Effect of repeated mass drug administration with praziquantel and track and treat of taeniosis cases on the prevalence of taeniosis in Taenia solium endemic rural communities of Tanzania

Braae, Uffe Christian; Magnussen, Pascal; Ndawi, Benedict; Wendy, Harrison; Lekule, F.P.; Johansen, Maria Vang

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Title: Effect of repeated mass drug administration with praziquantel and track and treat of taeniosis cases on the prevalence of taeniosis in *Taenia solium* endemic rural communities of Tanzania

Uffe Christian Braae 1,*, Pascal Magnussen 1,2, Benedict Ndawi 3, Wendy Harrison 4, Faustin Lekule 5, Maria Vang Johansen 1

Affiliations: 1 Section for Parasitology and Aquatic Diseases, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, DK-1870 Frederiksberg, Denmark, 2 Centre for Medical Parasitology, Faculty of Health of Medical Sciences University of Copenhagen, DK-1353 Copenhagen, Denmark, 3 Bora Professional Consultancy Services, Iringa, Tanzania, 4 Faculty of Medicine, School of Public Health, Imperial College London, United Kingdom, 5 Faculty of Agriculture, Sokoine University of Agriculture, Morogoro, Tanzania

* Corresponding author: Uffe Christian Braae, Section for Parasitology and Aquatic Diseases, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, DK-1870 Frederiksberg, Denmark. E-mail address: braae@sund.ku.dk

Abstract

This study evaluated the effect of mass drug administration (MDA) with praziquantel administered to school-aged children (SAC) combined with ‘track and treat’ of taeniosis cases in the general population on the copro-antigen (Ag) prevalence of taeniosis. The study was conducted in 14 villages in Mbozi and Mbeya district, Tanzania. SAC made up 34% of the population and received MDA with praziquantel (40 mg/kg) in 2012 (both districts) and in 2013 (Mbozi only). Three cross-
sectional population-based surveys were performed in 2012 (R0), 2013 (R1), and 2014 (R2). In each survey approximately 3,000 study subjects of all ages were tested for taeniosis using copro-Ag-ELISA. In total 9,064 people were tested and copro-Ag-ELISA positive cases were offered treatment 6-8 months after sampling. The copro-Ag prevalence of taeniosis was significantly higher (X²-test, p=0.007) in Mbozi (3.0%) at R0 compared to Mbeya (1.5%). Twelve months after MDA in both districts (R1), the copro-Ag prevalence had dropped significantly in both Mbozi (2.0%, p=0.024) and in Mbeya (0.3%, p=0.004), but the significant difference between the districts persisted (X²-test, p<0.001). Ten months after the second round of MDA in Mbozi and 22 month after the first MDA (R2), the copro-Ag prevalence had dropped significantly again in Mbozi (0.8%, p<0.001), but had slightly increased in Mbeya (0.5%, p=0.051), with no difference between the two districts (X²-test, p=0.51). The taeniosis cases tracked and treated between round R0 and R2 represented 9% of the projected total number of taeniosis cases within the study area, based on the copro-Ag prevalence and village population data. Among SAC in Mbozi, infection significantly decreased at R1 (p=0.004, OR 0.12 CI: 0.02-0.41) and R2 (p=0.001, OR 0.24, CI: 0.09-0.53) when comparing to R0. In Mbeya infection significant decreased at R1 (p=0.013, OR 0.14, CI: 0.02-0.55), but no difference was found for R2 (p=0.089), when comparing to R0 among SAC. This study showed that school-based MDA with praziquantel in combination with ‘track and treat’ of taeniosis cases significantly reduced the copro-Ag prevalence of taeniosis, and that annual MDA was significantly better than single MDA. The persistence of taeniosis cases illustrates that a One Health approach must be emphasized for effective control.
Keywords:
Risk factors; Track and treat; *Taenia solium*; Taeniosis; Mass Drug Administration (MDA); Copro-Ag prevalence; Preventive chemotherapy treatment

1. Introduction

*Taenia solium* taeniosis/cysticercosis is a growing and persistent problem in most sub-Saharan countries where pigs have been domesticated (Braae et al., 2015). Since humans are the sole definitive host of this zoonotic tapeworm, treatment of individuals with taeniosis is essential in the control of *T. solium* infection. Efficacious control tools for *T. solium* are available, but an algorithm for optimal tool combination for effective control is now needed. No studies assessing the effect of mass drug administration (MDA) with praziquantel on *T. solium* have been performed on the African continent to date. However, MDA campaigns have previously been used to treat humans in Latin America in efforts to control *T. solium*. Most studies have used porcine cysticercosis prevalence as an indicator of effect (Cruz et al., 1989; Garcia et al., 2006; Keilbach et al., 1989), and others have used prevalence of taeniosis as the measure of treatment effect. Diaz-Camacho et al. (1991) reported from Mexico that a reduction from 1% to 0% in taeniosis prevalence based on stool microscopy was seen one year after MDA with praziquantel (10 mg/kg). However, this was based only on four positive individuals at baseline, a detection methodology with low sensitivity, and no control group or statistical analysis. Another study from Mexico using copro-antigen (Ag)-ELISA and stool microscopy showed a borderline significant (p=0.06) drop from 1.1% to 0.5% six months after a single round of community wide MDA with praziquantel (5 mg/kg) (Sarti et al., 2000). In this study the prevalence remained at 0.5% 42 months post intervention. However, no comparison with a control group was made. Allan et al. (1997) reported from Guatemala that the
prevalence of taeniosis 10 months after MDA with niclosamide (coverage 75%) was, significantly
(p<0.001) reduced from 3.5% to 1%, based on copro-Ag-ELISA and stool microscopy. However,
no control group was included in this study. So far no studies have reported the effect of repeated
rounds of MDA.

Although theoretically controllable (Kyvsgaard et al., 2007) and declared eradicable by the
International Task Force for Disease Eradication in 1993, *T. solium* taeniosis/cysticercosis remains
a neglected zoonosis. This is not only due to a lack of available resources but also a lack of
information about its burden, transmission, and validation of simple intervention packages (WHO,
2010). The World Health Organisation (WHO) included *T. solium* cysticercosis as one of major
Neglected Tropical Diseases in 2010 and recommended MDA as the primary intervention strategy
against taeniosis (WHO, 2010), but to date no large scale taeniosis control programme has been
implemented in sub-Saharan Africa.

The drug praziquantel, which is effective against taeniosis at a dose as low as 5 mg/kg (Pawlowski,
1991), is also used in MDA campaigns against schistosomiasis at a dose of 40 mg/kg. Praziquantel
is being used extensively in a number of sub-Saharan African countries where schistosomiasis is
prevalent. In countries where schistosomiasis and taeniosis are co-endemic there is the possibility to
assess the impact on taeniosis of schistosomiasis control programmes using praziquantel. In
Tanzania, school-based MDA with praziquantel is carried out as part of the National
Schistosomiasis Control Programme (NSCP) in schistosomiasis endemic districts. This study aimed
to assess the effect of repeated rounds of school-based MDA with praziquantel (40 mg/kg) in
combination with treatment of all taeniosis cases, in two areas co-endemic for *T. solium* taeniosis/cysticercosis and schistosomiasis.

2. Methods

2.1 Study area

The study was carried out in Mbeya and Mbozi districts, Tanzania. The human population in 2012 was estimated to be 305,319 in Mbeya district and 446,339 in Mbozi district (URT, 2013a). School-aged children (SAC) and adults were defined as 4-15 years of age and 16 or above, respectively. In Mbozi and Mbeya districts SAC made up 35% and 33% of the total population, respectively (URT, 2013b). Both districts are rural areas with high numbers of pigs kept primarily on a smallholder level, with 31,190 pigs in Mbeya district and 117,483 pigs in Mbozi district in 2007/2008 (URT, 2012). *Taenia solium* taeniosis/cysticercosis is highly prevalent within the area (Braae et al., 2014; Komba et al., 2013; Mwanjali et al., 2013). No reports of bovine cysticercosis exist from the region and therefore the prevalence of *T. saginata* was expected to be negligible.

2.2 Study design and sample size

The NSCP carried out MDA with praziquantel to SAC in both districts between July and September 2012 and in Mbozi district only in September 2013. Children not enrolled in school were encouraged to go and get treatment at the schools during the MDA. Following the MDA three community-based cross-sectional surveys were conducted in 14 villages, eight in Mbeya district (total population 20,104) and six in Mbozi district (total population 18,025) (URT, 2013c). These 14 villages were purposively selected based on knowledge of porcine cysticercosis presence (Komba et al., 2013), and made up four communities in each district. The first survey (R0) was
carried out in February to March 2012, second survey (R1) in July to August 2013, and the last survey (R2) in July to August 2014 (Figure 1). A sample size of approximately 1500 individuals from each district was targeted with all inhabitants willing to participate from each community included, with approximately 375 people from each community. Meetings informing about the study was carried out in each community prior to each survey. Hereafter, the villages were visited and all individuals invited to participate in the study. Different collection points were set up within each village where participants were provided with a plastic container and requested to submit a stool sample. In each district an active “track and treat” strategy was implemented to subsequently treat taeniosis cases with a single dose of 2g niclosamide for adults and 50 mg/kg for children in October 2012 after the first survey and praziquantel (10mg/kg) in March 2014 after the second survey. Since the “track and treat” followed the MDA children found positive for taeniosis could have been treated twice.

2.3 Data collection and copro-Ag-ELISA

All study subjects were interviewed and asked to complete a short questionnaire in order to collect information on demographics, latrine availability, recent (12 months) anthelmintic treatments as part of an MDA, and presence of pigs in the household. Each participant provided one stool sample of which one gram of faeces was stored in 10% formalin at room temperature for analysis. Stool samples were analysed for T. solium/saginata antigens at the University of Lusaka in Zambia using a copro-Ag-ELISA assay (Allan et al., 1990) with slight modifications (Mwape et al., 2012). The copro-Ag-ELISA assay has been reported to have an approximate sensitivity and specificity of 85% and 92%, respectively (Praet et al., 2013).
2.4 Ethical considerations

Ethical approval was obtained from the National Institute for Medical Research (NIMR) reference number NIMR/HQ/R.8a/Vol. IX/1216. The study was also approved by the Imperial College Research Ethics Committee (ICREC), reference no. ICREC_11_3_6. Permission to conduct the study was sought through regional, district, and village authorities. Prior to each survey all study villages within the study area were visited and the community informed about the survey. Members of the community were given the opportunity to ask questions and seek more information about the study. Written informed consent was obtained from all participants after they were informed about the aim, risks, and benefits of the study. Individuals were informed about possible mild adverse effects of treatment such as stomach pain and nausea. If the person was under 18, consent was sought from a parent or guardian following assent from the participant. After each survey, and upon receiving the laboratory results, villages in the study area were visited, and village leaders, school headmasters and head teachers were informed about the results of the survey and copro-Ag-ELISA positive individuals were offered anthelminthic treatment within 6-8 months of sample collection. All people treated were provided with contact numbers of the district health officer and the medical doctor assigned to the treatment, and told to report any adverse events.

2.5 Statistical methods

Data were entered into EPI info 7 (http://wwwn.cdc.gov/epiinfo/7/) and transferred to an Excel spreadsheet (Microsoft Office Excel 2010®) from where it was imported into the statistical programme R (http://www.r-project.org). Logistical regression and univariate analyses based on district, were used for the statistical analyses with p-values considered significant if smaller than 0.05. Odds ratios (OR) and 95% confidence intervals (CI) are provide for significant factors.
3. Results

3.1 Study population

In total, 9,064 stool samples were collected, 3,029 at R0, 3,021 at R1, and 3,014 at R2 (Table 1). The overall copro-Ag prevalence of taeniosis in the population of both districts was 2.2% at R0, 1.2% at R1, and 0.7% at R2 (Figure 2). Among the study subjects found positive for taeniosis, 62 out of 67 and 29 out of 35 were tracked and treated when revisited after R0 and R1, respectively. Of the treated study subjects overall, 27 were from Mbeya district and 64 from Mbozi district. Based on the theoretical number of tapeworm carriers within the study area as the denominator (number of people per village multiplied with taeniosis copro-Ag prevalence) this corresponds to both 9% of all tapeworm carriers treated within each district and overall for both districts.

Logistic regression including data from the whole study period revealed a drop in infection at R1 (P<0.001, OR 0.49, CI: 0.32-0.74) twelve months after the MDA in both districts and at R2 ten months after the second round of MDA in Mbozi and 22 months after the first MDA (P<0.001, OR 0.38, CI: 0.22-0.62) when compared to R0 and controlling for age groups (SAC/Adults) and sex. Logistic regression analyses revealed that the risk of being infected with *T. solium* was significantly greater for males than females (p=0.004, OR 1.73, CI: 1.20-2.52). There was also an increased risk for adults compared to SAC throughout the study period (p<0.001, OR 2.39, CI: 1.64-3.50). Living in Mbozi district was associated with a higher risk of taeniosis infection compared to living in Mbeya district (p<0.001, OR 2.35, CI: 1.59-3.53).
From each of the three questionnaire surveys it was found that 54, 46, and 38 people, respectively, did not have access to a latrine and 606, 460, and 502, respectively, said they were keeping pigs. Neither lack of latrines or presence of pigs at the household could be associated with taeniosis in this study. During the study period there was a large drop in the number of people stating to have seen “worms” in their stool during the last 12 months, from 612 (20%) at R0 and 590 (20%) at R1, to 179 (6%) at R2. However, this could not be associated with infection.

3.2 District comparison

Between the two districts univariate analysis show that at baseline (R0), the copro-Ag prevalence of taeniosis was significantly higher ($X^2$-test, $p=0.007$) in Mbozi district (3.0%) compared to Mbeya district (1.5%). The copro-Ag prevalence had significantly dropped in both Mbozi district (2.0%, $p=0.024$) and in Mbeya district (0.3%, $p=0.004$) at R1, but the significant difference between the districts persisted ($X^2$-test, $p<0.001$). Ten months after the second round of MDA in Mbozi district and 22 month after the first MDA (R2), the copro-Ag prevalence had significantly dropped further in Mbozi district (0.8%, $p<0.001$), but had slightly increased in Mbeya district (0.5%, $p=0.051$). At this time point there was no difference in taeniosis copro-Ag prevalence between the two districts ($X^2$-test, $p=0.51$).

Analysis of the data from Mbozi district using logistic regression showed a decrease in infection at both R1 ($p=0.022$, OR 0.57, CI: 0.35-0.92) and R2 ($p=0.001$, OR 0.33, CI: 0.17-0.62) when compared to R0 and controlling for age groups and sex. The logistic regression further revealed males to be more associated with infection ($p=0.013$, OR 1.75, CI: 1.13-2.74) as well as adults ($p<0.001$, OR 2.84, CI: 1.80-4.55). The same analysis for Mbeya district showed a significant
decrease in infection from R0 to R1 (p=0.004, OR 0.24, CI: 0.08-0.58) and a significant decrease in
infection from R0 to R2 (p=0.047, OR 0.42, CI: 0.17-0.96). There was no association found
between infection and age groups (p=0.33) or sex (p=0.13).

3.3 School-aged children population comparison

Logistic regression analysis based on stool samples from SAC alone showed that in Mbozi district
infection significantly decreased at R1 (p=0.004, OR 0.12 CI: 0.02-0.41) and R2 (p=0.001, OR
0.24, CI: 0.09-0.53) when comparing to R0. There was no association between sex and infection.
The same analysis for Mbeya district among SAC showed that infection significant decreased at R1
(p=0.013, OR 0.14, CI: 0.02-0.55), but no difference was found for R2 (p=0.089), when comparing
to R0 (Figure 2). There was no difference between sexes. Overall, 95% (35/37) of positive SAC
were tracked and treated between R0 and R2. In Mbeya district all positive SAC were tracked and
treated after R0 (11/11) and R1 (2/2). In Mbozi district 20 SAC, equivalent to 91% (20/22) of
positives, were tracked and treated after R0, and all (2/2) SAC were tracked and treated after R1.
Based on the SAC population of the villages and the copro-Ag prevalence found at R0 and R1 this
corresponded to 13% of all taeniosis cases within the villages.

3.4 Adult population comparison

Focusing on the adult population, logistic regression showed that in Mbozi district there was no
difference in infection between R0 and R1 (p=0.65) or between R0 and R2 (p=0.11). Males where
more likely to be infected with a tapeworm (p=0.019, OR 1.99, CI: 1.13-3.60) compared to females.
In Mbeya district no difference could be seen between infection at survey R1 (p=0.15) or R2
compared to R0 (Figure 2). Nor could sex be associated with disease. Overall, 86% (54/63) of positive adults were tracked and treated between R0 and R2. Positive adults were all tracked and treated in Mbeya district after R0 (11/11) and R1 (3/3). In Mbozi district 20, equivalent to 87% (20/23) of the positive adults were tracked and treated after R0, and 22, equivalent to 79% (22/28) of the positive adults were tracked and treated after R1. Based on the adult population of the villages and the copro-Ag prevalence found at R0 and R1 this corresponded to 4% of all taeniosis cases within the villages.

4. Discussion
This is, to our knowledge, the first study assessing the effect of MDA of praziquantel on the copro-Ag prevalence of taeniosis in Africa, and the first to assess the effect of repeated MDA. The study showed that school-based MDA with praziquantel in combination with ‘track and treat’ of taeniosis cases significantly reduced the copro-Ag prevalence of taeniosis among SAC. It also demonstrated that annual MDA was significantly more effective in reducing taeniosis copro-Ag prevalence than a single MDA. Although no spill-over effect could be seen into the adult population it is likely that, due to the zoonotic nature of the parasite, this effect would take time to become evident. No measureable effect of ‘track and treat’ was seen in the adult population, and proportionately ‘track and treat’ was less successful in locating cases among the adult population compared to the SAC population.

The effect of MDA with praziquantel on taeniosis prevalence has been difficult to establish from previous studies due to lack of comparison groups (Carabin and Traoré, 2014). Overall for the entire study population it was not possible to detect a significant difference in copro-Ag prevalence
of taeniosis between two MDA schemes. However, this study showed an effect of school-based MDA in the target population, and when comparing two different treatment schemes, showed annual MDA to be better compared with a single MDA. The copro-Ag prevalence of taeniosis among SAC under the annual MDA remained at a significant lower level compared to baseline (R0) during both follow-up surveys (R1 and R2). The lack of difference found from baseline (R0) to 22 months after the MDA (R2) among the SAC that had only received MDA once supported annual MDA being better than a single MDA, but was in contrast to the long-term effect of MDA reported from Latin America by Sarti et al. (2000). It is unknown how fast the parasite returns to pre-treatment levels after intervention has stopped, but according to the model published by Kyvsgaard et al. (2007) it occurs rapidly. In Peru one study tried to estimate the effect of MDA of both humans (praziquantel) and pigs (oxfendazole) (Garcia et al., 2006). The effect was measured based on prevalence and incidence of porcine cysticercosis and the authors concluded that there was a small effect of the MDA, and infection pressure stabilised at decreased rates post treatment. The problem with measuring the effect of MDA on humans based on porcine cysticercosis prevalence is that, treatment might reduce the number of tapeworm carriers, but the environment may stay contaminated for an extended period of time (Ilsoe et al., 1990), diluting the immediate effect of the MDA depending on the transmission rate from the environment to pigs. Incidence of porcine cysticercosis may be a more feasible approach to quantify the environmental contamination (Gonzalez et al., 1994; Ngowi et al., 2008). The drop in reports of “worms” seen in the stool of the study subjects in our study was consistent with the downward trend in taeniosis copro-Ag prevalence, but might also be due to reduction in Ascaris infection. Although school-based MDA only targets a proportion of the total population, it presents fewer logistical challenges and might be more feasible and in some cases cost-effective compared to
community-based MDA. In Mbozi and Mbeya districts SAC constitutes a relatively large proportion (34%) of the general population and might therefore be a cost-effective alternative to community-based MDA. The lack of a measurable spill-over from the school-based MDA, into the adult population could be due to the limited time scale of this study. An important limitation of the study was the low sample size among the adult population towards the end of the study. This resulted in any potential spill-over effect of the school-based MDA into the adult population, being difficult to detect. It was not determined whether a specific SAC had been treated with praziquantel as part of the NSCP, or to what extent the same SAC were repeatedly sampled during the surveys.

‘Track and treat’ was successfully carried out, and only few individuals did not receive treatment, the majority of whom were adults. Proportionately, fewer theoretical adult cases (4%) within the study area were treated compared to SAC (13%). No significant decrease in copro-Ag prevalence was seen throughout the study among the adults. This could be due to the small sample size of the last survey (R2). It would be interesting to see if tripling the sample size of adults, thereby increasing the theoretically number of cases treated using ‘track and treat’ from 4% to 12%, reaching the approximate proportion of that in SAC (13%), would yield similar results as was seen among SAC in this study. This may provide a more accurate indication of the potential benefit of MDA versus ‘track and treat’.

Males were generally found to be more at risk of *T. solium* infection than females in this study. However, this is in contrast to a study from Latin America where females were more likely to have taeniosis (Allan et al., 1996). This difference might be explained by cultural differences that exist
between the regions, resulting in differences in risk behaviour among men and women, which influence the transmission of the parasite.

In Guatemala, Allan et al. (1996) found a peak in taeniosis prevalence among the 30-39 year olds. Similar in this study, the increased risk among adults suggests either increased risk behaviour with age, increased household exposure with repeated infection, or that T. solium is long lived meaning prolonged exposure is linked with greater risk of infection. In contrast, the low prevalence of taeniosis suggests that either, the tapeworm has a much shorter life span than anecdotally suggested in the literature, or establishment of infection is a rare event. This could be for two reasons; 1) infected pork with viable cysts is infrequently ingested, or 2) ingested cysts rarely result in establishment of a tapeworm. If the incidence rate of infections is extremely low, then the treatment of positives carried out in both districts after each survey could have great impact on the results and cloud the potential effect of an MDA.

Pigs or latrine presence and frequency of usage were not associated with infection. Although, these are logical risk factors, the study was restricted since the information was based on questionnaires and not observations which would have given the data more validity. Also, pigs are not slaughtered and consumed within the households, but are traded and consumed at local markets, resulting in general risk to the public consuming pork and not necessarily to the residents of households rearing infected pigs. There was a difference in the ratio of pigs and humans between the two districts, but on a village level the differences are expected to be minuscule, because some villages keep pigs while others do not. Therefore the numbers vary on the district level, but less so on a village level.
Results from this study demonstrate, for the first time, that repeated administration of praziquantel using a school based approach and part of an established schistosomiasis control programme, can have a significant impact on \( T. \text{solium} \) prevalence. This is an important finding as integration of treatment of neglected diseases will ensure that the limited resources allocated will be used in the most cost-effective manner. Increased availability of donated praziquantel, currently for the treatment of schistosomiasis, could also make a contribution to the control of \( T. \text{solium} \) in the future, but using praziquantel at the dose recommended for schistosomiasis treatment might increase the risk of seizures in people who are suffering from human cysticercosis, and still needs to be evaluated in co-endemic areas (Braae et al., 2015). It is also clear that due to the zoonotic potential of \( T. \text{solium} \), control and eventually elimination will require a One Health approach consisting of a combination of control tools from both the human and veterinary public health sectors. An algorithm with those control tools is therefore still needed. Further monitoring of prevalence and the establishment of incidence rates of taeniosis in co-endemic areas along with the combination of other control tools should pave the road forward to elimination.

5. Acknowledgements

The study was conducted under the Bill and Melinda Gates foundation funded ICTC-project (Integrated Control of Taeniosis/Cysticercosis in sub-Saharan Africa). To all inhabitants of Mbeya and Mbozi districts who agreed to participate and thereby made the study possible. To the field team for all the hours spend in the field. To Drs Chummy Sikasunge and Kabemba Evans Mwape from the University of Zambia for their technical assistance with the copro-Ag-ELISA assays.

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Figure legends:

**Figure 1:** Schematic illustration of the study design with school-based mass drug administration (MDA) with praziquantel (40 mg/kg) to school-aged children (SAC) aged 4-15, ‘track and treat’ of taeniosis positive people within the whole population, and three cross-sectional data collection points on taeniosis prevalence (R0 Feb/Mar 2012, R1 Jul/Aug 2013, and R2 July/Aug 2014).
**Figure 2:** Taeniosis prevalence in school-aged children (SAC) aged 4-15, adults (>15), and both groups in the two districts Mbozi and Mbeya during the three cross-sectional surveys R0, R1, and R2. Asterisk (*) illustrates significant difference with baseline (R0) based on logistic regression with *p=0.01-0.05 and **p=0.001-0.009. The error bars depict the 95% binomial confidence intervals based on sample size and number of positives.

### Table legends:

**Table 1:** Study population characteristics, and prevalence of taeniosis in the two districts Mbozi and Mbeya during the three surveys.

<table>
<thead>
<tr>
<th>Survey</th>
<th>R0</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>District</td>
<td>Mbozi</td>
<td>Mbeya</td>
<td>Mbozi</td>
</tr>
<tr>
<td>Stool samples</td>
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<td>1510&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1500&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Males %</td>
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<td>50</td>
<td>52</td>
</tr>
<tr>
<td>Females %</td>
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<td>48</td>
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<tr>
<td>Children (4-15)</td>
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<td>Adults (16-87)</td>
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<td>Taeniosis (%)</td>
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<td>11 (1.3)</td>
<td>2 (0.3)</td>
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<tr>
<td>Taeniosis in adults (%)</td>
<td>23 (4.1)</td>
<td>11 (1.8)</td>
<td>28 (3.6)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Sex was not recorded for 7 individuals
<sup>b</sup>Sex was not recorded for 9 individuals
<sup>c</sup>Sex was not recorded for 2 individuals
<sup>d</sup>Sex was not recorded for 1 individual