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Long-term monitoring of 10 selected pathogens in wild boar (*Sus scrofa*) in Sierra Nevada National Park, southern Spain

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ABSTRACT

Wild boar (*Sus scrofa*) populations are increasing in the Iberian Peninsula, and population management must include disease management and control. In this study, the epidemiology of 10 selected pathogens (Aujeszky's disease virus – ADV, porcine reproductive and respiratory syndrome virus – PRRSV, porcine influenza virus, porcine circovirus, porcine parvovirus, *Erysipelotrix rhusiopathiae*, *Leptospira pomona*, *Chlamydia/Chlamydiaceae* sp., *Salmonella* sp. and *Mycobacterium bovis*) in the wild boar population in Sierra Nevada National Park (SNNP), an open unfenced area, is reported, taking into account wild boar population abundance variation in space and time in an open unfenced environment. A total of 1103 wild boar were sampled in 141 hunting events randomly carried out for sampling in seven hunting seasons (October to February from 2002–2003 to 2009–2010 (except 2007–2008). Prevalence was overall lower than those previously reported for fenced wild boar populations in Spain, but all the pathogens analyzed except PRRSV were considered endemic in the SNNP. ADV, *E. rhusiopathiae* and total pathogen prevalence were positively correlated to wild boar density. Prevalence in the positive areas was significantly higher in females for ADV, *E. rhusiopathiae*, *L. pomona*, *Chlamydia/Chlamydiaceae* sp. and *Salmonella* sp., and in males for *M. bovis*. This longitudinal study provides the first data on the health status of the relatively unmanaged and low density wild boar population of SNNP. It is concluded that non-intensively managed wild boar populations are able to maintain the circulation of several pathogens, even in low prevalences and in open unfenced areas with natural density variation both in time and space.

1. Introduction

During the last decades, wild boar has experienced a population and distribution range increase in Europe, including the Iberian Peninsula (Fruzinski, 1995; Leránoz and Castién, 1996), although population oscillation and decrease in open mountain areas have been reported (Sarasa and Sarasa, 2013). Wild boar can act as a reservoir

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species for a number of pathogens (Parra et al., 2006), and population management must include health issues in order to improve disease management and control (Naranjo et al., 2008). Intensive management of wild boar for hunting purposes in south-central Spain, which usually includes estate fencing and supplementary feeding, increases the prevalence and transmission of diseases such as bovine tuberculosis, Aujeszky's disease, and type 2 porcine circovirus (Gortázar et al., 2006; Vicente et al, 2004, 2005, 2006).

Wild boar home range is very variable, and depends on season, food availability, reproductive status, presence of refuge areas and risk avoidance (Thurfjell et al., 2009). Spatial distribution may also be affected by forest fragmentation (Virgós, 2002). Wild boars are mainly sedentary (Spitz et al., 1984), but they may travel long distances sporadically (Andrezejewski and Jezierski, 1978). Population and migratory movements are highly variable depending on the location (Sarasa and Sarasa, 2013), which may have an effect on the spread of diseases (Vieira-Pinto et al., 2011). Wild boars may aggregate depending on refuge search, food availability, and social behaviour within their matriarchal social structure, and this aggregation has a determining role in the epidemiology of several diseases (Vicente et al., 2005).

The objective of this study is to describe the spatial distribution and temporal evolution of the prevalence of antibodies against nine selected pathogens and *Mycobacterium bovis* detection in the wild boar population in Sierra Nevada National Park (SNNP), taking into account wild boar density variation in space and time in an open unfenced environment.

2. Materials and methods

2.1. Study area

The SNNP is an 86,210 ha protected area, belonging to 44 municipalities and surrounded by the 88,965 ha of the Sierra Nevada Natural Park, accounting for a total of 175,175 ha which form the Sierra Nevada Natural Space (SNNS). For the purpose of this study, only the 26,588 hectares of continuous forest within the SNNP, composed of large continuous pine tree (*Pinus* sp.) reforestations, dense scrubland areas and oak patches (*Quercus* sp.), were considered. The SNNS is an open unfenced area with a Spanish ibex (*Capra pyrenaica*) population of around 17,500 individuals, which move in the SNNP and the surrounding Natural Park. In the 44 municipalities of the SNNP, more than 76,000 domestic ruminants (38,927 sheep, 32,047 goats and 5985 cattle) graze extensively in spring and summer (3–5 months). Conversely, there is practically no domestic pig in the SNNS. Cattle herds without the officially tuberculosis-free status (as defined in the EU Directive 64/432/EEC) are present in 13 out of the 44 municipalities of the SNNP. There is no supplementary feeding for wild boar, and hunting is permitted only for management purposes, which allowed sample collection for this study.

2.2. Sample collection

A total of 1103 wild boars (395 males and 708 females) were sampled in 141 hunting events carried out in seven hunting seasons (October to February 2002–2003, 2003–2004, 2004–2005, 2005–2006, 2006–2007, 2008–2009 and 2009–2010), in areas with known wild boar high density. Mean wild boar age, assessed according to Borgo et al. (2007), was over 24 months, ranging from 6 to 60 months and sex was biased towards females (males 35.81%, females 64.19%). Blood was collected by heart puncture, placed in tubes without anticoagulant and allowed to clot at room temperature until its arrival to the laboratory. Then it was centrifuged at 1200 × *g* for 15 min within 24 h from its collection, and sera frozen at −18 °C until analysis. For *M. bovis* analyses, tonsils and submandibular and retropharyngeal-parotid lymph nodes were collected and frozen also at −18 °C. Pulmonary parenchyma was also collected when tuberculosis-like lesions were observed. Sample size allows a good (3–5%) or moderate (5–10%) accuracy for an expected prevalence lower and higher than 10%, respectively (Epidat 3.1, Xunta de Galicia, Spain).

2.3. Sample analysis

Table 1 shows the serological analyses performed to detect antibodies against nine of the selected pathogens included in this study. The diagnosis of *M. bovis* infection

Table 1
Serological techniques used to detect antibodies against selected pathogens in wild boars from the SNNP.

Pathogen	Technique	Reactive origin
Aujeszky's disease virus (serovar 1)	ELISA	Ingenasa©
Porcine reproductive and respiratory syndrome virus	ELISA	Ingenasa©
Porcine influenza virus	Inhibition of haemagglutination	Infectious Diseases Department, University of Murcia
Porcine circovirus type 2	ELISA	Ingenasa©
Porcine parvovirus	Inhibition of haemagglutination	Infectious Diseases Department, University of Murcia
Erysipelothrix rhusiopathiae	ELISA	Ingenasa©
Leptospira pomona	Lysis microagglutination	Infectious Diseases Department, University of Murcia
Chlamydia/Chlamydiaceae sp.	Plate microagglutination	Vetoquinol©
Salmonella sp. (serovar C)	Plate agglutination	Microkit©