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Short Communication

Uses and limitations of faecal egg count for assessing worm burden in wild boars

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ABSTRACT

The most widely used technique to assess helminth infection in both domestic and wild mammals is the faecal egg count (FEC). Most efforts to test the reliability of FEC as a proxy for parasite load are in small ruminant studies and limited work has evaluated the use of FEC in pigs. The aim of this study was to explore whether FEC is a reliable indicator of helminth load, and to evaluate the effects of sample storage on FEC accuracy in 59 wild boars. Though FEC was useful for assessing most helminth infections (e.g., *Metastrongylus* spp., *Ascaris suum*, *Trichuris suis*), stomach nematodes were often missed. The accuracy of FEC decreased over time, and thus it is recommended that samples be processed within 5 days of collection.

1. Introduction

Gastrointestinal nematodes can be an important cause of growth impairment, diarrhoea, dehydration and post-weaning death and they have recently been highlighted as a neglected challenge to both indoor and outdoor pig production systems (Roepstorff et al., 2011). Faecal egg count (FEC) is a simple, non-invasive reference technique used to quantify parasitic burden in a broad range of domestic and wild vertebrate species, although some studies indicate that its accuracy may be affected by technical (Cringoli et al., 2004) and seasonal variations and host and helminth biological factors (Villanua et al., 2006). FEC relies on the relationship between adult worm burden and the number of eggs per gram of faeces. The McMaster Method (McMM) is the most widely used FEC technique to assess endoparasite burden in small and large ruminants among others.

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Little is known about the limitations of FEC in pigs one of the most common livestock. This lack of information is probably due to the low infestation burden due to modern intensive production and the routine use of anthelmintics in extensive production. In fact, to date, only two studies have assessed the utility of FEC as a proxy for adult *Ascaris suum* (Pereckiene et al., 2007) or *Oesophagostomun* spp. (Christensen et al., 1995) burdens in domestic pigs.

In recent years, swine production in Europe has gone from tethered or single-stalled sows to loose housing, hence increasing the exposure of pigs to infective phases of parasites (Eijck and Borgsteede, 2005; Roepstorff et al., 2011). Along the same lines, free-range pig production is gaining importance on the African continent (Kagira et al., 2012), making the assessment of worm burden by FEC indispensable. There is also an increasing interest in assessing the health status of wild boars (*Sus scrofa*), the wild counterpart of domestic pigs, due to the natural or artificial expansion of populations. The helminthofauna of wild boars is typically greater than in domestic pigs, especially for those parasites with indirect life cycles (de-la-Muela et al., 2001). Consequently, it is important to understand the uses and limitations of FEC as a proxy for endoparasite burden in wild boars, especially when veterinarians are working under field conditions and samples cannot be analysed as quickly as required.

In the present study, and taking the advantage of the total parasitological assessment of lung and gastrointestinal nematode load in 59 harvested wild boars, we: (I) evaluated the sensitivity of FEC as a diagnostic method and explored whether FEC is a reliable indicator of nematode load; and (II) assessed the effects of sample storage on the accuracy of FEC.

2. Material and methods

2.1. Sampling procedure

Fifty-nine wild boar samples were obtained from three study areas in Spain: 30 from the National Game Reserve Ports de Tortosa i Beseit, (28,587.87 ha, 40°48.11″ N, 0°20.35″E) and 4 from the campus of the Universitat Autònoma de Barcelona (41°30′1.21″N, 2 6′ 27.86″E), both in Catalonia northeast Spain; and 25 from Oropesa de Toledo, Toledo (central Spain; 39°55′15.55″ N, 5°10′32.33″ E). Animals were either captured by box trap and chemically euthanized or hunter-harvested (no approval was needed from any Ethics committee since the animals used in the present study were not sacrificed for research purposes, but we took advantage of the harvested animals for this aim. The harvested wild boar have been legally hunted (shot) or box-trapped in their own habitat by authorised gamekeepers and hunters within the framework of an annual hunting plan by the Regional authority in charge of livestock and wildlife management).

Lungs and complete digestive tracts (stomach, small and large intestine) were collected in individual bags, until they reached the laboratory and then frozen at −20 °C until subsequent examination. Rectal faeces were also collected for later analysis. All samples were transported in cold boxes (4 °C) until laboratory analysis.

2.2. Coprological analysis

The first FEC of 20 wild boars was performed after a maximum of 48 h under refrigeration, day 0, and was subsequently repeated on days 5, 12 and 20 after collection. After the first coprology (day 0), faeces were kept in the laboratory at room temperature (ranging from 17 to 25 °C and 30 to 50% relative humidity). A concentration method (McMM, MAFF, 1986) with 33% zinc sulphate (1.18 sg) was used for egg quantification, displaying a lower detection limit of 50 eggs per gram (e.p.g.) of faeces. To minimise false negatives, a test tube flotation technique was also used (Roepstorff and Nansen, 1998). Eggs were identified by their morphological characteristics with a microscope (Thienpont et al., 1979).

2.3. Adult worm identification

Trachea, lungs and digestive tract (stomach, small and large intestines) were dissected and washed to obtain adult worms (Roepstorff and Nansen, 1998). These were collected in 5 mm–500 μm sieves. Helminths were transferred in 70% ethanol solution for conservation. The worms were later immersed in lactophenol, observed under stereo microscope (10×, 40× or 100× magnification) and identified using Gassó et al. (2014) for lung nematodes and Frontera et al. (2009) for gastrointestinal helminths.

2.4. Statistical analysis

For exploring the accuracy of FECs, the intensity of infestation of different nematode species estimated after necropsy was correlated to the FEC made on the day of sampling (day 0). Specific linear regressions were carried out for those species identifiable by egg morphology (e.g., *Metastrongylus* spp., *A. suum* and *Trichuris* sp.) and for those nematode species with unidentifiable eggs (called “strongyle eggs”): *Oesophagostomum–Hyostrongylus–Globocephalus*-type eggs and Spiruid-type eggs for *Physocephalus* sp. and *Ascarops* sp. (Straw et al., 2006).

Changes in FEC in the same sample over time were estimated using non-parametric Kruskal–Wallis ANOVA with FEC as the response variable and time (days 0, 5, 12 and 20) as a fixed factor. This analysis was performed for eggs of specific species e.g., *A. suum*, *Trichuris suis* and *Metastrongylus* spp., and for those nematode species with unidentifiable eggs (e.g., spiruid and strongyle eggs). All statistical analyses were performed using R software version 3.2.1 (R Development Core Team, 2015).

3. Results and discussion

Prevalence and intensity of helminth infestation based on FEC and adult worm species are shown in Table 1.

The necropsy (adult worms found) revealed that all individuals were infested with 1–8 (mean = 4.5) helminth species: 13 helminth species, 12 nematodes and