

High Efficacy of Combined Albendazole and Ivermectin Treatment Against Gastrointestinal Nematodes in Vervet Monkeys and Baboons

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Summary

Conventional treatment that eliminates other gastrointestinal nematodes has failed to show adequate efficacy against *Trichuris trichura* in non-human primates (NHPs). We investigated the efficacy of albendazole and ivermectin against natural infestation of nematodes in non human primates. 18 vervet monkeys (*Chlorocebus aethiops*) and 21 baboons (*Papio anubis*) were divided into three treatment groups comprising of 6 vervets and 7 baboons per group. Albendazole (ABZ, 7.5mg/kg) was administered orally, and ivermectin (IVM, 300µg/kg) subcutaneously, each for three consecutive days. Group I animals were treated with a combination of albendazole and ivermectin, Group II ABZ alone, while Group III animals were treated with IVM alone. Faecal samples were collected at 0, 7, 14 and 28 days post treatment (dpt) and analysed for the presence of faecal eggs using the McMaster and formol ether acetyl (FEA) methods. Faecal egg count reduction percentage (FECR (%)) and cure rate (CR (%)) i.e. percentage of faecal egg negative individuals after treatment) were used to determine the efficacy of the treatment regimens. The FEA method was found to be a more sensitive assessment method than the McMaster technique. When both methods were used the helminths observed included *Trichuris trichura* (100% in both NHPs) and strongyles (29.4% in vervets and 28.6% in baboons). In vervets, the FECR of *T. trichura* at 28 dpt was 100% (Group I), 75% (Group II) and 0% (Group III) while the CR (at the same time point) was 100% (Group I), 60% (Group II) and 0% (Group III). In baboons, the FECR% and CR% of *T. trichura* at 28dpt, for groups I, II, III was 100%, 100%, 0%, respectively. All the three drug regimens were curative (100%) of strongyles at 28 dpt. It is concluded that a combined ivermectin and albendazole treatment for 3 days is effective in treating *T. trichura* and strongyles infections in vervet monkeys and baboons. Further trials should be conducted using a bigger sample size as well as in other primates including humans.

Introduction

Non-human primates (NHPs) are important pre-clinical animal models in biomedical research, mainly through vaccine and drug development for diseases such as schistosomiasis, trypanosomiasis, leishmaniasis, malaria and HIV/AIDS, amongst others (Farah et al., 2005). These NHPs harbour a variety

of gastrointestinal parasites which can cause blood loss, tissue damage, spontaneous abortion, congenital malformations and death (Gillespie, 2006). Apart from causing clinical disease the nematodes may confound research data and thus it is important that experimental animals be parasites free. Most of the nematodes such as *Trichuris trichura* are also potentially zoonotic. Previous studies conducted at the Institute of Primate Research (IPR) have recorded a high prevalence of important gastrointestinal helminthes (in up to 65%) and protozoa (up to 30%) in animals in captivity (Muriuki et al., 1998). The main helminth parasites comprise *Strongyloides*

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fulleborni, *Trichuris trichiura*, *Oesophagostomum* spp., *Trichostrongylus* sp., *Enterobius vermicularis*, *Schistosoma mansoni* and *Streptopharagus* spp.

Most of the currently registered anthelmintics are effective against the gastrointestinal nematodes but have minimal effect against *T. trichiura* in humans and animals (Geary *et al.*, 2010). Worldwide, it is estimated that 1,049 million persons harbour *T. trichiura*, including 114 million preschool-age children and 233 million school-age children. Indeed, the prevalence of *T. trichiura* is high and may reach 95% in children in many parts of the world where protein energy malnutrition and anaemias are also prevalent, and access to medical care is limited (Stephenson *et al.*, 2000). This has called for development of newer drugs as well as combinations of existing drugs to treat these resistant parasites. The anthelmintic effects of benzimidazoles such as albendazole and mebendazole are related to inhibition of parasite tubulin and are parasitostatic rather than parasitocidal (Olsen *et al.*, 2009). This implies that for resistant nematodes, adequate doses need to be given for a number of days to produce sustained cure rates. Ivermectin has been shown to have poor to moderate efficacy against *T. trichura* infections in humans and NHPs (Moncayo *et al.*, 2008; Wang *et al.*, 2008). Ivermectin has been shown to act through inhibition of glutamate-gated Cl⁻ channels leading to paralysis of the pharynx and body wall muscles of the nematodes (Geary *et al.*, 2010). A recent large scale trial showed that a combined use of albendazole and ivermectin for three days was effective in treatment of *T. trichiura* (Keiser & Utzinger, 2008). In the current study, we tested the efficacy of an albendazole and ivermectin combination for control of intestinal worms in African green monkeys (*Chlorocebus aethiops*) and baboons (*Papio anubis*) housed at IPR.

Materials and Methods

Study site

The study was conducted at the Department of Animal Sciences in the Institute of Primate Research, a directorate of the National Museums of Kenya,

Karen, approximately 20km from Nairobi. The study protocol was reviewed and approved by the Institutional Review Committee (IRC) of IPR.

Study animals and experimental design

18 Vervet monkeys (*Chlorocebus aethiops*, *syn. Cercopithecus aethiops*, Africa green monkeys) and 21 baboons (*Papio anubis*) naturally infected with helminths were used in the study. The animals were caught from the wild using a procedure described by Moinde *et al.*, (2004). Following capture, the baboons and vervet monkeys were housed in group cages for two and six weeks respectively to allow acclimatization to captivity. Thereafter they were housed individually in single stainless steel cages to allow individual monitoring during quarantine. The animals were fed with fresh vegetable such as carrots, kale, sweet potatoes, maize, to supplement the commercial diet (Monkey pellets®, Unga Feeds, Nairobi, Kenya). Water was provided *ad libitum*. They were maintained at an ambient room temperature of between 18 and 25°C. The compartments housing the subjects were cleaned with soap and water every day and disinfected with sodium hypochlorite (1%) twice a week. The animals were screened for tuberculosis, simian immunodeficiency virus (SIV), salmonellosis, shigellosis, ecto- and endo-parasites.

Fresh faecal samples were collected from the experimental animals at 0, 7, 14 and 28 days post treatment. The samples were then analyzed for nematode eggs using both the McMaster (MAFF, 1986) and formol ether acetyl methods (Chesbrough, 1998). Formol ethyl acetate sedimentation (FEA) is a semi-quantitative method commonly used in both human and veterinary medicine, while McMaster method (MM) is a quantitative method used in veterinary medicine. The samples were also analyzed for protozoan parasites using the FEA method. Based on the faecal egg counts by MM, 18 vervet monkeys and 21 baboons were selected and allocated to the following three treatment groups. Each treatment group comprised 6 vervets and 7 baboons.

Table 1. Groups and treatments administered to the vervets and baboons.

Groups	Drug and dosage
I	Albendazole (7.5mg/Kg orally x 3 days) and Ivermectin (300µg/kg subcutaneously x 3 days)
II	Ivermectin (300µg/Kg subcutaneously x 3 days)
III	Albendazole (7.5mg/Kg orally x 3 days)

Legend: Albendazole (Valbazen®, Bayer Ltd, Kenya) and Ivermectin (Emectin®, South Korea).

Analysis of data

Data were entered into Ms Excel worksheets from which tables and graphs on the frequency of the occurrence of parasites were then developed. A comparison of the percentage of experimental animals positive by either method was used to determine which method was more sensitive. The egg counts based on the MM were used to determine the efficacy of the anthelmintics using the faecal egg count reduction test (FECRT). The FECRT formula described by Varady & Corba, (1999) was used thus; $[\{\text{Pretreatment EPG minus Post treatment EPG}\} / \text{Pretreatment EPG}] * 100$

EPG = nematode eggs per gram faeces

Cure rate was also calculated and was defined as percentage egg-negative (using MM and/or FEA) individuals after treatment.

Descriptive statistics for the other parasites are presented as tables and graphs.

Results

Prevalence

At day 0, the nematode eggs identified from the vervets were those of whipworms (*Trichuris trichiura*) and/or strongyles with the proportion of infected monkeys varying according to the method of identification analysis. The prevalence of *T. trichiura* in monkeys as determined by the MM and FEA methods was 58.8% (EPG range = 200-3000) and 100%, respectively. Further, the prevalence of strongyles as determined by either method was 23.5% (EPG range=200-3000). In baboons, the prevalence of strongyles as determined by MM and FEA was 14% (EPG = 200-600) and 29%, respectively while that

of *T. trichiura*, as determined by both methods was 100% (EPG range= 200-5400). Taking into consideration the sensitivities of the two methods the prevalence of the nematodes of interest in the vervet monkeys and baboons was 100% for *T. trichiura* and 29% for strongyles.

Efficacy as determined by FECR%

The efficacy of the various anthelmintics regimens administered to the vervets and baboons is shown in Table 2. Figure 1 shows the effects of the anthelmintics regimens on the mean EPG values of *T. trichiura* in vervets and baboons. In vervets, treatment with combination of albendazole and ivermectin (Group I), caused a drastic decline of nematode EPG and an efficacy of 100% was observed at 28 dpt. Ivermectin (Group II) did not have any efficacy against *T. trichiura* at 28 dpt but was effective (100%) against strongyles. . The efficacy of albendazole (Group III) against *T. trichiura* and strongyles at 28 dpt was 75% and 80% respectively. In baboons, both combination (Group I) and albendazole (Group III) treatments had high efficacy (100%) at 28 dpt against *T. trichiura* and strongyles. However, ivermectin alone (Group II) did not appear to have any effect (0%) against *T. trichiura* but it was effective (100%) against strongyles.

Cure rate

The cure rate (%) (when both MM and FEA methods were used) following administration of the three anthelmintics regimens is shown in Table 3. In vervets, the combination treatment was most effective (100% for both nematodes) while ivermectin

Table 2. Faecal egg reduction percentage (%) in monkeys and baboons treated with various anthelmintics regimens.

Groups	Parasite	FECR% at various days post treatment					
		7dpt		14dpt		28dpt	
		Ver	Pan	Ver	Pan	Ver	Pan
I	<i>Trichuris trichura</i>	100	100	100	100	100	100
	Strongyles	100	100	100	100	100	100
	Total	100	100	100	100	100	100
II	<i>Trichuris trichura</i>	100	81	12.5	62	0	0
	Strongyles	100	100	100	100	100	100
	Total	100	81	0	62	0	0
III	<i>Trichuris trichura</i>	69	100	75	100	75	100
	Strongyles	100	100	100	100	100	100
	Total	75	100	80	100	80	100

Key: The monkeys were treated with combination of albendazole and ivermectin (Group I), ivermectin (Group II) and Albendazole (Group III); dpt= days post treatment; FECR%= Fecal egg reduction percentage; Ver= Vervets, Pan = Baboons.

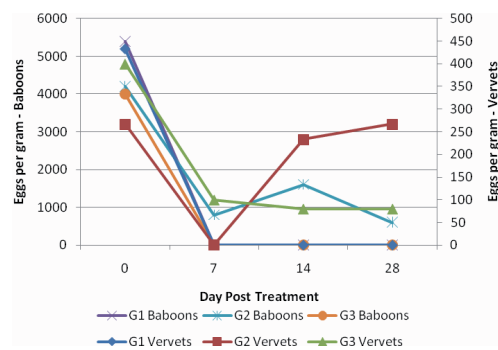


Figure 1. Mean eggs per gram (EPG) values of faeces for *T. trichura* infections in vervets and baboons following treatment with various anthelmintics regimens.

tin was the least (0% for *T. trichiura* at 28 dpt). In baboons, combination (Group I) and albendazole (Group III) were equally effective (100%) in con-

trolling both nematodes at 28 dpt. Similar to the vervets, ivermectin was least effective (0% at 14 and 28 dpt) against *T. trichiura*.

Discussion

The current study aimed at investigating the efficacy of a combination of albendazole and ivermectin in treating gastro-intestinal nematodes in vervets and baboons. The NHPs were caught from the wild and thus were naturally infected with the parasites including *T. trichiura* and strongyles; both species are the most common pathogenic parasites in NHPs (Muriuki et al., 1998). In the current study, the formal ethyl acetate method was more sensitive than the McMaster method in detection of nematode parasites. Each method has its own advantages. McMaster technique is a floatation method which allows quantification of egg counts and has been extensively used in determining the intensity of infection before treatment as well as in evaluation of ef-

Table 3. Cure rate percentage in vervets and baboons treated with various anthelmintics.

Groups	Parasites	Cure rate (%) at different sampling periods					
		7dpt		14dpt		28dpt	
		Ver	Pan	Ver	Pan	Ver	Pan
I	<i>Trichuris trichiura</i>	100	100	100	100	100	100
	Strongyles	100	100	100	100	100	100
	Total	100	100	100	100	100	100
II	<i>Trichuris trichiura</i>	50	28.6	50	0	0	0
	Strongyles	100	100	100	100	100	100
	Total	50	16.7	50	0	0	0
III	<i>Trichuris trichiura</i>	60	100	60	100	60	100
	Strongyles	100	100	100	100	100	100
	Total	60	100	60	100	60	100

NB: The monkeys were treated with combination of albendazole and ivermectin (Group I), ivermectin (Group II) and Albendazole (Group III); dpt = days post treatment; Ver= Vervet, Pan = Baboons; Cure rate was defined as percentage egg-negative (using McMaster and/or Formol ethyl acetate method) animals after treatment.

ficacy of anthelmintics (Cole *et al.*, 1992; Kagira *et al.*, 2003). However, it is not suitable for identification of parasites which require sedimentation such as flukes and protozoan parasites. These parasites can easily be identified when using a concentration method such as formol ethyl acetate.

In vervets and baboons, the study showed that a single administration of ivermectin for 3 days was not effective against *T. trichiura* but was highly effective against the strongyles. Ivermectin was also observed to have high efficacy against *Oesophagostomum* spp and *S. fulleborni* in macaques and rhesus monkeys respectively (Wang *et al.*, 2008, Brack & Rietschel, 1986), but due to the limited number of animals with nematodes in the current study, the comparison of anthelmintics against strongyles is not conclusive. The efficacy of ivermectin against *Trichuris* spp in humans is low and ranges between 0 and 11% (Beach *et al.*, 1999; Belizario *et al.*, 2008). However, the use of ivermectin (annual or twice-

annual) in control of onchocerciasis for 15–17 years reduced substantially the level of *T. trichiura* infections in humans in Ecuador (Moncayo *et al.*, 2008). In NHPs, a single dose of ivermectin at 0.3mg/kg caused an efficacy of 97% against *T. trichiura* in macaques at 11 days post treatment (Wang *et al.*, 2008). This high efficacy in macaques could have been due to the short monitoring period (11 days) compared to the current study where the vervets and baboons were monitored for 28 days. It is quite possible that ivermectin causes temporary suppression of egg-laying by the female parasites, which was even manifested in the current study at 7 and 14 dpt. It is widely reported that Ivermectin can temporarily reduce the fecundity of female nematodes, but not necessarily death and the recovering parasites may continue to lay eggs (Petersen *et al.*, 1996). The current study showed that a 3 day regimen of albendazole treatment was fully effective against *T. trichiura* in baboons, but it was less effective in

vervets. The cure rate of a single dose of albendazole or mebendazole against *T. trichiura* in humans ranges from 28% to 36% (Keiser & Utzinger, 2008). Thus, although single dose albendazole use is advocated for treatment of gastrointestinal nematodes, resistant nematodes such *T. trichiura* and *Strongyloides* spp are not affected by the drugs and thus continue to affect the human populations (Olsen et al., 2009). Similarly, in NHPs, single dose albendazole is curative for all the nematodes apart from strongyloides and trichuris and thus these resistant parasites remain in the animals and often compromise research protocols. Benzimidazole drugs such as albendazole bind to beta-tubulin and thereby inhibit dimerization with alpha-tubulin and subsequently the polymerization into microtubules (Lacey & Gill, 1994). It has been observed that since albendazole action is parasitostatic, and *Trichuris* are located in the colon (which is difficult to access by most drugs) adequate doses need to be given for at least 3 days to produce sustained cure rates of *Trichuris* spp and *Strongyloides* spp (Geary et al., 2010). In the current study, the 3 day treatment using albendazole was only fully effective in baboons. This showed that in vervets, the *T. trichiura* population was less susceptible when compared to that in baboons. Further, the differences could have been due to variation in the pharmacodynamics of the drug in the two NHPs.

The observed high efficacy of combined administration of ivermectin and albendazole against *T. trichiura* has also been reported in humans (Belizario et al., 2003; Olsen, 2007). Single-dose therapy with albendazole (400mg/kg) plus ivermectin (200µg/kg) produced a 'cure rate' (79 %) and an egg-reduction rate (94%) which were significantly higher than the corresponding rates produced by albendazole alone (Olsen, 2007). The combination has been shown to be efficacious for other nematode infections such as filariasis and treatment rarely results in side-effects outside those commonly associated with a therapeutic effect of an anthelmintic (Beach et al., 1999). Although there is an absence of efficacy standards for human anthelmintics (Geary et

al., 2010), veterinary regulations require efficacy of 95% against a particular species before registration (Coles et al., 1992; Kagira et al., 2003). Results of this study show that the combined ivermectin and albendazole treatment against *T. trichiura* meets the later criterion. It would be important to repeat the study in a larger group of animals with a view of elucidating the pharmacokinetics and toxicity of the drug combinations.

In conclusion, the study has shown that repeated administration of albendazole (7.5mg/kg) and ivermectin (200µg/kg) for 3 days is highly effective in treating *T. trichura* infections in vervet monkeys and baboons. The combination regime was also effective against the strongyles, but the number of nematode positive monkeys was low and thus future studies should also test the efficacy of this regimen against a wider spectrum of nematodes. Apart from affecting the gastrointestinal nematodes, the combination therapy will also be effective in control ectoparasites which are susceptible to ivermectin.

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