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Research paper

Lung parasites of the genus *Metastrongylus* Molin, 1861 (Nematoda: Metastrongilidae) in wild boar (*Sus scrofa* L., 1758) in Central-Italy: An eco-epidemiological study

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ABSTRACT

The respiratory tracts of 57 wild boars (*Sus scrofa* L. 1758) hunted in central Italy during the 2011/2012 hunting season were examined to detect the presence of lung worms. Fifty-five out of 57 animals (96,5%) were positive. Five species of *Metastrongylus* were detected and their prevalence was as follows: *Metastrongylus asymmetricus* Noda, 1973 (91.2%), *Metastrongylus confusus* Jansen, 1964 and *Metastrongylus salmi* Gedoelst, 1923 (87.7%), *Metastrongylus apri* Gmelin, 1790 (80.7%), *Metastrongylus pudendotectus* Vostokov, 1905 (70.2%). In most cases multi-species infection was observed. The highest parasite load was found in young animals (<1 year old). The *Metastrongylus* genus sex ratio (M/F) had a range from 1:4.8 to 1:1.5 in favor of females. The Simpson and Shannon–Wiener indices showed a moderate uniformity in parasite community composition. The Fager index highlighted a high degree of affinity among all pairs of selected parasites. The whole parasite population showed an aggregate distribution. Our findings confirm that these parasites are widespread in the wild boar population. The establishment of outdoor domestic pig farming in the same area of the game preserve could pose the risk of infection to domestic animals. Further studies will be needed to understand the factors involved in the presence and prevalence of the intermediate host as well as the population dynamics of *Metastrongylus* spp.

1. Introduction

During the past century the wild boar (*Sus scrofa* L. 1758) population in Italy fluctuated reaching its minimum after the Second World War. In the 1960s, the species was repopulated through the uncontrolled introduction of Central-Europe wild boars and hybrids characterized by high prolificacy. This fact, together with the depopulation of rural sites and the reduction of natural predators, caused the wild boar population to sprawl in some areas, bringing about losses in agriculture and farming that required official damage control (Riga et al., 2011). Furthermore, the repopulation led to an alteration of the endemic wild boar population in Italy, and in fact genetic purity of such species is now very rare (Scandura et al., 2005). Today, wild boars are present in 95 out of 107 Italian provinces; their population is estimated at around 300,000–500,000 heads (Carnevali et al., 2009).

As the practice of outdoor pig farming has been constantly growing over the past years, domestic pigs could interact with wild boars more easily. In order to properly assess the risks for farmed animals, a deeper knowledge of wildlife health is strongly needed. Furthermore, as the wild boar and domestic pig belong to the same species and have similar morphology, ethology and diseases, the wild boar can be a valuable model for the study of the host-parasite interaction without the influence of anthropogenic factors.

Verminous pneumonia or metastrongylosis is an important respiratory disease affecting domestic pigs and wild boars. The genus *Metastrongylus* Molin, 1861 includes 6 species reported around the world: *Metastrongylus apri* Gmelin, 1790 (syn. *Metastrongylus elongatus* Dujardin, 1845); *Metastrongylus salmi* Gedoelst, 1923; *Metastrongylus pudendotectus* Vostokov, 1905; *Metastrongylus confusus* Jansen, 1964; *Metastrongylus asymmetricus* Noda, 1973 and *Metastrongylus madagascariensis* Chabaud and Gretillat, 1956. The latter was found only in pigs from Madagascar (Chabaud and Gretillat, 1956); *M. apri*, *M. salmi* and *M. pudendotectus* are

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commonly reported wordwide with variable frequency (Macchioni et al., 1988; Eslami and Farsad-Hamdi, 1992; Manfredi et al., 1996; Epe et al., 1997; Biddau et al., 2003; De Sousa et al., 2004; Järvis et al., 2007; Nosal et al., 2010; Senilk et al., 2011; Da Silva and Műller, 2012). *M. confusus* and *M. asymmetricus* are not that widespread and usually associated with low prevalence and abundance (Macchioni et al., 1988; Eslami and Farsad-Hamdi, 1992; Manfredi et al., 1996; Epe et al., 1997; Biddau et al., 2003; De Sousa et al., 2004; Järvis et al., 2007; Nosal et al., 2010; Senilk et al., 2011; Da Silva and Műller, 2012).

Lung parasites of the *Metastrongylus* genus produce fully larvated eggs in the host respiratory system. Eggs come up to the boar's mouth when the animal coughs or sneezes and are then swallowed and passed with the feces; in the soil the eggs will hatch and release L1 larvae that are ingested by earthworms, which are the intermediate hosts. The final host acquires the parasite by ingesting infected earthworms which are very abundant in their environment (Anderson, 2000).

Currently, *Metastrongylus* spp. is uncommon in intensively farmed pigs whereas its prevalence is very high in wild boars (Gadomska, 1981; Macchioni et al.,1988; Hubert and Henry, 1989; Manfredi et al., 1996; Epe et al., 1997; De-la-Muela et al., 2001; Biddau et al., 2003; De Sousa et al., 2004; Järvis et al., 2007; Nosal et al., 2010; Senilk et al., 2011; Da Silva and Műller, 2012; García-González et al., 2013; Navarro-Gonzalez et al., 2013). The infection is considered one of the most important selective factors in wild boar, increasing the mortality of weaker young and adult animals, causing dyspnoea, bronchopneumonia and permanent weight loss. In addition, infection inflicts tissue damage that allows the co-occurance of opportunistic infections from viruses and bacteria (Da Silva and Műller, 2012). In this study, we investigated lung worms of a wild boar population from Central-Italy using an eco-epidemiological approach.

2. Materials and methods

2.1. Study area and sampled population

During two consecutive hunting seasons (2011 and 2012, from November the 1st up to January the 31st), a total of 57 wild boars (36 male and 21 female) were collected from hunters in a game preserve in the Central-Italy, in the province of Viterbo. The boundaries of the hunting preserve (2415 ha), located near Musignano (42°25′53.54″N; 11°43′01.42″E), are irregular in shape: they cover about 10 km from East to West, and reach a maximum width of 2 km from North to South. The area has irregular orographic landscape features as well, varying from 71 to 437 m above sea level (a.s.l.) (Fig. 1).

Nineteen percent of the territory is zoned for agricultural use (cereals and fodder), while the remaining areas is zoned both for extensive breeding (cattle and horses) and hunting purposes (wild boar, roe deer, red deer), or for growing oak forests (*Quercus* spp.).

Surrounded by fences, the preserve hosts approximately 970 wild boars (0.4 heads/ha) that are supplementarily fed with cereals and fodder produced in the same preserve. Feeding is provided on the ground; no feeders are used. Data from each wild boar (culling site, sampling time, sex and age) were recorded. Animals were divided into 3 age groups juveniles (<1 year), subadults (1–3 years) and adults (>3 years) (Table 1), both on the basis of coat color and of tooth development (Matschke, 1967; Boitiani and Mattei, 1991; Genov and Massei, 1991). Animals weight ranged from 8 to 120 kg.

2.2. Parasites collection

The lungs from each animal were collected, then labeled and stored in plastic bags at −20 °C until processing. After thawing at room temperature for 24 h, the samples were inspected to detect gross lesions. The lungs were then divided into left and right lung and the weight of each lung was recorded. The bronchial tree was completely dissected from the main bronchi to the smallest bronchioles; all nematodes found were fixed in 70% ethanol and cleared with lacto-phenol to allow for gender and species identification as well as counting, according to specific morphometric keys (Dujardin, 1845; Gedolest, 1923; Jansen, 1964; Noda, 1973).

2.3. Data analysis

The infection parameters described (prevalence, mean abundance, mean intensity) were based on the terminology of Bush et al. (1997). The sex ratio, related to the whole parasite community and to each single species, were calculated. Pearson's chi-squared (χ^2) test was applied to compare prevalence among sex, age classes and left/right lung. The non-parametric Kruskall–Wallis and Wilcoxon Signed-Rank tests (Ròzsa et al., 2000) were used to calculate the differences between quantitative variables (mean abundance and mean intensity).

A linear regression analysis was performed to evaluate the influence of the parasite load on the number of *Metastrongylus* species found: the number of parasite species found in each sample was used as dependent variable, while the whole number of parasites was considered an independent variable.

Furthermore, in order to describing and analyzing the parasite community ecology, as in the Magurran (1988) study, the following ecological indices were calculated**:** species richness (S), Simpson index (D) (Simpson, 1949) and Shannon–Wiener index (H) (Shannon, 1948) to evaluate the degree of community diversity; Fager index to evaluate the affinity between different species of parasites (Fager, 1957); *k* value as an indicator of parasites distribution (Poulin, 2007).

The data were analyzed using SPSS® ver.16. Values of $p \leq 0.05$ were considered statistically significant.

3. Results

3.1. Gross examination of lungs

The left lung had a mean weight of 180.53 g (±13.97 SE) and a range 57–425 g, while the right lung had a mean weight of 215.7 g (±14.96 SE) and a range of 71–413 g.

Hemorrhagic lesions due to firearm wounds were the most frequently observed lesions. Areas of lobular emphysema and fibrotic lesions were found, in some cases, on the edge of the caudal lobes. Solid and greyish scattered nodules between 1 and 3 mm in diameter were observed in the heavily infected lungs. The carefully dissected nodules harbored one or more adult worms. Bronchial wall thickening and catarrhal inflammation were observed. Fibrinous pneumonia or atelectasis were identified, in some cases, in the apical part of the lobe (Fig. 2).

3.2. Helminth species, prevalence, mean abundance and mean intensity

Fifty-five out of 57 (96.5%) wild boars were positive for lung worms; 9077 helminths belonging to the genus *Metastrongylus*, were collected. Five species were morphologically identified (Fig. 3) and their prevalence was as follows: *M. asymmetricus* 91.2%, *M. salmi* 87.7%, *M. confusus* 87.7%, *M. apri* 80.7%, *M. pudendotectus* 70.2%. The prevalence, mean abundance, mean intensity of lung