Tuberculosis-Associated Death among Adult Wild Boars, Spain, 2009–2014

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We investigated adult Eurasian wild boar (*Sus scrofa*) survival and death in 2 tuberculosis-endemic populations with different harvest pressure in Spain. Overall, tuberculosis accounted for 30% of total deaths. Increased survival in protected areas has direct implications for wild boar management and tuberculosis control.

Eurasian wild boar (*Sus scrofa*) population dynamics and hunting strategies might influence the persistence of disease (*1*). Determining the death rates for wild boar and unfolding the relative contribution of several causes of death and their nature (additive vs. compensatory death) is key to predicting the effects of harvesting, predation, and disease on population dynamics over time and to develop disease control–oriented hunting strategies. In central and northern Europe, the effect of disease-mediated death on wild boars is relatively low, whereas predation, winter starvation, and especially hunting play more important roles (*2,3*). In Mediterranean regions, natural death from summer starvation during droughts has been described, but most deaths are attributed to hunting (*4*); no information is available about rates of disease-related death among wild boars.

Animal tuberculosis (TB) caused by the *Mycobacterium tuberculosis* complex (MTC) is a reemerging multihost infectious disease (*5*). In Spain, the Eurasian wild boar is regarded as the key wildlife MTC maintenance host; its infection prevalence rates are >50% in Mediterranean areas that have dense wild boar populations (*6*). Up to one third of wild boar piglets might become infected during their first 6 months of life (*7*). In half of MTC-infected wild boars, generalized lesions develop that affect the lungs, particularly in juveniles (12–24 months of age). In adults (>2 years), the observed proportion of wild boars with generalized TB decreases, suggesting some degree of TB-driven death among juveniles (*6,8*). TB is a sporadic cause of death among wild boars (*9*), but no data are available about its actual contribution to mortality.

In the context of growing and expanding wild boar populations and of increasing concern about the effect of wild boar infections (*10*), we hypothesized that TB could be a major component of total wild boar death in Spain and have implications for TB control and wildlife management. We aimed to 1) describe the rates and causes of adult wild boar death and 2) compare the total death and its causes in 2 TB-endemic regions that differ in harvest pressure.

The Study

We compared 2 settings: a mosaic of game estates and a protected area. Montes de Toledo (MT) is a mountain chain in the central Spanish plateau whose large game estates are mainly devoted to recreational hunting. Harvest is conducted by dog-driven hunts; average annual extraction quota is 2.26 wild boar/km^2 and no age or sex are selected (i.e., extraction is random). Doñana National Park (DNP) is a protected area on the Atlantic coast of southern Spain. Harvest is part of population control management because no recreational hunting is allowed within the park; this modality has a minimal extraction capacity (1.11 wild boar/km^2) and targets wild boar seen by park rangers (random and opportunistic). Wild boars, except piglets, have no natural predators (occasionally stray dogs) in the study areas.

During 2009–2014, we captured (*11*) and fitted very high frequency global positioning system–global system for mobile communications (VHF-GPS-GSM) collars (Microsensory, Spain) to 45 free-ranging adult wild boars (24 from MT and 21 from DNP; online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/22/12/16-0677-Techapp1.pdf) following Animal Experimentation legislation (PR-2015-03-08). We collected serum and tested it for antibodies to MTC by using ELISA (89.6% sensitivity [*12*]). Post-release monitoring was programmed to acquire 1 GPS location per hour. We monitored the animals daily for death (alarm set at 12 h of inactivity) to promptly retrieve carcasses and assess the cause of death. The 18 retrieved carcasses underwent a full postmortem examination, and tissue samples (pooled lymph nodes and lung) were submitted for culture (online Technical Appendix).

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We detected serum antibodies to MTC in 35 (78%) of 45 GPS-collared wild boars. We found no differences in MTC serum antibody prevalence between study sites ($p>0.05$ by Fisher exact test) and no differences in survival time between antibody-positive and -negative wild boars ($p>0.05$ by Mann-Whitney U test). MTC infection was confirmed by culture in 13 (72%) of 18 wild boars for which postmortem results were available. The 9 wild boars that died of generalized TB had severe lesions in >1 anatomic region; >70% of lung tissue was affected (online Technical Appendix Figure 1).

We assessed total survival probability and the main causes of death (Figure). The mean annual death rate (45.48% ± 5.6% SE overall) was higher in the regularly hunted MT (56%) than in the protected DNP (34%). Overall, harvest accounted for half (53%) of total annual deaths, whereas TB contributed to 30% of deaths. The remaining 17% of deaths were caused by predation (stray dogs in DNP) and unknown causes (Table). The mean annual death rate for adult wild boars caused by harvest was significantly higher in MT (40%) than in the protected DNP (8%; Fisher exact test, $p = 0.011$). However, death from TB did not differ between MT (12%) and DNP (14%; $p>0.05$ by Fisher exact test) (online Technical Appendix Table).

Mean survival time was twice as long in DNP (average 672 ± 96 days) as in MT (297 ± 41 days; Mantel-Cox, $\chi^2 = 11.42$, 1 d.f.; $p = 0.001$). Kaplan-Meier survival probabilities and causes of death are detailed by study area in online Technical Appendix Figure 2. Two death peaks were found, 1 in summer (July) associated with TB and 1 in autumn (October–January) associated with harvest (online Technical Appendix Figure 3).

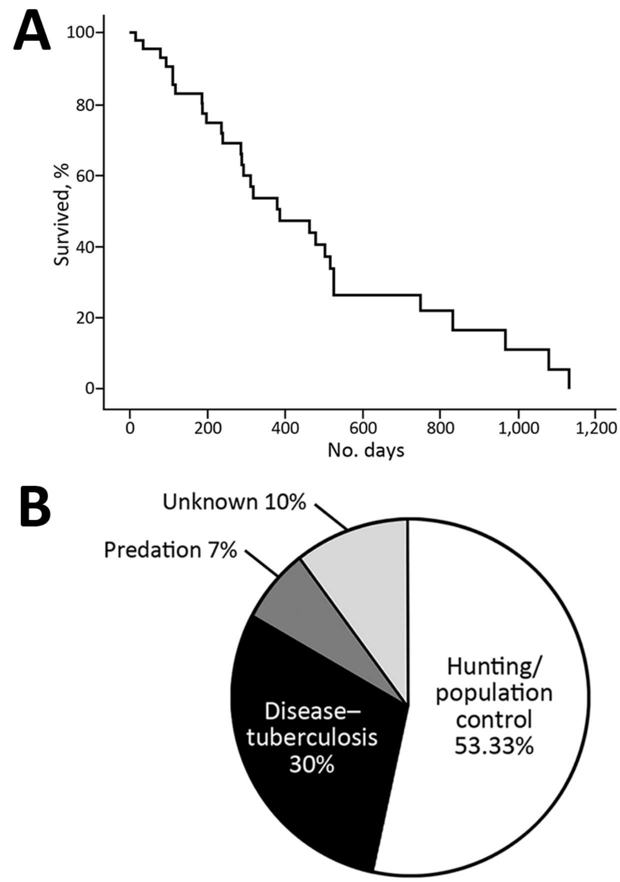

Figure. Total survival probability and the main causes of death among wild boars (*Sus scrofa*), Spain, 2009–2014. A) Kaplan-Meier survival curve representing the proportion of adult wild boars alive over time for all the animals studied. B) Percentage of each cause of death among wild boars (i.e., when considering only all dead animals)

Conclusions

The results confirmed our hypothesis that TB causes a substantial proportion of deaths among adult wild boars in TB-endemic Mediterranean areas of Spain. This information is relevant for TB control at the wildlife–livestock interface and for understanding wild boar and TB dynamics under different harvest pressure.

Severely diseased wild boars, with advanced generalized TB lesions affecting large proportions of the lung, probably are important shedders of MTC (super-shedders [*13*]). The higher survival rate for MTC super-shedders in protected areas, such as DNP, resulting from a low harvest pressure might contribute toward explaining the extremely high spread of TB in sites where risky artificial management, such as feeding, is absent (*5*).

The large proportion of natural death from TB (30% of deaths) contrasts with results obtained in other parts of Europe (total natural death rate 3% [*3*]), although this finding is consistent with differences in TB prevalence within Europe (*14*). Previous findings suggest that TB-induced death is relevant in subadults but decreases in adults (*6,8*), hence, deaths of juvenile wild boars deserves special study. However, given the chronic nature of TB and the early reproduction of wild boars, TB is unlikely to substantially contribute to wild boar population regulation. In fact, we observed a mean annual death rate of 45%, which is below the recommended annual harvest or death rate of 65% needed to maintain stable wild boar populations (*3,15*).

Two additional aspects about hunting and wild boar TB deserve attention. First, increased hunting might contribute to TB control in wild boars by reducing population size and by reducing survival of super-shedders. Hunting bans should therefore be reconsidered in protected areas in which TB is a concern. Second, TB causes a substantial loss of adult (trophy) wild boars, thus reducing the profitability of the hunting industry. Hunters should therefore actively engage in TB control.

Table. Causes of death of GPS-collared adult wild boars (*Sus scrofa*), Spain, 2009–2014*

Variable	Study site: Montes de Toledo†	Study site: Doñana National Park‡	Total
No. GPS-collared wild boars	24	21	45
Mean survival time ± SE, d§	297.28 ± 40.91	672.78 ± 96.53	470.43 ± 58.69
Mean annual survival rate ± SE, %¶	44.17 ± 7.55	66.35 ± 7.73	54.52 ± 5.60
Mean annual mortality rate ± SE, %¶	55.83 ± 7.55	33.64 ± 7.73	45.48 ± 5.60
Proportion of deaths, %			
From harvest	72.22	25	53.33
From tuberculosis	22.22	41.67	30.00
From other causes	5.56	33.93	16.67

*GPS, global positioning system.
†High harvest pressure.
‡Low harvest pressure.
§Mantel-Cox log-rank, χ^2 = 11.42, 1 d.f., p = 0.001.
¶Mann-Whitney U test, U = –1.994, p = 0.046.

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References

1. Kramer-Schadt S, Fernández N, Thulke HH. Potential ecological and epidemiological factors affecting the persistence of classical swine fever in wild boar *Sus scrofa* populations. Mammal Rev. 2007;37:1–20. http://dx.doi.org/10.1111/j.1365-2907.2007.00097.x
2. Jedrzejewski W, Jedrzejewska B, Okarma H, Ruprecht AL. Wolf predation and snow cover as mortality factors in the ungulate community of the Bialowieza National Park, Poland. Oecologia. 1992;90:27–36. http://dx.doi.org/10.1007/BF00317805
3. Keuling O, Baubet E, Duscher A, Ebert C, Fischer C, Monaco A, et al. Mortality rates of wild boar *Sus scrofa* L. in central Europe. Eur J Wildl Res. 2013;59:805–14. http://dx.doi.org/10.1007/s10344-013-0733-8
4. Massei G, Genov PV, Staines BW, Gorman ML. Mortality of wild boar, *Sus scrofa*, in a Mediterranean area in relation to sex and age. J Zool (Lond). 1997;242:394–400. http://dx.doi.org/10.1111/j.1469-7998.1997.tb05813.x
5. Gortázar C, Torres MJ, Vicente J, Acevedo P, Reglero M, de la Fuente J, et al. Bovine tuberculosis in Doñana Biosphere Reserve: the role of wild ungulates as disease reservoirs in the last Iberian lynx strongholds. PLoS One. 2008;3:e2776. http://dx.doi.org/10.1371/journal.pone.0002776
6. Vicente J, Barasona JA, Acevedo P, Ruiz-Fons JF, Boadella M, Diez-Delgado I, et al. Temporal trend of tuberculosis in wild ungulates from Mediterranean Spain. Transbound Emerg Dis. 2013;60(Suppl 1):92–103. http://dx.doi.org/10.1111/tbed.12167
7. Che' Amat A, González-Barrio D, Ortiz JA, Díez-Delgado I, Boadella M, Barasona JA, et al. Testing Eurasian wild boar piglets for serum antibodies against *Mycobacterium bovis*. Prev Vet Med. 2015;121:93–8. http://dx.doi.org/10.1016/j.prevetmed.2015.05.011
8. Díez-Delgado I, Boadella M, Martín-Hernando M, Barasona JA, Beltrán-Beck B, González-Barrio D, et al. Complex links between natural tuberculosis and porcine circovirus type 2 infection in wild boar. BioMed Res Int. 2014;2014:765715. http://dx.doi.org/10.1155/2014/765715
9. Segalés J, Vicente J, Luján L, Toussaint MJM, Gruys E, Gortázar C. Systemic AA-amyloidosis in a European wild boar (*Sus scrofa*) suffering from generalized tuberculosis. J Vet Med A Physiol Pathol Clin Med. 2005;52:135–7. http://dx.doi.org/10.1111/j.1439-0442.2005.00703.x
10. Ruiz-Fons F. A review of the current status of relevant zoonotic pathogens in wild swine (*Sus scrofa*) populations: changes modulating the risk of transmission to humans. Transbound Emerg Dis. 2015;n/a. http://dx.doi.org/10.1111/tbed.12369
11. Barasona JA, López-Olvera JR, Beltrán-Beck B, Gortázar C, Vicente J. Trap-effectiveness and response to tiletamine-zolazepam and medetomidine anaesthesia in Eurasian wild boar captured with cage and corral traps. BMC Vet Res. 2013;9:107. http://dx.doi.org/10.1186/1746-6148-9-107
12. Boadella M, Lyashchenko K, Greenwald R, Esfandiari J, Jaroso R, Carta T, et al. Serologic tests for detecting antibodies against *Mycobacterium bovis* and *Mycobacterium avium* subspecies paratuberculosis in Eurasian wild boar (*Sus scrofa scrofa*). J Vet Diagn Invest. 2011;23:77–83. http://dx.doi.org/10.1177/104063871102300111
13. Santos N, Santos C, Valente T, Gortázar C, Almeida V, Correia-Neves M. Widespread environmental contamination with *Mycobacterium tuberculosis* complex revealed by a molecular detection protocol. PLoS One. 2015;10:e0142079. http://dx.doi.org/10.1371/journal.pone.0142079
14. Gortazar C, Delahay RJ, Mcdonald RA, Boadella M, Wilson GJ, Gavier-Widen D, et al. The status of tuberculosis in European wild mammals. Mammal Rev. 2012;42:193–206. http://dx.doi.org/10.1111/j.1365-2907.2011.00191.x
15. Genov PW, Massei G, Kostova W. Die nutzung des wildschweins (*Sus scrofa*) in Europa in theorie und praxis. Z Jagdwiss. 1994;40:263–7.

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Technical Appendix

Statistical Analyses

Death rates were explored by estimating the daily probability of survival (*P*) according to method proposed by Kenward (*1*). Annual death rates were calculated as $1 - P^{365}$, which is the probability that an individual boar will die in any given year. In addition, Kaplan-Meier survival curves were used to compute the probabilities of death occurring, given those animals that had died, along with those animals with "censored" observations (i.e., still alive or nontransmitting collars) at the conclusion of the study (*2*). We calculated the annual death rates and Kaplan-Meier survival curves for the entire population, in addition to those for Montes de Toledo and Doñana National Park independently. We used the Mantel-Cox log-rank, Mann-Whitney U, and Fisher exact test analyses at a level of significance of 0.05 to test survival differences between areas, sex, and according to the tuberculosis test at the time of the first capture. Data were analyzed by using IBM SPSS statistical package version 20 (IBM Corporation, Somar, NY, USA).

References

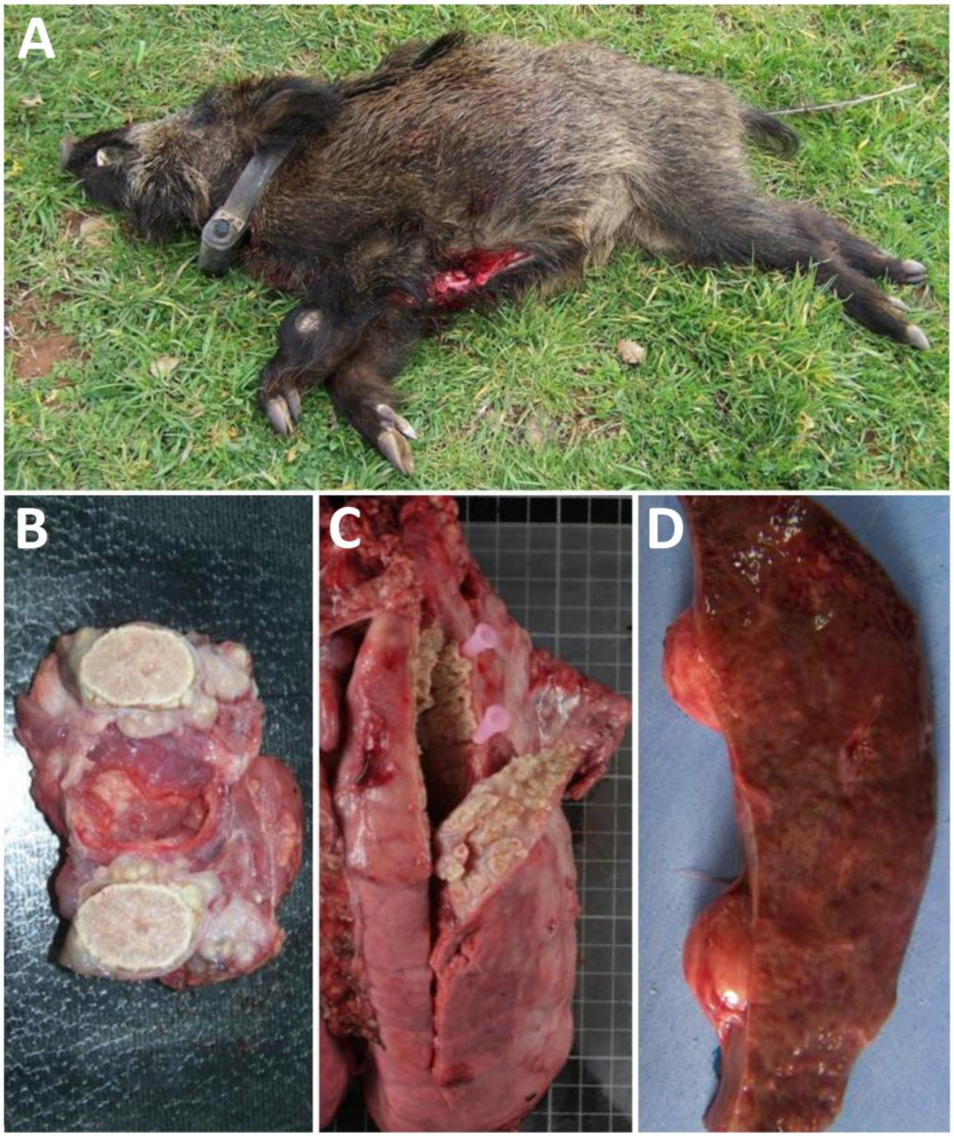
1. Kenward RE. A manual for wildlife radio tagging. San Diego: Academic Press; 2001.

2. Nuss K, Warneke M. Life span, reproductive output, and reproductive opportunity in captive Goeldi's monkeys (*Callimico goeldii*). Zoo Biol. 2010;29:1–15. PubMed

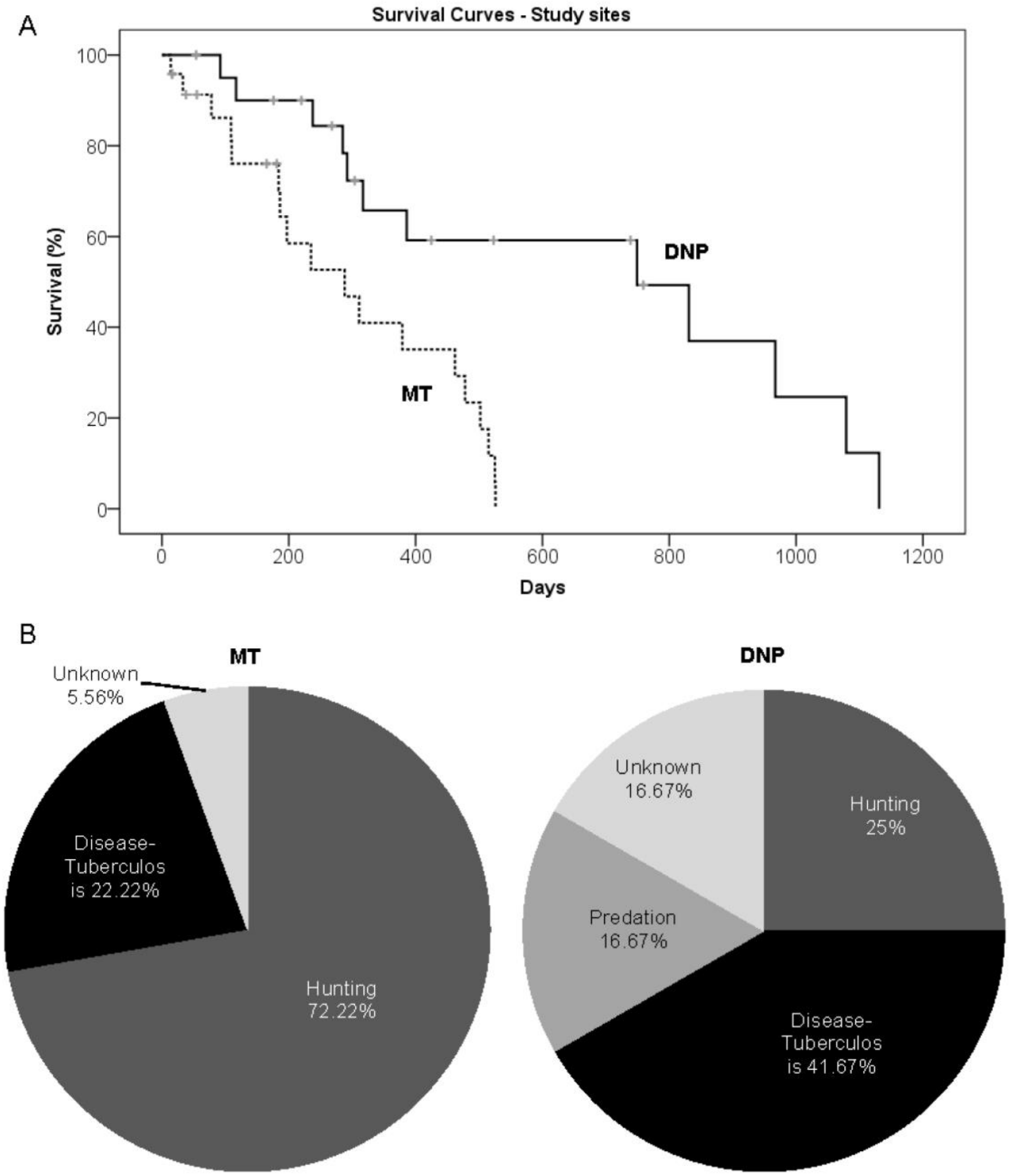
Technical Appendix Table. List of GPS-collared adult wild boars (*Sus scrofa*), Spain, 2009–2014

Animal ID	Study area	Sex	Cause of death or monitoring discontinued	No. days monitored	*Mycobacterium tuberculosis* ELISA
1	MT	F	Disease–tuberculosis	33	Positive
2	MT	F	Disease–tuberculosis	109	Positive
3	MT	F	Hunting	502	Positive
4	MT	F	Hunting	186	Positive
5	MT	F	Hunting	462	Positive
6	MT	F	Hunting	197	Positive
7	MT	F	Hunting	379	Negative
10	MT	F	Unknown	515	Negative
8	MT	F	Monitoring discontinued	16	Positive
9	MT	F	Monitoring discontinued	181	Positive
11	MT	M	Disease–tuberculosis	110	Positive
12	MT	M	Disease–tuberculosis	288	Positive
13	MT	M	Hunting	78	Positive
14	MT	M	Hunting	235	Positive
15	MT	M	Hunting	525	Negative
16	MT	M	Hunting	478	Positive
17	MT	M	Hunting	311	Negative
18	MT	M	Hunting	526	Negative
19	MT	M	Hunting	14	Negative
20	MT	M	Hunting	184	Positive
21	MT	M	Monitoring discontinued	55	Positive
22	MT	M	Monitoring discontinued	165	Positive
23	MT	M	Monitoring discontinued	38	Negative
24	MT	M	Monitoring discontinued	16	Negative
25	DNP	F	Disease–tuberculosis	285	Negative
26	DNP	F	Disease–tuberculosis	238	Positive
27	DNP	F	Disease–tuberculosis	749	Positive
28	DNP	F	Population control	92	Positive
30	DNP	F	Unknown	117	Positive
31	DNP	F	Unknown	386	Positive
29	DNP	F	Monitoring discontinued	54	Positive
32	DNP	F	Monitoring discontinued	739	Positive
33	DNP	M	Disease–tuberculosis	292	Positive
34	DNP	M	Disease–tuberculosis	317	Positive
35	DNP	M	Population control	831	Positive
36	DNP	M	Population control	967	Positive
37	DNP	M	Predation	1131	Positive
38	DNP	M	Predation	1079	Positive
39	DNP	M	Monitoring discontinued	304	Positive
40	DNP	M	Monitoring discontinued	220	Positive
41	DNP	M	Monitoring discontinued	176	Positive
42	DNP	M	Monitoring discontinued	523	Positive
43	DNP	M	Monitoring discontinued	759	Negative
44	DNP	M	Monitoring discontinued	268	Positive
45	DNP	M	Monitoring discontinued	425	Positive

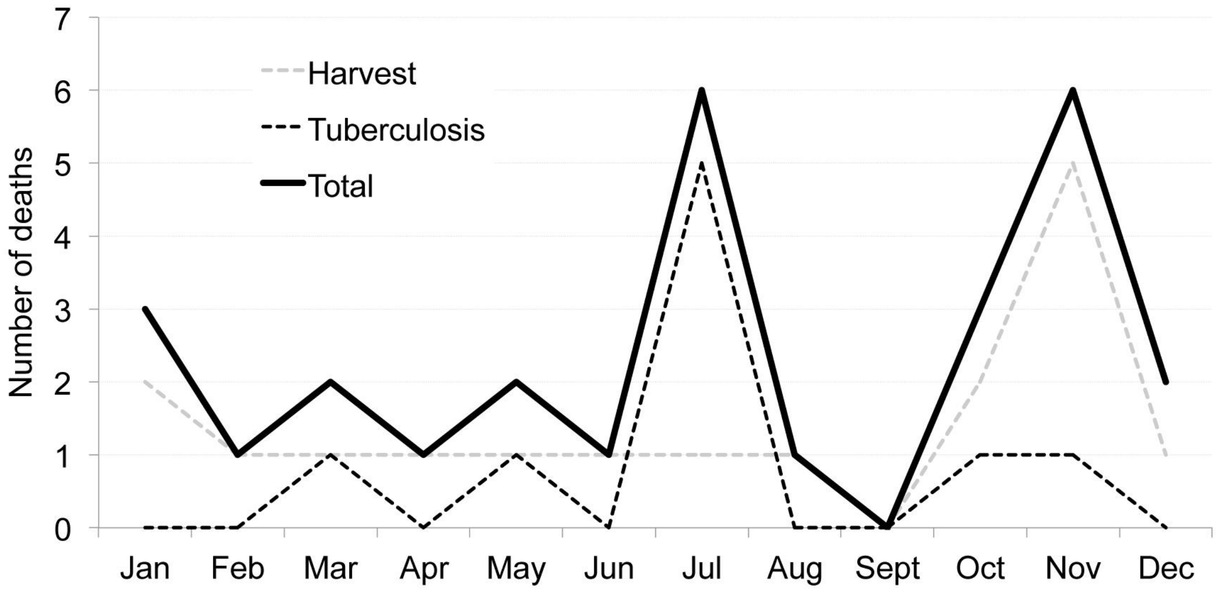
DNP, Doñana National Park; GPS, global positioning system; ID, identification; MT, Montes de Toledo.



Technical Appendix Figure 1. A) Global positioning system–collared wild boar (*Sus scrofa*) found dead as the result of generalized tuberculosis on a cattle farm in the Montes de Toledo, Spain. B) Calcified and caseous lesions in the mandibular lymph node (B), lung (C), and spleen (D) of this boar.



Technical Appendix Figure 2. A) Kaplan-Meier survival curves representing the proportion of free-ranging wild boars (*Sus scrofa*) alive over time for all the animals studied in each area. Differences in probability of survival between Montes de Toledo (MT) and Doñana National Park (DNP) are significant (Mantel-Cox χi^2 = 11.42, 1 d.f., p = 0.001). Tick marks on each curve indicate a specific censored animal, with some ticks overlapping each other. B) Percentage of each cause of death among wild boars (i.e., when considering only all dead animals) in MT and DNP.



Technical Appendix Figure 3. Monthly death distribution of global positioning system–collared adult wild boars (*Sus scrofa*) by main known causes, Spain, 2009–2014.