procedure described in Barasona et al. (2017b).

Results

Within the potential TB reservoir species detected in the 1397 recorded visits, cattle ($n = 789$), pigs ($n = 453$), goats ($n = 37$), sheep ($n = 92$), wild boar ($n = 11$), and red deer ($n = 15$) were identified, and all of them were captured using mineral supplements at some point. Livestock species were the main users of mineral supplements compared with wild ungulates, and their presence was predominant in PR, VR, AR, and UI (Mann–Whitney test $P < 0.05$ in any case) (Supplementary Material 1). The presence of wild ungulates at MPs was infrequent in terms of days visited (26 of the 315 days analyzed) and in terms of number of visits per visited day (number of visits on these 26 days ranged from 1 to 3 for red deer, with 1.5 visits on average, and wild boar only visited MPs once each day it appeared). No significant differences between red deer and wild boar were found in terms of the activity parameters (Mann–Whitney test $P > 0.05$ in any case).

Regarding seasonality, there were no significant differences in wild boar or red deer activity parameters between spring and fall (Wilcoxon test $P > 0.05$ in any case). However, we identified seasonal differences in some cases for domestic species (Cattle VR, AR, and UI were significantly higher in fall, while Goat PR and VR, Pig PR, and Sheep PR, VR, and AR were significantly higher in spring). In the Supplementary Material 1, we show which species where absent per season and farm, where livestock management determined the presence or absence of a given species in the area.

There were also no significant differences in wild boar or red deer activity parameters depending on mineral supplement type (hanging artificial mineral block or natural salt rock on the ground), and neither for cattle (Wilcoxon test $P > 0.05$ in any case). Goat and sheep mineral supplement type preference could not be consistently analyzed because their presence was limited to specific farms, but we identified significant differences for pig (Wilcoxon test $P < 0.05$ in any case). These results are consistent with our empirical observations, since during picture visualization, it was possible to verify that wild boar and pig were not able to lick directly from mineral supplements on Farm 1, where the mineral blocks were hanging at least at 1 m from the ground (Fig. 1). However, wild boar and

Fig. 1 Example of different species interacting with a hanging mineral block MP. The species (from left to right and from top to bottom) are goat, cattle, wild boar, Iberian pig, and red deer

pigs were attracted by the mineral remnants on the ground, since they showed rooting behavior immediately below the hanging mineral block.

Regarding the direct licking on mineral supplements (Supplementary Material 2), cattle was the most frequent species in absolute terms (527 "licking" visits) and goat was the species with higher relative use (93.90% of the visits). Red deer "licked" MPs in 8/15 visits, and wild boar in 3/11 visits. No statistical differences were found in licking behavior (number of "licking" visits and proportion of visits with "licking" behavior) between seasons for red deer nor wild boar (Mann–Whitney test $P > 0.05$ in any case).

The results of generalized lineal mixed models (Table 2) showed that Wild boar VR significantly and positively associated with the time spent by cattle in MPs (Cattle UI). The red deer model showed that in those MPs with no hunting pressure (the MPs in farms with just "livestock land use" and no "hunting land use"), red deer activity was significantly higher. The model also evidenced that VR was lower ($p = 0.05$) in MPs further from the forest/scrubland patches. No seasonal differences in specific VR were observed neither in wild boar nor red deer. Regarding hierarchical variance partitioning, the independent effects that explained the most variance (> 10% of the total variance) in the species VR were, in descending order, for eild boar, Cattle UI (34.06%), and distance from the nearest forest/scrubland patch (17.28%), and for red deer, distance from the nearest forest/scrubland patch (24.89%), pig UI (14.82%), and land use (13.37%) (Supplementary Material 4).

As for the DUP, domestic species were mainly seen during daytime at MPs, while wildlife showed a nocturnal pattern (Fig. 2).

Regarding the interactions, 42 direct interspecific events (two species in the same visit) were identified at MPs, 41 of which were livestock-livestock interactions: 21 cattle-pig, 15 cattle-sheep, and 5 cattle-goat interactions. The only direct livestock-wildlife interaction occurred in fall season at 3:58 a.m. between one cow and four wild boar. Direct intraspecific interactions were recorded in 878 visits (62.85%), corresponding to those composed by more than one individual of the same species (Supplementary Material 5).

In addition, 29,632 indirect interactions were recorded, from which 24,726 (83.44%) were intraspecific interactions, and 4906 (16.56%) were interspecific interactions (Table 3). Wild ungulates were involved in 503 indirect interspecific interactions (10.25% of the interspecific interactions), from which about half (266) presented wildlife-livestock directionality (58 deer-cattle, 41 deer-pig, 147 wild boar-cattle, 13 wild boar-sheep, and 7 wild boar-goat), 80 occurring during spring season, and 186 during fall. Time lapse between wildlife-livestock interactions ranged between 1 h 35 min and 77 h 53 min, with 42 h 19 min on average. Only 15 first-order wildlife-livestock interactions were recorded (3.05% of the interspecific interactions, 5.63% of those presenting wildlife-livestock directionality), ranging from 1 h 35 min and 45 h 40 min, with 9 h 54 min on average (Fig. 3). All 266 wildlife-livestock interactions originated from 21 visits (10 out of 15 red deer visits and all 11 wild boar visits).

All analyzed swabs tested negative to the presence of MTC DNA. Positive controls performed correctly, so all the PCRs amplified.

Discussion

Throughout this work, we have been able to verify how all present ungulate reservoirs, both domestic and wild, have been recorded licking on the mineral supplementation points. We recorded less activity and use of MPs by wild ungulates compared to livestock. As indicative, in terms of the proportion of days when wildlife activity was detected, our values (red deer mean = 2.53% of PCT days, SD = 15.73; wild boar mean = 3.48% PCT days, SD = 18.36) contrast with the 36% and the 28% of days previously reported for red deer and wild boar for other extensive farm resources such as water points, in the same study area (Carrasco-García et al. 2016). This suggests that wildlife is not as strongly attracted

Table 2 Best Poisson mixed effects models for wild boar and red deer daily visit rate (VR) in mineral supplementation points. Significance is marked with an asterisk

VR Models	Wild boar				Red deer			
	Estimate	S.E	*z*	*p*	Estimate	S.E	*z*	*p*
Intercept	−4.741	1.451	−3.267	0.001*	−3.505	2.902	−1.208	0.227
Distance to wildlife cover	−0.002	0.002	−0.992	0.321	−0.012	0.006	−1.946	0.052
CattleUI	0.002	0.001	2.129	0.033*	0.001	0.002	0.660	0.509
SheepUI	0.009	0.001	1.600	0.109				
PigUI					0.004	0.003	1.186	0.236
Mineral supplement type (Natural on the ground)	−2.369	2.222	−1.067	0.286				
Wild boar VR in wildlife trails	−2.904	2.099	−1.383	0.167				
Land use (no hunting)					8.556	3.152	2.715	0.006*

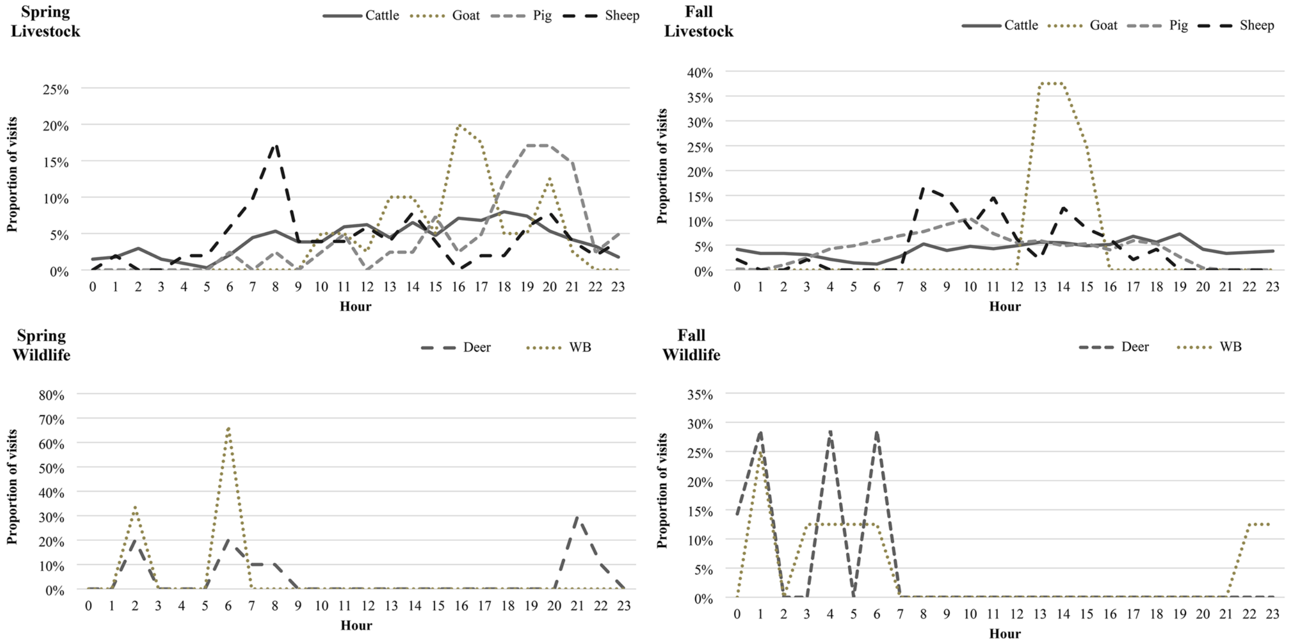

Fig. 2 Livestock (above) and wildlife (below) daily use profile at mineral supplementation points during spring and fall seasons assessed as the proportion of visits (separately for each species) to mineral supplementation points by hour of the day. The proportion of visits is with respect to the individual total of each species. For a better understanding, data summary in terms of box plots are provided in Supplementary Material 3

to mineral resources as it is to water (Kukielka et al. 2013). Additionally, we observed that red deer and wild boar VR were higher in wildlife trails than in MPs, and these species were not detected at all the farms in MPs even if they were present in the farms. These findings contrast with the natural attractiveness of this type of resources for North American cervids looking for supplemental dietary Na (Lavelle et al. 2014), and with the behavior reported for red deer in French farm facilities, where salt licking was the most frequently detected behavior and where they performed the longer visits (Payne et al. 2016).

Red deer and wild boar access to, and needs of, mineral have not yet been characterized in our study area. As indicative, saline soils and halophilous vegetation that can be found in uncultivated areas of central Spain (Bernáldez et al. 1989) may reduce the needs of mineral from anthropogenic sources, and therefore, the behavior of the wild ungulates towards human-borne mineral supplementation devices intended for livestock use. However, the large number of indirect intraspecific interactions (mainly for cattle and pig) reinforces the hypothesis that mineral supplementation is a potential source for disease transmission.

Consistently with previous studies in extensive cattle farms in SCS (Kukielka et al. 2013; Carrasco-García et al. 2016), domestic reservoirs presented a wider and more diurnal activity pattern at MPs than wild reservoirs. Thus, our results indicate that livestock species, for which mineral supplement were intended, were the main users and presented

Table 3 Number of total indirect interactions in mineral supplementation points from extensive cattle farms using a critical time window of 78 h. The average indirect interactions per MP and week, excluding the days when they were not present, are presented in brackets

		First species					
		Cattle	Goat	Pig	Sheep	Red deer	Wild boar
Second species	Cattle	16,431 (432.9)	158 (26.3)	478 (95.6)	1342 (112.9)	58 (2.0)	147 (3.9)
	Goat	159 (19.9)	103 (17.2)	0	0	0	7 (2.3)
	Pig	954 (190.8)	0	7375 (351.2)	0	41 (4.6)	0
	Sheep	1312 (118.7)	0	0	795 (73.2)	0	13 (1.3)
	Red deer	37 (1)	0	65 (7.2)	0	19 (0.3)	4 (0.1)
	Wild boar	103 (2.8)	3 (1.5)	0	17 (1.7)	8 (0.1)	3 (0.1)

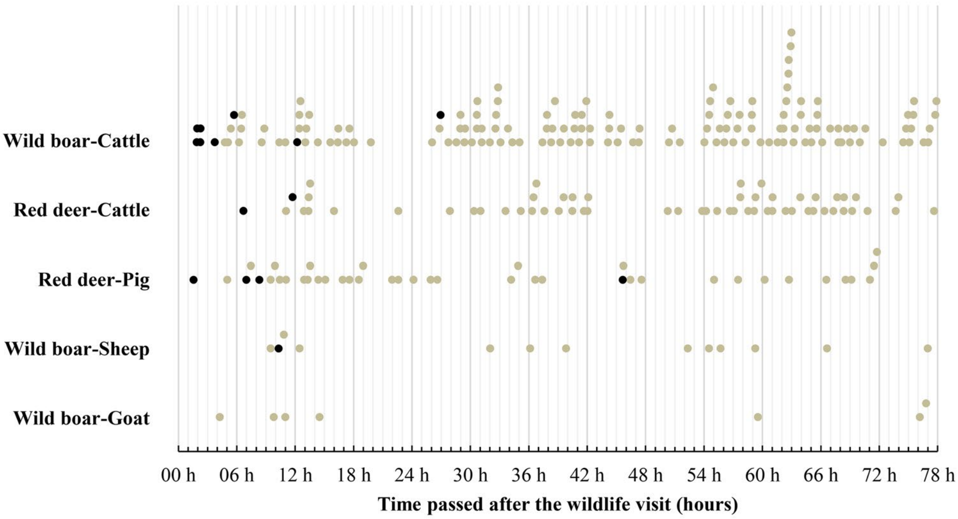

Fig. 3 Time-lag plot for indirect wildlife-livestock interactions in MPs. Time passing between a wildlife visit and the following livestock visit that generates the interaction is represented by dots. The first-order interactions, in which livestock visited the MP is after wildlife, are marked as black dots ($n=15$; first order interactions;) and grey dot interactions are second order or higher ($n=251$; a livestock visit preceded by as many livestock visits as the order of magnitude indicates, after the wildlife visit that has generated the interaction). Interactions are grouped by hour ranges within the critical time window (0 to 78 h). First interaction in the hour range is presented in the baseline, and subsequent interactions in the same hour range are presented in successively upper lines to facilitate visualization

a diurnal use pattern, while wild ungulates presented a nocturnal-crepuscular use pattern, with limited overlapping with livestock.

In our study, wildlife-livestock interactions were mostly determined by the intense influx of livestock to the MPs, and not by the presence/abundance of wild ungulates. The best fitted model generated for wild boar VR suggested that the presence of cattle in MPs is attractive to wild boar, similarly to results previously described between feral swine and cattle in Texas (Cooper et al. 2010), and between wild boar and cattle in SCS in other types of aggregation points (Carrasco-García et al. 2016). This indicates that there is no indirect inter-species avoidance and suggests that wild boar may find attractive resources associated with cattle presence, like the presence of invertebrates in cattle manure piles (Baubet et al. 2003; Acevedo et al. 2019). This is similar to what occurs in other wild reservoir species, such as the badger in the UK (Woodroffe et al. 2016). The best fitted model generated for red deer VR evidenced that MPs in farms without hunting activity were more visited than those farms in which red deer is hunted (Table 2). It is widely demonstrated that cervid species exhibit a behavioral disturbance in presence of hunting pressure, leading to a temporal and spatial restriction in their movement, and affecting their natural disposition to search for resources (Bonnot et al. 2013; Lone et al. 2015; Little et al. 2016). However, hunting pressure needs to be investigated in greater depth in order to develop management measures, and to clarify how it may affect other relevant factors mediating the epidemiology of TB (apart than the use of mineral supplementation points). Additionally, it shows an strong and negative correlation between the distance from the MP to wildlife cover and the presence of red deer on MPs (Table 2) in accordance with previous studies in SCS (Carrasco-García et al. 2016). All those evidences were supported by the hierarchical variance partitioning of the models, further revealing a relevant contribution of the distance from MP to wildlife cover on the total variance of wild boar VR, and pig UI on the total variance of red deer VR, maybe related with the behavioral plasticity of wild boar, and the disturbance in the behavior of red deer by the hunting activity, respectively. This evidences that further research is needed in the field of spatial behavior of wild boar and red deer in extensive cattle farms, and its interaction with livestock spatial behavior and pasture management.

Additionally, the absence of MTC positive swabs is consistent with the low activity detected for wildlife in the studied mineral blocks, along with brevity of the visits and the shortage of visits with “licking” behavior performed by wild reservoirs (including the inability of the wild boar to reach devices located more than one meter high). Domestic species could also potentially contribute to the presence of MTC DNA in MPs, although the development of large TB lesions and MTC excretion is normally prevented by regular TB eradication campaigns (at least 2 in a year), which eliminate animals that are positive to the skin test. Whereas this technique has been described to have a good sensitivity, it is possible that low levels of MTC were undetectable using our sampling protocol. A recent study established that approximately a minimum of one-third of TB positive wild boar randomly captured in our study area are potential MTC shedders (Barasona et al. 2017a). Controlled experiments evaluating the survival of *M. bovis* and other members of the MTC in periodically eroded mineral blocks exposed to Mediterranean environmental conditions and its potential for disease transmission are still needed.

All this, together with the fact that we recorded 266 indirect wildlife-livestock interactions, all of them derived from 21 unique wildlife visits, suggests that mineral supplementation points are mainly drivers for within species (rather than between species interspecific) interactions and that this resource is less attractive to wildlife comparing to other environmental sources of MTC in our study area, such as water ponds. This raises the possibility that, by controlling the low number of wildlife visits, most of the interspecific interactions with greater potential for MTC (or other pathogens) transmission at mineral supplementation points can be prevented. This requires implementing specific measures in the context of integral biosecurity plans at the wildlife-livestock interface:

(i) Withdrawing mineral supplementation overnight may prevent most visits by wild ungulates. It can be carried out by mechanically removing the mineral supplement or using a device that can be closed (by a lid or trapdoor).
(ii) Placing the mineral supplement at least 1-m high may prevent its use by wild boar, although not by red deer.
(iii) Establishing mineral supplementation points in open pastures far from wildlife cover may reduce visits by red deer, and to a lower extent, wild boar.
(iv) Segregating the use of MPs for the different livestock species making use of them, to prevent interspecific interactions, and hindering the indirect transmission of pathogens.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10344-021-01493-3.

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Author contribution Jordi Martínez-Guijosa, Christian Gortázar, Pelayo Acevedo, and Joaquín Vicente conceived and designed the study. Material preparation and data collection were performed by Jordi Martínez-Guijosa. Laboratorial analysis was performed by Maria José Torres. Data analysis and interpretation were performed by Jordi Martínez-Guijosa, Adrían López-Alonso, and Joaquín Vicente. Jordi Martínez-Guijosa and Adrían López-Alonso wrote the paper, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated for this study can be found in the Mendeley Data repository (https://doi.org/10.17632/bndtp9sx7w.1). Link: https://data.mendeley.com/datasets/bndtp9sx7w/1.

Declarations

Ethics approval The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as there were not sample collection from animals or humans.

Conflict of interest The authors declare no competing interests.

References

Acevedo P, Prieto M, Quirós P et al (2019) Tuberculosis epidemiology and badger (Meles meles) spatial ecology in a hot-spot area in atlantic spain. Pathogens 8:292. https://doi.org/10.3390/pathogens8040292

Barasona JA, Torres MJ, Aznar J et al (2017a) DNA detection reveals Mycobacterium tuberculosis complex shedding routes in its wildlife reservoir the eurasian wild boar. Transbound Emerg Dis 64:906–915. https://doi.org/10.1111/tbed.12458

Barasona JA, Vicente J, Díez-Delgado I et al (2017b) Environmental presence of Mycobacterium tuberculosis complex in aggregation points at the wildlife/livestock interface. Transbound Emerg Dis 64:1148–1158. https://doi.org/10.1111/tbed.12480

Baubet E, Ropert-Coudert Y, Brandt S (2003) Seasonal and annual variations in earthworm consumption by wild boar (S*us scrofa scrofa L.*). Wildl Res 30:179–186. https://doi.org/10.1071/WR00113

Berentsen AR, Miller RS, Misiewicz R et al (2014) Characteristics of white-tailed deer visits to cattle farms: Implications for disease transmission at the wildlife-livestock interface. Eur J Wildl Res 60:161–170. https://doi.org/10.1007/s10344-013-0760-5

Bernáldez FG, Benayas JMR, Levassor C, Peco B (1989) Landscape ecology of uncultivated lowlands in Central Spain. Landsc Ecol 3:3–18. https://doi.org/10.1007/BF00157752

Böhm M, Hutchings MR, White PCL (2009) Contact networks in a wildlife-livestock host community: identifying high-risk individuals in the transmission of bovine TB among badgers and cattle. PLoS One 4 https://doi.org/10.1371/journal.pone.0005016

Bonnot N, Morellet N, Verheyden H et al (2013) Habitat use under predation risk: hunting, roads and human dwellings influence the spatial behaviour of roe deer. Eur J Wildl Res 59:185–193. https://doi.org/10.1007/s10344-012-0665-8

Burnham KP, Anderson DR (2004) Model selection and multimodel inference. Springer

Carrasco-García R, Barasona JA, Gortazar C et al (2016) Wildlife and livestock use of extensive farm resources in South Central Spain: implications for disease transmission. Eur J Wildl Res 62:65–78. https://doi.org/10.1007/s10344-015-0974-9

Cooper SM, Morgan Scott H, De La Garza GR et al (2010) Distribution and interspecies contact of feral swine and cattle on rangeland in south Texas: implications for disease transmission. J Wildl Dis 46:152–164. https://doi.org/10.7589/0090-3558-46.1.152

Cowie CE, Hutchings MR, Barasona JA et al (2016) Interactions between four species in a complex wildlife: livestock disease community: implications for Mycobacterium bovis maintenance and transmission. Eur J Wildl Res 62:51–64. https://doi.org/10.1007/s10344-015-0973-x

Drewe JA, O'Connor HM, Weber N et al (2013) Patterns of direct and indirect contact between cattle and badgers naturally infected with tuberculosis. Epidemiol Infect 141:1467–1475. https://doi.org/10.1017/S0950268813000691

Fine AE, Bolin CA, Gardiner JC, Kaneene JB (2011) A study of the persistence of Mycobacterium bovis in the environment under natural weather conditions in Michigan, USA. Vet Med Int 2011:1–12. https://doi.org/10.4061/2011/765430

Fitzgerald SD, Kaneene JB (2013) Wildlife reservoirs of bovine tuberculosis worldwide: hosts, pathology, surveillance, and control. Vet Pathol 50:488–499. https://doi.org/10.1177/0300985812467472

Gormley E, Corner LAL (2018) Pathogenesis of Mycobacterium bovis Infection: the badger model as a paradigm for understanding tuberculosis in animals. Front Vet Sci 4:247. https://doi.org/10.3389/fvets.2017.00247

Gortázar C, Che Amat A, O'Brien DJ (2015) Open questions and recent advances in the control of a multi-host infectious disease: animal tuberculosis. Mamm Rev 45:160–175. https://doi.org/10.1111/mam.12042

Gortázar C, Ferroglio E, Lutton CE, Acevedo P (2010) Disease-related conflicts in mammal conservation. Wildl Res 37:668–675. https://doi.org/10.1071/WR10031

Humblet MF, Boschiroli ML, Saegerman C (2009) Classification of worldwide bovine tuberculosis risk factors in cattle: A stratified approach. Vet Res 40 https://doi.org/10.1051/vetres/2009033

Kaneene JB, Hattey JA, Bolin CA et al (2017) Survivability of *Mycobacterium bovis* on salt and salt-mineral blocks fed to cattle. Am J Vet Res 78:57–62. https://doi.org/10.2460/ajvr.78.1.57

Krebs JR, Anderson RM, Clutton-Brock T et al (1998) Badgers and bovine TB: Conflicts between conservation and health. Science 279(80):817–818. https://doi.org/10.1126/science.279.5352.817

Kukielka E, Barasona JA, Cowie CE et al (2013) Spatial and temporal interactions between livestock and wildlife in South Central Spain assessed by camera traps. Prev Vet Med 112:213–221. https://doi.org/10.1016/j.prevetmed.2013.08.008

Lavelle MJ, Henry C, LeDoux K et al (2015) Deer response to exclusion from stored cattle feed in Michigan, USA. Prev Vet Med 121:159–164. https://doi.org/10.1016/j.prevetmed.2015.06.015

Lavelle MJ, Kay SL, Pepin KM et al (2016) Evaluating wildlife-cattle contact rates to improve the understanding of dynamics of bovine tuberculosis transmission in Michigan, USA. Prev Vet Med 135:28–36. https://doi.org/10.1016/j.prevetmed.2016.10.009

Lavelle MJ, Phillips GE, Fischer JW et al (2014) Mineral licks: motivational factors for visitation and accompanying disease risk at communal use sites of elk and deer. Environ Geochem Health 36:1049–1061. https://doi.org/10.1007/s10653-014-9600-0

Little AR, Webb SL, Demarais S et al (2016) Hunting intensity alters movement behaviour of white-tailed deer. Basic Appl Ecol 17:360–369. https://doi.org/10.1016/j.baae.2015.12.003

Lone K, Loe LE, Meisingset EL et al (2015) An adaptive behavioural response to hunting: Surviving male red deer shift habitat at the onset of the hunting season. Anim Behav 102:127–138. https://doi.org/10.1016/j.anbehav.2015.01.012

Moustakas A, Evans MR (2015) Coupling models of cattle and farms with models of badgers for predicting the dynamics of bovine tuberculosis (TB). Stoch Environ Res Risk Assess 29:623–635. https://doi.org/10.1007/s00477-014-1016-y

Payne A, Chappa S, Hars J et al (2016) Wildlife visits to farm facilities assessed by camera traps in a bovine tuberculosis-infected area in France. Eur J Wildl Res 62:33–42. https://doi.org/10.1007/s10344-015-0970-0

Core Team R (2019) A language and environment for statistical computing. R Found. Stat. Comput. 2:https://www.R--project.org

Santos N, Almeida V, Gortázar C, Correia-Neves M (2015) Patterns of Mycobacterium tuberculosis-complex excretion and characterization of super-shedders in naturally-infected wild boar and red deer. Vet Res 46:129. https://doi.org/10.1186/s13567-015-0270-4

Santos N, Richomme C, Nunes T et al (2020) Quantification of the animal tuberculosis multi-host community offers insights for control. Pathogens. https://doi.org/10.3390/pathogens9060421

Silk MJ, Drewe JA, Delahay RJ et al (2018) Quantifying direct and indirect contacts for the potential transmission of infection between species using a multilayer contact network. Behaviour 155:731-757. https://doi.org/10.1163/1568539X-00003493

Triguero-Ocaña R, Laguna E, Jiménez-Ruiz S et al (2020) The wildlife-livestock interface on extensive free-ranging pig farms in central Spain during the "montanera" period. Transbound Emerg Dis. https://doi.org/10.1111/tbed.13854

VerCauteren KC, Lavelle MJ, Campa H (2018) Persistent spillback of bovine tuberculosis from white-tailed deer to cattle in Michigan, USA: Status, Strategies, and Needs. Front Vet Sci 5:301. https://doi.org/10.3389/fvets.2018.00301

Woodroffe R, Donnelly CA, Ham C et al (2016) Badgers prefer cattle pasture but avoid cattle: implications for bovine tuberculosis control. Ecol Lett 19:1201–1208

Woodroffe R, Donnelly CA, Ham C et al (2017) Use of farm buildings by wild badgers: implications for the transmission of bovine tuberculosis. Eur J Wildl Res 63:6. https://doi.org/10.1007/s10344-016-1065-2

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x

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