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AUJESZKY'S DISEASE IN HUNTED WILD BOAR (*SUS SCROFA*) IN THE IBERIAN PENINSULA

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ABSTRACT: Aujeszky's disease (AD, pseudorabies) eradication programs in domestic pigs are implemented in several European countries where AD virus (ADV) circulates in local wild boar (*Sus scrofa*), making studies on ADV infection dynamics in wild boar increasingly relevant. The objective of our study was to characterize ADV dynamics in wild boar at a site in central Portugal and compare this site to three enzootic sites in central Spain. A total of 235 wild boar were sampled during the hunting season 2014–15. We collected serum, tissues (oropharyngeal tonsils and trigeminal and sacral ganglia), and swabs (oral, nasal, and genital) and analyzed these samples to detect ADV antibodies (enzyme-linked immunosorbent assay) and DNA (PCR). An overall seroprevalence of 42.6% was found (range 12.7–57.7%), being highest in adults (54.1%; 72/133). Overall, 2.8% (3/108) oral, 6.4% (7/109) nasal, and 12.8% (12/94) genital swabs were PCR positive. We found 20.4% (20/98) of the wild boar had at least one positive swab and were considered shedders. We found ADV in tissues of five animals; of 111 tonsils, three (2.7%) were PCR positive. Trigeminal (2/48; 4%) and sacral (2/53; 4%) ganglia collected in central Portugal, pertaining to three animals, were positive for ADV DNA. Logistic regression models showed that seroprevalence was influenced by site and age, whereas ADV shedding was influenced by site. Our study describes patterns of ADV infection in wild boar in Portugal and shows that wild boar also pose a risk, albeit lower than that in central Spain, for the eradication of AD from extensively managed domestic pigs in Portugal.

Key words: Aujeszky's disease, infection dynamics, PCR, pseudorabies, serology, shedding, wild boar (*Sus scrofa*).

INTRODUCTION

Aujeszky's disease (AD), or pseudorabies, is defined by the World Organisation for Animal Health (OIE) as an "...infection of domestic pigs or captive wild pigs which are under direct human supervision or control" (OIE 2019; page 1, article 8.2.1). It is a compulsory notifiable disease in European Union (EU) member states (Council of the European Union 1964). Most member countries have established official control and eradication programs, and many regions or territories are already free of AD, whereas others are in different phases of achieving this goal (CEC 2008; European Commission Health & Consumers Directorate-General 2016). The status of AD-freedom of a member state or region can be considered and maintained, despite established AD infection in wild boar, as long as measures are in place that prevent transmission from wild boar to domestic pigs (European Commission Health & Consumers Directorate-General 2016). In the Iberian Peninsula, AD is under specific eradication programs in domestic pigs in both Spain and Portugal, and freedom from disease is expected in the near future in the whole Peninsula. In Spain, AD has been eradicated in domestic pigs; no

cases have been reported at least in the last 5 yr in intensive pig premises; only very sporadic cases are reported in extensively managed pigs exposed to wild boar (*Sus scrofa*) in the country, and mandatory vaccination is performed as a preventive measure in the whole country (Ministerio de Agricultura, Pesca y Alimentacion 2018). Extensive production of pigs is traditional in southwestern Spain as well as on the other side of the border into central and southeastern Portugal.

Eurasian wild boar is the predominant wild pig type in Europe (Müller et al. 2011). The local densities of wild boar vary geographically and temporally, as do AD virus (ADV) infection patterns within Europe (Vicente et al. 2005; Pannwitz et al. 2012; Denzin et al. 2013; Lipowski et al. 2017; Caruso et al. 2018; Casades-Martí et al. 2020). Molecular analyses have shown different genotypes of ADV circulating in sympatric wild boar compared to domestic pigs (*Sus scrofa domesticus*; Müller et al. 2010). Although considered minor, the risk of spillover of ADV from wild boar to domestic pig herds, potentially interfering with control or eradication programs, is generally acknowledged and monitoring is recommended (Boadella et al. 2012a; Vicente-Rubiano et al. 2014). Additional harm by spillover events from wild boar to carnivores, such as hunting dogs or even endangered wildlife species such as the Iberian lynx (*Lynx pardinus*), underline the importance of monitoring ADV in wild boar populations (ProMED-mail 2010, 2014; Masot et al. 2017; Cano-Terriza et al. 2019).

The prevalence and dynamics of ADV in local wild boar populations has been intensively studied in Spain (Ruiz-Fons et al. 2007; Boadella et al. 2012a; Vicente-Rubiano et al. 2014; González-Barrio et al. 2015), but publicly available information on neighboring Portugal is lacking. Therefore, the objective of our study was to characterize ADV infection dynamics in hunted wild boar in central Portugal and to compare it to that in central Spain where ADV is endemic in the wild boar population.

MATERIALS AND METHODS

Study areas and sampling

Our study was carried out in four hunting areas in central Spain and Portugal. In Spain, two areas were located in the Toledo Mountains (QM and ED), and one in the valley of the Guadiana River (RF). These Spanish sites have already been described (González-Barrio et al. 2015; Casades-Martí et al. 2020). In Portugal, the study site focused on Idanha-a-Nova county (IN), a major wild boar hunting area. The Spanish and Portuguese sites are not contiguous. We collected samples from hunter-harvested wild boar in commercial hunting events during the hunting season 2014–15. Biologic samples were individually collected aseptically into sterile containers and transported refrigerated to the laboratory. Sterile swabs were employed to collect oral, nasal, and genital secretions. Samples of oropharyngeal tonsils and trigeminal and sacral nerve ganglia were taken. Blood was obtained by puncture of the cavernous dura mater sinus (Arenas-Montes et al. 2013). For each animal, age was based on tooth eruption patterns and sex was recorded (Saenz de Buruaga et al. 1991). Age categories included piglets when <6 mo, juveniles when 6–12 mo, subadults when 12–24 mo, and adults when >2 yr.

Serology and PCR

Following centrifugation of blood samples at 3,000 × G for 10 min, serum was separated and stored at –20 C until testing. Antibodies to the glycoprotein E antigen of ADV were detected by a commercial enzyme-linked immunosorbent assay according to instructions (IDEXX PRV/ADV gI Ab Test, IDEXX Inc., Westbrook, Maine, USA) and as previously described for wild boar (Ruiz-Fons et al. 2006). The enzyme-linked immunosorbent assay readings, categorized as doubtful by the manufacturer's protocol, were considered positive. Swabs and tissue samples were stored at –80 C until testing. Different commercial DNA extraction kits were used for swabs (DNeasy Blood and Tissue, Qiagen®, Hilden, Germany) and tissues (NucleoSpin™ Tissue, Macherel-Nagel, Düren, Germany; see González-Barrio et al. 2015). Extracted DNA was quantified by spectrophotometry (Nanodrop™ ND-1000, Thermo Fisher Scientific, Wilmington, Delaware, USA) and adjusted to a concentration of 50 ng/μL for PCR. A nested PCR was used to amplify fragments of the ADV gene codifying the highly conserved glycoprotein B (gB) as described, with slight modifications (Ruiz-Fons et al. 2007).

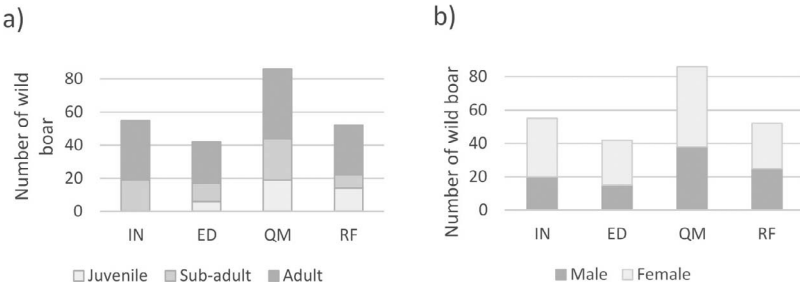

FIGURE 1. Wild boar sampled for Aujeszky's disease virus (ADV) antibodies and viral DNA during the hunting season of 2014–15 in central Spain and Portugal by a) age and b) sex. Study sites in Spain included areas in the Toledo Mountains (QM and ED) and in the valley of the Guadiana River (RF), and in Portugal to the county of Idanha-a-Nova (IN).

Statistical analysis

Descriptive analyses were carried out using SPSS version 25.0 (IBM Corporation, Armonk, New York, USA). Bivariate analyses included chi-square tests to assess the relationship between proportions between nominal variables and the Fisher's exact test when expected cell counts were <5. We defined ADV shedders as having at least one PCR-positive swab. Clopper-Pearson 95% confidence intervals (CI) were calculated with EPITOOLS (Sergeant 2011). Multivariate analyses consisted of binary logistic regression using the backward stepwise method based on likelihood ratio using SPSS version 25.0 (IBM). Two models were fitted with individual antibody presence/absence and shedding as dependent (outcome) variables, respectively. Site, age, and sex were included as explanatory variables.

RESULTS

A total of 235 wild boar was sampled at the four study sites (Fig. 1). Only one piglet was sampled at ED, so this age category was merged to that of juveniles, which then included all wild boar with <12 mo of age. No juveniles were hunted at the IN site.

The results of ADV serology and PCR in swabs and tissues are shown in Table 1. Seroprevalence ranged from 12.7% (95% CI: 5.3–24.5) at site IN to 57.7% (95% CI: 43.2–71.3) at RF. Seroprevalence was lowest in subadults (22.2%, 95% CI: 12.7–34.5) and highest in adults (54.1%, 95% CI: 45.23–62.8). The differences in seroprevalence among sites (χ^2: 32.388, df=3, $P<0.001$) and age classes (χ^2: 18.155, df=2, $P<0.001$) were statistically significant. No statistically significant difference was observed between ADV seroprevalence in males compared to females (χ^2: 0.523, df=1, $P=0.47$). Shedding of ADV was observed in 20 of 98 wild boar (20.4%; 95% CI: 12.4–29.7). Of these shedders, 13 wild boar had one of three swabs positive, five wild boar had one of two swabs positive, and two wild boar had two of two swabs positive. Shedding was highest at the RF site (55.6%, 95% CI: 30.8–78.5) and lowest at QM (5.9%, 95% CI: 0.2–28.7). Univariate analysis showed that the proportion of subadult shedders was significantly lower than that of juveniles ($P<0.01$) and adults ($P<0.05$). No difference in ADV shedding between male and female was observed (χ^2: 0.656, df=1, $P=0.418$).

We found ADV in tissues of five wild boar (Tables 1, 2). Tonsils were collected from all study sites, and three of 111 tonsils (2.7%, 95% CI: 0.6–7.7) were found PCR positive. Trigeminal and sacral ganglia samples were collected only from study site IN. Two of 48 trigeminal nerve ganglia (4%, 95% CI: 0.5–14.3) and two of 53 sacral nerve ganglia (4%, 95% CI: 0.5–13) were PCR positive. As wild boar number 140 was positive in both nerve ganglia (Table 2), these four positive nerve ganglia samples corresponded to three animals.

The results of the multivariable logistic regression models are shown in Table 3. The variable 'sex' was excluded as it did not improve the fit of the model. According to Model 1, the highest odds of seropositivity were observed at sites QM and RF, being 14.3 (95% CI: 5.4–37.9) and 12.8 (95% CI: 4.5–

TABLE 1. Prevalence of Aujeszky's disease virus (ADV) antibodies and viral DNA in swabs and tissues of wild boar (*Sus scrofa*). Wild boar was sampled in the hunting season of 2014–15 in central Spain and Portugal. Study sites in Spain included areas in the Toledo Mountains (QM and ED) and in the valley of the Guadiana River (RF), and in Portugal the county of Idanha-a-Nova (IN). Results are displayed as number positive/number tested and in parentheses the percentage and 95% Clopper-Pearson exact confidence intervals (%; 95% CI).

		ADV shedding		
	Seroprevalence	Oral	Nasal	Genital
Study site				
IN	7/55 (12.7; 5.3–24.5)	1/55 (1.8; 0.1–9.7)	0/55 (0; 0–6.5)	4/46 (13.0; 4.9–26.3)
ED	15/42 (35.7; 21.6–52.0)	0/17 (0; 0–19.5)	1/17 (5.9; 0.2–28.7)	3/17 (0; 0–19.5)
QM	49/87 (56.3; 45.3–67.0)	0/19 (0; 0–17.7)	1/19 (5.3; 0.1–26.0)	0/17 (0; 0–19.5)
RF	30/52 (57.7; 43.2–71.3)	2/17 (11.8; 1.5–36.4)	5/18 (27.8; 9.7–53.5)	5/14 (35.7; 12.8–64.9)
Age				
Juvenile	15/39 (38.5; 23.4–55.4)	0/15 (0; 0–21.8)	4/17 (23.5; 6.8–49.9)	3/15 (20.0; 4.3–48.1)
Subadult	14/63 (22.2; 12.7–34.5)	0/32 (0; 0–10.9)	0/32 (0; 0–10.9)	1/30 (3.3; 0.1–17.2)
Adult	72/133 (54.1; 45.3–62.8)	3/61 (4.9; 1.0–13.7)	3/60 (5; 1.0–13.9)	8/49 (16.3; 7.3–29.7)
Sex				
Male	39/98 (39.8; 30.0–50.2)	2/49 (4.1; 0.5–14.0)	5/49 (10.2; 3.4–22.2)	5/44 (11.4; 3.8–24.6)
Female	61/137 (44.5; 36.0–53.3	1/59 (1.7; 0.1–9.1)	2/60 (3.3; 0.4–11.5)	7/50 (14.0; 5.8–26.7)
Total	101/236[c] (42.6; 36.4–49.4)	3/108 (2.8; 0.6–7.9)	7/109 (6.4; 2.6–12.8)	12/94 (12.8; 6.8–21.2)

[a] ADV shedders = wild boar with at least one PCR positive result in oral, nasal and/or genital swabs.

[b] Nerve ganglia; sampled only at site IN (Portugal).

[c] Total values for serology vary according to available information. Site: 101/236 (42.6%; 36.4–49.4); age: 101/235 (43.0%; 36.6–49.6); sex: 100/235 (42.6%; 36.2–49.2).

36.0) times higher, respectively, than at site IN. The odds of seropositivity in adults was 4.3 (95% CI: 1.9–9.6) times higher than in juveniles. Model 2 shows that the odds of ADV shedding at sites ED and QM were similar to that of the reference category IN. The highest odds of shedding were observed at RF, being 6.1 (95% CI: 1.5–25.0) times higher than at IN. Virus shedding of subadult wild boar was 0.08 (95% CI: 0.01–0.9) times that of juveniles. The odds of ADV shedding of adults did not differ statistically from that of juveniles.

To describe the ADV infection status, animals were classified into four categories based on their individual serologic and shedding results (Fig. 2). Of the 98 hunted wild boar for which this information was available, there were 11 seropositive shedders, nine seronegative shedders, 16 nonshedding seropositive, and 62 nonshedding seronegative. The five wild boar with positive tissues (as shown in Table 2) were included into the categories accordingly: one seropositive shedder, two nonshedding seropositive, and two nonshedding seronegative. At site RF, >55% wild boar were shedding as compared to <25% at IN, ED, and QM. Of these shedders, ≤50% of sites ED and RF were seropositive compared to >80% seropositive at IN and QM. The percentage of nonshedding was highest at QM (94%) and IN (89%). Within these nonshedders, the proportion of seronegatives was highest at IN (97.5%) and in subadults (93%) compared to the remaining sites (56–63%) and age categories (≤80%).

DISCUSSION

Knowledge on the epidemiology of ADV in local wild boar is relevant for assessing the risk of disease transmission to domestic pigs as well as for hunting and conservation purposes, such as wild boar translocations and the health of sympatric carnivores (Ruiz-Fons et al. 2007;

TABLE 1. Extended.

ADV shedding	ADV detection in tissues		
Shedders[a]	Tonsils	Trigeminal[b]	Sacral[b]
5/46 (10.9; 3.6–23.6)	2/51 (3.9; 0.5–13.5)	2/48 (4.2; 0.5–14.3)	2/53 (3.8; 0.5–13.0)
4/17 (23.5; 6.8–49.9)	0/20 (0; 0–16.8)	Not available	Not available
1/17 (5.9; 0.2–28.7)	1/20 (5.0; 0.1–24.9)	Not available	Not available
10/18 (55.6; 30.8–78.5)	0/20 (0; 0–16.8)	Not available	Not available
6/16 (37.5; 15.2–64.6)	1/18 (5.6; 0.1–27.3)	Not available	Not available
1/30 (3.3; 0.6–16.7)	1/32 (3.1; 0.1–16.2)	0/15 (0; 0–21.8)	0/17 (0; 0–19.5)
13/52 (25.0; 14.0–39.0)	1/61 (1.6; 0.0–8.8)	2/33 (6.1; 0.7–20.2)	2/36 (5.6; 0.7–18.7)
11/46 (23.9; 12.6–38.8)	3/52 (5.8; 1.2–16.0)	1/17 (5.9; 0.2–28.7)	0/20 (0; 0–16.8)
9/52 (17.3; 08.2–30.3)	0/59 (0; 0–6.1)	1/31 (3.2; 0.1–16.7)	2/33 (6.1; 0.7–20.3)
20/98 (20.4; 12.9–29.7)	3/111 (2.7; 0.6–7.7)	2/48 (4.2; 0.5–14.3)	2/53 (3.8; 0.5–13.0)

Boadella et al. 2012b; Masot et al. 2017; Cano-Terriza et al. 2019). Previous studies have shown ADV to circulate enzootically in central Spain (Vicente et al. 2005; Ruiz-Fons et al. 2007; Boadella et al. 2012a; González-Barrio et al. 2015). Our study confirmed the circulation of ADV among wild boar in central Portugal, adding to what is known in Europe (Müller et al. 2011; Meier et al. 2015). Further geographic distribution, as well as the circulating genotypes, remain to be determined for Portugal (Müller et al. 2010; Verin et al. 2014).

Our results illustrate clear differences among the studied populations. Seroprevalence was higher at the study sites in Spain compared to the one site in Portugal and was higher in adults compared to younger animals. This is in agreement with previous studies, suggesting that cumulative exposure explains increasing seropositivity with age (Vicente et al. 2005; Verin et al. 2014; Lipowski et al. 2017; Caruso et al. 2018). Shedding differed

TABLE 2. Characterization of the five wild boar (*Sus scrofa*) with PCR-positive tissues for Aujeszky's disease virus. Wild boar were sampled in the hunting season of 2014–15 in the Toledo Mountains of central Spain (QM) and in Idanha-a-Nova county (IN) in Portugal. Seropositivity and Aujeszky's disease virus shedding are shown as positive (+) and negative (–). NA = not available.

Wild boar ID no.	Tissues			Serology	Shedding[b]			Site	Sex	Age
	Tonsils	Trigeminal[a]	Sacral[a]		Oral	Nasal	Genital			
8	+	NA	–	–	–	–	–	IN	Male	Subadult
48	–	–	+	–	–	–	–	IN	Female	Adult
140	–	+	+	+	–	–	NA	IN	Female	Adult
141	+	+	–	+	–	–	–	IN	Male	Adult
173	+	NA	NA	+	–	+	–	QM	Male	Juvenile

[a] Nerve ganglia; sampled only at site IN (Portugal).

[b] Swabs tested by PCR.

TABLE 3. Logistic regression modeling of the effect of age of wild boar (*Sus scrofa*) and study site on seropositivity (Model 1) and on shedding (Model 2) of Aujeszky's disease virus. Variables with statistical significance are shown in boldface type. Study sites in Spain included areas in the Toledo Mountains (QM and ED) and in the valley of the Guadiana River (RF), and in Portugal the county of Idanha-a-Nova (IN).[a]

Outcome variable	Explanatory variables	Coefficient	Odds ratio	SE	95% Confidence intervals		*P*-value
					Lower	Upper	
Serology Model 1	Site						
	IN (ref)	—	—	—	—	—	0.000
	ED	**1.512**	**4.537**	**0.538**	**1.579**	**13.031**	**0.005**
	QM	**2.661**	**14.307**	**0.497**	**5.398**	**37.916**	**0.000**
	RF	**2.549**	**12.789**	**0.528**	**4.548**	**35.962**	**0.000**
	Age						
	Juveniles (ref)	—	—	—	—	—	0.000
	Subadults	−0.238	0.788	0.475	0.311	2.001	0.617
	Adults	**1.451**	**4.268**	**0.414**	**1.895**	**9.613**	**0.000**
ADV shedding Model 2	Site						
	IN (ref)	—	—	—	—	—	0.017
	ED	0.690	1.993	0.851	0.376	10.560	0.417
	QM	−1.119	0.327	1.236	0.029	3.680	0.365
	RF	**1.810**	**6.109**	**0.718**	**1.494**	**24.970**	**0.012**
	Age						
	Juveniles (ref)	—	—	—	—	—	0.126
	Subadults	**−2.497**	**0.082**	**1.237**	**0.007**	**0.930**	**0.044**
	Adults	−0.574	0.563	0.777	0.123	2.582	0.460

[a] — = reference category.

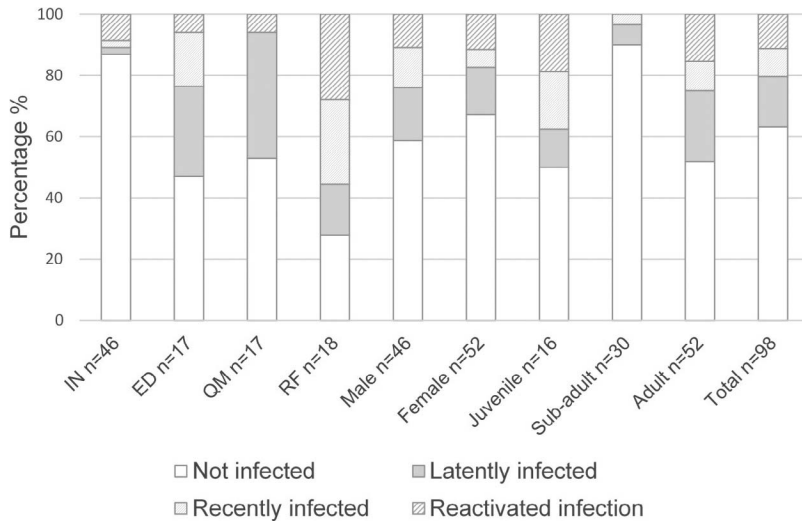

FIGURE 2. Aujeszky's disease virus (ADV) infection categories of 98 wild boar (*Sus scrofa*) per site, age, and sex categories. Individual animals were classified based on their ADV shedding and serology profile. Animals with at least one ADV PCR-positive swab were classified as shedders. Sampling occurred in the hunting season of 2014–15. Study sites in Spain included areas in the Toledo Mountains (QM and ED) and in the valley of the Guadiana River (RF), and in Portugal the county of Idanha-a-Nova (IN).

also according to site; we found the highest shedding prevalence (56%) at the RF site. The highest proportion of ADV positivity was found in genital swabs (13%) compared to nasal and oral swabs (<6%), further strengthening the importance of the genital route in transmission (Verin et al. 2014; González-Barrio et al. 2015). In our study, the numbers of swabs tested in some categories were rather low, and the resulting uncertainty (large CIs) needs to be taken into account when interpreting the findings. Although the crude values of seroprevalence and shedding differed between female and male wild boar, as in previous studies (Ruiz-Fons et al. 2007; Caruso et al. 2018) this difference was not statistically significant.

To gain insight into the latently infected state, the presence of ADV in tissues was analyzed (Ruiz-Fons et al. 2007; OIE 2018). Interestingly, a rather low proportion of infected animals was found, with approximately 3% PCR-positive tonsils and 4% PCR-positive sacral and trigeminal nerve ganglia, respectively. Taken together, these PCR-positive tissues pertained to only five wild boar; that is, some individuals accounted for more than one positive tissue type. These values are much lower than the >35% found in feral swine in the US (Romero et al. 2003). A possible explanation could be methodological, as those authors targeted the TK gene of ADV as opposed to the gB gene that we used. Further explanations include the different geographic, host, and epidemiologic contextualization. Using the same nested PCR targeting gB in wild boar populations in central Spain, positive results were observed in 24% of tonsils and 6% of trigeminal ganglia; in 7% of the animals both tonsils and trigeminal ganglia were positive (Ruiz-Fons et al. 2007). Although the PCR findings in trigeminal ganglia are similar to ours, the high virus prevalence of tonsils clearly contrasts our findings. Considerable temporary variations of ADV infection dynamics may explain these differences (Casades-Martí et al. 2020). The findings by Ruiz-Fons et al. (2007) showed that wild boar with positive trigeminal ganglia tend to be seropositive, potentially reflecting latent infection, whereas those with positivity only in tonsils may reflect recent infection in which seroconversion had not yet occurred. This appears to be supported by our study, although the large uncertainty associated with the low numbers of positive samples warrants care in any extrapolations.

Different infection dynamics of ADV based on serology, shedding, and/or PCR-positive tissues have been previously described (Ruiz-Fons et al. 2007; Verin et al. 2014; González-Barrio et al. 2015). By combining the serologic and shedding status of individual wild boar, we established four ADV infection categories. These categories can, despite some minor discrepancies, be interpreted as follows: 1) Nonshedding seronegative animals as not infected, 2) seropositive nonshedding as latently infected, 3) seronegative shedders as recently infected, and 4) seropositive shedders as reactivated latent infections (Ruiz-Fons et al. 2007; OIE 2018). Our overall findings show that the 63% of wild boar were not infected, 16% were latently infected, 9% were recently infected, and 11% were shedders from reactivated infections. These findings were similar to the values of 62%, 14%, 7%, and 17%, respectively, of a recent study in Tuscany (Verin et al. 2014). Despite the totals being similar, very different pictures were obtained when comparing populations, for example sites IN versus RF. At IN, the vast majority (87%) was seronegative nonshedding wild boar, but ADV DNA was found in tissues in two of these seronegative animals (numbers 8 and 48; Table 2). The remaining 13% of wild boar from IN were seropositive or shedders, or both, indicating an overall low infection pressure. The situation at RF was nearly the opposite. Here, the vast majority (72%) was seropositive, shedders, or both, and only 28% were apparently not infected. At RF over 50% of the wild boar were found shedding, regardless of serologic status, indicating recent/active as well as concomitant reactive infections leading to potentially high transmission rates.

The OIE (2018) considers latently infected pigs as those harboring noninfective virus in trigeminal and other nerve ganglia. These

animals are generally seropositive but do not shed any virus until the infection is reactivated and the virus is shed again (see Casades-Martí et al. 2020). We found that two (animals number 140 and 141) of the 46 wild boar in IN in which all samples could be collected were seropositive and PCR positive in trigeminal ganglia.

How can the different infection patterns observed in this study among sites be explained? Risk factors for increased ADV transmission have been analyzed before (e.g., Ruiz-Fons et al. 2008). Major drivers include wild boar individual traits (age, sex) as well as density and aggregation; for example, in intensively managed hunting estates that are fenced and where supplemental feeding is practiced (Acevedo et al. 2007). High densities and aggregation might increase individual stress and promote reactivation of infections in latently infected wild boar. Hunting and physiologic mating stress may also promote the reactivation of latent infections (Casades-Martí et al. 2020). Once ADV infection is reactivated, transmission to naïve individuals may occur. Our understanding of the effects of wild boar aggregation and density on ADV transmission is currently unclear. Whereas at large spatial scales increasing densities favored ADV transmission (Acevedo et al. 2007), time series data in particular wild boar populations did not show relevant effects of variable local densities (Boadella et al. 2012a; Casades-Martí et al. 2020). We cannot discuss aggregation and density effects for our study, as these were not measured at IN. However, our findings resemble those observed in Italy, where a seroprevalence of 65% was found in wild boar in the enclosed La Mandria Park in contrast to the 10% of free-ranging wild boar in that same region (Caruso et al. 2018). Population parameters are essential if these effects are to be studied and should be included in future studies.

According to international trade rules, "A member country should not impose trade bans in response to a notification of ADV in wild and feral pigs" (OIE 2019; page 1, article 8.2.1). In EU member states or regions, freedom from AD can be achieved even if infection is known to be established in wild boar, as long as measures have been implemented to prevent any transmission of ADV from wild boar to domestic pigs (CEC 2008; European Commission Health & Consumers Directorate-General 2016). From April 2021 onward, the new EU Animal Health Law shall apply (CEC 2016). While AD is currently not part of its Listed Diseases, this can be amended anytime by the Commission and Delegated Acts.

In conclusion, AD infection dynamics differed considerably in hunted wild boar at the study sites in Portugal versus Spain. In Portugal, we show for the first time the patterns of ADV infection in wild boar. Given that wild boar may shed ADV, transmission to domestic pigs, most probably under extensive production, is feasible; therefore, potential pig-wild boar interactions should be considered in the eradication of AD in Portugal. We recommend monitoring of AD in wild boar as a means of estimating where and when transmission might occur.

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