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Application of a Poisson distribution quality control measure to the analysis of two human hookworm drug treatment studies in Ghana

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ABSTRACT

We examined faecal egg count reduction tests (FECRTs) conducted with hookworm-infected humans in Ghana in 2007 (study 1) and 2010 (study 2) in order to explore aspects of the test analysis. Some subjects showed increased FEC following drug treatment. This occurred mostly in <150 epg pre-treatment FEC subjects. We sought a means to remove 'erroneous' negative drug efficacy cases from the FECRT analysis. Pre- and post-treatment FECs from negative drug efficacy cases were examined to determine whether they represented replicates from a single randomly distributed sample, that is, if they were consistent with a Poisson distribution. Cases where the post-treatment FEC was greater than that expected if it and the pre-treatment sample had been taken from a single random distribution of eggs were excluded from the FECRT. We suggest that these cases most likely represent non-random distribution of eggs in stools, day-to-day variations in egg excretion, or worm patency onset after drug treatment, and hence are not accurate measurements of drug efficacy. This led to exclusion of the most extreme negative drug efficacy cases, with significant increases in overall drug efficacy for study 1 (81.6% vs 89.2%) and study 2 (86.7% vs 89.4%). Excluding FEC <150 individuals from the analysis also increased the study 1 efficacy (81.6% vs 88.9%), however, this resulted in the exclusion of 45% of the study subjects, compared to the exclusion of just 5% using the Poisson distribution method. While low FEC subjects are excluded from livestock FECRTs, the significant prevalence of such subjects in human FECRTs suggests that their exclusion may not be practical. Hence, we suggest that the influence of low FECs can be minimised by excluding 'erroneous' negative efficacy cases using a simple Poisson distribution analysis.

1. Introduction

With the implementation of mass drug administration programmes for the control of human soil transmitted helminths (STHs) there is increasing interest in ensuring that drug resistance can be detected should it emerge. While some *in vitro* bioassay and molecular-based methods for testing drug sensitivity have been examined with reference to STHs and benzimidazole drugs (for example, Albonico et al., 2004, 2005; Diawara et al., 2009; Kotze et al., 2009; Humphries et al., 2013) they are yet to be validated as useful tools for resistance detection in field settings. Hence, the only current means to monitor drug sensitivity in the field is the faecal egg count reduction test (FECRT). This test involves a comparison of faecal egg counts (FECs) in human subjects pre- and post-drug treatment. While such tests have been used for many years in the livestock health sphere to examine changes in drug sensitivity in gastrointestinal parasites, they have only more recently been applied to human STHs. Consequently, a number of recent reports have examined aspects of their design, and made recommendations as to how the tests should be used for human STHs (Levecke et al., 2011a,b; Vercruysse et al., 2011a,b), and the WHO has recently issued revised guidelines for assessing drug efficacy against schistosomiasis and STHs (WHO, 2013).

One feature of livestock FECRTs is the recommendation to exclude animals with low FEC for statistical reasons (Presidente, 1985; Coles et al., 2006), as low FECs are not accurately measured with existing egg counting techniques which lack sensitivity. The

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recommended minimum FECs are 150 for sheep, goats and horses, and 100 for cattle (FECs are usually lower in cattle than in the other species). Satisfying this constraint for inclusion does not generally present a great difficulty in terms of recruitment of suitable animals for livestock studies. However, subject recruitment and compliance issues are more difficult in the human field than for livestock drug efficacy studies. The difficulty in recruiting suitable individuals for human FECRTs is further exacerbated by the high prevalence of subjects with low infection levels at some field sites described in recent reports; for example, over the seven field sites described by Vercruysse et al. (2011a), human subjects with light hookworm infections (that is, epg < 1999, from Montresor et al., 1998) made up 94% of the total number of infections. Further, the proportion of the total study populations at the various sites that had FEC < 150 varied from 6% in Brazil, up to 49% in Cameroon, and 67% in Vietnam. Hence, it may be far more difficult to adhere to a FEC > 150 inclusion limit for human FECRTs compared to the relative ease of following this rule in livestock FECRTs.

Vercruysse et al. (2011a) reported on the influence of inclusion of low pre-treatment subjects on human FECRT data. They found that drug efficacy calculated as the mean of the FECRs in individual study subjects was highly affected by excluding subjects with pre-treatment FECs of <150. However, the inclusion of FEC <150 subjects did not affect the FECRT outcomes at each of their seven field sites when the FECR was calculated on the basis of group means. This indicated that, for their data sets, the responses of the low FEC subjects did not distort the overall study outcomes as long as the analysis was performed at the group level. However, in demonstrating the significant effect of low FEC subjects on FECRT outcomes when calculated using individual FECRs, this study indicated the potential for the influence of low FEC subjects to be significant in terms of the group mean-based FECR if they reached dominant proportions in a study population.

As a part of ongoing efforts to refine human STH FECRTs in order to ensure that they are reproducible, and hence will be able to detect changes in drug sensitivity over time, we aimed to assess the influence of low pre-treatment FECs on the analysis of data from two recently conducted human hookworm FECRTs. We examined data sets collected during trials of albendazole against human hookworms conducted in Ghana in 2007 and 2010 (Humphries et al., 2011, 2013). These studies were of interest as they had been carried out in populations dominated by subjects with low infection intensities (from Montresor et al., 1998). We compared three different analytical approaches for estimating drug efficacy from FECRTs: (a) group FEC averages, (b) group averages excluding subjects with pre-treatment FEC less than 150 epg, and (c) group averages after the use of a Poisson distribution method to identify and exclude subjects with 'erroneous' negative drug efficacy values. We also utilized duplicate egg counts from the 2010 field study in order to examine the variability in FEC at low infection intensities.

2. Materials and methods

2.1. FECRT data

We examined data from two recent FECRTs conducted in Kintampo North Municipality, Ghana. The studies were denoted 'study 1' (conducted in 2007, Humphries et al., 2011) and study 2 (conducted in 2010, Humphries et al., 2013). Both studies were approved by the Yale Human Investigations Committee and the Institutional Review Board of the Noguchi Memorial Institute for Medical Research (NMIMR) at the University of Ghana. The two studies are summarised below:

(i) Study 1: Egg counts were performed on 258 subjects using the Kato–Katz method (WHO, 1996) on single faecal samples from each subject (egg count sensitivity of 37). Positive counts were recorded for 116 subjects, and 102 of these were treated with albendazole. Post-treatment feacal samples were received from 95 subjects during the period 14–21 days after the drug treatment.

(ii) Study 2: Egg counts were performed on duplicate faecal samples received from 258 subjects using the Kato-Katz method (WHO, 1996) (egg count sensitivity of 24, with two replicate samples per subject, i.e. for the total eggs counted per subject 1 egg = 12 epg). At least one positive count was recorded for 121 subjects, and all of these were treated with albendazole. Duplicate follow up samples were requested from all subjects, and received from 94 subjects during the period 14–21 days after the drug treatment. At least one follow up sample was received from 112 subjects. A total of 84 subjects provided two pre- and two post-treatment samples and were therefore used in the subsequent FECRT analysis.

2.2. Infection intensity

We examined the infection intensities across subjects in Studies 1 and 2 by subdividing the pre-treatment FEC data into infection intensity classes according to the criteria of Montresor et al. (1998) for human hookworms: light, 1–1999 epg, moderate 2000–3999, and high >3999. In addition, we applied a further subdivision of the light infection category into <150 and 150–1999 as the <150 group was of particular interest given the WAAVP guidelines for exclusion of animals with FEC <150 from livestock FECRTs (described by Coles et al., 2006).

2.3. Analysis of drug efficacy

FECRT outcomes for both studies were determined using the R package "eggCounts" (Paul, 2013) via the user friendly web interface at http://www.math.uzh.ch/as/?calc. The approach is based on Bayesian methods, and assumes firstly that the observed egg count is subject to Poisson errors (magnified by the dilution factor), and secondly that the between animal counts are over dispersed (negative binomial or zero inflated). The user enters the pre-treatment and post-treatment FECs, and the dilution factor used to calculate the FECs. Bayesian inference is done via Markov chain Monte Carlo sampling. The output includes the FECR with 95% Confidence Intervals (CIs).

We examined the FECRT data using, (a) the full data sets from studies 1 and 2, as well as, (b) in separate groups showing pre-treatment FECs of < or >150 epg, as we were interested specifically in the effect of excluding FEC <150 epg individuals on the FECRT outcomes. Within study 1 or study 2, drug efficacies derived from these different groups were considered to be significantly different if their 95% CIs did not overlap.

2.4. Poisson distribution quality control test for negative drug efficacy data

We utilised a Poisson distribution-based method (modified from Torgerson et al., 2012) to examine cases in which drug efficacy was negative (FEC increased after drug treatment) as a means of applying a quality control measure to the data set. The FEC results for individual subjects at the pre- and post-treatment time points ($n = 2$ for study 1, and $n = 4$ for study 2) were analysed to determine if the group of two or four values for each subject followed a Poisson distribution. The index of dispersion (ID = variance/mean) was calculated for each subject, and then compared