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Research paper

Comparison between McMaster and Mini-FLOTAC methods for the enumeration of *Eimeria maxima* oocysts in poultry excreta

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ABSTRACT

Monitoring *Eimeria* shedding has become more important due to the recent restrictions to the use of antibiotics within the poultry industry. Therefore, there is a need for the implementation of more precise and accurate quantitative diagnostic techniques. The objective of this study was to compare the precision and accuracy between the Mini-FLOTAC and the McMaster techniques for quantitative diagnosis of *Eimeria maxima* oocyst in poultry. Twelve pools of excreta samples of broiler chickens experimentally infected with *E. maxima* were analyzed for the comparison between Mini-FLOTAC and McMaster technique using, the detection limits (dl) of 23 and 25, respectively. Additionally, six excreta samples were used to compare the precision of different dl (5, 10, 23, and 46) using the Mini-FLOTAC technique. For precision comparisons, five technical replicates of each sample (five replicate slides on one excreta slurry) were read for calculating the mean oocyst per gram of excreta (OPG) count, standard deviation (SD), coefficient of variation (CV), and precision of both aforementioned comparisons. To compare accuracy between the methods (McMaster, and Mini-FLOTAC dl 5 and 23), excreta from uninfected chickens was spiked with 100, 500, 1,000, 5,000, or 10,000 OPG; additional samples remained unspiked (negative control). For each spiking level, three samples were read in triplicate, totaling nine reads per spiking level per technique. Data were transformed using log10 to obtain normality and homogeneity of variances. A significant correlation ($R = 0.74$; $p = 0.006$) was observed between the mean OPG of the McMaster dl 25 and the Mini-FLOTAC dl 23. Mean OPG, CV, SD, and precision were not statistically different between the McMaster dl 25 and Mini-FLOTAC dl 23. Despite the absence of statistical difference ($p > 0.05$), Mini-FLOTAC dl 5 showed a numerically lower SD and CV than Mini-FLOTAC dl 23. The Pearson correlation coefficient revealed significant and positive correlation among the four dl ($p \leq 0.05$). In the accuracy study, it was observed that the Mini-FLOTAC dl 5 and 23 were more accurate than the McMaster for 100 OPG, and the Mini-FLOTAC dl 23 had the highest accuracy for 500 OPG. The McMaster and Mini-FLOTAC dl 23 techniques were more accurate than the Mini-FLOTAC dl 5 for 5,000 OPG, and both dl of the Mini-FLOTAC were less accurate for 10,000 OPG counts than the McMaster technique. However, the overall accuracy of the Mini-FLOTAC dl 23 was higher than the McMaster and Mini-FLOTAC dl 5 techniques.

1. Introduction

Coccidiosis is an intestinal disease caused by protozoans of the genus *Eimeria*, and is one of the most important livestock diseases worldwide (Blake and Tomley, 2014), with a global impact predicted to be over $3 billion USD per year in commercial poultry production (Williams, 1999; Dalloul and Lillehoj, 2006). In chickens, coccidiosis is caused by up to seven *Eimeria* species: *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella*. Birds become infected by ingesting sporulated oocysts, which release sporocysts that invade intestinal epithelial cells (Allen and Fetterer, 2002), causing subsequent impairment of nutrient absorption. In addition, coccidiosis is a predisposing factor for the development of other economically relevant diseases, such as necrotic enteritis (Dahiya et al., 2006; Collier et al., 2008).

Monitoring *Eimeria* shedding has become increasingly important due to the recent restrictions on antibiotic use within the poultry industry. Therefore, there is a need for the implementation of more precise and accurate quantitative diagnostic techniques. Quantitative detection of *Eimeria* oocyst shedding in commercial poultry production systems is often used for monitoring control programs, such as vaccination in the field, in which the McMaster technique has been the

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method of choice (Hodgson, 1970). Additionally, studies looking for drugs and novel feed additives targeting the reduction of oocyst shedding in poultry have primarily used the McMaster technique for this purpose (Allen et al., 1997; Almeida et al., 2012; Kim et al., 2013; Markazi et al., 2017). Recently, the Mini-FLOTAC was developed as a new method for quantitative diagnosis of intestinal parasites in various mammalian hosts (Cringoli et al., 2017). This technique has been seen as an alternative to the McMaster method, especially in cases when higher accuracy and precision are necessary (Noel et al., 2017; Scare et al., 2017).

To our knowledge, no work has been done to assess the performance of the Mini-FLOTAC for quantitative diagnosis of *Eimeria* spp. oocysts in poultry. However, there is a single study comparing the Mini-FLOTAC to the McMaster technique for the quantitative diagnostic of *Eimeria* spp. oocysts in goats (Silva et al., 2013). In this study, the mean oocyst per gram of excreta (OPG) detected with the Mini-FLOTAC detection limit (dl) 20 technique was higher than the McMaster dl 15 technique with similar precision, as measured by the coefficient of variation (CV); however, it showed lower CV than the McMaster dl 50 technique (Silva et al., 2013). In other livestock species, numerous studies have compared the Mini-FLOTAC to other routinely-used fecal egg counting techniques including the McMaster, Cornell-Wisconsin, and/or Fecpak, and have found that the Mini-FLOTAC recovers a more accurate number of parasite ova, and has greater repeatability in comparison to other methods (Levecke et al., 2012; Barda et al., 2013; Godber et al., 2015; Noel et al., 2017; Scare et al., 2017).

Regardless of the methodology used, quantitative techniques must have high sensitivity, specificity, accuracy, precision, reproducibility, and the capacity to diagnose and monitor parasitic infections (Cringoli et al., 2010). In poultry, the McMaster technique is the gold standard for the enumeration of *Eimeria* oocysts (Hodgson, 1970); however, we hypothesized that the Mini-FLOTAC would have higher accuracy and better precision than the McMaster technique. Thus, the objectives of this study were to compare the precision, accuracy, sensitivity and specificity of the Mini-FLOTAC and McMaster techniques, including different dl of the Mini-FLOTAC technique for the enumeration of *E. maxima* oocysts in poultry excreta.

2. Material and methods

2.1. Birds

The animal care and use procedures followed the Guide for the Care and Use of Agricultural Animals in Research and Teaching of the Federation of Animal Science and Society (Federation of Animal Science and Societies, 2010). One-day-old Cobb broiler chickens (696) were raised on floor pens (58 birds in each pen, with 12 pens, and 0.09m^2/bird), and fed a diet devoid of anticoccidial drugs until 14 days of age. On day 14, the birds were experimentally infected by oral gavage with *E. maxima* (~5000 sporulated oocysts/bird). The suspension of *E. maxima* oocysts was provided by Dr. Lorraine Fuller from the Department of Poultry Science of the University of Georgia. The estimation of the number of inoculated oocysts was done by counting the number of oocysts in three aliquots of 10 µL of solution, and extrapolating it to 1mL. On day 28, a pool of 50 g of excreta was randomly collected from each pen (total of 12 pens) into Ziploc bags, thoroughly homogenized by hand, and stored at 4 °C until analysis. Our samples were acquired in an opportunistic manner as we had access to excreta samples of birds experimentally infected with *E. maxima* for a necrotic enteritis study (Bortoluzzi et al., 2017).

2.2. Comparison of precision of the Mini-FLOTAC and McMaster

To compare the precision of the Mini-FLOTAC dl 23 and McMaster dl 25 techniques, five replicates, from one prepared excreta slurry, of 12 pooled excreta samples were read, in a total of 60 readings per method. The Mini-FLOTAC procedure (Cringoli et al., 2017) was performed using the manufacturer's protocol. The Mini-FLOTAC apparatus is comprised of two devices, the Fill-FLOTAC and reading chamber. The Fill-FLOTAC is a clear, plastic container with a capacity of 70 mL, with an enclosed homogenization and filtration system, and the reading chamber is comprised of the bottom chamber portion, top reading disk, and key for turning the apparatus.

One gram of excreta was weighed into the Fill-FLOTAC, and 45 mL of sodium nitrate solution with a 1.25 specific gravity (sg) was used (Zajac and Conboy, 2012). This sg was chosen because it showed a higher recovery rate of *Eimeria* oocysts in feces of domestic ruminants (Cringoli et al., 2010). The mixture was homogenized using the Fill-FLOTAC apparatus before filling the chambers. The Mini-FLOTAC slide was allowed to rest on the lab bench for ten minutes before rotating the top piece of the reading disc. All readings were performed under light microscopy (100×).

For the McMaster dl 25 technique (60 readings), two grams of excreta and 13 mL of sodium nitrate solution (1.25 sg) were used (Zajac and Conboy, 2012). The mixture was well homogenized with a tongue depressor and strained through one layer of cheesecloth. The solution was quickly pipetted into the McMaster slide, which was read under light microscopy (100×). Only oocysts observed within the grids of each side of the chamber were counted.

2.3. Comparison of precision of the different Mini-FLOTAC detection limits

Six additional pooled excreta samples were collected to compare the different dl (5, 10, 23, and 46) of the Mini-FLOTAC technique, and each sample was read 5 times (30 reads per each dl). One or five grams of excreta were weighed into the Fill-FLOTAC apparatus, 45 mL of sodium nitrate was added to obtain the dl 23 or 5, respectively. The same procedure described above was used. Both sides of the Mini-FLOTAC slide were read to obtain the dl 5 and 23, and only one side of the dl 5 and 23 chambers were read to obtain the dl 10 and 46, respectively. This experiment was conducted to compare the performance of the Mini-FLOTAC technique when reading only one side of the slide, because it has been reported that Mini-FLOTAC takes twice the time of the McMaster technique (Noel et al., 2017).

2.4. Comparison of accuracy

To assess accuracy of the Mini-FLOTAC dl 5, dl 23, and the McMaster dl 25, excreta of laying hens, fed a diet devoid of anticoccidial drugs and raised in cages, were confirmedly negative for *Eimeria* spp.. Samples were confirmed negative by performing the three aforementioned techniques, on three samples that were read in triplicate (totaling 27 reads). The excreta samples were then spiked with different OPG concentrations (100, 500, 1,000, 5,000, and 10,000) using sporulated oocysts of *E. maxima* from a pure culture from same source stated above. Three samples per OPG count, were read in triplicate, totaling 135 reads (3 techniques, 3 samples, 3 replicates, and 5 spiked OPG counts).

The number of oocysts in the inoculum was determined by counting the total number of oocysts/µL. The volume of inoculum to be added to the excreta slurry was calculated, and added using a micropipette. The micropipette tips were washed 10 times with the flotation solution to ensure that all oocysts were added to the slurry. Accuracy was calculated according to Noel et al. (2017), using the formula: Accuracy = (OPG observed / OPG expected) *100%. The test validity of each technique was calculated according to Thrushfield (2005). For sensitivity, we used the formula: true positive/(true positive + false negative), and for specificity, the formula: true negative/(true negative + false positive).