Short communication

High prevalence and intensity of *Stephanurus dentatus* in a population of wild boar (*Sus scrofa*) in south-western Spain

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Abstract

In the period from October 2016 to February 2017, the urinary tracts of 390 wild boars (*Sus scrofa*) from four areas of south-central Spain (102 from Doñana National Park, 150 from Sierra Morena and Toledo Mountains, 84 from Sierra Nevada and 54 from Sierra de Cazorla, Segura y Las Villas Natural Park) were examined for the presence of adult specimens of *Stephanurus dentatus* (Nematoda: Strongyloidea). This parasite was only detected in the wild boar population of Doñana National Park, with high prevalence (76.5 ± 4.2%; 78/102), mean intensity (43.2 ± 4.4) and mean abundance (33.1 ± 3.8). Juvenil wild boar had significantly lower prevalence and abundance than subadult and adult wild boar. The intensity of infection was significantly higher in male than in female wild boar. The detection of a focus of *S. dentatus* infestation in the wild boar population in Doñana National Park will provide further opportunities for understanding the epidemiology of this parasite.

Keywords: Distribution; Epidemiology; *Stephanurus dentatus;* Spain; Wild boar

Stephanurus dentatus (Nematoda: Strongyloidea) is a renal nematode of suids from tropical and subtropical countries. This parasite has a direct life cycle; the host

51 becomes infected by ingesting free-living larvae, a paratenic host (earthworm) or
52 percutaneously. Larvae are sensible to desiccation, but can survive in moist soils for
53 several months (Olsen, 1977). Migrating larvae affect mainly the hepatic parenchyma,
54 while adults establish in the urinary system (Islam et al., 2015). Stephanurosis is
55 responsible for important economic losses in warm regions with traditional pig
56 production systems (Islam et al., 2015) and is also important in wild pig populations
57 (*Sus scrofa domesticus* and their hybrids) (Cauquil et al., 2013). However, there is a lack
58 of information regarding the distribution and epidemiology of this parasite in wild boar
59 (*Sus scrofa*) populations.
60
61 *S. dentatus* had been detected by the authors in wild boars in Doñana National
62 Park (DNP), south-west Spain (37º0'N, 6º30'W), since 2007 (Moratal et al.,
63 unpublished). The aims of this study were: (1) to assess the presence of *S. dentatus* and
64 to describe the epidemiological features of this parasitic infection in wild boar in DNP;
65 and (2) to evaluate the presence of *S. dentatus* in other wild boar populations from south
66 and central Spain.
67
68 The study was carried out in four areas of south and central Spain: (1) DNP, one
69 of the most important protected wetlands in Europe; (2) Sierra Morena (SM) and the
70 Toledo Mountains (TM), comprising two mountain ranges connected by the Guadiana
71 River valley in the south of the central Spanish plateau; (3) Sierra de Cazorla, Segura y
72 las Villas Natural Park (SC), a protected area in Jaén province (38º30'N, 2º45'W); and
73 (4) Sierra Nevada Natural and National Park (SN), in Almería and Granada provinces
74 (37º07'N, 3º14'W) (Fig. 1). DNP is the wettest of this aresa, with an annual relative
75 humidity of 65-70%.

Taking advantage of an annual official culling program in DNP, 102 wild boars (*Sus scrofa*) were sampled between October and December 2016 following a protocol approved by the Animal Experiment Committee of Castilla-La Mancha University and the Doñana Office of Research Coordination. In addition, 150 animals from SM and TM, 84 from SN and 54 from SC were sampled during the authorized hunting regular season (October-February) of 2016-2017. Sex was recorded and age was assessed on the basis of tooth eruption pattern (Saenz de Buruaga et al., 2001): (1) juveniles (6 to < 12 months); (2) subadults (12-24 months); and (3) adults (>24 months). Perirenal fat, kidneys and ureters were collected and frozen at -20 ºC until dissection. Nematodes were collected in 70% ethanol, counted and identified on the basis of morphology following the descriptions of Olsen (1977) and Skryabin (1991).

The prevalence, abundance and intensity of *S. dentatus* adults were determined according to Bush et al. (1997). To assess statistical differences by sex and age categories, three generalised linear models (GLMs) were parameterised; for *S. dentatus* prevalence (binominal variable; 0 absence, 1 presence), we considered a binomial error distribution and a logit link function (n = 102). The parasitic abundance and intensity were logarithmically transformed and modelled following a normal distribution and an identity link function (n = 102 and n = 78, respectively); overdispersion was detected with raw and negative binomial data, whereas it was not detected with the normal distribution and the log-transformed data. Sex, age category (categorical variables) and the interaction between both were included as explanatory variables (model features in Table 1). The statistical analyses were performed with SPSS Statistics v22 (IBM).

100 *S. dentatus* was detected in wild boars from the DNP, but not in animals from
101 the other three areas sampled. The prevalence (±standard error, SE) of *S. dentatus* in
102 DNP was 76.5 ± 4.2% (78/102), the mean intensity was 43.2 ± 4.4 nematodes per
103 infected individual (n = 78; range 1-199), and the mean abundance 33.1 ± 3.8
104 nematodes per individual (n = 102).
105
106 There were no statistically significant differences between wild boar sexes in
107 prevalence or abundance. However, the intensity was significantly higher in males than
108 female wild boar (Wald = 4.13, $P < 0.05$; Fig. 2a). Prevalence (Wald = 11.74, $P < 0.05$)
109 and abundance (Wald = 16.95, $P < 0.05$) were significantly lower for juvenile wild
110 boar, compared to subadult and adult wild boar. No significant differences between age
111 categories were detected for intensity (Fig. 2b).
112
113 The results of this study show that DNP is an area with a high prevalence of *S.*
114 *dentatus* in wild boar, which contrasts with the absence of this parasite in three other
115 areas of the Iberian Peninsula. These findings match the high prevalences reported in
116 domestic pigs in tropical endemic areas, for example in some southern states of
117 the USA (78-94%; Hale, 1986), and in wild pigs in New Caledonia (64.3%; Cauquil et
118 al., 2013). The high prevalence and parasitic intensity in DNP are probably related to
119 environmental conditions, particularly high humidity (Barasona et al., 2014), which is
120 higher in DNP than in the three other areas included in this study (Fig. 1).
121
122 The prevalence and abundance of *S. dentatus* in juvenile wild boar was
123 significantly lower than in older animals, which can be explained by: (1) the long
124 prepatent period of *S. dentatus*, which may last 9-16 months (Batte et al., 1960); and (2)

125 the shorter time, and hence lower probability, of contacting free-living larvae, of
126 juvenile wild boar. No age-related effect was detected for parasitic intensity, which
127 could indicate that the available space in the urinary system for adult parasites to
128 establish is independent of age.
129
130 Male wild boar harboured consistently higher parasitic loads than female wild
131 boar across all ages. This relation between sex and microparasite infestation is common
132 in vertebrate hosts and can be explained by factors such as differences in behaviour
133 between the sexes or the immunosuppressive effect of testosterone (Poulin, 1996).
134
135 The identification of a focus of infestation of wild boar with *S. dentatus* in DNP
136 will provide further opportunities for investigating the epidemiology of this parasite,
137 including the assessment of environmental risk factors, such as e.g. humidity.
138
139 **Conflict of interest statement**
140 None of the authors of this paper has a financial or personal relationship with
141 other people or organisations that could inappropriately influence or bias the content of
142 the paper.
143
144 **Acknowledgements**
145 This work was supported by the Spanish Ministerio de Economía y
146 Competitividad (MINECO; grant AGL2016-76358-R). Pelayo Acevedo and Samantha
147 Moratal were supported by the MINECO and the Universidad de Castilla-La Mancha
148 (UCLM) through a 'Ramón y Cajal' contract (RYC-2012-11970) and a predoctoral
149 fellowship (FPU16/02036), respectively. Preliminary results were presented at the 35th

meeting of the Wilflife Fauna Ecopathology Study Group (Groupe d'Étude sur l'Ecopathologie de la Faune Sauvage de Montagne/Grupo de Estudios de Ecopatología de Fauna Salvaje de Montaña, GEEFSM), Cofrentes Muela de Cortes, Valencia, Spain, 1st-4th June 2017.

References

Barasona, J.A., Latham, M.C., Acevedo, P., Armenteros, J.A., Latham, A.D.M., Gortázar, C., Carro, F. Soriguer, R.C., Vicente, J., 2014. Spatiotemporal interactions between wild boar and cattle: Implications for cross-species disease transmission. Veterinary Research 45, 122.

Batte, E.G., Harkema, R., Osborne, J.C., 1960. Observations on the life cycle and pathogenicity of the swine kidney worm (*Stephanurus dentatus*). Journal of American Veterinary Medical Association 136, 622-625.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. The Journal of Parasitology 83, 575-583.

Cauquil, L., Barrière, P., Hüe, T., 2013. Prevalence of *Stephanurus dentatus* in feral pig (*Sus scrofa*) in New Caledonia. Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux 66, 131-135.

Hale, O.M., 1986. Internal parasite infections influence feeding cost of swine. Feedstuffs 1, 1-14.

Islam, A., Anisuzzaman, Roy, J.R., Yasin, Md.G., Labony, S.S., Hossain, Md.M., Alim, Md.A., 2015. Swine kidney worm in Bangladesh: An abattoir survey. Eurasian Journal of Veterinary Science 31, 222-226.

S. Moratal, M.R. Ruiz de Ybáñez, P. Barroso, J. Martínez-Guijosa, R. Triguero, P. Acevedo, J.E. Granados, C. Martínez-Carrasco, J. Viente. Aspectos epidemiológicos de *Stephanurus dentatus* en jabalíes (*Sus scrofa*) del Parque Nacional de Doñana, sudeste de España. Libro de Resúmenes 35º Encuentro Grupo de Estudios de Ecopatología de la Fauna Slavale de Montaña (GEEFSM) Cofrentes, Muela de Cortes, Valencia, Spain, 1-4 June 2017 (Abstract)

Olsen, O.W., 1977. Parasitología animal II. Platelmintos, acantocéfalos y nematelmintos. Aedos, Barcelona, 719 pp.

Poulin, R., 1996. Sexual inequalities in helminth infection: A cost of being male? The American Naturalist 147, 287-295.

193 Saenz de Buruaga, M., Lucio, A.J., Purroy, F.J., 2001. Reconocimiento de sexo y edad
194 en especies cinegéticas. Ediciones Leonesas S.A., León, 127 pp.
195 Skryabin, K.I., 1991. Key to parasitic nematodes. Volume 3: Strongylata. EJ Brill,
196 Leiden, 912 pp.
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Table 1

Results of generalised linear models (GLMs) for *Stephanurus dentatus* prevalence, abundance and intensity.

(a)	Parameter		*B*[a]	*SE*	*Wald*	*p*
	Sex	Male	-0.31	0.89	0.12	0.73
	Age	Juvenile	-3.70	1.08	11.74	<0.01
		Subadult	-0.33	1.06	0.09	0.76
	Sex*Age	Male*Juvenile	2.15	1.32	2.66	0.10
		Male*Subadult	0.05	1.60	0.001	0.98
(b)	Parameter		*B*[a]	*SE*	*Wald*	*P*
	Sex	Male	0.16	0.17	0.83	0.36
	Age	Juvenile	-0.96	0.23	16.95	<0.01
		Subadult	0.06	0.21	0.08	0.78
	Sex*Age	Male*Juvenile	0.33	0.31	1.11	0.29
		Male*Subadult	0.13	0.35	0.15	0.70
(c)	Parameter		*B*[a]	*SE*	*Wald*	*p*
	Sex	Male	0.25	0.12	4.13	0.04
	Age	Juvenile	0.004	0.31	<0.001	0.99
		Subadult	0.14	0.15	0.83	0.36
	Sex*Age	Male*Juvenile	-0.37	0.36	1.04	0.31
		Male*Subadult	0.17	0.25	0.44	0.51

[a] Parameter estimates (B) were calculated considering a reference value of zero for female level in the variable ‘sex’ and adult level in the variable ‘age’.

207 **Figure legends**

208

209 Fig. 1. Map of peninsular Spain showing the study areas and the prevalence of
210 *Stephanurus dentatus* in each area. The annual average relative humidity is shown in
211 different tonalities of green (adapted from the Instituto Nacional de Meteorología,
212 Ministerio de Medio Ambiente. Atlas Nacional de España, IGN). DNP: Doñana
213 National Park; SC: Sierra de Cazorla, Segura y Las Villas Natural Park; SN: Sierra
214 Nevada Natural and National Park; SM: Sierra Morena; TM: Toledo Mountains.

215

216 Fig. 2. Prevalence (▬), abundance (▭) and intensity (▭) of *Stephanurus dentatus* in
217 Doñana National Park, Spain, according to: (a) age category and (b) sex class. Error bar,
218 standard errrors A, a and 1 differ significantly from B, b and 2, respectively (post-hoc
219 Tukey test).