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Improving the detection of anthelmintic resistance: Evaluation of faecal egg count reduction test procedures suitable for farm routines

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ABSTRACT

The faecal egg count reduction test (FECRT) is the main method of detection of anthelmintic resistance (AR) in nematodes of veterinary importance. However, although the FECRT is standardised, the diagnostic performance of this method has not been fully characterised. In this survey Monte Carlo routines were used to simulate the estimation of faecal egg count reduction (FECR) with several FECRT protocols that were performed under different field and laboratory conditions. The goal was to determine, from a practical viewpoint, the most suitable protocols for farm routines and to evaluate the diagnostic performance of FECRTs across different parasitological scenarios with several levels of AR. The simulated field procedures included variations in the sample size and the sampling (or not) of a control group, whereas the simulated laboratory procedures comprised group mean individual-based vs. composite-based FECR estimations and variations in the egg detection threshold of the McMaster technique. For composite procedures, the random weight variations among individual samples and an increased number of McMaster chamber counts were also simulated. The results showed that FECRTs were moderately affected by inaccuracy but crucially affected by imprecision, and both parameters were clearly dependent on the parasitological conditions and the laboratory and field procedures used. An individual-based FECRT method performed without a control group was the most appropriate to quantify the AR, whereas a composite-based method with a control group was the easiest method for discriminating susceptible and resistant parasite populations. More interestingly, the diagnostic performance of the simulated FECRT methods was low for the procedures that are currently recommended by the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) and for many of the typical field and laboratory procedures that are currently implemented. Therefore, the results suggest that the diagnostic performance of the FECRT should be re-evaluated, and the recommendations of the W.A.A.V.P. should be redefined. Finally, theoretic critical conditions for FECRT procedures have been defined to improve future AR surveys and to allow the interpretation of FECRT results with the necessary caution according to the diagnostic performance expected for every FECRT procedure and parasitological scenario.

1. Introduction

The widespread use of anthelmintic drugs, particularly to control nematode infections in grazing livestock, is causing the development of anthelmintic resistance (AR). The increasing incidence of AR in the nematodes in livestock

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in many parts of the world is becoming a global problem (Sangster and Gill, 1999; Jabbar et al., 2006).

The detection and monitoring of AR are vital to slow the spread of this phenomenon and to preserve the efficacy of the available anthelmintics (Coles et al., 2006). The faecal egg count reduction test (FECRT), which was the first AR-detection test to be developed (Boersema, 1983; Presidente, 1985), is considered the best initial screening method for anthelmintic resistance in the field (Johansen, 1989), and it is, therefore, the most widely used method for detecting and monitoring the presence of AR in nematodes both in the field and in research studies.

According to this method, a comparison is made by counting the numbers of eggs before and after anthelmintic treatment. The World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) recommends a standardised FECRT protocol for the detection of AR in nematodes of veterinary importance (Coles et al., 1992). However, despite this attempt of standardisation the FECRT, the lack of validation and calibration for this method has prompted the development of a relatively large number of variations on this protocol (see revision in supplementary data).

Numerous studies have been performed to compare the diagnostic performance of FECRT protocols that were conducted under different field and laboratory conditions. These studies have shown that the presence of methodological variability makes the interpretation and comparison of results from different surveys (or even within a single survey) a difficult task, mainly because the field and laboratory conditions determine the accuracy and precision of the FECRT estimations (Maingi et al., 1996; Cabaret and Berrag, 2004; Torres-Acosta et al., 2005; Cringoli et al., 2007; Levecke et al., 2012b).

Because the empirical validation and calibration of FECRT protocols are challenging due to the laboratory work and the extremely difficult fieldwork required (McKenna, 1990), a systematic exploration of the effects of protocol variation in a wide range of scenarios is only possible using mathematical modelling approaches (Torgerson et al., 2005; Dobson et al., 2009, 2011; Levecke et al., 2012a). The above-cited works have remarked upon the complexity of the factors that set the conditions for the diagnostic performance of the FECRT and the relationships between these factors. However, because all of these studies were partial approaches, the main conclusion of them was that more comprehensive studies are required to characterise the complex interplay between the factors inherent to both study design (sample sizes and FEC methods) and host–parasite interactions (the levels of egg excretion and aggregation across the host population).

As a response, the primary goal of this work was to explore the diagnostic performance of several FECRT protocols using Monte Carlo simulations on a broad spectrum of field and laboratory procedures to define the theoretic conditions that are critical to the improvement of future AR surveys and to the interpretation of FECRTs. Model outcomes were explored to evaluate diagnostic performances of FECRT to quantify AR across a broad spectrum of scenarios but, also, to discriminate resistant parasite populations from susceptible ones. A secondary goal was to present useful information to researchers and technicians to select the best FECRT protocol for implementation in farm routines or AR monitoring programs as a function of available logistical resources or required diagnostic performance. To accomplish these goals, both field and laboratory procedures were simulated on a gradient from the least laborious to the most laborious to evaluate the diagnostic performance of several FECRT protocols comprising combinations of field and laboratory procedures with different cost-labour levels.

2. Materials and methods

2.1. Simulation of parasitological conditions

Following previous studies, it was assumed that the individual FECs within a herd are aggregated and can be adequately modelled using a negative binomial distribution (NBD) with two parameters: the arithmetic mean FEC (m), measured as eggs per gram (epg), and the inverse degree of aggregation (k) (Morgan et al., 2005; Dobson et al., 2009). The model simulated seven levels of mean FEC (m = 50, 100, 200, 500, 1000, 5000 and 10,000 epg) to model both natural and experimental infections, which can produce extremely high FEC values. Similarly, although the observed k values in commercial herds usually range from near 0 to 2.5 (Grenfell et al., 1995; Dobson et al., 2009), the model also simulated a wider range of k values (k = 0.1, 0.2, 1, 2, 2.5, and 5).

Although the k parameter values usually track the m values in naturally infected herds (Grenfell et al., 1995), all of the combinations of the seven levels of m and the six degrees of k were modelled, which yielded 42 different NBDs. This approach was used to separately estimate the effects of both parameters on the model outcomes. When it was necessary to derive practical results, however, the simulated parasitological conditions were grouped into the following three parasitological scenarios: low egg excretion ($50 \leq m \leq 200$; $k = 0.2$); moderate egg excretion ($200 \leq m \leq 1000$; $k = 1$); and high egg excretion ($1000 \leq m \leq 10{,}000$; $k = 2$). These three scenarios were derived from extrapolations of the results of Grenfell et al. (1995) with respect to k and m covariation in natural populations.

In addition, nine levels of the true anthelmintic efficacy or true FECR (E_t) produced by anthelmintic treatment were simulated to reproduce nine levels of AR: two for high-AR scenarios (E_t = 30 and 45%) and seven for medium-to-low-AR scenarios (E_t = 60, 80, 85, 90, 95, 99 and 99.9%). These levels were used to achieve a finer characterisation of the diagnostic performance of the FECRT under moderate and, especially, low AR levels.

2.2. Simulation of field sampling procedures

The spectrum of the simulated field sampling procedures was mainly chosen to minimise the disturbance to the farm owners. For this reason, animals were randomly included in the sample with no previous information about their parasitological status. To simulate the sampling procedures and to follow the procedure of Dobson et al. (2009),