Application for Classification of

Albendazole 200mg Tablets

To General Medicine

As an Anthelmintic

In Total Doses Not Exceeding 400mg

55th Medicines Classification Committee

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Introduction

Albendazole is included in the WHO List of Essential Medicines 19th Edition April 2015. [1] Albendazole and mebendazole have been extensively used worldwide for more than 30 years.

Albendazole is a benzimidazole carbamate with anthelmintic effects against tissue parasites. It is thought to exhibit its anthelmintic effect by inhibiting tubulin polymerisation. Albendazole is poorly absorbed in humans (<5%) and rapidly undergoes first-pass metabolism in the liver and is generally not detected in the liver. The active metabolite albendazole sulfoxide is thought to exert the product's pharmacological properties. Albendazole sulfoxide has a half-life of approximately 8.5 hours. Absorption of albendazole is increased around 5-fold if taken with a fatty meal. Albendazole is almost exclusively eliminated through the liver. [2]

Mebendazole has been classified in New Zealand as a Pharmacy-Only Medicine in packs containing 600mg or less since 1990.[3] Mebendazole is also a benzimidazole carbamate anthelminitic with around 20% systemic absorption and rapid first-pass liver metabolism. Absorption is increased with a high fat meal. Maximum plasma concentrations reached 2-4 hours after administration. Approximately 95% is excreted unchanged in the faeces. [4]

Overview

Albendazole is not yet registered in New Zealand but the 400mg tablets are supplied and reimbursed under s29 of the Medicines Act. PHARMAC's Sole Supply Tender List for 2015-16 is seeking supply of albendazole 200mg tablets and solution, which Te Arai BioFarma are able to supply and will be seeking Medsafe registration.

Twenty years ago many scientists considered drug resistance in livestock helminths an unimportant phenomenon. High prevalence of AR, often exceeding 50%, have now been reported in all parts of the world for gastrointestinal helminths of sheep, goats, and horses

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kept in industrial livestock systems. In recent years, several reports of apparent failures in the treatment of human schistosomes and nematodes have been published. The recent reports on possible emerging drug resistance in human nematodes and schistosomes do not provide conclusive evidence for the increase of innately tolerant strains or for the appearance of newly mutated resistant strains. However, they strongly suggest that such tolerant or resistant strains can and do exist and that these strains may emerge more prominently under drug pressure (hookworm in Australia, schistosomes in Egypt). [5]

In a community-based, open-label, assessor-blinded randomsied controlled trial, 314 individuals five years of age or older were treated for soil-transmitted helminths with either single dose albendazole (400mg) or mebendazole (500mg) versus triple dose. The cure rate for the single dose regimen was 69% albendazole and 29% for mebendazole with egg eradication rates of 97% versus 84% respectively. For the triple dose regimen the cure rates were 92% for albendazole versus 54% for mebedazole with egg eradication rates of 99.7% and 96% respectively. [6]

Adverse events included headache (n=3; all mebendazole), abdominal cramps (n=3; 2 mebendazole, 1 albendazole) and the closely related ‘‘full stomach’’ (n=2; mebendazole), and waist pain (n =1; albendazole). Two individuals each reported vomiting, including production of A. lumbricoides worms (1 albendazole, 1 mebendazole), diarrhea (2 mebendazole), fatigue (1 albendazole, 1 mebendazole), and chills (2 mebendazole). Vertigo (albendazole), throat pain (albendazole), fever (mebendazole), and a swollen face (mebendazole) were each reported once.

None of the study participants requested medical interventions as adverse events were mild and self-limiting. [6]

A randomised open-label trial 200 children infected with hookworm were treated with either single-dose 400mg albendazole or single-dose 500mg mebendazole. The cure rates of children that became egg free were 36.0% for albendazole and 17.6% for mebendazole

(p=0.01). Monitoring of children within 3 hours after drug administration revealed no drug-related adverse events, neither in the albendazole nor in the mebendazole group. Hence, both treatments were well tolerated. [7]

In a second study 1,186 children aged 4-19 years were randomised to either albendazole 600mg or mebendazole 600mg to examine the efficacy at 4-month and 6-month intervals on soil-transmitted helminths. The cure rates for albendazole were 92.4% for hookworm infection, 83.5% for *Ascaris lumbricoides*, and 67.8% for *Trichuris trichiura* were superior to Mebendazole with cure rates of 50% and 55.0% (respectively) for hookworm, 79.6% and 97.5% for *A. lumbricoides,* and 60.6% and 68.3% for *T. trichiura* infection (p=0.0001). [8]

A meta-analysis of 20 randomised controlled trials examined the efficacy of albendazole compared with mebendazole in treating soil-transmitted helminths. The efficacy of single-dose oral albendazole and mebendazole against hookworm infections were 72% (95% CI,59%-81%; 742 patients) and 15% (95%CI,1%-27%; 853 patients) respectively. Albendazole was well tolerated. In 11 studies included in our meta-analysis, no significant adverse events were reported following albendazole administration. One trial carried out in the Philippines reported nausea and diarrhoea in 2 and 1 individuals, respectively. There was no indication whether or not adverse events were assessed in the remaining 2 randomized placebo-controlled trials included in our meta-analysis. Mebendazole was well tolerated. In 3 trials, no adverse events were observed. One study reported abdominal discomfort in 6 of 45 children who were treated with 500-mg mebendazole. No information on adverse events was given in the remaining 2 studies. [9]

Conclusion

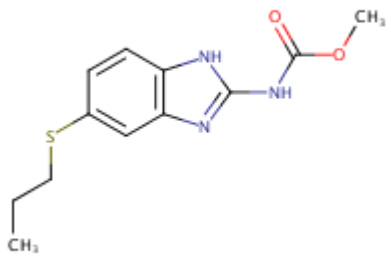
Albendazole is from the same chemical class as mebendazole with similar pharmacological properties. Albendazole is at least as effective as mebendazole against soil-transmitted helminths and superior to mebendazole against hookworms. The adverse event profiles for both albendazole and mebendazole are very similar and unremarkable. Albendazole has been in use worldwide for more than 30 years with no signals of unexpected or serious

adverse events and offers an alternative anthelmintic particularly when resistance is a concern. For these reasons we propose albendazole in doses not exceeding 400mg be classified Pharmacy Only.

PART A

1. International Non-proprietary Name (or British Approved Name or US Adopted Name) of the medicine.

Name: albendazole



2. Proprietary name(s).

Albenda

3. Name of the company / organisation / individual requesting a reclassification.

Te Arai BioFarma Limited

Auckland, New Zealand

4. Dose form(s) and strength(s) for which a change is sought.

Dose Form: Tablet

Strength: albendazole 200mg

5. Pack size and other qualifications.

2 x 200mg tablets per pack.

6. Indications for which change is sought.

The treatment of a variety of intestinal nematode, cestode and trematode infections including pinworms, threadworms, whipworms, roundworms, tapeworms, hookworms and hydatid disease.

7. Present classification of the medicine.

Currently albendazole is not classified in New Zealand since there is no registered product.

8. Classification sought.

This application seeks to classify albendazole up to 400mg as Pharmacy Only.

9. Classification status in other countries (especially Australia, UK, USA, Canada).

Australia: On the General Schedule for Medical and Nurse Practitioners.

Canada: OTC for cattle

10. Extent of usage in New Zealand and elsewhere (eg, sales volumes) and dates of original consent to distribute.

Albendazole does not currently have marketing approval in New Zealand. However, albendazole 400mg tablets are supplied under s29 of the Medicines Act and reimbursed for hydatid disease.

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11. Labelling or draft labelling for the proposed new presentation(s).

A copy of the proposed labelling is available in Appendix 1.

12. Proposed warning statements if applicable.

Albendazole should not be given to pregnant women or women thought to be pregnant. Effective contraception should be taken during and within one-month after treatment. Prior to starting treatment women of childbearing age should take a pregnancy test. In human field trials of albendazole 17 women in the first trimester of pregnancy were inadvertently given a single oral dose of 400 mg/person without any adverse effects on mother or child being apparent.

Albendazole should not be taken while breast feeding.

Albendazole may cause dizziness and caution should be exercised if driving or operating machinery.

Caution should be exercised in treating people with liver disease.

Care should be taken in patients taking oral contraceptives, anticoagulants, oral anti-diabetes medications or theophylline.

13. Other products containing the same active ingredient(s) and which would be affected by the proposed change.

No other products containing the same active ingredient are registered in New Zealand. Therefore, no other products would be affected by the proposed change.

Part B

1. **A statement of the benefits to both the consumer and to the public expected from the proposed change.**

The public gain a second choice of anthelmintic medication at Pharmacy Only level. One that

has similar safety to the well-established mebendazole, but which has superior cure rates against hookworms and mebendazole-resistant helminths. Both hookworms and mebendazole-resistant helminths are present in Australia, Pacific Islands, Asia and countries of origin of most refugees. Eradication of hookworms and mebendazole-resistant helminths is an important issue for the community at large.

The public are already well accustomed to the availability and use of Pharmacy Only mebendazole and the ability to access such medicines without a prescription charge. It would not be in the community's interests to have a financial barrier in the case of albendazole but not mebendazole.

Albendazole is on the WHO List of Essential Medicines and this application would make it reasonably available for empiric use.

2. Potential risk of harm to the consumer as a result of the proposed change, and factors to mitigate this risk.

There is no possibility of community harm resulting from wider use of albendazole.

The benefits of albendazole being classified as Pharmacy Only medicine significantly outweigh the risk. A Pharmacy Only medicine classification is logical given the Pharmacy Only classification of second generation antihistamines and presents a low risk to a level at least equivalent to Pharmacy Only mebendazole.

3. Ease of self-diagnosis or diagnosis by a pharmacist for the condition indicated.

Definitive diagnosis of helminth infestation is via faecal egg pathology. This test is represents a barrier to effective therapy given the availability of an effective, well-tolerated Pharmacy Only treatment. Symptoms or helminth infestation are often non-specific such as anaemia, tiredness or anal irritation. Mebendazole is used empirically upon suspicion of helminth infestation. [10]

However, in the case of refugees entering New Zealand faecal screening for parasites is a routine part of their health screening. Refugee Health Care: A handbook for health professionals 2012 by the Ministry of Health states: [10]

"some helminths infections (strongyloides, opisthorchis, schistosomiasis) may be asymptomatic, and persist for many years before causing serious disease…Maintain a low threshold of suspicion for these conditions and refer appropriately."

- ***General ill health, weight loss****: leishmaniasis, TB, worms/parasites*
- ***Muscle pain/ limb pain****: cysticercosis, leptospirosis, malaria, schistosomiasis*
- ***Eosinophilia****: cysticercosis, dracunculiasis, filiariasis, hydatid disease, onchocerciasis/loiasis parasites, trichinella, visceral larvae migrans (toxocara)*
- ***Pyrexia of unknown origin****: trypanosomiasis, leishmaniasis, loiasis/onchocerciasis*

4. Relevant comparative data for like compounds.

Albendazole is from the same chemical class as mebendazole with similar pharmacological properties. Albendazole is at least as effective as mebendazole against soil-transmitted helminths and superior to mebendazole against hookworms. The adverse event profiles for both albendazole and mebendazole are very similar and unremarkable. Albendazole has been in use worldwide for more than 30 years with no signals of unexpected or serious adverse events and offers an alternative anthelmintic particularly when resistance is a concern.

Mebendazole has been classified Pharmacy Only in doses of no more than 600mg since 1990. [3]

In a community-based, open-label, assessor-blinded randomsied controlled trial, 314 individuals 5 years of age or older were treated for soil-transmitted helminths with either single dose albendazole (400mg) or mebendazole (500mg) versus triple dose. The cure rate for the single dose regimen was 69% albendazole and 29% for mebendazole with egg eradication rates of 97% versus 84% respectively. For the triple dose regimen the cure rates were 92% for albendazole versus 54% for mebedazole with egg eradication rates of 99.7% and 96% respectively. [5]

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None of the study participants requested medical interventions as adverse events were mild and self-limiting. [5]

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A meta-analysis of 20 randomised controlled trials examined the efficacy of albendazole compared with mebendazole in treating soil-transmitted helminths. The efficacy of single-dose oral albendazole and mebendazole against hookworm infections were 72% (95% CI,59%-81%; 742 patients) and 15% (95%CI,1%-27%; 853 patients) respectively. Albendazole was well tolerated. In 11 studies included in our meta-analysis, no significant adverse events

were reported following albendazole administration. One trial carried out in the Philippines reported nausea and diarrhoea in 2 and 1 individuals, respectively. There was no indication whether or not adverse events were assessed in the remaining 2 randomized placebo-controlled trials included in our meta-analysis. Mebendazole was well tolerated. In 3 trials, no adverse events were observed. One study reported abdominal discomfort in 6 of 45 children who were treated with 500-mg mebendazole. No information on adverse events was given in the remaining 2 studies. [9]

5. Local data or special considerations relating to New Zealand.

New Zealand is at an increased risk of immigrants and refugees entering the healthcare system infected with hookworms or mebendazole-resistant helminths. Albendazole provides an empiric alternative with superior cure rates.

6. Interactions with other medicines.

Albendazole, like mebendazole, induces liver enzymes of the P450 system. Other medicines that may reduce the effectiveness of albendazole are: anticonvulsants, levamisole and ritonavir. Other medicines that may increase levels of the active metabolite albendazole sulfoxide and therefore potentially increase the risk of side effects are: cimetidine, dexamethasone and praziquantel. However, the risk is no greater than with mebendazole and it must be noted that the level of use of cimetidine, dexamethasone and praziquantel in New Zealand are quite low (cimetidine around 2,400 packs annually; dexamethasone around 15,000 packs annually; and, praziquantel around 90 packs annually according to IMS).

7. Contraindications and precautions.

Albendazole should not be given to pregnant women or women thought to be pregnant. Effective contraception should be taken during and within one-month after treatment. Prior to starting treatment women of childbearing age should take a pregnancy test. In human field trials of albendazole 17 women in the first trimester of pregnancy were inadvertently

given a single oral dose of 400 mg/person without any adverse effects on mother or child being apparent.

Albendazole should not be taken while breast feeding.

Albendazole may cause dizziness and caution should be exercised if driving or operating machinery.

Caution should be exercised in treating people with liver disease.

Care should be taken in patients taking oral contraceptives, anticoagulants, oral anti-diabetes medications or theophylline.

8. Possible resistance

A 2003 report by Meat and Wool Innovation Ltd noted that the resistance to benzimidazole anthelmintics in sheep was around 60% and in Western Australia up to 100%. [11] This report also notes that both sheep and beef in New Zealand are infected with the hookworm species *Bunostromum trigonocephalum*, *Bunostromum phlebotomum* while sheep, beef, goats and deer are infected with *Haemonchus contortus* which most closely resembles the human hookworm. The report states that the longer the duration of action of an anthelmintic the greater the risk of resistance developing. Thus, short-acting oral anthelmintics such as albendazole are least likely to put pressure on the development of resistant strains, especially when used as a once only medication as proposed in tis application.

The risk of resistance to albendazole is no greater than to any other member of the benzimidazole anthelmintics including mebendazole. The Meat and Wool Innovation Ltd Report notes that once resistance to a member of the class has been established there is little evidence of reversion. This supports a strategy of using the most effective member of a class as first line therapy.

9. Adverse events

A meta-analysis of 20 randomised controlled trials albendazole was well tolerated. In 11 studies included in the meta-analysis, no significant adverse events were reported following albendazole administration. One trial carried out in the Philippines reported nausea and diarrhoea in 2 and 1 individuals, respectively. There was no indication whether or not adverse events were assessed in the remaining 2 randomized placebo-controlled trials included in our meta-analysis. Mebendazole was well tolerated. In 3 trials, no adverse events were observed. One study reported abdominal discomfort in 6 of 45 children who were treated with 500-mg mebendazole. No information on adverse events was given in the remaining 2 studies. [9]

Data from large clinical studies were used to determine the frequency of very common to rare undesirable reactions. The frequencies assigned to all other undesirable reactions (i.e. those occurring at < 1/1000) were mainly determined using post-marketing data and refer to a reporting rate rather than a true frequency. The following convention has been used for the classification of frequency: Very common ≥1/10 Common ≥1/100 to < 1/100 Rare ≥ 1/10,000 to < 1/1000.

Very rare < 1/10,000 Blood and the lymphatic system disorders Uncommon: Leucopenia Very rare: Pancytopenia, aplastic anaemia, agranulocytosis Patients with liver disease, including hepatic echinococcosis, appear to be more susceptible to bone marrow suppression. Immune system disorders **Uncommon**: Hypersensitivity reactions including rash, pruritus and urticaria Nervous system disorders **Very common**: Headache Common: Dizziness Gastrointestinal disorders Common: Gastrointestinal disturbances (abdominal pain, nausea, vomiting) Gastrointestinal disturbances have been associated with albendazole when treating patients with echinococcosis. Hepato-biliary disorders Very common: Mild to moderate elevations of hepatic enzymes Uncommon: Hepatitis Skin and subcutaneous tissue disorders Common: Reversible alopecia (thinning of hair, and moderate hair loss) Very rare: Erythema multiforme, Stevens-Johnson syndrome General disorders and administrative site conditions Common: Fever

10. Potential for abuse or misuse.

There are no reported cases of fatal overdose.

In case of overdose, gastric lavage and general supportive measures should be undertaken.

Conclusion

Albendazole is from the same chemical class as mebendazole with similar pharmacological properties. Albendazole is at least as effective as mebendazole against soil-transmitted helminths and superior to mebendazole against hookworms. The adverse event profiles for both albendazole and mebendazole are very similar and unremarkable. Albendazole has been in use worldwide for more than 30 years with no signals of unexpected or serious adverse events and offers an alternative anthelmintic particularly when resistance is a concern.

Resistance to benzimidazole anthelmintics in sheep in New Zealand was around 60% in 2003 and in Western Australia up to 100%. [11] Both sheep and beef in New Zealand are infected with the hookworm species *Bunostromum trigonocephalum*, *Bunostromum phlebotomum* while sheep, beef, goats and deer are infected with *Haemonchus contortus* which most closely resembles the human hookworm. The longer the duration of action of an anthelmintic the greater the risk of resistance developing. Thus, short-acting oral anthelmintics such as albendazole are least likely to put pressure on the development of resistant strains, especially when used as a once only medication as proposed in this application.

The risk of resistance to albendazole is no greater than to any other member of the benzimidazole anthelmintic class, including mebendazole. The Meat and Wool Innovation Ltd Report notes that once resistance to a member of the class has been established there is little evidence of reversion. This supports a strategy of using the most effective member of a class as first line therapy.

For these reasons we propose albendazole 200mg tablets in doses not exceeding 400mg be classified Pharmacy Only.

References:

1. WHO Model List of Essential Medicines. 19th Edition. August 2015.
2. Albendazole. MHRA SmPC. 2014.

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10. Ministry of Health. Refugee Health Care: A Handbook for health professionals. 2012.
11. Rattray P V. Helminth Parasites in the New Zealand Meat & Wool Pastoral Industries : A Review of Current Issues. MEAT & WOOL INNOVATION LTD. September 2003.

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Appendix 1. Proposed Labelling

ALBENDA® Tablets

Albendazole

WHAT IS IN THIS LEAFLET

Please read this leaflet carefully before you start using ALBENDA.

This leaflet answers some common questions about ALBENDA. It does not contain all the available information. It does not take the place of talking to your doctor or pharmacist.

All medicines have risks and benefits. Your pharmacist or doctor will be able to advise you about the risks and benefits of using ALBENDA.

If you have any concerns about using this medicine, ask your pharmacist or doctor. Keep this leaflet with the medicine. You may need to read it again.

WHAT IS ALBENDA USED FOR

ALBENDA contains the active substance albendazole.

Albendazole belongs to a group of medicines known anthelmintics, which are effective against certain worms which are parasitic in humans.

ALBENDA is effective against threadworm or pinworm, roundworm, whipworm, tapeworm and hookworm among others.

ALBENDA is thought to kill these worms by causing them to starve. The eggs, larvae and adult worms are affected.

Ask your doctor or pharmacist if you have any questions about why ALBENDA has been prescribed for you.

BEFORE YOU USE ALBENDA

When you must not use it

Do not use ALBENDA

- If you have had an allergic reaction to albendazole or any of the other ingredients contained in this medicine. Albendazole is also contained in

ALBENDA. The ingredients are listed at the end of this leaflet. Signs of an allergic reaction may include an itchy skin rash, shortness of breath and swelling of the face or tongue.

- if you have taken albendazole before and became unwell, tell your doctor or pharmacist before taking the first dose.
- you are allergic to medicines similar to albendazole such as mebendazole (Sqworm, Vermox) or thiabendazole (Mintezol).
- YOU KNOW OR SUSPECT YOU ARE PREGNANT. Pregnancy must be avoided (ie use effective contraceptive measures) during treatment, and for one month after stopping ALBENDA.
  In order to avoid taking ALBENDA during early pregnancy, treatment with ALBENDA should only be started during the first week of having your period or after a negative pregnancy test.
- you are breast feeding. Your baby can absorb albendazole from breast milk if you are breast feeding. Breast feeding should be stopped while taking ALBENDA, and for at least 5 days after finishing treatment.
- the expiry date printed on the pack has passed.
- the packaging is torn or shows signs of tampering.

ALBENDA is not recommended for children under 2 years of age.

Do not give this medicine to anyone else; your doctor or pharmacist has prescribed it specifically for you and your condition.

Do not take ALBENDA if any of the above apply to you. If you are not sure, talk to your doctor or pharmacist before taking ALBENDA.

Do not use this medicine after the expiry date printed on the pack or if the packaging is torn or shows signs of tampering. In that case, return it to your pharmacist.

Before you start to use it

Talk to your doctor or pharmacist before taking ALBENDA:

- you are or think you may be pregnant or if you intend to become pregnant.
  Pregnancy must be avoided (ie use effective contraceptive measures) during treatment, and for one month after stopping ALBENDA. In order to avoid taking ALBENDA during early pregnancy, treatment with ALBENDA should only be started during the first week of having your period or after a negative pregnancy test.
- you are breast feeding. Breast feeding should be stopped while taking ALBENDA, and for at least 5 days after finishing treatment.
- you have any liver problems.

If any of the above apply to you talk to your doctor or pharmacist before taking ALBENDA. Check with your doctor or pharmacist if you are not sure.

Children and adolescents
ALBENDA should not be taken by children under 2 years of age.

If you require further advice, you should talk with your doctor or pharmacist.

Taking other medicines

The effects of some medicines may be affected if other medicines are used at the same time. You should therefore tell your doctor or pharmacist if you use other medicines regularly, have used other medicines until recently or wish to use other medicines at the same time as ALBENDA. This includes those medicines that you buy without a prescription. Your doctor or pharmacist will be able to tell you if any problems could occur when taking ALBENDA with other medicines.

If you have not told your doctor or pharmacist about any of these things, tell him/ her before you start using ALBENDA.

Driving and using machines
Be careful driving or operating machinery until you know how you react to ALBENDA.

ALBENDA may cause dizziness in some people.

HOW TO USE ALBENDA

Follow all directions given to you by your doctor and pharmacist carefully.

These directions may differ from the information contained in this leaflet.

If you do not understand the instructions on the end of this leaflet, ask your doctor or pharmacist for help.

How much to use

Your doctor or pharmacist will advise how many doses are needed each day, and for how long you will need to take ALBENDA. The usual dose for adults and children older than 2 years of age, is two ALBENDA tablets as a single dose.

For other conditions the dose prescribed by your doctor or pharmacist may be different. You should take the full course of tablets, and not just stop when you feel better.

Your doctor may need to see you two to four weeks after taking the dose or course. This is to make sure that ALBENDA has worked. A second dose or course of ALBENDA is sometimes needed.

How to use

You will be told whether to take the tablets with food or on an empty stomach, and it is important you follow these instructions. ALBENDA tablets are usually taken on an empty stomach. In some conditions the tablets may need to be taken after food.

ALBENDA tablets may be taken crushed or chewed or swallowed whole. No special laxative or fasting is needed.

If you forget to take ALBENDA

Take the missed tablets as soon as you remember. If you have been prescribed more than a single dose, do not try to make up for missed doses by taking more than two tablets at a time.

Taking more than the prescribed dose can increase the chance of unwanted side effects.

WHILE YOU ARE USING ALBENDA

Things you must do

Tell your doctor if you become pregnant while taking ALBENDA.

Tell your doctor or pharmacist you are taking ALBENDA, before starting any other medicines.

Some medicines may affect the way other medicines work.

You may require monitoring of your liver function or white blood cell counts. Patients with liver disease may be monitored more closely.

If you are having a blood test done, tell your doctor you are taking ALBENDA.

Keep any follow up appointments with your doctor. It may be necessary to check that ALBENDA has worked. A second dose or course of ALBENDA is sometimes needed.

Tell any other doctors, dentists, and pharmacists who treat you that you are using this medicine.

Things to be careful of

Be careful driving or operating machinery until you know how ALBENDA affects you.

SIDE EFFECTS

Tell your doctor or pharmacist as soon as possible if you do not feel well while you

are using ALBENDA.

All medicines can have side effects. Sometimes they are serious, most of the time they are not. You may need medical attention if you get some of the side effects.

Do not be alarmed by these lists of possible side effects. You may not experience any of them. Ask your doctor or pharmacist to answer any questions you may have.

Stop taking ALBENDA and see a doctor straight away if you notice any of the following serious side effects – you may need urgent medical treatment:

- swelling of limbs, face, mouth or throat
- shortness of breath or breathing difficulties
- hives or severe skin reactions

These are signs of a severe allergic reaction to ALBENDA. Allergy to ALBENDA is rare.

Tell your doctor if you experience any of the following and they worry you:

- headache or dizziness
- vomiting or feeling sick, stomach pains or diarrhoea
- mild skin rash or itchiness

Tell your doctor immediately if you notice any of the following:

- fever
- bone pain
- headache
- tiredness, shortness of breath, looking pale.
- frequent infections
- unusual bleeding or bruising.
- yellowing of the skin and eyes, also called jaundice, dark coloured urine and/or light coloured stools.
- infections of the throat, mouth, skin or nasal passage.
- Seizures
- Blurred or abnormal vision
- Unusual behaviour
- Unusual numbness or weakness
- Unusual taste, smell or hearing

Tell your doctor or pharmacist if you notice anything else that is making you feel unwell.

IF YOU USE TOO MUCH (overdose)

Immediately telephone your doctor or the National Poisons Centre (telephone 0800 POISON or 0800 764 766), or go to accident and emergency at your nearest hospital, if you think that you or anyone else may have taken too much ALBENDA.

Do this even if there are no signs of discomfort or poisoning.

AFTER USING ALBENDA

Storage

Keep your tablets in the original pack until it is time to take them.
Keep this medicine out of the sight and reach of children.
Do not use this medicine after the expiry date which is stated on the blister.
Store below 25˚C.

Disposal

Do not throw away any medicines via wastewater or household waste. Ask your pharmacist how to throw away medicines you no longer use. These measures will help protect the environment.

PRODUCT DESCRIPTION

What it looks like

ALBENDA tablets are provided in blister packs containing 2 tablets.

Ingredients

Active Ingredient

The active substance is Albendazole. Each tablet contains 200 mg of Albendazole.

Inactive Ingredient

The other ingredients are starch, lactose, cross linked starch, povidone, saccharin sodium, polysorbate, cellulose, vanilla and blood orange flavours, stearic acid, magnesium stearate, aerosol, sodium lauryl sulfate, hypromellose, talc, titanium dioxide and polyethylene glycol.

SPONSOR DETAILS

ALBENDA is supplied in New Zealand by:

Te Arai Consumer,
a division of Te Arai BioFarma Ltd
P.O Box 46205
Herne Bay, Auckland 1147
New Zealand

This leaflet was prepared in January 2016.

Reference 1.

valproic acid (sodium valproate)	***Injection:*** *100 mg/ mL in 4- mL ampoule; 100 mg/ mL in 10- mL ampoule.*
6. ANTI-INFECTIVE MEDICINES	
6.1 Anthelminthics	
6.1.1 Intestinal anthelminthics	
albendazole	**Tablet (chewable):** 400 mg.
levamisole	**Tablet:** 50 mg; 150 mg (as hydrochloride).
mebendazole	**Tablet (chewable):** 100 mg; 500 mg.
niclosamide	**Tablet (chewable):** 500 mg.
praziquantel	**Tablet:** 150 mg; 600 mg.
pyrantel	**Oral liquid:** 50 mg (as embonate **or** pamoate)/ mL. **Tablet (chewable):** 250 mg (as embonate **or** pamoate).
6.1.2 Antifilarials	
albendazole	**Tablet (chewable):** 400 mg.
diethylcarbamazine	**Tablet:** 50 mg; 100 mg (dihydrogen citrate).
ivermectin	**Tablet (scored):** 3 mg.
6.1.3 Antischistosomals and other antitrematode medicines	
praziquantel	**Tablet:** 600 mg.
triclabendazole	**Tablet:** 250 mg.
Complementary List	
*oxamniquine**	***Capsule:*** *250 mg.* ***Oral liquid:*** *250 mg/5 mL.* ** Oxamniquine is listed for use when praziquantel treatment fails.*
6.2 Antibacterials	
6.2.1 Beta-lactam medicines	
amoxicillin	**Powder for oral liquid:** 125 mg (as trihydrate)/5 mL; 250 mg (as trihydrate)/5 mL **[c]**. **Solid oral dosage form:** 250 mg; 500 mg (as trihydrate).
amoxicillin + clavulanic acid	**Oral liquid:** 125 mg amoxicillin + 31.25 mg clavulanic acid/5 mL AND 250 mg amoxicillin + 62.5 mg clavulanic acid/5 mL **[c]**. **Tablet:** 500 mg (as trihydrate) + 125 mg (as potassium salt).

Reference 5.

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Drug Resistance in Human Helminths: Current Situation and Lessons from Livestock

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INTRODUCTION

In recent years, several reports of apparent failures in the treatment of human schistosomes and nematodes have been published (33, 81, 116, 132). Although the interpretation and the implications of these studies are still being debated, they have led to an increased awareness of the potential problem of anthelmintic resistance (AR) in the treatment and control of human helminths.

In view of the short but worrying history of AR in livestock, such concerns are not superfluous. At present, AR is the most important disease problem of the sheep-farming industry in Australia, South Africa, and possibly South America (140, 146, 147). Twenty years ago, however, many scientists considered drug resistance in livestock helminths an unimportant phenomenon. High prevalences of AR, often exceeding 50%, have now been reported in all parts of the world for gastrointestinal helminths of sheep, goats, and horses kept in industrial livestock systems. Surprisingly, up to now very few problems with AR have been noticed in cattle helminths (58). Table 1 summarizes the helminth species and the anthelmintic classes most frequently involved.

Even multiple drug resistance is not uncommon in helminths of veterinary importance. In parts of Paraguay (95) and South Africa (140), helminths are resistant to all available broad-spectrum anthelmintics and farmers have started to give up sheep farming because of insurmountable problems with AR (138).

For purposes of discussion, AR is defined as a heritable reduction in the sensitivity of a parasite population to the

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TABLE 1. Main helminth species of livestock for which drug resistance has been reported

Host	Parasite	Resistance to[a]:		
		BZ	LEV-MOR	AVM-MIL
Sheep and goats	*Haemonchus contortus*	+		+
	Ostertagia spp.	+	+	+
	Trichostrongylus spp.	+	+	+
Horses	*Cyathostomes*	+		

[a] BZ, benzimidazoles; LEV-MOR, levamisole-morantel; AVM-MIL, avermectins-milbemycins.

action of a drug. The reduction is expressed as the decrease of the frequency of individual parasites affected by exposure to the drug, compared to the frequency observed in the same population upon initial or prior to exposure (31). Although not unequivocal but generally considered the most adequate, this definition encompasses two biologically distinct but not always distinguishable processes: (i) existing drug-tolerant parasite lines may become more frequent, particularly under drug pressure, and (ii) previously susceptible parasites may undergo genetic mutations, possibly induced by drug exposure, and be selected under drug pressure.

The term "tolerance" refers to the innate unresponsiveness of a parasite to a drug, independent of prior exposure to that drug or to others belonging to the same class.

In advancing the cause for the widespread use of drugs to control human helminths, Cerami and Warren (20) believed that "helminths are less likely to develop resistance or would do so more slowly" compared to other infectious agents because they multiply at a lower rate. This assumption has certainly not appeared valid for livestock helminths, justifying caution in the treatment of human helminths as well. AR may not be a medical problem yet, but for all we know the few reports so far may represent only the tip of an iceberg. Veterinary experiences have shown that the problems becomes apparent only when it is too late and reversion to susceptibility is no longer possible (31). Individual treatment failures may often remain unnoticed, since most helminth infections lead only to subclinical disease. Epidemiologically, there have been few efforts so far to examine or monitor the problem. The development of drug resistance, and AR in particular, usually follows a sigmoidal pattern: a long period of incubation with only a few scattered cases is followed by a sudden explosion of the problem (145). Once AR becomes apparent, it may very quickly become a major problem in both clinical and preventive medicine.

For more than a decade, veterinary researchers have drawn the attention of the medical community to the risk of AR development in human helminths, such as schistosomes and hookworms (26, 28, 62, 128). Drawing from the lessons and errors in their own field, they urged medical workers to use anthelmintics more carefully in order to avoid or at least to delay the development of AR. Nevertheless, the widespread drug use for the control of schistosomiasis, onchocerciasis, and geohelminths has been increasingly advocated by scientists and international organizations, with drug companies willing to offer assistance (1, 17, 113, 151). In light of these issues, in this paper we critically review the available evidence for drug resistance of human helminths at present and discuss the prospects for the future, taking the veterinary experiences into account.

REPORTS ON DRUG RESISTANCE IN HUMAN HELMINTHS: A CRITICAL ANALYSIS

Early reports on possible resistance to santonin in *Ascaris lumbricoides* (86) and diethylcarbamazine (DEC) in *Onchocerca volvulus* (143) were not well documented and cannot be assessed for accuracy and relevance. In this section, we concentrate on the more recent and better documented reports on AR of human nematodes (hookworms) and trematodes (schistosomes). AR of human cestodes has not yet been reported. Also, livestock cestodes do not seem to develop drug resistance easily; only a single report of drug resistance in tapeworms of sheep (*Moniezia expansa*) has been published (144).

Drug Resistance in Nematodes

Use of anthelmintics. The main drugs used to treat human nematodes nowadays are mebendazole, albendazole, pyrantel pamoate, and levamisole for intestinal nematodes, ivermectin (IVM) for onchocerciasis, and DEC alone or DEC-albendazole and IVM-albendazole combination treatments for filariasis (1, 35, 154). Depending on local epidemiology, availability, and cost, these drugs have been widely available in most health care systems for the curative treatment of clinical cases for many years. In addition, the use of anthelmintics is now being strongly advocated in a preventive, population-based way as well (1, 17, 113, 151, 155). It is estimated that some 1.3 to 2.0 billion people in the world suffer from helminth infections. Although direct mortality is low, intestinal helminth infections are believed to contribute to "general morbidity." Both intestinal helminths and schistosomiasis have been associated with anemia, stunted growth, poor nutritional status, and reduced physical and intellectual abilities (17, 18, 151); onchocerciasis has been associated with severe itching, skin diseases, poor health, and even reduced chances for marriage. By providing single-dose anthelminthics on a regular basis to entire populations or high-risk groups (such as schoolchildren and pregnant women), it is hoped to reduce both morbidity and transmission. It has even been proposed to combine albendazole, IVM, and praziquantel (PZQ) at a low dose in a single tablet and to distribute it to virtually all school-age children in the developing world (148, 149). The proponents of these strategies recognize the risk of emergence of AR but usually judge it to be insignificant. As mentioned above, veterinary experiences dictate otherwise. The recently published reports on AR in human helminths must thus be taken seriously, yet examined critically.

Problems of defining drug resistance in hookworms. It should first be noted that complete cure of hookworm infection (and most other helminth infections for that matter) is usually not achieved with any drug. Depending on the dosage and the coprological method applied (with lack of standardization and control methods being a noteworthy problem), cure rates as low as 61% (400 mg) and 67% (800 mg) for albendazole, 0% (single dose) and 23% (repeated dose) for levamisole, 30% (single) and 37% (repeated) for pyrantel pamoate, 27% for thiabendazole, 19% (single) and 45% (repeated) for mebendazole have been reported (35, 88).

Thus, at least some hookworm populations show some degree of (innate) tolerance to at least one of the drugs currently in use. The different susceptibilities of the two species *Ancylostoma duodenale* and *Necator americanus* is well established. Most probably, the degree of tolerance varies regionally, even locally, within a species.

Second, the results of field trials depend critically on the coprological methods used. The number of hookworm eggs per

gram (EPG) measured by the Kato-Katz method, commonly used for schistosomes, is unreliable if not strictly standardized. This method consists of measuring 25 to 50 mg of sieved stools in a punched template, after which the sample is allowed to clear with glycerin. Since hookworm eggs tend to dissolve quickly and uncontrollably, the slides must be examined within 30 to 60 min of preparation (96, 110). In the field, however, Kato slides are often difficult to read, unless the thick fecal smear has been allowed to clear for at least several hours, particularly when the feces are hard or dark or when quantities over 25 mg are examined, such as in the commonly applied Kato-Katz technique (83, 106, 109, 131). To quantify hookworm eggs correctly and certainly to compare the number of EPG between individuals or groups or over time, the method must be strictly followed. Qualitative methods, such as $ZnSO_4$ flotation or Ridley's formol-ether concentration, allow only semiquantitative determinations at best. The most sensitive method, stool culture, is laborious and also only semiquantitative. It is noteworthy, however, that the few therapeutic trials in which this method was applied have resulted in considerably lower cure rates than were reported with other methods, and this holds for most of the drug regimens in use (88). Finally, even correctly measured egg counts or EPGs must be interpreted cautiously, since they are only an indirect measure of worm counts (the actual outcome indicator of transmission and treatment) and are subject to inter- and intraindividual variations (38, 74).

In contrast to veterinary helminthology, in which methods and cutoff values to define AR are well established and standardized (27), there are no such guidelines in human helminthology. In vitro methods for the biological confirmation of AR have not been developed or validated for human nematodes. Also, the local endemic situation and the timing of follow-up are of paramount importance in tests for the detection of AR, and this is true in different ways for different species and drugs. In endemic situations, people (particularly children) who were cured are reinfected quickly and may reach the pretreatment level of infection within a few months. Moreover, they may carry prepatent infections which are affected by some drugs but not by others such as mebendazole, which is hardly absorbed.

Therapeutic trials for treatment of human helminths demand rigorous statistical methods, since the worms are overdispersed (i.e., a large number of worms are present in a small proportion of the hosts) within a population due to physiological, immunological, ecological, and behavioral factors. The study and control populations must therefore be large enough and randomly selected, and upon analysis any cluster bias must be excluded. A few "wormy" people in one or another group may lead to fatal flaws in the analysis of the results (3, 18). Clearly, lack of validated methods and reference data, many confounding factors, and complex statistics complicate the interpretation of low drug efficacy.

Reports of drug resistance in hookworms. Two recent publications have invoked AR as the probable cause of failure of anthelmintic treatment of human hookworms. Both are community-based studies in field conditions, not clinical observations. De Clercq et al. (33) described a failure of mebendazole to treat *N. americanus* in Mali, whereas Reynoldson et al. (116) reported poor efficacy of pyrantel pamoate against *A. duodenale* in northwestern Australia. The salient features of both reports are summarized in Table 2. The authors mentioned other possible causes of reduced drug sensitivity of the hookworms such as a genetic change in the susceptibility of the local strain of hookworms (i.e., not through selection pressure by the drug) or host factors (such as local diets) which might have altered the pharmacodynamic properties of the drug. However, some features which were present in one or both localities are suggestive of possible drug resistance. Since regions in Mali and Australia are remote, relatively isolated areas with probably a rather limited influx of infected foreigners, local helminth populations may have been isolated with little dilution or replenishment by (susceptible) helminths from elsewhere. Under these circumstances, AR would develop more rapidly, because of the lack of influx of susceptible genotypes (2).

The possible development of resistance to mebendazole in human hookworms (Mali study) would not altogether be surprising, since benzimidazoles (BZ) are known to be relatively good selectors of AR (8, 118). In helminths of livestock, BZ resistance has appeared quickly and spread easily (31). On the other hand, the drug pressure in the Mali community was not especially noteworthy, as far as data are available (no history of previous mass treatments).

Pyrantel/morantel resistance in livestock helminths developed mainly as cross-resistance due to widespread use of levamisole (125). In the Australian study (116), there might be a plausible case for intense pyrantel pressure having led to specific resistance: it had been used for passive case detection as well as active community treatment for decades. Albendazole, which had not previously been used in this population before, worked perfectly, thereby also validating the hypothesis.

The hypothesis of drug resistance in the Australian situation was inspired by clinical suspicion of resistance in an area where pyrantel pamoate had been used for a considerable length of time in the community. The reported efficacy of pyrantel pamoate (cure rate [CR] 13%; egg reduction rate [ERR], 46%) at the given (relatively low and single) dose and for the particular species is below those documented elsewhere, although CR as low as 19% have been described (35). The reported ERR is based on Kato slides from a small number of subjects and may therefore be biased. The study did not include an untreated control group, a necessity for the correct interpretation in light of egg output variations or statistical bias due to aggregation. The follow-up period of 7 days was relatively short, and no in vitro confirmation was attempted. In conclusion, the situation and the data are suggestive but fall short from providing conclusive evidence.

In the Mali study, drug resistance was discovered within the context of a research project on schistosomiasis. Since there was no history of intense treatment or clinical suspicion of drug resistance, the local situation was not different from any other area of endemic infection in Africa. Single-dose mebendazole treatment is known to be of low efficacy, with a reported CR as low as 18% and an ERR as low as 46% (35). Few data are available from sub-Saharan Africa. Therefore, the low CR and ERR in the treated groups may be due to a general low susceptibility of African hookworms to that drug regimen, as well as to local resistance. Also, pyrantel, the control drug used, is known to have little activity against human hookworms (88). Furthermore, the Mali study relied on Kato-Katz slides from "overnight samples that were processed and examined on the same day" (33), which may have led to some overclearing of the slides and consequent underestimation of hookworm egg counts. The 4-week interval between treatment and examination was too long to distinguish treatment failure from rapid reinfection and/or maturing prepatent infections, particularly in a relatively high-transmission area and for a drug such as mebendazole, which does not affect immature infections.

Both a negative and a placebo group were included, showing ERRs of 37.5 and 32.5%, respectively. This may be considered suggestive of the poor efficacy of mebendazole but also of statistical and methodological bias. The in vitro confirmation

TABLE 2. Important features of reports on treatment failures of human hookworm infections[a]

Characteristic	Mali (*N. americanus*)	Australia (*A. duodenale*)
Helminth species		
Initial prevalence and transmission	High	Moderate
Previous drug exposure	In health centers	Community treatment
Anthelmintic drug	Mebendazole (Vermox)	Pyrantel (Combantrin)
Dose	500 mg/person	10 mg/kg
Treatment regimen	Single dose	Single dose
Study design		
No. of subjects	103	29
Random selection of subjects	Yes	Yes
Control group, other drug	Pyrantel	Albendazole
Control group, no treatment	Yes	No
Placebo	Yes	No
Coprological method	Kato-Katz	ZnSO4 flotation + Kato
EPG[b] after treatment (wk)	4	1
Cure rate (%)[c]		
Treated group	22.9	13.3
Control group, no treatment	25.0	ND[e]
Control group, other treatment	44.8	100
Placebo group	22.6	ND
Egg reduction rate (%)[d]		
Treated group	−6.5(increase)	−46.1(increase)
Control group, no treatment	39.5	ND
Control group, other treatment	75.0	100.0
Placebo group (vitamin C)	32.7	ND
In vitro assay (drug resistance)	Egg hatch test	ND

[a] Data from references 33 and 116.
[b] EPG, eggs per gram of feces.
[c] Percentage of treated (infected) persons becoming negative after treatment.
[d] Percent reduction of EPG after treatment compared to EPG before treatment.
[e] ND, not done.

of the Mali results was based on the egg-hatching technique, accepted in veterinary medicine but not yet standardized for human hookworms. A 50% reduction of egg hatchability was found compared to a laboratory strain; it is unclear if this difference is statistically or biologically significant. Strain differences, processing of the field samples, delays during transport, etc., may have affected the results. Again, this study is at best suggestive, but does not provide conclusive evidence for reduced mebendazole efficacy. This study has since been repeated using a more rigorous study design, in which the efficacies of three anthelmintics (mebendazole, albendazole, and pyrantel) against *N. americanus* were compared (121a). Participants were examined 10 days after treatment. After controlling for the drift in the fecal egg counts (opposite trends in male and female subjects) in the placebo-treated subset, age, sex, fasting, and intensity of infection, single-dose mebendazole (500 mg) treatment showed efficacies (ERR) ranging from 60.9 to 89.9%, depending on the method used for the evaluation of the results. The efficacies obtained using albendazole (single dose of 400 mg) and pyrantel (12.5 mg/kg) ranged from 92.1 to 99.7% and 4.8 to 89.7% respectively (121a). These results are more or less consistent with those reported elsewhere (35, 88). Thus, it remains a matter of conjecture whether pyrantel and mebendazole lack efficacy against *N. americanus* or whether resistance is beginning to develop.

In conclusion, AR in human hookworms might already be present, but the evidence to date is doubtful. Future studies should be carried out under well-controlled conditions and using standardized methods for trial design, calculation of summary data relating to drug efficacies, and statistical analysis to confirm the presence or absence of drug resistance in these or other human hookworms populations (121a). Ideally, clear hypotheses, standard protocols (in vivo as well as in vitro), and indisputable cutoff values should be established by a governing body and/or multidisciplinary groups of scientists, such as has been the case in veterinary medicine by the World Association for the Advancement of Veterinary Parasitology (WAAVP).

However, the doubts about the reported data should not lead to optimism or complacency. If anything, the critical review of these and earlier data shows that tolerance traits are indeed present in many hookworm populations. Even without taking into account the possibility of mutations, experience in veterinary practice suggests that these traits might quickly and irreversibly become dominant in helminths under drug pressure.

Drug Resistance in Schistosomes

Use of antischistosomal drugs. Praziquantel (PZQ) is the most common drug for the treatment of human schistosomiasis (32, 89, 155), since it is active against all the *Schistosoma* species (*Schistosoma mansoni*, *S. haematobium*, *S. japonicum*, *S. intercalatum*, and *S. mekongi*). In the field, particularly in community treatment, the usual dosage is 40 mg/kg of body weight in a single dose; higher dosages or split regimens result in lower compliance (89). In hospitalized patients, particularly for *S. japonicum* and *S. mekongi*, and for heavy infections with the other species, the recommended dose is 30 mg/kg, up to

three times daily, for two consecutive days (32, 35, 89). The drug is safe, with few or limited side effects; in heavy infections with *S. mansoni*, acute abdominal cramps and bloody diarrhea are frequent but always transient. CR with 40 mg/kg are usually between 70 and 90%; ERR are above 90% (32, 71, 89).

In endemic conditions, reinfection is the rule rather than the exception, particularly in children, who are heavily exposed and appear to be (innately or immunologically) more susceptible to infection than adults (72). Nevertheless, when the intensity and duration of infection decrease, treatment considerably reduces individual pathology and community morbidity (89, 155).

Several brands and generic formats of PZQ are now on the market. Although there is no indication so far that substandard products are a problem (103), some products are of unclear origin; it is advisable to select reputed production or wholesale companies complying with international quality control procedures. International competition has brought the initial high price back to about 40 cents per average dose. The World Health Organization has therefore recently called for a major effort to bring the drug within reach of all primary health care systems (101).

In several countries with major endemic infections, the drug is not only widely available for treatment but is also being actively distributed to prevent or control disease ("morbidity control"). Community-based treatment after active screening, through indiscriminate mass treatment, or in specific target groups is now the major control strategy in Egypt, China, Brazil, the Philippines, and several other countries (89, 155). For example, all school-age children and millions of adults are screened and, if necessary, treated every 6 to 12 months in Egypt. In high-prevalence areas, treatment is now given indiscriminately to the entire population (46). Out of concern for the appearance of drug resistance under such high drug pressure, an elaborate national monitoring system has been set up in which stool samples from apparent treatment failures are referred to regional research centers and subjected to in vivo and in vitro tests.

Oxamniquine, used at a dosage of 15 to 40 mg/kg, is active only against *S. mansoni*, with CR (>80%) and ERR (>95%) usually somewhat higher than with PZQ (71, 155). Although by and large a safe drug, oxamniquine may have troublesome side effects in some individuals, such as drowsiness, severe dizziness, and seizures. It is used mostly in Brazil and is not on the market any more in most of Africa because of the commercial dominance of PZQ.

Metrifonate is another, inexpensive drug, active only against *S. haematobium*, that was available until recently, but it is no longer available for the treatment of schistosomiasis.

Thus, there is presently only one general schistosomicide available, PZQ. The single available alternative, oxamniquine, is active only against *S. mansoni*. The emergence of resistance is therefore a frightening prospect, not only for disease control or prevention but also for curative use in clinical practice.

Reports on resistance to schistosomicides. As with nematodes, it should first be noted that CR and ERR in therapeutic trials with any drug for human schistosomes rarely reach 100%, even in situations where reinfection is excluded (32, 71). Moreover, reported cure rates considerably overestimate real CR. Many light infections (with EPGs below the detection limit of the coprological techniques) that persist after treatment are not detected by the usual diagnostic methods but require repeated or very sensitive examinations (37, 70). Thus, the recommended doses of schistosomicides should be considered subcurative (41). In light of these data, it is safe to assume that in schistosome populations, some individual parasites are tolerant to the drug to some degree, at least at the usual dosages.

Unlike for nematodes, robust parasitological methods for the measurement of egg counts are available for schistosomes, such as the Kato-Katz method for fecal eggs and urine filtration for urinary schistosomiasis (83, 106, 155). Moreover, the detection and quantitation of circulating antigens in blood and urine have added another quantitative tool for the evaluation of drug efficacy (34). On the other hand, day-to-day variation of egg output and antigen levels is substantial; e.g. the coefficient of variation of EPGs in seven consecutive stool examinations varied between 28 and 245% (50), and the relation between worm numbers in the blood and egg counts in excreta is even more indirect and statistically complex than for nematodes (37, 70).

Resistance of schistosomes to oxamniquine is undisputably documented, both in vivo and in vitro (23, 25). Epidemiologically, the phenomenon has remained remarkably limited to scattered areas in Brazil. Possibly, the resistance trait is disadvantageous to parasite survival and/or reproduction of schistosomes; also, the mutation may actually be induced by exposure of individual schistosomes to oxamniquine (16). Combined, these factors would explain a self-limiting process even under drug pressure. Since the use of oxamniquine is by and large confined to Brazil and since it is being replaced by PZQ, oxamniquine resistance is not considered to be a major problem.

Recent reports on the possible development of resistance to PZQ have generated much more unrest, particularly since this drug is at the basis of current control strategies aimed at the reduction of morbidity through population-based treatment (152, 153, 155). The first field report came from a new, intense, and epidemic focus in northern Senegal (72, 132). In a community with extremely high prevalences and intensities of infection, a CR of only 18% was observed using PZQ, much lower than is usually reported from other (even comparably intense) foci (132). However, ERR were still over 80%. Heavy initial infections, intensive transmission, prepatent parasites, and immunological naivety were considered the most likely explanations for these low CR. The possibility of drug resistance or tolerance could not be ruled out, however. Another hypothesis was that in such an epidemic focus, a clonal parasite population may have sprung from a few tolerant worms.

The matter was further investigated in a systematic series of field studies, the results of which can be summarized as follows. (i) The low CR with PZQ at 40 mg/kg (18 to 36%) in the field were confirmed in four more study cohorts, consisting of various age and infection-intensity groups, in different seasons, with different timings of follow-up surveys, and with circulating antigen detection (72, 130, 137). (ii) CR remained abnormally low when the dose was increased to two consecutive doses of 30 mg/kg at a 16-h interval (73). CR for oxamniquine at 20 mg/kg in a single dose, however, were normal (84%) (132). (iii) CR with PZQ at 40 mg/kg rose to normal when the treatment was repeated after 2 to 4 months and were also normal in children originating from the area of endemic infection but living in an urban area with no transmission (108; A. Mbaye, D. Engels, L. Tchuente, and B. Gryseels, unpublished results). (iv) The efficacy of PZQ could be related to age and pretreatment intensity but not to other host factors, including behavioral and immunological parameters (137). (v) Application of a statistical model relating egg counts more accurately to worm numbers showed that the poor CR could be explained by the initial high intensity of infection, even if over 95% of the worms were killed (S. J. de Vlas, D. Engels, A. Mbaye, and B. Gryseels, Schistosomiasis Res. Project Conf. Proc., p. 211, 1998).

The overall conclusion that may be drawn from these observations is that there is no convincing field evidence of reduced susceptibility of *S. mansoni* to PZQ and that the observed low CR may be explained by the specific epidemiological situation. Unfortunately, there is no reliable in vitro test available to determine PZQ resistance. In fact, a major problem in developing such a test is precisely the lack of a reference schistosome strain that is resistant.

Several experimental in vivo studies have recently been conducted to unravel the problem in Senegal. In short, these studies have shown the following. (i) It was possible to select from a mixture of *S. mansoni* strains kept for years in the laboratory a parasite population that was almost insensitive to PZQ treatment (51). However, it is probable that this result can be explained by the experimental protocol, in which mice were treated after 35 days of infection. Parasite lines with a slower maturation time would not yet be susceptible to PZQ at that time and would be selected under drug pressure as a "resistant" strain (22). (ii) In the same protocol, a "wild" Senegalese strain appeared to be less susceptible to PZQ (53). Remarkably, this observation was not consistent with the high ERR observed in the field, indicating reduced susceptibility at most. Again, it is quite probable that the result was an artifact of the early treatment of the infected mice. Subsequent studies with experimental treatment after 60 days of infection showed a markedly improved efficacy, albeit lower than in other geographical strains (52). (iii) In another laboratory, schistosomes isolated from Senegalese patients who had undergone several treatments but still (or again) excreted eggs did not show any reduction in susceptibility to PZQ (21, 22).

The consistent field observation of low cure rates with PZQ can apparently be explained statistically by the high initial worm burdens and possibly heavy immature infections (against which PZQ is not very effective), in combination with the inherent limits of the diagnostic system. Biologically, this hypothesis is supported by the high levels of circulating antigen (indicating heavy infections) and the results of repeated treatments and treatment in areas with no endemic infection, which gave normal cure rates. The "normal" results with oxamniquine can statistically be explained by a somewhat stronger inherent schistosomicidal effect. The results of the mouse experiments are conflicting; the only methodologically indisputable observation on reduced susceptibility is the geographical strain difference (52). Although geographical differences in drug susceptibility have not been described for PZQ, they are well known for hycanthone and oxamniquine, even leading to region-specific dosage recommendations (4, 32).

If anything, these studies lead to the conclusion that only a very substantial reduction in susceptibility can be detected reliably by current field methods. Laboratory confirmation is still compromised by the lack of standardization and reference material. The international effort to establish at least some tentative protocols and to coordinate the collection of data and material is therefore most welcome (114, 157).

Other, well-documented clinical and experimental reports come from Egypt, an area of endemic infection which, due to extensive drug usage, would seem predestined for the appearance of PZQ resistance. A nationwide monitoring system was set up to detect and investigate cases in which PZQ did not lead to cure, even after repeated treatment (9, 46). From several dozen cases, largely clustered in one geographical area, parasites were isolated that showed a reduced susceptibility in mice and in vitro compared to Egyptian reference strains (10, 81, 81a). Again, the lack of standardized methods, particularly in vitro, do not yet allow definite conclusions. At the very least, however, the possibility that less susceptible strains are (and possibly always were) present and are emerging more prominently under drug pressure cannot be excluded (10).

Conclusions

The recent reports on possible emerging drug resistance in human nematodes and schistosomes do not provide conclusive evidence for the increase of innately tolerant strains or for the appearance of newly mutated resistant strains. However, they strongly suggest that such tolerant or resistant strains can and do exist and that these strains may emerge more prominently under drug pressure (hookworm in Australia, schistosomes in Egypt) or under specific circumstances (schistosomes in Senegal). Perhaps even more important, the published studies show that available tools, methods, and reference materials are so far insufficient to detect problems of AR in a timely fashion, if at all. Therefore, we will review in more detail the knowledge of the veterinary world, which has a longstanding experience with AR, and analyze how it can be used to clarify and possibly remediate the situation in humans.

DRUG RESISTANCE IN LIVESTOCK HELMINTHS AND ITS RELEVANCE FOR HUMAN HELMINTHS

As described above, AR in livestock is now a well established fact. Several contributing factors have been identified and studied.

Contributing Factors for the Development of Resistance

High treatment frequency. Barton (6) and Martin et al. (98, 99) have shown in well-controlled trials that a high treatment frequency selects for resistance more strongly than do less frequent dosing regimens. There is also strong evidence that resistance develops more rapidly in regions where animals are dewormed regularly. Serious problems with AR in *Haemonchus contortus* were reported in some humid tropical areas where 10 to 15 treatments per year were used to control this parasite in small ruminants (42).

Drug resistance, however, can also be selected at lower treatment frequencies, especially when the same drug is used over many years. Several authors (7, 19, 29, 59) have reported the development of drug resistance even when only two or three treatments were given annually. This observation is important, since similar treatment frequencies are advocated for the control of intestinal nematodes in humans (17, 115, 148, 151).

Single-drug regimens. Often a single drug, which is usually very effective in the first years, is continuously used until it no longer works. In a survey of sheep farmers in the United States, Reinemeyer et al. (112) found that one out of every two flocks were dosed with a single anthelmintic until it failed. Long-term use of levamisole in cattle also led to the development of resistance, although the annual treatment frequency was low and cattle helminths seem to develop resistance less easily than do worms in small ruminants (58, 61). Frequent use of IVM without alternation with other drugs has also been reported as the reason for the fast development of resistance in *H. contortus* in South Africa and New Zealand (127, 139). In the light of these data, the frequent and continuous use of single drugs such as albendazole for the control of intestinal helminths, IVM for onchocerciasis, or PZQ for schistosomiasis in humans may raise concern. The quickness with which AR to BZ in livestock nematodes has spread is described above; if similar strategies are to be applied in humans, there is no reason why the same problems would not arise as well.

Because resistance of *H. contortus* in sheep and goats to

IVM has been widely reported (31), Shoop (127) has warned of the risk of AR problems in the onchocerciasis control programs in western Africa, which are increasingly based on periodic community-based treatment with ivermectin (113). Although the initial objectives of drug-based control strategies in schistosomiasis and helminthiasis were restricted to the reduction and prevention of disease in humans, they are now also advocated for the control and even interruption of transmission (17, 113, 156). Two IVM treatments per year for a period of at least 10 years are recommended to interrupt transmission of *O. volvulus* among humans (156). In countries such as Egypt, active antischistosomal community treatment with PZQ has been going on for more than a decade already and will be continued, even intensified, for the foreseeable future (46). AR probably will not develop as easily in helminths with an indirect life cycle (having the multiplicative part of their cycle in arthropods or molluscs) as in directly transmissible intestinal helminths. However, given sufficient time, intensive treatment strategies such as in Egypt may provide opportunities for resistant strains to appear and/or become dominant.

Targeting and timing of mass treatment. Prophylactic mass treatments of domestic animals have certainly contributed to the widespread development of AR in helminths. Although no data are available from experimental studies, computer models (5) indicate that the development of resistance is delayed when 20% of the flock is left untreated. This approach would ensure that the progeny of the worms surviving treatment will not consist only of resistant worms. Given the well-known overdispersed distribution of helminths, leaving part of the group untreated, especially the members carrying the lowest worm burdens, should not necessarily reduce the overall impact of the treatment.

In worm control in livestock, regular moving of the flocks to clean pastures after mass treatment and/or planning to administer treatment in the dry seasons is common practice to reduce rapid reinfection. However, these actions result in the next helminth generation that consists almost completely of worms that survived therapy and therefore might contribute to the development of AR (128, 134). For example, Coles et al. (29) reported problems with AR in the helminths of sheep and goats on some small Greek islands which suffered from extended drought; in contrast, no AR developed under similar management and deworming practices on the mainland.

In contrast to livestock, where nearly 100% of the animals of the herd or the flock are treated, population compliance is usually less than 80% in community-based mass treatment of humans: people are absent, not interested, ill, or pregnant. Often, compliance decreases further after the first few treatments, if only because of the reduction of morbidity. Moreover, populations are often not stable, and there may be an influx of neighboring or traveling communities (47, 48). Timing of treatment in dry, low-transmission periods has been proposed (155). In some areas of China, synchronized treatment of cattle and humans is applied in the hope of reducing transmission (121). However, such strategies are difficult to apply, if only because of organizational and logistical problems.

It may be hoped (but not guaranteed) that these typically human factors will delay (but not prevent) the occurrence and spread of AR in humans. However, if regular treatments are focused mainly on school-age children (intestinal worms) or in isolated communities (onchocerciasis), groups in which participation is well controlled and even reinforced and in which transmission may occur in a relatively closed ecological system, the situation and risks may be not that different from those in livestock.

Underdosing. Underdosing is generally considered an important factor in the development of drug resistance, because subtherapeutic doses might allow the survival of heterozygous resistant worms (128). Several laboratory experiments have shown that underdosing indeed contributes to the selection of resistant or tolerant strains (43, 78). Some indirect field evidence further supports this assumption. Recently, it was shown that the bioavailability of BZ and levamisole is much lower in goats than in sheep and that goats should be treated with dosages 1.5 to 2 times higher than those given to sheep (77). For many years, however, sheep and goats were given the same anthelmintic doses. The fact that AR is very frequent and widespread in goats may be a direct consequence. Recent modeling exercises suggest that the field situation of AR is not always as simple (129). Depending on the initial frequency of the resistance alleles, there might be a range of dose levels where underdosing promotes resistance and a range of dose levels where it actually impedes resistance.

Although further research on the impact of underdosing on resistance development is necessary, current knowledge advises against the use of subcurative dosages. To reduce the costs of anthelmintic treatment campaigns in developing countries, the use of lower dosages than the recommended therapeutic ones has been advocated (151). Such practices should clearly be avoided. As shown above, most of the currently applied anthelmintics are in fact subcurative in at least part of the population. This is considered acceptable for morbidity control, but in the long run such strategies may contribute to the development of AR as well.

Underdosing in humans occurs widely in many developing countries. Drugs are commonly shared or used at half (or less) the normal doses by poor families. Furthermore, generic products of substandard quality, repacked and/or reformulated products, and expired drugs are widespread in pharmacies and general markets. Also, the presence of poor-quality drugs has been documented in human as well as in veterinary medicine (104, 126, 141). Human drugs, especially antibiotics and anthelminthics, are produced by a large number of unlicensed companies all over the world. Quality control of these drugs is usually lacking.

Mechanisms of Drug Resistance

Benzimidazoles. The best known mechanism of resistance is the one to BZ. No information is available about the resistance mechanisms present in BZ-resistant human hookworms, but veterinary helminthologists have studied BZ resistance of *H. contortus* in detail. The BZ exert their anthelmintic activity by binding to β-tubulin, which interferes with the polymerisation of the microtubuli. Several authors (9, 120) showed that there is an extensive polymorphism of the β-tubulin gene in susceptible *H. contortus* populations. Roos et al. (120) proved that selection for resistance to BZ is accompanied by a loss of alleles at the locus of β-tubulin isotype 1. Kwa et al. (91) nicely demonstrated that resistance to BZ is correlated with a conserved mutation at amino acid 200 in β-tubulin isotype 1 (with Phe being replaced by Tyr).

The same mutation was shown to occur in BZ-resistant fungi such as *Aspergillus nidulans* and *Venturia inaequalis* (82, 85). The functional importance of this amino acid substitution was shown by heterologous expression of the β-tubulin isotype 1 (isolated from BZ-susceptible *H. contortus*) in BZ-resistant *Caenorhabditis elegans*. Expression of the *H. contortus* gene altered the phenotype of transgenic *C. elegans* from resistant to susceptible. Conversely, when Phe was replaced by Tyr at

amino acid position 200 of this gene by in vitro mutagenesis, the reverting activity was lost (92).

A second resistance mechanism was identified in some *H. contortus* populations showing higher levels of resistance and in which a deletion of the β-tubulin isotype 2 locus was shown (120). However, Beech et al. could not confirm this in other BZ-resistant *H. contortus* populations (9). These authors also showed that changes in allele frequencies rather than novel rearrangements induced by exposure to the drug explained changes associated with BZ resistance. A similar stepwise selection of BZ resistance also occurs in some *Trichostrongylus colubriformis* and *Ostertagia circumcincta* populations (45, 68). Furthermore, Kerboeuf et al. (84) recently provided indirect evidence that P-glycoproteins (P-gp) also play a role in BZ resistance in *H. contortus*. P-gp are involved in multidrug resistance in mammalian tumor cells, *Leishmania*, and *Plasmodium* and in resistance to toxic compounds in *C. elegans*. Rhodamine 123, a P-gp transport probe, associated with the reversal agent verapamil (an inhibitor of multidrug resistance-associated proteins), gave significantly higher levels of fluorescence in eggs from *H. contortus* resistant to BZ and IVM than in susceptible eggs. These results confirm those obtained with biological drug assays using both anthelmintics and verapamil and reinforce the probability of a P-gp-like dependent efflux in nematode eggs, which could be involved in resistance to xenobiotics. However, Kwa et al. (90), using a P-gp gene probe from *H. contortus*, were not able to correlate polymorphism to any of the (multi)drug resistances examined in different *H. contortus* populations. It should be noticed that the DNA used by Kwa et al. (90) was prepared from pooled L3 larvae and not from individual parasites, so that no estimates of allele frequencies could be made (2). Since at least 14 P-gp genes seem to be present in *C. elegans*, it is also possible that P-gp other than those characterized by Kwa et al. (90) or multidrug resistance-associated proteins might be involved in drug resistance. Blackhall (personal communication) recently found that the same gene, encoding a P-gp which is responsible for resistance to IVM and moxidectin, is also involved in BZ resistance.

Since specific BZ resistance seems to be due to similar point mutations in several fungi and nematodes of veterinary importance, it is not unlikely that it would be relevant for resistance in human nematodes as well. Since similar molecules are used in human and veterinary medicine, it would be worthwhile to look for the presence of these point mutations in human helminths as well.

Levamisole. Levamisole and the related anthelmintics pyrantel and morantel are cholinergic agonists with a selective action on nematode receptors. The mechanism of resistance to levamisole has not yet been elucidated. Sangster (122) thoroughly reviewed the pharmacology of levamisole resistance. It is thought to be caused either by a reduction of the number of nicotinic acetylcholinesterase receptors or by a decreased affinity of these receptors for the drug. Hoekstra et al. (79) were able to clone the gene *Hca 1*, encoding the nicotinic acetylcholinesterase receptor from *H. contortus*. Although polymorphism at the amino acid level could be demonstrated, these authors could not find evidence that alleles at this locus were involved in selection for resistance to levamisole. A similar gene, *tar-1*, was identified on the X chromosome in *T. colubriformis* (150). However, although statistical comparison of allele frequencies from individual male and female worms was consistent with sex linkage of *tar-1*, no correlation was found with levamisole resistance status.

Ivermectin. IVM and other macrocyclic lactones affect gastrointestinal nematodes by causing starvation and/or paralysis by opening chloride channels, which are thought to be associated with alfa-subunits of glutamate-gated ion channels located on muscles of the pharynx and possibly the somatic musculature (122). Rohrer et al. (117) compared IVM-resistant and -susceptible *H. contortus* populations and found that resistance is not due to an alteration in the binding of IVM to glutamate gated chloride channel receptors. Nevertheless, Blackhall et al. (13) did report that one allele of the putative alfa-subunit gene is associated with resistance to the drug. Recently, Blackhall et al. (12) reported considerable genetic variation of a P-gp locus in *H. contortus*. In several drug-selected strains of the parasite, selection for the same allele was observed. Using different approaches, Xu et al. (158) and Sangster et al. (124) came to the conclusion that P-gp might be involved in resistance to IVM in this helminth species. Other mechanisms of resistance may be present as well, as suggested by Gill et al. (64) and Gill and Lacey (65). The latter described five possible types of resistance to IVM in *H. contortus* based on different behavior in in vitro tests (larval development assay and L3 motility tests), different sensitivity to paraherquamide (an anthelmintic with a completely different structure and different binding sites from IVM), and different inheritance (in at least two of the five resistance types). Gill and Lacey (65) also suggested that the mechanism of resistance to IVM might be different from one species of helminth to another, because the critical events leading to expulsion have been shown to be different, e.g., when *O. ostertagi* is compared to *H. contortus* and *T. colubriformis*. Further research is needed to confirm these observations, to which the relevance to human *O. volvulus* is at present not clear.

Antischistosomal drugs (oxamniquine and praziquantel). The mechanism of action of oxamniquine is closely associated with its irreversible inhibition of nucleic acid synthesis in schistosomes (23). Based on cross-breeding experiments using susceptible and drug-selected schistosome strains exhibiting stable resistance, Cioli et al. (24) suggested that oxamniquine is not bioactivated in resistant worms, allowing them to survive the drug action. The activating enzyme, which is present in sensitive and absent in resistant schistosomes, seems to be a sulfotransferase. There is no clear understanding of the mode of action of PZQ, which also hampers the elucidation of possible mechanisms of resistance to PZQ. Redman et al. (111) have reviewed the existing knowledge and consider the PZQ-induced Ca^{2+} influx across the tegument as vital in the effect of this drug. However, the mechanisms leading to this alteration in Ca^{2+} homeostasis are not clear at all (22).

Genetics of Drug Resistance

Nematodes. Nematode parasite populations are genetically heterogeneous and thus able to respond to selective pressures, i.e., anthelmintic drugs (67). Widespread drug pressure will favor and select parasite lines carrying tolerance or resistance alleles. The rate at which resistance spreads in the parasite population depends on many factors. One key factor is the proportional contribution that helminths surviving therapy will make to the next generation. This contribution is influenced by the drug pressure (frequency and timing of treatment), the drug efficacy, the gene flow (the introduction of susceptible genotypes from elsewhere), the generation time and fecundity of the worms, the frequency of resistance alleles prior to drug use, the number of genes involved, and the dominance or recessiveness of these genes. Since it is quite difficult to set up experiments to examine the influence of these different factors, several mathematical models have been developed to simulate the development of AR in gastrointestinal helminths (5, 63, 128, 129). Although these models have their limitations and

must certainly be interpreted with caution (39), models such as the one of Barnes et al. (5) concerning *Trichostrongylus colubriformis* in grazing sheep provide interesting insights. The model allowed up to three genes for drug resistance, each with two alleles, that were combined independently under random mating. Worms of all genotypes were assumed to be equally fit in the absence of the anthelmintic. The initial frequency of resistance alleles in the worm population was assumed to be very low and was set at 0.01%. To examine the effect of using either mixtures of two drugs or rotations of a single one, two independent genes for resistance to two drugs (with different mechanisms of action) were simulated, with resistance being codominant and each drug killing 99, 50, and 10% of worms of homozygous susceptible (SS), heterozygous (RS), and homozygous resistant (RR) genotypes, respectively. The simulations were run for a period of 20 years, with treatment once a year for the ewes and three times a year for the lambs. These resulted in little development of resistance when the two drugs were used together (mixture). Substantial resistance, however, developed for all rotation strategies, 1-, 5-, and 10 yearly, with slowest development of AR in the annual rotation strategy. Assuming equal initial drug efficacy and equal resistance allele frequency, resistance developed more rapidly if it was determined by a single gene than when two or more genes were involved. Furthermore, resistance evolved fastest when it was dominant, slower when it was codominant, and slowest when it was recessive. When 20% of the flock was never treated, resistance was delayed at the expense of worm control.

It should be noted, however, that this and most other models are deterministic, ignoring the overdispersed distribution of free-living and parasitic helminth stages. Smith et al. (129) used a stochastic model to examine the effect of aggregated parasite distributions on parasite mating probabilities and the spread and maintenance of rare (resistant) genotypes. They concluded that spatial heterogeneity in transmission might be a significant force in promoting the spread of resistant genotypes, at least when infection levels are low.

When modelling exercises are compared with current knowledge of genetics of AR in helminths of livestock, the most striking and alarming observation is the high frequencies of resistance alleles observed in untreated populations of livestock helminths of veterinary importance. Beech et al. (9) analyzed individual genotypes of susceptible *H. contortus* before any exposure to BZ and reported initial frequencies of resistance alleles of 46 and 12% at the isotype 1 and isotype 2 β-tubulin loci, respectively. Anderson et al. (2) suggested that similar high frequencies associated with IVM resistance might occur in unselected lines of the same helminth species. The numbers of Beech et al. (8) may be overestimations, but they indicate that resistance alleles in untreated helminth populations of livestock—and maybe also humans—might be much more common than is usually assumed in the theoretical models.

Contradictory reports have been published regarding the number of genes involved in AR and their dominance or recessiveness. The available information, mainly on *H. contortus*, has been summarized by Anderson et al. (2). BZ resistance in this parasite seems to be polygenic; at least two, possibly three, genes with recessive alleles are involved. Levamisole resistance in *H. contortus* and *T. colubriformis* is probably due to one single major gene or gene cluster, the alleles of which are autosomal recessive for the former and sex-linked recessive for the latter (2). Resistance to IVM in *H. contortus* appears to be mediated by a single gene or gene complex with primarily dominant effects. IVM resistance might thus develop quite fast, as appears to be confirmed by field observations in South Africa, where IVM resistance in *H. contortus* developed after only three treatments (139). Avermectin and mylbemycin resistance is now widespread in *H. contortus* and *O. circumcincta* of small ruminants all over the world but remarkably not in *T. colubriformis* (123).

Obviously, these veterinary experiences and findings are of considerable relevance to humans. The presence of tolerant strains to anthelmintics in any parasite population has been demonstrated; as far as biological observations and statistical extrapolations allow, the proportion of innately resistant helminths is on the order of percentages (10^{-2}), not of 10^{-3} or less, as previously thought. Virtually all strategies proposed and implemented to date for human intestinal helminth control are based on a single-drug approach, without combination or rotation, and at a minimal frequency of once a year for a considerable length of time. Although the situation with livestock is different from that of humans and the results or simulations cannot be automatically extrapolated, the biological, epidemiological, and pharmaceutical similarities are of concern. Research should focus on genetic and related phenotypic similarities with relevance to AR in livestock and human helminths. Modeling and simulation studies, which have been applied to advance the cause for large-scale treatment programs in humans (17, 113) should be used to project possible side effects and AR in particular.

Trematodes. The genetics of resistance of schistosomes to oxamniquine are quite well known, but this is not the case for PZQ. In contrast to the development of classical drug resistance in helminths, which spreads gradually through a population as a consequence of selection of resistant phenotypes present at low frequency, resistance to hycanthone-oxamniquine appeared universally in the first filial progeny of parasites exposed to the drug (16). This strongly suggests that resistance is induced rather than selected from preexisting forms (16). The crossbreeding experiments of Cioli et al. (23, 25) and Pica-Mattocia et al. (107) have clearly shown that oxamniquine resistance is controlled by a single autosomal recessive gene. Resistance to oxamniquine does not appear to spread easily within communities but, rather, tends to remain limited to individual cases. According to Cioli et al. (25), this could be due to a selective disadvantage of resistant schistosomes in the absence of drug pressure. The fact that resistance is induced rather than selected might also contribute to this phenomenon.

Little is known about the genetic or biochemical background of possible resistance to PZQ. Recently, genetic differences have been demonstrated between a laboratory strain of *S. mansoni* selected for resistance to PZQ and the parent susceptible strain (105). Although these authors did not detect any major genomic rearrangements in these strains, they showed that mRNA encoding a fragment of the subunit 1 of cytochrome *c* oxidase was overexpressed about 5- to 10-fold in the resistant strain compared to the susceptible one. Further research is necessary to examine whether a similar phenomenon is also present in field strains suspected of resistance to PZQ and whether other genes are also differentially expressed in resistant strains of *S. mansoni*.

Detection of Drug Resistance

Fecal egg count reduction test. The most commonly used test to detect problems of anthelmintic resistance is the fecal egg count reduction test (FECRT), which compares the egg count before and after treatment with an anthelmintic drug. A standardized protocol for the FECRT is available for the detection of anthelmintic resistance in nematodes of veterinary

importance (27). In small ruminants, fecal samples are taken from two groups of at least 15, preferably young animals, which have been bred on the farm and not treated in the previous 8 to 12 weeks. Animals are randomly distributed into a treatment and a control group. Fecal samples are collected 10 to 14 days after treatment. To reduce the workload, no pretreatment samples may be taken; it has been shown that comparing treatment and control groups posttreatment is as reliable as comparing pre- and posttreatment samples. Egg counts are performed using a standardized McMaster method (27). The EPG in the feces of the control group should be higher than 150 to allow valid comparison. The following formula is used to calculate the percent reduction of the EPG: ERR = $100(1 - X_t/X_c)$, where X is the arithmetic mean EPG and c and t indicate the control and treated groups respectively. According to the guidelines of the WAAVP, drug resistance in helminths of small ruminants is considered to be present when ERR <95% and the lower 95% confidence interval is below 90%. If only one of both criteria is met, resistance is suspected (27).

This protocol could help guide the development of a standard approach for AR in humans, but modifications must be made because of significant differences between animals and humans. To start with, the objective is different: in livestock, the test is used as a routine local confirmation of known AR. In humans, the challenge is still to demonstrate that AR exists at all. Furthermore, study populations of humans are much more heterogeneous than are those of animals: there is a loss of compliance in follow-up, sample collection is not evident, and individual behavior (concerning exposure as well as health-seeking behavior) can have an important impact on the test parameters. Finally, the infecting worm species are different and require other coprological methods.

Taking into account the methodological problems experienced in the past in defining drug resistance in human helminths (see "Problems of defining drug resistance in hookworms" above), a standard protocol to detect AR in humans under field conditions could include a number of standard elements, such as study groups and parasitological methods.

(i) Study groups. Studies to confirm suspected drug resistance, particularly for a compound for which this has not yet been convincingly reported, should include at least a treatment group (with the compound under study) and a nontreated group (possibly placebo). Preferably, a "positive" control group, treated with another, nonrelated and presumably efficacious drug should also be included. The drugs should be of undisputable origin and quality, and adequate dosages should be used, i.e., those recommended for clinical use, not the subcurative doses applied for community-based morbidity control, with individual dosages adapted to actual body weight. The tablets must be swallowed under direct observation; particularly in young children appropriate syrup or suspension formats should be used. People who vomit or have severe diarrhea shortly after treatment should be excluded from the cohort. Apart from toxicity reasons, pregnant women and people with systemic illnesses should also be excluded, since pharmacological and immunoparasitological dynamics may be disturbed. Pharmacodynamical studies are not essential from the start but should be conducted before conclusions about drug resistance are made.

Sample sizes should be determined using a statistical power analysis based on a quantified hypothesis; i.e., for each tested anthelmintic, a normal and an abnormal CR and ERR should be defined beforehand. As is made clear by the above discussion, there are currently no generally accepted normal rates. An international concerted action to determine reference data would be useful.

The study group composition must be statistically similar for age, sex ratio, and pretreatment mean egg count, and this includes averages as well as distribution. Children and adults should be considered different populations. Other possible confounding factors which may lead to differential exposure patterns, such as socioeconomic class, occupation, school attendance, and religion, must be avoided as well. The groups should ideally be selected from one more or less homogeneous population (e.g., one village) and should be studied simultaneously to avoid spatial and temporal variations of transmission. None of the study subjects should have received treatment with the drug or a related compound in the previous 3 (nematodes) to 12 (schistosomes) months, since such subjects may be in the process of "rebuilding" their parasite load.

Given these requirements and the unavoidable dropout rate of study subjects, initial sample sizes should probably be not less than 50 children or adults in each study group, if only to validly test the distribution pattern of the egg counts. The pretreatment egg counts should be sufficiently high to allow meaningful statistical interpretation, taking into account the detection level of the coprological method.

All ethical conditions must be met: fully informed consent of subjects and/or their parents; treatment of negative controls immediately after follow-up or earlier if clinically necessary; monitoring and management of side effects; and permission of local and national health authorities.

(ii) Parasitological methods. A standardized egg-counting technique should be used to determine individual egg counts. For schistosomes, *Ascaris* and *Trichuris*, the Kato technique can be used in a standard way, as described by Katz et al. (83), Peters et al. (106), or Polderman et al. (109). Slides should preferably be stored for later reference and quality control.

For hookworms, utmost care must be taken to validate and standardize the Kato technique. Martin and Beaver (96) recommended reading the slides after 30 min and not later than after 60 min. This was based on only a few clinical samples, however. In the field, stool consistency and transparency can vary widely between individuals and communities. In any case, Kato slides based on stool samples of more than 25 mg, such as the standard Kato-Katz, can hardly be read after only 1 h (106) and are thus not suitable for standardized quantitative hookworm research. Reading all slides within a narrow window of time after preparation requires a rigidly organized and supervised field setup. Ideally, an adapted Kato technique in which hookworm eggs are preserved or alternative methods comparable to the veterinary FECRT should be developed. There is a great need for the development, optimization, and validation of a standard protocol, without which further field studies on AR in hookworms will remain severely handicapped.

Fecal helminth egg counts show strong day-to-day, interindividual, and intraindividual variations, both for nematodes and for schistosomes (50, 69, 75). To obtain more accurate schistosome egg counts at the individual level, a minimum of three stool samples must be examined (49, 50).

If the focus is on CR (e.g., to establish fully curative doses), the most sensitive coprological (qualitative) methods should be used in conjunction with the quantitative ones, such as glycerine sedimentation for schistosomes and cultures for hookworms (8). The qualitative methods are essential in at least a subsample to determine the exact species involved.

The statistical interpretation of mean egg counts is complicated. Scientific accuracy demands the use of models which relate the egg count to worm burdens, the underlying outcome parameter of treatment. Direct use of EPG assumes a proportional relationship, which is far from the biological and statistical truth. Practical statistical tools to that end are not readily

available and have so far only been developed for schistosomes (38). For simplicity, mean EPGs can be used for a first crude analysis and may be sufficient to reject the hypothesis of resistance. As shown by the Senegalese experiences with PZQ, however, more sophisticated analysis is essential before definite and far-reaching conclusions can be drawn. In veterinary science, arithmetic mean egg counts are preferred over geometric mean counts because they are more sensitive and allow an earlier detection of resistance (102). This may be justified in situations where AR is known to exist and needs only to be confirmed in a particular situation. Statistically, however, arithmetic means are by no means valid, due to the strongly aggregated helminth egg counts, which usually follow a negative binomial distribution (3). Geometric means are more appropriate although not yet ideal, since the distribution patterns change after intervention.

The interval between treatment and sampling should be adapted to each parasite species and to the drugs used. For example, for the evaluation of the efficacy of BZ in treating hookworms, a period of about 2 weeks is appropriate. A longer period would allow immature or even new infections to become patent, while a shorter one may overestimate efficacy, since some drugs temporarily suppress egg production without killing the worms.

For schistosomes, the problem of distinguishing active from immature or even past infections is somewhat more complicated. Since the worms live in the blood vessels, eggs follow a long and difficult path from this intravascular location to the outside world and may be excreted up to 6 to 8 weeks or even longer after their production. The Kato method does not distinguish dead from live eggs. On the other hand, immature infections, which are not affected by PZQ, can become patent days after successful cure of adult worms. Newly contracted infections may result in egg excretion within 4 to 6 weeks. The ideal solution for this dilemma would be to consider only patients outside the area of endemic infection and to evaluate cure after 8 to 12 weeks or even longer. In practice, this can be done only for tourists, who usually have uncharacteristically light infections. A pragmatic and generally accepted compromise is to evaluate cure in an area of endemic infection after 5 to 6 weeks of treatment (71, 72, 114). However, the results will always have to be interpreted in the light of possible reinfection (including maturation of prepatent infections) in high-transmission areas. If possible, treatment trials should take place in a non- or low-transmission season.

The quantification of circulating antigens, particularly in serum, can be a useful complementary tool (34). Cure can be assessed within a few days to a week after treatment, and so is much less sensitive to rapid reinfection. However, antigen detection cannot fully replace egg counts, since 5 to 30% of the infections are still missed (34); the assay is not commercially available and requires much more laboratory infrastructure than does the egg count method.

It may be clear from the above that valid data to confirm AR in the field requires considerable expertise in parasitology and epidemiology, well-trained field teams, careful organization, and strict quality control and that it is vital for further studies to improve and establish appropriate methods and standard protocols (157).

Laboratory tests for detection of resistance in livestock helminths. A variety of different laboratory tests have been described for the detection of AR in livestock helminths (31). Those which are most commonly used and which might be applied to detect AR in human helminths are briefly described here.

(i) Egg hatch test. The egg hatch test is an in vitro test, which is used only for the detection of BZ resistance in livestock helminths; it is based on the ovicidal activity of this group of molecules. The original test was described by Le Jambre (94); a standardized protocol was adopted by the WAAVP (27). Freshly collected fecal samples (within 3 h of being shed) are needed to obtain reliable data. If this is not possible, samples must be stored anaerobically; this storage does not influence the outcome of the test, at least for the major gastrointestinal helminths of small ruminants (80). Helminth eggs are purified and incubated with a series of concentrations of thiabendazole (TBZ). This compound was selected because it dissolves readily in dimethyl sulfoxide and because side resistance is usually present with other members of the BZ group. After 24 h, the number of hatched larvae is counted. When resistance develops, the ovicidal activity decreases, which results in a higher percentage of eggs that hatch. Based on vast experience with the test, WAAVP considers resistance to be present when the 50% effective dose is $\geq$0.1 μg/ml (27). This in vitro test has the advantage of requiring only one fecal sample. However, several authors have reported poor correlations between the results of the FECRT and the egg hatch test for helminths of livestock (14, 42).

Unfortunately, the FECRT and the egg hatch test detect resistance only when at least 25% of the worm population carries resistance genes, as shown by artificial infection of animals with mixtures of helminth populations with a known level of AR (97). Since reversion to susceptibility is considered to be possible only as long as resistance genes are present in less than 5% of the helminth population (119), FECRT and egg hatch assays allow the detection of AR only when it is too late to interfere. Field and experimental data for helminths of livestock indeed indicate that reversion to susceptibility to anthelmintic drugs in livestock helminths rarely occurs once resistance has been confirmed (31).

(ii) Larval development assay. The larval development assay is more laborious and time-consuming than the egg hatch test but allows the detection of resistance to the major broad-spectrum anthelmintic classes, including the avermectins-mylbemycins. It was originally described by Coles et al. (30) and further improved by several others (66, 93) and is now commercially available (DrenchRite; Horizon Technology). In the larval development assay, nematode eggs or L1 larvae are exposed to different concentrations of anthelmintics incorporated into agar wells in a microtiter plate. The effect of the drugs on the subsequent development into L3 larvae is measured. The results correlate well with those of in vivo tests. It is claimed that this test is more sensitive than FECRT and egg hatch test and detects AR when about 10% of the worm population carries resistance genes (40), but this remains to be proven.

(iii) Larval motility or paralysis test. Several in vitro assays to detect resistance to BZ, macrocyclic lactones or levamisole-morantel have been described which are based on the motility of larvae (31). For the latter group of anthelmintics, a clear-cut distinction between susceptible and resistant strains is not always possible (60, 142). A similar motility test has been used to evaluate the sensitivity of *O. volvulus* microfilariae to ivermectin (135). To render the interpretation more objective, a micromotility meter has been developed (11). Folz et al. (55, 56) used this apparatus to detect drug resistance in *H. contortus* and *T. colubriformis*, but other authors have found it less reliable (142; S. Geerts, unpublished results).

(iv) PCR. The first specific primers to detect drug-resistant parasitic nematodes were developed by Kwa et al. (91). These primers discriminated between heterozygous and homozygous

BZ-resistant *H. contortus* for the alleles in question (β-tubulin isotype 1), even when these genotypes are phenotypically indistinguishable, and could also identify BZ-resistant *T. colubriformis*. According to Roos et al. (120), PCR detected 1% of resistant individuals within a susceptible worm population, a tremendous improvement over other in vivo and in vitro tests.

Recently, Elard et al. (44) developed a more simplified method for the diagnosis of BZ-resistant *O.* (*Telodorsagia*) *circumcincta*. Using four primers (two allele-specific and two nonallele-specific ones) in the same PCR, adult worms were characterized for the mutation of residue 200 of isotype 1 β-tubulin. The technique has now been refined for use on a single worm, egg, or larva (M. H. Roos, personal communication). Since the frequencies of alleles associated with anthelmintic drug resistance might be quite high even in susceptible populations, it is indeed important to examine DNA from individual parasites. If DNA is prepared from pooled parasites, the association between particular alleles is likely to be obscured (2).

Since the same mutation is responsible for BZ resistance in many parasitic nematodes, this method may provide a means of investigating the frequencies of alleles bearing it in a wide range of animal and human intestinal nematodes.

Another interesting development is the availability of a P-gp gene probe for *Onchocerca volvulus* (90). Since it has been shown that P-gp plays a role in resistance to BZ and IVM in *H. contortus* (12, 84, 158), it can be expected that the same resistance mechanism might develop in many other helminths, including *O. volvulus*.

Laboratory tests for detection of resistance in human helminths. Apart from the use of the egg hatch test for hookworms in the Mali study (33), in vitro tests for AR in human nematodes have so far not been developed, adapted, or validated. A major problem is obviously the lack of reference resistant strains. If these were available, the egg hatch test and the larval development assay, as well as the promising new PCRs, could probably easily be validated for human hookworms.

Laboratory tests for schistosomicide resistance, in particular to PZQ, consist mainly of measuring worm count reduction after treatment in experimentally infected mice. First, it must be stressed that white mice are highly unnatural hosts for schistosomes; these large blood-dwelling worms are giant foreign bodies in the tiny murine blood vessels. Proportionally, a single schistosome in a mouse (blood volume, 5 ml) corresponds to 10,000 worms in an adult (blood volume, 5 liters). Few mice survive high worm counts long enough to allow therapeutic trials, and so the statistical power is inherently limited. Mouse-based experiments are laborious and subject to considerable methodological pitfalls, including those involving different strain maturation times (9, 22, 52). Laboratory strains are usually maintained using eggs derived from livers of mice that have been infected for 5 to 6 weeks, resulting in the selection of parasites which mature much more rapidly, and become susceptible to PZQ much earlier, than natural strains. As mentioned above, such bias probably explains the first reports on induced and "natural" PZQ resistance in the laboratory (51, 53). Also, it is not easy to isolate homogeneous parasites, resistant or not. Usually, mice are infected with a mixture of cercariae from at least five snails to obtain bisexual, productive infections. These snails have in turn usually been exposed to three to five miracidia, often resulting in mixed infections. These miracidia, even if isolated from stools of one person not responding well to treatment, stem from an unknown number and variety of adult worm couples, of which only one or a few may be (partly) tolerant to the drug. Confirming and assessing drug resistance in such a model is thus a most tedious and tricky task. The standard protocol proposed by Fallon et al. (54), based on procedures and recommendations by Cioli in a series of European Community-supported consensus meetings in Leiden, The Netherlands, is a valuable basis for better standardization, but this mouse model remains difficult to handle and interpret.

There is thus a great need for in vitro tests. Adult schistosomes can be cultured in artificial media, providing an excellent opportunity for straightforward in vitro exposure tests for individual worms. Such tests are much more accurate, reproducible, and feasible than mouse experiments, and they allow the screening of a great number of individual worms and well defined isolates. It has allowed the in-depth research of resistance to hycanthone and oxamniquine (16, 23). However, for PZQ, the test cannot be established as long as there is no convincing resistant reference strain (D. Cioli, personal communication).

Therefore, the main priority in research on AR in human schistosomes and nematodes is to conduct field studies in communities where clinical and/or epidemiological suspicion warrants the investments needed, to isolate as many individual parasites as possible from noncured patients, and to confirm the results in animal models. Once such strains are established and consolidated, in vitro tests can be validated. These will then in turn allow much wider and faster testing of field isolates and in-depth research of the biology and genetics of AR in human helminths.

CONCLUSIONS AND RECOMMENDATIONS

There is as yet no unequivocal evidence that resistance to commonly used anthelmintics in humans is an emerging problem, either through new mutations or by the selection of innately tolerant strains. However, experiences with other infectious agents, particularly those with the quick and dramatic spread of AR in livestock, should warn the medical world against the widespread use of anthelmintics for the control of helminths.

The projected conditions in drug-based human helminth control may be different from those in livestock: the transmission dynamics are more complex (particularly for schistosomes and filariae); treatments may be less frequent, and coverage may be lower; different strategies can be proposed to reduce the appearance or selection of resistant helminth strains. However, these are all hypothetical and optimistic assumptions, which may delay but probably will not avoid the appearance of AR. The biological, epidemiological, and pharmaceutical similarities between human and livestock helminths are so great that optimism may amount to complacent neglect. In livestock, the problem is mainly economic, which is bad enough. In humans, widespread AR would be a serious public health problem. At present, our only certainty is the striking lack of adequate tools to detect AR in human helminths and the inability to remedy the problem once it is detected. The perspective is indeed extremely worrying. For major helminths affecting humans, there are a few drugs available which are both safe and efficacious; since the commercial benefits are low, there is little or no investment in research on new molecules.

If drug-based strategies are implemented, the following guidelines may delay the development of resistance. (i) The intervention should be targeted and justified. Indiscriminate mass treatment (without any previous screening of the population) should be applied only in areas and groups where the impact of helminths and the benefits expected outweigh the

costs and burden on the health system and where it can be integrated in a sustainable package of health care. Such a cost-benefit calculation must be made by local and national health authorities, taking into account a whole range of qualitative and quantitative parameters, for which no clear-cut model is available.

(ii) Other control measures should be incorporated. Although health education programs, construction of latrines, improved water supply, etc., are much more difficult to implement are than treatment programmes, they have a much wider impact on public health, improve the sustainability of the helminth control, and allow the number of treatments to be reduced in the long run. Mass treatment is easy and popular but can reduce the commitment to more fundamental advances in the improvement of the living conditions of the local population.

(iii) The number of treatments should be reduced. The most efficient way to delay the development of drug resistance remains the reduction of the selection pressure by the drugs, in particular the number of treatments, preferably to one per year at most. It is obvious that a reduced treatment frequency should be combined with other control measures (see above) to maximize its effect. Two or three treatments a year, as advised by Albonico et al. (1), were already sufficient to induce the development of AR in some livestock helminths.

(iv) Exposure of the whole parasite population to the drug should be avoided. As suggested by simulation models, limiting the exposure of the whole helminth population should delay the development of AR. Targeted treatment, e.g., aimed at schoolchildren, is preferable to indiscriminate mass treatment, although even in such programs over 50% of the parasite population may be exposed to anthelmintics (2). Timing of treatment to occur during low-transmission seasons may seem efficient in terms of reinfection but may contribute to the development of AR.

(v) The correct dosage should be used. The use of lower dosages of anthelmintics for morbidity control programmes has been advocated to reduce costs but should be avoided to prevent or delay AR. In fact, the costs of drugs make up only a minor part of treatment programs (87). Some of the currently recommended drug dosages, including PZQ at 40 mg/kg, IVM at 150 μg/kg, mebendazole at 500 mg, and albendazole at 200 mg and even 400 mg, are actually subcurative. Although the administration of higher doses might increase costs, the useful life of the drugs may be extended, a worthwhile investment.

Incorrect dosages due to substandard or counterfeit anthelmintics must and can be avoided by imposing adequate quality standards on wholesale suppliers for national health care systems and special control programs. Obviously, there is also an urgent need for drug quality control systems in the private and public curative sector.

(vi) Simultaneous or rotational use of different drugs should be implemented. The simultaneous use of two or more drugs with different mechanisms of action is able to postpone the development of resistance to each of the drugs used (15, 76, 133). The cost increase is a serious obstacle, however. A less effective alternative is the rotation of drugs belonging to different classes. In any case, strategies which depend exclusively on administration of one single drug during many consecutive years, as in current onchocerciasis and schistosomiasis control programs, seem bound to result in resistance problems.

(vii) The development of drug resistance should be monitored. Monitoring the development of AR should be an obligatory part of large-scale worm control programs. As made clear in this review, standardized reliable tests to detect AR are not yet available.

The most appropriate strategy would therefore seem not to embark on control strategies based on the widespread and frequent use of anthelmintics and to restrict their use to curative medicine and possibly targeted interventions in very-high-risk groups or areas, which can be identified through rapid appraisal methods or through the regular health information system. To that end (and many others), reinforcement of the general primary health care systems should be the first priority in the control of human helminths. Meanwhile, the most important scientific challenge is to develop the appropriate tools, methods, and protocols to reliably and quickly detect the appearance of drug resistance in human helminths.

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PLoS one

Efficacy of Single-Dose and Triple-Dose Albendazole and Mebendazole against Soil-Transmitted Helminths and *Taenia* spp.: A Randomized Controlled Trial

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Abstract

Background: The control of soil-transmitted helminth (STH) infections currently relies on the large-scale administration of single-dose oral albendazole or mebendazole. However, these treatment regimens have limited efficacy against hookworm and *Trichuris trichiura* in terms of cure rates (CR), whereas fecal egg reduction rates (ERR) are generally high for all common STH species. We compared the efficacy of single-dose *versus* triple-dose treatment against hookworm and other STHs in a community-based randomized controlled trial in the People's Republic of China.

Methodology/Principal findings: The hookworm CR and fecal ERR were assessed in 314 individuals aged ≥5 years who submitted two stool samples before and 3–4 weeks after administration of single-dose oral albendazole (400 mg) or mebendazole (500 mg) or triple-dose albendazole (3×400 mg over 3 consecutive days) or mebendazole (3×500 mg over 3 consecutive days). Efficacy against *T. trichiura*, *Ascaris lumbricoides*, and *Taenia* spp. was also assessed. Albendazole cured significantly more hookworm infections than mebendazole in both treatment regimens (single dose: respective CRs 69% (95% confidence interval [CI]: 55–81%) and 29% (95% CI: 20–45%); triple dose: respective CRs 92% (95% CI: 81–98%) and 54% (95% CI: 46–71%)). ERRs followed the same pattern (single dose: 97% *versus* 84%; triple dose: 99.7% *versus* 96%). Triple-dose regimens outperformed single doses against *T. trichiura*; three doses of mebendazole – the most efficacious treatment tested – cured 71% (95% CI: 57–82%). Both single and triple doses of either drug were highly efficacious against *A. lumbricoides* (CR: 93–97%; ERR: all >99.9%). Triple dose regimens cured all *Taenia* spp. infections, whereas single dose applications cured only half of them.

Conclusions/Significance: Single-dose oral albendazole is more efficacious against hookworm than mebendazole. To achieve high CRs against both hookworm and *T. trichiura*, triple-dose regimens are warranted.

Trial Registration: www.controlled-trials.com ISRCTN47375023

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Introduction

Hundreds of millions of people are infected with the common soil-transmitted helminths (STHs), namely hookworms (*Ancylostoma duodenale* and *Necator americanus*), *Ascaris lumbricoides* and *Trichuris trichiura*, many by multiple species concurrently [1–5]. *Taenia* spp. infections are also widespread [6,7]. STHs and taeniasis/cysticercosis belong to the neglected tropical diseases (NTDs) and are responsible for mainly chronic and often inconspicuous morbidity [8,9]. Iron-deficiency anemia, malnutrition, and impaired physical and cognitive development have all been attributed to STH infections [1,5,10]. *Taenia solium* cysticercosis is a major cause of epilepsy and other neurological disorders in developing countries [11,12].

The current strategy for STH control in highly endemic areas focuses on morbidity control through large-scale administration of single-dose anthelminthics to at-risk populations, particularly school-aged children [9,13,14]. Due to the zoonotic nature of

taeniasis/cysticercosis, its control must also include the veterinary sector [6,15–17]. At present, only four drugs are recommended by the World Health Organization (WHO) for treating STH infections [13,18]. The global STH control relies on two of them – albendazole and mebendazole – both benzimidazole carbamates. Albendazole [19] and mebendazole [20] display a broad spectrum of activity and are administered orally, usually at a single dose of 400 mg and 500 mg, respectively [13,18,21]. Children below the age of 1 year and pregnant women in the first trimester of pregnancy are not eligible for treatment [13].

Albendazole and mebendazole have been extensively used worldwide for more than 30 years, both as stand-alone treatments and, more recently, in combination with other drugs, e.g., praziquantel (against schistosomiasis and food-borne trematodiasis) or ivermectin (against lymphatic filariasis) [9,22–24]. Surprisingly though, only few clinical trials compared the efficacy of albendazole and mebendazole against STHs. Rather, availability, cost, drug donation programs, and policy instead of the local parasite spectra and evidence determine the choice of which anthelminthic drug is deployed. Justification for the indiscriminate use of either drug is derived from high egg reduction rates (ERRs) achieved with both albendazole and mebendazole, and the assumption that morbidity is a function of infection intensity [25,26]. However, a recent meta-analysis of randomized placebo-controlled single-dose drug efficacy trials pointed to a marked superiority of albendazole over mebendazole against hookworm, high efficacy (in terms of cure rate [CR]) of both drugs against *A. lumbricoides*, and disappointing efficacy of either drug against *T. trichiura* [18]. Few data are available regarding ERRs.

The aim of this randomized controlled trial was to assess the efficacy of standard single-dose *versus* triple-dose oral albendazole and mebendazole against hookworm and other STH infections in a highly endemic but virtually benzimidazole-naïve population in the People's Republic of China (P.R. China).

Methods

The protocol for this trial and the supporting CONSORT checklist are available as supporting information; see Protocol S1 and Checklist S1.

Study Area, Study Period, and Participants

The study was conducted between October and December 2008 in Nongyang, a village located in Menghai county, Yunnan province, P.R. China. Details of the study area, population and epidemiological characteristics, including the prevalence of STHs, *Taenia* spp., and intestinal protozoa, have been described before [3,27,28]. The local prevalence of each *A. lumbricoides*, hookworm, and *T. trichiura* exceeded 85% in a survey conducted in 2006 [3]. Upon completion of the 2006 survey, compound mebendazole (mebendazole 100 mg/tablet+levamisole hydrochloride 25 mg/tablet, 2 tablets per day for 3 consecutive days) was distributed to the village population. No further interventions took place until the present study.

Ethics

The study was approved by the Ethics Committee of Basel (no. 294/08) and the Academic Board of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention in Shanghai (no. 2008091701). The trial was registered with Current Controlled Trials (identifier: ISRCTN47375023). The study objectives and procedures were discussed with the village head, village committee, and local health care officials who informed the residents. Individuals who were interested to participate signed an informed consent form in Chinese (parents or legal guardians in case of minors aged 5–17 years). Upon study completion, albendazole was provided for treatment of study participants found to be infected at evaluation, drop-outs, sick individuals upon recovery, and pregnant women once beyond the first trimester.

Interventions, Trial Medication, and Outcome Measures

The trial was designed as a community-based open-label, outcome assessors-blinded randomized controlled trial with four arms: (i) single-dose albendazole (400 mg), (ii) single-dose mebendazole (500 mg), (iii) triple-dose albendazole (3×400 mg, given over 3 consecutive days), and (iv) triple-dose mebendazole (3×500 mg, given over 3 consecutive days). No placebo drugs were given to individuals assigned to single dose treatment (open label).

Albendazole (Zentel®; lot no 08060407) was commercially obtained from Sino-American Tianjin SmithKline and French Laboratories Ltd., a Chinese joint venture of GlaxoSmithKline Plc. Mebendazole (Vermox®; lot nos. 8CL4F00 and 7CL8900), produced by Johnson & Johnson/Janssen-Cilag S.p.A., was provided by the WHO regional office in Hanoi, Vietnam.

The primary outcome considered was CR against hookworm 3–4 weeks following dosing. Changes in hookworm infection intensity, as determined by ERR, and efficacy against *A. lumbricoides* and *T. trichiura* served as secondary outcomes. Additionally, the effects of all four treatment regimens on *Taenia* spp. were assessed.

Eligibility Criteria and Sample Size

Eligible for inclusion were all residents of Nongyang aged 5 years and above. The following exclusion criteria were applied: presence of diagnosed or perceived chronic disease or other conditions likely to interfere with anthelminthic treatment (e.g., hypersensitivity to anthelminthics), pregnancy (verbally assessed at enrolment and again before treatment), recent history of anthelminthic treatment, and participation in other trials (within 1 month).

The intended sample size at enrolment was 370 individuals, based on the following assumptions: a total of 176 individuals (44 in each of the four treatment arms) would be needed to detect differences in the CR following different treatments for the cure of hookworm infections with 80% power using a 2-sided statistical test with an α-level of 0.05 and CRs of albendazole and mebendazole against hookworm infections of 75% and 45%. According to Keiser and Utzinger [18], the respective CRs are 78% and 23%; the higher estimate for the CR of mebendazole was employed in order to include a safety margin. The local prevalence of hookworm infections was assumed to be 60% and compliance was estimated to be 80%. Recruitment was to be stopped once 400 individuals had been enrolled.

Field and Laboratory Procedures

Families were contacted in batches of 20–30 (~80–120 potential participants) based on family registry numbers. Interested family members were invited to the local primary school for further information and enrolment. No monetary compensation was offered for participation. Participants answered a short questionnaire investigating demographic and health-related issues, and were given a stool collection container labeled with a unique identifier and their full name. The ability of all study participants to recognize their collection container was determined, and the importance of using the own receptacle emphasized. Each morning, filled containers were collected, and a new container

handed out with the aim to obtain two stool samples from each participant.

Stool samples were forwarded to a nearby laboratory and processed on the collection day. First, samples were visually inspected for adult *A. lumbricoides* and *Taenia* spp. proglottids. Second, two 41.7 mg Kato-Katz thick smears [29] were prepared from each sample. Depending on the ambient temperature and considering over-clearance of hookworm eggs, slides were read within 30–90 min of preparation [30]. At least 5% of the daily diagnoses were cross-checked by the principal investigator. Procedures for the evaluation of the treatment efficacy commenced 3 weeks post-treatment, lasted 2 weeks, and involved all participants given at least one drug dose. The same approach was adhered to as during the baseline survey.

Randomization

All participants who had submitted at least one stool sample during the baseline survey were randomly assigned either to the albendazole or the mebendazole arm of the study. In an independent randomization step, single or triple dose treatment using two computer-generated random sequences of 0 and 1 which were aligned with the list of participants in ascending order of their identification numbers. The eligible individuals were neither stratified by age nor sex before randomization.

Drug Administration

For each day of treatment, an envelope of the type locally used to hand out drugs was labeled with the name, identification number, and number of treatment, loaded with the appropriate drugs, and sealed. The distribution teams directly observed drug intake after asking about acute health problems and pregnancy status. Study participants had been reminded not to drink alcohol on treatment days and to report emerging health problems to the study physician (a medical doctor from a nearby hospital who visited the village each morning after drug distribution), any member of the research team, or the head of the village. On the second morning – 36 hours after the first dosing – all participating households were visited and participants actively solicited to report any potential adverse events. Reported health problems were classified by the study physician and graded by severity according to a pre-defined scale.

Statistical Analysis

Data were double-entered in EpiData version 3.1 (EpiData Association; Odense, Denmark) or Microsoft® Excel 2002 (Microsoft; Redmond, USA). After removing discrepancies, the datasets were aligned, and the accuracy of the merged database verified against the original data through random cross-checking. All analyses were performed on a per-protocol basis. Only participants with complete datasets were included.

Baseline and post-treatment prevalences were estimated, and CRs determined for each study arm. The extent of prevalence reductions and differences in CRs between groups were explored, using a 2-sided 2-sample test of proportions, which tests the equality of proportions using large-sample statistics. For each participant, the species-specific helminth infection intensity at baseline and at treatment evaluation was calculated and expressed as eggs per gram of stool (EPG), based on the arithmetic mean of the quadruplicate Kato-Katz thick smear readings, multiplied by a factor 24. Arithmetic and geometric means and ERRs were calculated according to Montresor et al. [31]. Confidence limits for the ERR were calculated using a bootstrap re-sampling method with 2000 iterations. Significant treatment group differences were defined by non-overlapping 95% confidence limits. For all tests, a p-value of 0.05 was considered the limit of statistical significance, and 95% confidence intervals (CIs) were calculated as appropriate. Statistical analyses were done in STATA version 10.1 (StataCorp LP; College Station, USA), bootstrap confidence intervals were calculated using R 2.9.1.

Results

Participant Flow and Baseline Characteristics

As detailed in Figure 1, at least one stool sample was available from 378 people who were randomly assigned to one of the four treatment arms. Among them, 314 (83%) could be included in the final analysis. The composition of all four groups with regard to sex and age was comparable and baseline prevalences of *A. lumbricoides*, *T. trichiura*, hookworm and *Taenia* spp. were 90%, 75%, 73% and 11%, respectively, with no differences among the four treatment arms (Table 1).

Efficacy Against Hookworm and Other STHs

A single dose of albendazole cured 69% (95% CI: 55–81%) of the hookworm infections, while single-dose mebendazole only cured 31% (95% CI: 20–45%), significantly less (Table 2 and table 3). Triple doses of either drug were significantly more efficacious than single-dose regimens, but the difference between the two drugs persisted: triple-dose albendazole cured significantly more hookworm infections (92%, 95% CI: 81–98%) than triple-dose mebendazole (58%, 95% CI: 46–71%).

Triple-dose mebendazole exhibited the highest reduction in *T. trichiura* prevalence (CR: 71%), followed by triple-dose albendazole (56%). Single dose applications were found to be significantly less efficacious (mebendazole: 40%, albendazole: 34%). In both cases, the differences between drug-specific CRs were not statistically significant. As expected, both albendazole and mebendazole cleared most of the *A. lumbricoides* infections with observed CRs ranging between 93% and 97%. The efficacies of albendazole and mebendazole were comparable. Triple-dose treatment tended to be slightly more efficacious than single-dose treatment, but the difference was not statistically significant. For *Taenia* spp., a single dose of either drug cured about one half of the infections; triple-dose administration cured all infections.

Table 4 and 5 (and in greater detail the Figure S1) show the baseline EPGs and changes following treatment. In general, the efficacy regarding ERRs followed a similar pattern as that of CRs. Albendazole outperformed mebendazole in terms of hookworm ERR, whereas mebendazole tended to be more efficacious against *T. trichiura*. Triple-dose regimens exhibited significantly higher ERRs against both parasites. All treatments resulted in ERRs>99.9% against *A. lumbricoides*. The median hookworm egg count in the 228 infected participants was 84 EPG at baseline and 30 EPG in those 92 still infected after treatment. The administration of three doses of albendazole resulted in the highest ERR against hookworm (99.7%; 95% CI: 99–99.9%). Single-dose albendazole with an ERR of 97% (95% CI: 95–99%) performed as well as triple-dose mebendazole (96%, 95% CI: 93–98%). A single dose of mebendazole resulted in an ERR of only 84% (95% CI: 73–90%). For *T. trichiura*, the administration of triple doses resulted in an ERR of 97% for mebendazole, and 94% for albendazole. With ERRs of 83% and 77%, respectively, single doses performed significantly worse.

Adverse Events

Thirteen study participants (4.1%) reported between one and five adverse events following drug administration, mostly in the morning of the third drug distribution day (about 12 hours after

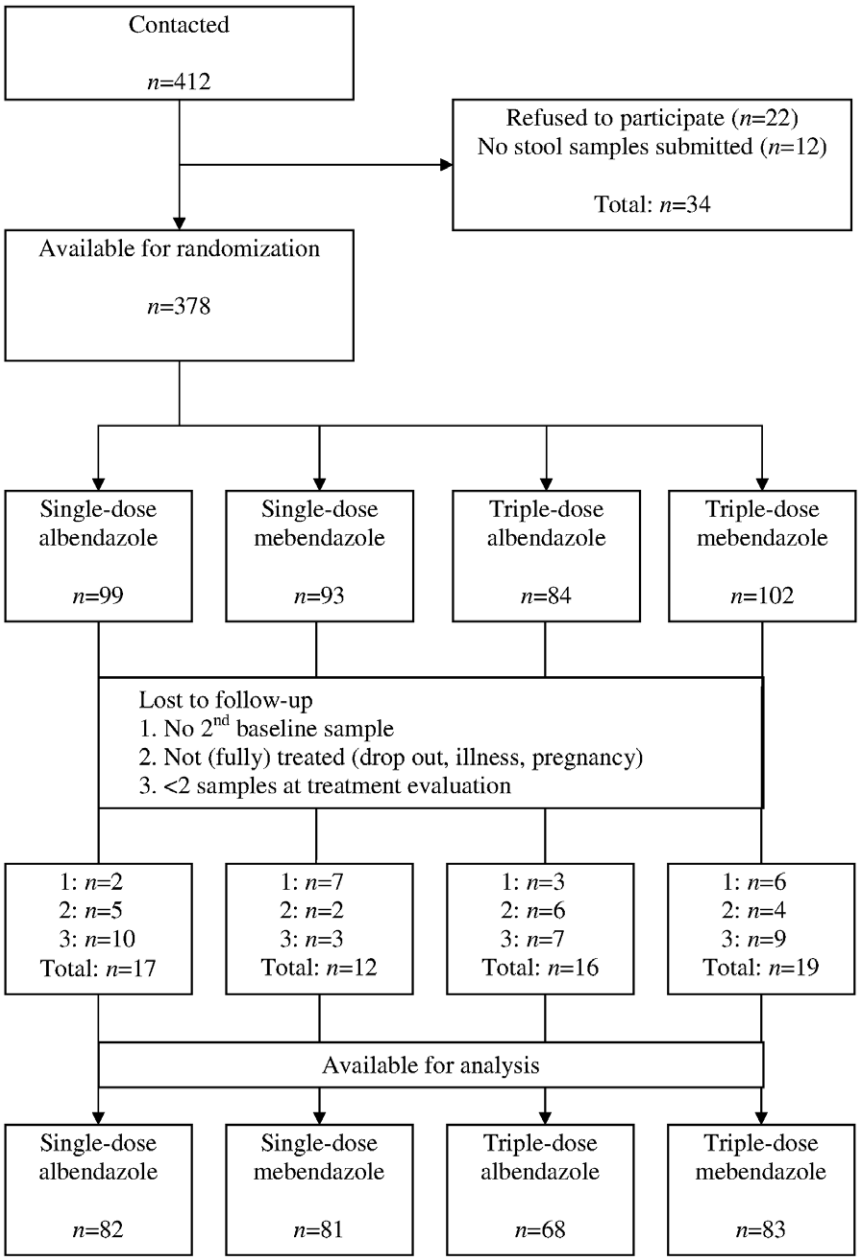

Figure 1. Participation and drop-out at various stages in a trial assessing the efficacy of anthelminthic drugs. Participation and causes for drop-out at various stages in a randomized controlled trial assessing the efficacy of single-dose *versus* triple-dose albendazole and mebendazole against STH infections and *Taenia* spp. in a Bulang ethnic minority community in Yunnan province, P.R. China in late 2008.
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the administration of the second dose, if given) and upon active questioning. Four of these individuals were treated with a single dose (3 with mebendazole, 1 with albendazole) while the remaining nine were treated with triple mebendazole ($n = 5$) or triple albendazole ($n = 4$). One symptom was reported by nine individuals, two symptoms by two individuals (1 treated with triple albendazole, 1 with triple mebendazole), three symptoms by one individual (triple mebendazole) and five symptoms by one individual (triple mebendazole). Adverse events included headache ($n = 3$; all mebendazole), abdominal cramps ($n = 3$; 2 mebendazole, 1 albendazole) and the closely related "full stomach" ($n = 2$; mebendazole), and waist pain ($n = 1$; albendazole). Two individuals each reported vomiting, including production of *A. lumbricoides* worms (1 albendazole, 1 mebendazole), diarrhea (2 mebendazole), fatigue (1 albendazole, 1 mebendazole), and chills (2 mebendazole). Vertigo (albendazole), throat pain (albendazole), fever (mebendazole), and a swollen face (mebendazole) were each reported once. None of the study participants requested medical interventions as adverse events were mild and self-limiting. More women than men reported adverse events (ten women among whom four treated with albendazole and six treated with mebendazole *versus* three men; $P = 0.046$) but there was no significant association between the report of adverse events and age, drug, or number of treatments according to the Fisher's exact test.

Table 1. Demographic characteristics of the participants in a trial assessing the efficacy of anthelminthic drugs.

	Total	Single-dose albendazole	Single-dose mebendazole	Triple-dose albendazole	Triple-dose mebendazole
Total n (%)	314 (100)	82 (100)	81 (100)	68 (100)	83 (100)
Sex: Female n (%)	151 (48.1)	35 (42.7)	39 (48.2)	36 (52.9)	41 (49.4)
Age n (%)					
- 5–14 years	42 (13.4)	9 (11.0)	8 (9.9)	14 (20.6)	11 (13.3)
- 15–24 years	88 (28.0)	26 (31.7)	20 (24.7)	19 (27.9)	23 (27.7)
- 25+ years	184 (58.6)	47 (57.3)	53 (65.4)	35 (51.5)	49 (59.0)
Parasite n (%)					
- Hookworm (95% CI)	228 (72.6; 67.7–77.5)	55 (67.1)	58 (71.6)	50 (73.5)	65 (78.3)
- *Ascaris lumbricoides* (95% CI)	284 (90.4; 87.2–93.7)	78 (95.1)	71 (87.7)	63 (92.6)	72 (86.7)
- *Trichuris trichiura* (95% CI)	234 (74.5; 69.7–79.3)	65 (79.3)	63 (77.8)	48 (70.6)	58 (69.9)
- *Taenia* spp. (95% CI)	33 (10.5; 7.1–13.9)	10 (12.2)	6 (7.4)	7 (10.3)	10 (12.0)

Demographic characteristics and baseline helminth prevalence of the study participants in a randomized controlled trial assessing the efficacy of single-dose and triple-dose albendazole *versus* mebendazole against STH infections and *Taenia* spp. in a Bulang ethnic minority community in Yunnan province, P.R. China, stratified by treatment arm.

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Discussion

This randomized controlled trial comparing the efficacy of single and triple dose albendazole and mebendazole confirmed that single oral albendazole is more efficacious than mebendazole against hookworm infections [18,32]. It also corroborated that triple-dose regimens result in significantly higher CRs than recommended and widely used single-dose regimens [13,33]. A single dose of mebendazole only cured 31% of the hookworm infections, while the highest CR, after triple albendazole, was 92%. Even triple administration of mebendazole was less efficacious than a single dose of albendazole. Keiser and Utzinger's meta-analysis [18] estimated a CR of only 15% after single-dose mebendazole, and a value comparable to that found in the present study after single-dose albendazole (present study: 69%, meta-analysis: 72%). With regard to ERRs, all four drug regimens resulted in significant reductions among those infected at baseline. A triple dose of mebendazole was significantly more efficacious than a single dose.

The number of *T. trichiura* infections in each treatment arm was significantly, though only moderately reduced, in line with previous findings [18,33,34]. As expected, triple doses resulted in higher CRs than a single dose regardless of the drug. Worryingly, the highest CR observed was only 71% following triple-dose mebendazole. Single and triple doses of mebendazole resulted in higher ERRs than the respective number of albendazole administrations. With regard to *A. lumbricoides* infections, high CRs were observed for both drugs even at a single dose;

Table 2. Prevalences and cure rates in a trial assessing the efficacy of anthelminthic drugs (hookworm and *Ascaris lumbricoides*).

	Single-dose albendazole ($n=82$)	Single-dose mebendazole ($n=81$)	Triple-dose albendazole ($n=68$)	Triple-dose mebendazole ($n=83$)
Hookworm				
Prevalence at baseline [% (n)]	67.1 (55)	71.6 (58)	73.5 (50)	78.3 (65)
Prevalence after treatment [% (n)]	20.7 (17)	50.6 (41)	5.9 (4)	36.1 (30)
New positives at evaluation	0	1	0	3
Cure rate [% (95% CI)][excluding new positives at evaluation]	69.1 (55.2–80.9)	31.0 (19.5–44.5)	92.0 (80.8–97.8)	58.5 (45.6–70.6)
Difference between drug-specific cure rates [% (95% CI)]	38.1 (21.0–55.1)***	Reference	33.5 (19.4–47.7)***	Reference
Difference single- vs. triple-dose cure rates [% (95% CI)]	Reference	Reference	22.9 (8.6–37.2)**	27.4 (10.5–44.3)**
Ascaris lumbricoides				
Prevalence at baseline [% (n)]	95.1 (78)	87.7 (71)	92.6 (63)	86.7 (72)
Prevalence after treatment [% (n)]	3.7 (3)	6.2 (5)	2.9 (2)	6.0 (5)
New positives at evaluation	0	0	0	0
Cure rate [% (95% CI)][excluding new positives at evaluation]	96.1 (89.1–99.2)	93.0 (84.3–97.7)	96.8 (89.0–99.6)	93.1 (84.5–97.7)
Difference between drug-specific cure rates [% (95% CI)]	3.2(−4.1–10.5)	Reference	3.8 (−3.5–11.1)	Reference
Difference single- vs. triple-dose cure rates [% (95% CI)]	Reference	Reference	0.7 (−5.4–6.8)	0.1 (−8.2–8.4)

Cure rates following single-dose and triple-dose albendazole *versus* mebendazole against STH infections and *Taenia* spp., and comparisons between treatment arms.
* P value$<$0.05, ** P value$<$0.01, *** P value$<$0.001.

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Table 3. Prevalences and cure rates in a trial assessing the efficacy of anthelminthic drugs (*Trichuris trichiura* and *Taenia* spp.).

	Single-dose albendazole ($n=82$)	Single-dose mebendazole ($n=81$)	Triple-dose albendazole ($n=68$)	Triple-dose mebendazole ($n=83$)
Trichuris trichiura				
Prevalence at baseline [% (n)]	79.3 (65)	77.8 (63)	70.6 (48)	69.9 (58)
Prevalence after treatment [% (n)]	53.7 (44)	49.4 (40)	32.4 (22)	25.3 (21)
New positives at evaluation	1	2	1	4
Cure rate [% (95% CI)][excluding new positives at evaluation]	33.8 (22.6–46.6)	39.7 (27.6–52.8)	56.2 (41.2–70.5)	70.7 (57.3–81.9)
Difference between drug-specific cure rates [% (95% CI)]	−5.8 (−22.5–10.8)	Reference	−14.4 (−32.7–3.8)	Reference
Difference single- vs. triple-dose cure rates [% (95% CI)]	Reference	Reference	22.4 (4.3–40.5)*	31.0 (14.2–47.8)***
***Taenia* spp.**				
Prevalence at baseline [% (n)]	12.2 (10)	7.4 (6)	10.3 (7)	12.0 (10)
Prevalence after treatment [% (n)]	7.3 (6)	4.9 (4)	0 (0)	1.2 (1)
New positives at evaluation	1	1	0	1
Cure rate [% (95% CI)][excluding new positives at evaluation]	50.0 (18.7–81.2)	50.0 (11.8–88.2)	100 (59.0–100)	100 (69.2–100)
Difference between drug-specific cure rates [% (95% CI)]	0 (NA)	Reference	0 (NA)	Reference
Difference single- vs. triple-dose cure rates [% (95% CI)]	Reference	Reference	50.0 (19.0–80.1)*	50.0 (10.0–90.0)*

Cure rates following single-dose and triple-dose albendazole *versus* mebendazole against STH infections and *Taenia* spp., and comparisons between treatment arms.
* P value$<$0.05, ** P value$<$0.01, *** P value$<$0.001.
doi:10.1371/journal.pone.0025003.t003

observations that are in line with systematic reviews and meta-analysis [18,33].

Attention was paid to enhance the sensitivity of STH diagnosis by examining multiple Kato-Katz thick smears before and after drug administration [3,35,36]. The low number of "new" infections found at treatment evaluation (Table 2 and table 3) indicates that a high sensitivity had been achieved despite the rather low density of hookworm and *T. trichiura* eggs. Because of the low *Taenia* spp. prevalence and since the study was not designed to evaluate treatment efficacy against this parasite, the respective results should be interpreted with caution. The conventional indicator for the successful cure of *Taenia* spp. infections – i.e., recovery of the scolex – is no definitive proof whenever individuals harbor several worms, and is difficult to perform outside an institutional setting. We focused on the presence of proglottids and eggs.

An open-label trial design was adhered to due to the complexities and high cost for implementing a double-blind trial in a field setting. We are confident that this did not negatively impact on the validity of the results since outcome assessors were blinded. One individual assigned to the triple albendazole group switched to the single-dose group, and in two instances the drug assignment was changed between members of the same family due to an initial mix-up. We used logistic regression to assess if our results were sensitive to the potential effect modifiers age and sex. Age was treated as a categorical variable (categories as in Table 1)

Table 4. Infection intensity and egg reduction rates in a trial assessing the efficacy of anthelminthic drugs (geometric mean).

	Single-dose albendazole	Single-dose mebendazole	Triple-dose albendazole	Triple-dose mebendazole
Hookworm [n]	55	58	50	65
EPG at baseline (geometric mean)	69	73	90	86
EPG after treatment (geometric mean)	2	12	0.3	3
ERR; difference in geometric mean [%; (95% CI)]	97.3 (95.2–98.7)[b]	83.6 (72.9–90.3)[a]	99.7 (99.1–99.9)[c]	96.4 (93.3–98.2)[b]
***Ascaris lumbricoides* [n]**	78	71	63	72
EPG at baseline (geometric mean)	8,442	7,855	6,485	8,435
EPG after treatment (geometric mean)	0.1	0.5	0.2	0.2
ERR; difference in geometric mean [%; (95% CI)]	$>$99.9 ($>$99.9–100)[a]	$>$99.9 ($>$99.9–$>$99.9)[a]	$>$99.9 ($>$99.9–100)[a]	$>$99.9 ($>$99.9–$>$99.9)[a]
***Trichuris trichiura* [n]**	65	63	48	58
EPG at baseline (geometric mean)	58	47	68	55
EPG after treatment (geometric mean)	14	8	4	1
ERR; difference in geometric mean [%; (95% CI)]	76.7 (62.6–86.1)[a]	82.5 (71.0–89.6)[a,b]	94.0 (89.4–96.8)[b,c]	97.3 (94.9–98.8)[c]

Infection intensities among those infected at baseline expressed as EPG and ERR following single-dose and triple-dose albendazole *versus* mebendazole against STH infections, and comparisons between treatment arms. Different letters (a, b, c) designate significant differences of ERR between treatment arms, defined by non-overlapping 95% confidence limits (calculated by bootstrap resampling).
doi:10.1371/journal.pone.0025003.t004

Table 5. Infection intensity and egg reduction rates in a trial assessing the efficacy of anthelminthic drugs (arithmetic mean).

	Single-dose albendazole	Single-dose mebendazole	Triple-dose albendazole	Triple-dose mebendazole
Hookworm [n]	55	58	50	65
EPG at baseline [median (25%–75%)]	78 (30–180)	84 (36–180)	84 (30–210)	102 (30–216)
EPG after treatment [median (25%–75%) [n]]	30 (12–43) [17]	30 (18–126) [41]	36 (18–56) [4]	18 (12–72) [30]
***Ascaris lumbricoides* [n]**	78	71	63	72
EPG at baseline [median (25%–75%)]	9600 (3,576–24,504)	10,260 (4,476–18,744)	8736 (2,382–22,056)	7956 (4,608–19,050)
EPG after treatment [median (25%–75%) [n]]	18 (6–396) [3]	1488 (24–2,904) [5]	384 (18–750) [2]	6 (6–6) [5]
***Trichuris trichiura* [n]**	65	63	48	58
EPG at baseline [median (25%–75%)]	66 (24–138)	48 (18–144)	78 (36–132)	51 (18–138)
EPG after treatment [median (25%–75%) [n]]	48 (18–144) [44]	39 (18–57) [40]	30 (18–78) [22]	18 (6–30) [21]

Infection intensities among those infected at time of observation expressed as EPG and ERR following single-dose and triple-dose albendazole *versus* mebendazole against STH infections, and comparisons between treatment arms.

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and also as a continuous variable (in years). None of the analyses showed noteworthy differences between the crude and adjusted models with respect to the point estimates or CIs of the odds ratios. The sole exception was the treatment regimen (single dose *versus* triple dose) for which adjustment for sex and age showed stronger effects for both drugs in the case of *T. trichiura*.

The susceptibility of the two human hookworm species to albendazole is known to be unequal, with CRs for the more pathogenic *A. duodenale* higher than that for *N. americanus* [37]. Both hookworm species are endemic in P.R. China but the locally predominant species probably is *N. americanus* according to a polymerase chain reaction (PCR)-based [38] species identification performed in a neighboring area [39]. Multiple-species intestinal helminth infections are common [4] but no associations between species have been found and the high prevalence of multi-parasitism in the study population is unlikely to diminish the validity of the findings for other settings.

Two additional observations are worth discussing. First, the *A. lumbricoides* CR did not differ significantly ($p>0.05$) between infection-intensity classes as defined by WHO [40]. Second, the baseline prevalence of *A. lumbricoides* and hookworm was higher among females than males. At evaluation, the difference persisted for hookworm, but had disappeared for *A. lumbricoides*, probably owing to the high CR against the latter parasite. In the case of *T. trichiura*, comparable prevalences were found for males and females at baseline, but treatment with either drug reduced the prevalence in males more markedly than in females.

The raw data of our randomized controlled trial is provided as supplementary files (Data S1 and Codes S1). In the spirit of trial registration prior to conducting clinical research, of open-access publishing, and of evidence-based medicine, we believe that others might find our data useful (e.g. for subsequent meta-analysis of drugs used against STHs). We hope that other clinical investigators and research groups will follow our example.

In conclusion, single-dose albendazole and mebendazole are highly efficacious against *A. lumbricoides*, albendazole is superior to mebendazole for treating hookworm, and mebendazole slightly outperforms albendazole with regard to treating *T. trichiura*. To achieve high CRs against hookworm and *T. trichiura* infections, triple dose regimens should be considered. Yet, for *T. trichiura*, even triple doses only resulted in the cure of a bit more than half of the infections, a result corroborating previous reports [33,41]. Triple-dose treatment is commonly deemed unfeasible in the context of large-scale drug administration programs based on logistical and organizational considerations [42], an issue which needs careful attention. To justify rolling out triple dose treatment, the additional efforts and costs required to do so must be weighed against the benefit, i.e., the higher treatment efficacy, and hence the prevention of harm. From a patient perspective, triple dose treatment appeared acceptable in the present study. Our findings therefore underscore the need for discovery and development of novel drugs for the management of trichuriasis [21,33]. Until new drugs become available, it is recommended to investigate ways to boost the efficacy of existing anthelminthics, including combination therapy (e.g., albendazole or mebendazole plus ivermectin) [21,33,34] and multiple dosing [21,33]. The higher efficacy of triple doses for treating *Taenia* spp. infections further tips the balance in favor of triple dose schedules in certain areas. With regard to large-scale interventions, the present results call for a more nuanced approach than the standard single-dose mono-drug distribution. Indeed, our findings emphasize the need for careful assessment of the locally endemic STHs, and the adaptation of the employed anthelminthic drug regimens to the prevailing situation. In populations primarily parasitized by *A. lumbricoides* and/or hookworm infections, single or – in case of a high prevalence or high-intensity hookworm infections – triple-dose albendazole might suffice. Mebendazole treatment with one or better three doses should be adopted in areas with a high prevalence of *T. trichiura* (and possibly *A. lumbricoides*), but a lower number of hookworm infections. In areas where all three species are co-endemic, alternation between albendazole and mebendazole as well as co-administration of different anthelminthic drugs should be considered.

Supporting Information

Protocol S1 Trial protocol.
(DOC)

Checklist S1 CONSORT checklist.
(DOC)

Figure S1 Frequency distribution of baseline EPGs and changes following treatment.
(PNG)

Data S1 Raw data of the trial.
(XLS)

Codes S1 Codes to raw data of the trial.
(DOC)

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Author Contributions

Conceived and designed the experiments: PS JU J-XC X-NZ. Performed the experiments: PS Z-WD J-YJ HZ. Analyzed the data: PS JU JH. Contributed reagents/materials/analysis tools: PS JU Z-WD J-YJ HZ JH. Wrote the paper: PS JU JH.

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PLoS NEGLECTED TROPICAL DISEASES

Low Efficacy of Single-Dose Albendazole and Mebendazole against Hookworm and Effect on Concomitant Helminth Infection in Lao PDR

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Abstract

Background: Albendazole and mebendazole are increasingly deployed for preventive chemotherapy targeting soil-transmitted helminth (STH) infections. We assessed the efficacy of single oral doses of albendazole (400 mg) and mebendazole (500 mg) for the treatment of hookworm infection in school-aged children in Lao PDR. Since *Opisthorchis viverrini* is co-endemic in our study setting, the effect of the two drugs could also be determined against this liver fluke.

Methodology: We conducted a randomized, open-label, two-arm trial. In total, 200 children infected with hookworm (determined by quadruplicate Kato-Katz thick smears derived from two stool samples) were randomly assigned to albendazole (n = 100) and mebendazole (n = 100). Cure rate (CR; percentage of children who became egg-negative after treatment), and egg reduction rate (ERR; reduction in the geometric mean fecal egg count at treatment follow-up compared to baseline) at 21–23 days posttreatment were used as primary outcome measures. Adverse events were monitored 3 hours post treatment.

Principal Findings: Single-dose albendazole and mebendazole resulted in CRs of 36.0% and 17.6% (odds ratio: 0.4; 95% confidence interval: 0.2–0.8; $P = 0.01$), and ERRs of 86.7% and 76.3%, respectively. In children co-infected with *O. viverrini*, albendazole and mebendazole showed low CRs (33.3% and 24.2%, respectively) and moderate ERRs (82.1% and 78.2%, respectively).

Conclusions/Significance: Both albendazole and mebendazole showed disappointing CRs against hookworm, but albendazole cured infection and reduced intensity of infection with a higher efficacy than mebendazole. Single-dose administrations showed an effect against *O. viverrini*, and hence it will be interesting to monitor potential ancillary benefits of a preventive chemotherapy strategy that targets STHs in areas where opisthorchiasis is co-endemic.

Clinical Trial Registration: Current Controlled Trials ISRCTN29126001

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Introduction

Infections with the three common soil-transmitted helminths (STHs), *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm (*Ancylostoma duodenale* and *Necator americanus*), are a global public-health concern, particularly in areas where poor sanitation prevails [1,2]. STH infections are among the most widespread of the neglected tropical diseases (NTDs) [3]. Indeed, more than a billion people are currently infected with one or several STH species, even though growing efforts are underway to control these parasitic worm infections [4]. In terms of their estimated global burden, hookworm is the most important among the STHs, perhaps responsible for more than 20 million disability-adjusted life years (DALYs) among the estimated 600 million infected people worldwide [1,5]. Chronic hookworm infection cause intestinal blood loss and result in poor iron status and iron-deficiency anemia, particularly in children, and women in reproductive age [1,6,7]. As a consequence, permanent impair-

Author Summary

Parasitic worms remain a public health problem in developing countries. Regular deworming with the drugs albendazole and mebendazole is the current global control strategy. We assessed the efficacies of a single tablet of albendazole (400 mg) and mebendazole (500 mg) against hookworm in children of southern Lao PDR. From each child, two stool samples were examined for the presence and number of hookworm eggs. Two hundred children were found to be infected. They were randomly assigned to albendazole (n = 100) or mebendazole (n = 100) treatment. Three weeks later, another two stool samples were analyzed for hookworm eggs. Thirty-two children who were given albendazole had no hookworm eggs anymore in their stool, while only 15 children who received mebendazole were found egg-negative. The total number of hookworm eggs was reduced by 85.3% in the albendazole and 74.5% in the mebendazole group. About one third of the children who were co-infected with the Asian liver fluke *Opisthorchis viverrini* were cleared from this infection following albendazole treatment and about one forth in the mebendazole group. Concluding, both albendazole and mebendazole showed disappointingly low cure rates against hookworm, with albendazole performing somewhat better. The effect of these two drugs against *O. viverrini* should be studied in greater detail.

ment, including delayed physical and cognitive development, has been described [8].

In the absence of a vaccine, the global strategy to control STHs and other NTDs is to reduce morbidity through repeated large-scale administration of anthelmintic drugs, a strategy phrased preventive chemotherapy [9]. At present, the World Health Organization (WHO) recommends four drugs against STH infections, of which albendazole and mebendazole are the two most widely used drugs for preventive chemotherapy [10]. In 2008, in the Western Pacific Region, 33.4 million children were given anthelmintic drugs [11]. According to the Lao national scheme for school deworming, there is a treatment round at the beginning of the first semester (September–December) and in the second semester (January–April). Mebendazole (single 500 mg oral dose) is annually distributed to all school-aged children since 2005 [12]. Recent efforts have been made to provide mebendazole also to preschool-aged children through the Expanded Program on Immunization (EPI) and alongside vitamin A distribution campaigns [4,13]. However, the efficacy of mebendazole and albendazole against STH infections in Lao PDR remains to be determined, and such locally derived evidence is important to guide the national treatment policy.

The liver fluke *Opisthorchis viverrini* is co-endemic in Lao PDR, and particularly high prevalences have been observed in the southern provinces [14–17]. Praziquantel is the current drug of choice against *O. viverrini* [3]. Previous work has shown that multiple doses of albendazole also show some effect [18,19]. Hence, in areas where STHs and *O. viverrini* co-exist and preventive chemotherapy targeting STHs is under way, it will be interesting to monitor for potential ancillary benefits of this control strategy against opisthorchiasis.

The purpose of this study was to assess the efficacy of single-dose albendazole (400 mg) and single-dose mebendazole (500 mg) against hookworm infection among school-aged children in Lao PDR. In addition, the effect on other STHs (i.e., *A. lumbricoides* and *T. trichiura*) and *O. viverrini* in co-infected children was assessed. Our study complements a recent investigation, done in the People's Republic of China that compared single and triple dosing with albendazole and mebendazole against the three common STHs [20].

Methods

Ethics Statement

The research protocol (see Protocol S1) was approved by the Ethics Committee of Basel, Switzerland (EKBB; reference no. 146/08) and the Lao National Ethics Committee for Health Research (NECHR), Ministry of Health, Vientiane, Lao PDR (reference no. 170/NECHR). The trial is registered with Current Controlled Trials (identifier: ISRCTN29126001). Written informed consent was obtained from parents/legal guardians of eligible children. Participation was voluntary and children could withdraw from the trial at any time without further obligation.

At completion of the trial, all children of the two primary schools and participants who were still found positive for hookworm (or other STHs) were treated with albendazole (400 mg). *O. viverrini*-infected children were administered praziquantel according to national guidelines [21].

Study Area and Population

A randomized, open-label trial was carried out between February and March 2009 in two primary schools (Oudomsouk and Nongbok Noi) in Batieng district, Champasack province, southern Lao PDR. Children in the two schools were treated with mebendazole 5–6 months prior to the start of our study. The schools are located approximately 15 km southeast of Pakse town, on the Bolaven plateau at an altitude of approximately 1,000 m above sea level (geographical coordinates: 105°56′53″N latitude, 15°14′59″E longitude). The rainy season lasts from May to mid-October. A census done in 2007 revealed that 43,651 people lived in the 95 villages of Batieng district (Dr. Nanthasane Vannavong, Champasack Provincial Health Department; personal communication). More than three-quarter of the households (77.5%) lack appropriate sanitation. Drinking water is primarily obtained from unprotected boreholes and wells. Most villagers live on subsistence rice farming and rubber plantations (Dr. Nanthasane Vannavong, Champasak Provincial Health Department; personal communication). Infections with STHs and *O. viverrini* are common in Batieng district; a recent study revealed infection prevalences above 50% and above 20%, respectively [22].

Study Design

We designed a randomized, open-label trial comparing albendazole (single 400 mg dose) and mebendazole (single 500 mg dose) for treatment of hookworm infection. The sample size was calculated based on results of a meta-analysis on the efficacy of current anthelmintic drugs against common STH infections, which reported cure rates (CR; defined as percentage of helminth-positive individuals who became helminth-egg negative after treatment) of 75% and 15% for albendazole (400 mg) and mebendazole (500 mg), respectively against hookworm infection [10]. In order to account for the large variation (uncertainty) of the observed efficacy of mebendazole in the individual studies (CRs of 8–91% were found in the six randomized controlled trials), we more than tripled the mean efficacy of mebendazole (50% instead of 15%). Assuming superiority of albendazole (1-tailed test) and taking into account a 90% power, and an alpha error of 5%, we obtained a sample size of 85 children per treatment group. Furthermore, we assumed a drop-out rate of 15%, which resulted

in a total sample size of 200 hookworm-positive school-aged children.

Field and Laboratory Procedures

The teachers of the two primary schools, the children, and the staff of the National Institute of Public Health, Centre of Malaria, Parasitology and Entomology, Centre for Laboratory and Epidemiology, the Provincial Department of Health, the Provincial Hospital, and the Malaria Station of Champassak, and the village authorities were informed one week in advance on the study aims and procedures. Potential risks and benefits were explained to all children and their parents/guardians. An informed consent form was distributed to all parents/guardians and signed. Children assented orally.

At baseline screening the consenting children (n = 465) of the two schools, aged 6–12 years, provided two fresh stool samples within a period of 3 days. Stool containers were filled by children and transferred to a laboratory in the early morning (between 8 and 9 am). All collected specimens were worked up on the day of collection. From each stool sample, duplicate Kato-Katz thick smears were prepared on microscope slides, using standard 41.7 mg templates [23]. Kato-Katz thick smears were quantitatively examined under a light microscope for helminths with a 100× magnification. Slides were read within 30–45 min after preparation. A random sample of approximately 10% of the Kato-Katz thick smears were re-examined by two senior technicians for quality control purposes. In case of discrepancies (i.e., positive *vs.* negative results and egg counts differing by >10%), results were discussed with the respective technicians, and the slides re-examined until agreement was reached.

In addition, a questionnaire was administered to each participating child to obtain sociodemographic data (i.e., sex, age, parent's education and occupation, ethnic group, and sanitation infrastructure), and behavioral data (i.e., wearing shoes, sources of drinking water, food consumption, and personal hygiene). Hookworm-positive children (defined by the presence of at least one hookworm egg in one of the quadruplicate Kato-Katz thick smears examined per child) were invited for treatment (n = 200).

At enrollment, a clinical examination, which included measurement of weight (using an electronic balance measured to the nearest 0.1 kg), height (using a measuring tap fixed to the wall and measured to the nearest cm), and axcillary temperature (using battery-powered thermometers, measured to the nearest 0.01°C), anemia assessment (finger prick capillary blood sample) was conducted, and a medical history taken. Children were excluded if they had fever, or showed signs of severe malnutrition. Additional exclusion criteria were the presence of any abnormal medical condition such as cardiac, vascular, pulmonary, gastrointestinal, endocrine, neurologic, hematologic (e.g., thalassaemia), rheumatologic, psychiatric, or metabolic disturbances, recent history of anthelmintic treatment (e.g., albendazole, mebendazole, pyrantel pamoate, levamisole, ivermectin, and praziquantel), attending other clinical trials during the study, or reported hypersensitivity to albendazole or mebendazole.

At follow-up, 21–23 days after drug administration, two stool samples were collected from each child and transferred to a hospital laboratory within one hour after collection. Each stool specimen collected at follow-up was subjected to the same procedures as during the baseline survey. Hence, duplicate Kato-Katz thick smears were prepared from each stool sample, examined under a microscope within 30–45 min by experienced laboratory technicians, and helminth eggs were counted and recorded for each species separately. We adhered to the same quality control as during the baseline survey.

Randomization

Children were randomly assigned to a single dose of albendazole (400 mg) or mebendazole (500 mg), using a block randomization procedure (six blocks each containing four treatment allocations), generated by an independent statistician who was not otherwise involved in the trial. The sequence of blocks was determined using a random number table. In addition, schools were decoded by a researcher to assign children either to albendazole or mebendazole. Eligible children were randomly assigned and allocated to treatment by an epidemiologist. Children and drug administrators were not blinded for drug treatment. Laboratory personnel and clinicians monitoring the adverse events were blinded throughout the study.

Drugs and Adverse Events

Albendazole (400 mg; Albendazole®, South Korea) was obtained from the Ministry of Health, Vientiane, Lao PDR. Mebendazole (500 mg; Vermox®, Italy) was donated by Johnson & Johnson Pharmaceuticals, provided through the Ministry of Health and the Ministry of Education, Vientiane, Lao PDR. At treatment day, both groups received the drugs under direct medical supervision on an empty stomach. Children were monitored for at least 3 hours after drug administration and asked to report for any drug-related adverse events using a standard questionnaire administered and graded by study physicians.

Statistical Analysis

Data were double-entered and cross-checked in EpiData version 3.1 (EpiData Association; Odense, Denmark). Statistical analyses were performed with STATA, version 10.1 (Stata Corp.; College Station, TX, USA). Efficacy was calculated for both intention-to-treat (ITT) and per-protocol (PP) analyses. ITT analysis was based on the initial treatment intent. PP analysis included only those children who had complete data records (i.e., quadruplicate Kato-Katz thick smear reading before and after treatment, and full treatment compliance).

Infections with hookworm, *A. lumbricoides*, *T. trichiura*, and *O. viverrini* were grouped into light, moderate, and heavy infections, according to WHO guidelines (for STHs) and cut-offs put forward by Maleewong and colleagues and WHO (for *O. viverrini*) [24,25]. Infection intensity classifications are as follows: hookworm, 1–1,999 eggs per gram of stool (EPG) (light), 2,000–3,999 EPG (moderate), and ≥4,000 EPG (heavy); *A. lumbricoides*, 1–4,999 EPG (light), 5,000–49,999 EPG (moderate), and ≥50,000 EPG (heavy); and *T. trichiura* and *O. viverrini*, 1–999 EPG (light), 1,000–9,999 EPG (moderate), and ≥10,000 EPG (heavy).

Primary outcome measures were CR and egg reduction rate (ERR), the latter defined as the positive group's reduction of geometric mean (GM) fecal egg count at posttreatment, divided by the GM fecal egg count at pretreatment, multiplied by 100. Additionally, changes in class of infection intensities were determined following treatment. Negative binomial regression was applied to compare ERRs observed between both treatment groups. A Wilcoxen test was employed for the matched pair's analysis. We determined egg reduction rate ratio (ERRR) and 95% confidence interval (CI). Pearson's χ^2-test and Fisher's exact test, as appropriate, were used to assess the baseline binary characteristics between the treatment arms. Statistical significance was estimated using a likelihood ratio test (LRT). *P*-value below 5% was considered significant.

CONSORT checklist was followed to report on the trial (see Checklist S1).

Results

Study Cohort

Four hundred sixty-five school-aged children were enrolled in the baseline screening. Two hundred children (43.0%), 130 boys and 70 girls with a parasitologically confirmed hookworm infection, were randomly assigned to one of the two treatments. Data of these 200 children were included in the ITT analysis. The remaining 265 children were excluded because they had no hookworm eggs in their stool (n = 235) or provided only a single stool sample (n = 30). Overall, 171 children (85.5%) had complete baseline data, received treatment, and completed follow-up examinations, and hence PP analysis was performed on these children. Twenty-nine children (14.5%) were lost to follow-up, 18 in the mebendazole and 11 in the albendazole group (Figure 1). The 171 children with complete data records were included in the primary analysis. Their parents most commonly had completed primary school only (77.5% of parents for the albendazole group and 80.5% for the mebendazole group). The most common profession of patients' parents was farming with 49.4% and 62.2% for albendazole and mebendazole treatment groups, respectively. The two groups were similar in terms of household assets, source of drinking water and consumption of cooked foods as well as raw fish (data not shown). More specifically, the consumption of raw fish was reported by 61.8% and 58.5%, respectively, and included dishes like "Pa Dek" (fermented fish sauce), "Lap Pa", and "Koy Pa" (raw, fish-based dishes).

Baseline Characteristics

At baseline, characteristics of the two treatment groups were similar (Table 1), including age (albendazole recipients: mean (standard deviation, SD) age 8.4 (2.1) years; mebendazole recipients: 8.7 (2.1) years), weight (mean (SD) 23.8 (5.8) kg and 25.0 (5.9) kg, respectively), height (mean (SD) 123.8 (11.0) cm and 126.9 (11.0) cm, respectively), and hemoglobin (Hb) concentration (mean (SD) 11.8 (1.1) mg/dl and 11.9 (1.3) mg/dl, respectively). In both treatment groups, most children were diagnosed with a light hookworm infection (82.0%), whereas the remaining children had moderate or heavy infection intensities. The hookworm GM fecal egg counts in the mebendazole and albendazole groups were 707.0 and 859.1 EPG, respectively (Table 2).

The overall infection rates of *A. lumbricoides*, *O. viverrini* and *T. trichiura* were 34.0%, 48% and 45.0%, respectively. *O. viverrini* GM fecal egg counts were 84.9 EPG (albendazole) and 120.8 EPG (mebendazole) (Table 3).

Albendazole and Mebendazole Efficacy against Hookworm

In the ITT analysis, the CRs of albendazole and mebendazole against hookworm infection were 32.0% and 15.0%, respectively. Overall, 124 children (73%) remained hookworm-egg positive; 68 receiving albendazole and 85 in the mebendazole treatment group. Similar results were obtained with the PP analysis (Table 2). A statistically significant difference was observed when comparing the observed CRs using albendazole *vs.* mebendazole (OR = 0.4; 95% CI 0.2–0.8; $P = 0.01$). The hookworm GM fecal egg counts obtained at follow-up were 63.0 EPG in albendazole recipients and 147.3 EPG in mebendazole recipients (ITT analysis 96.5 EPG and 210 EPG, respectively). The respective ERRs for albendazole and mebendazole were

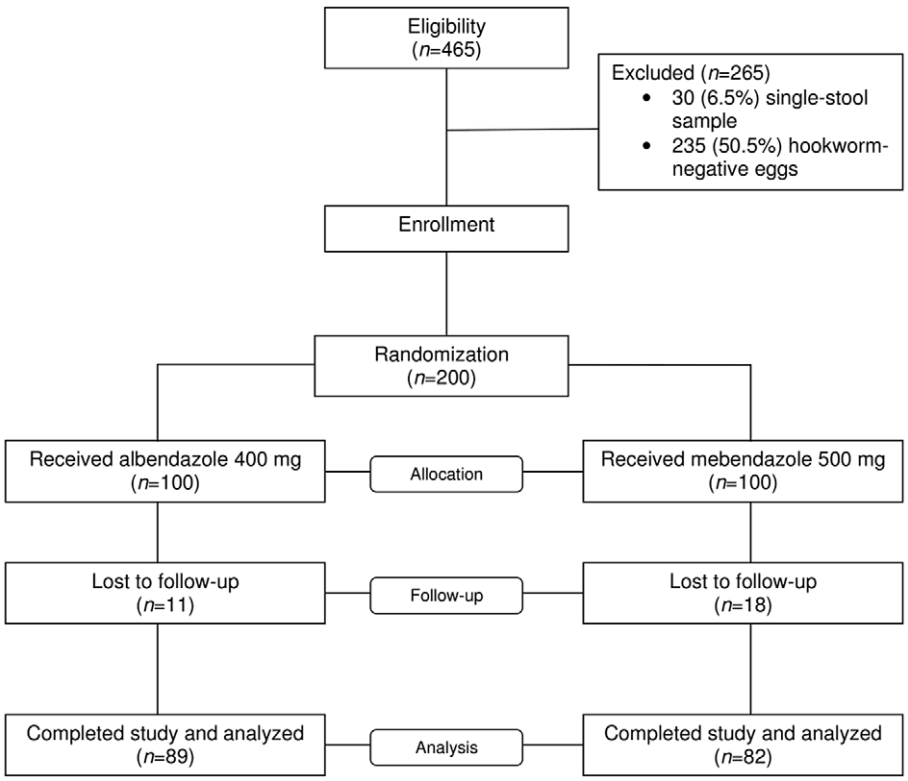

Figure 1. Flow chart detailing the study participation and compliance. Children who completed two stool samples were included in the final analysis for assessing the efficacy of single-dose albendazole (400 mg) and single-dose mebendazole (500 mg) treatment against hookworm and concomitant helminth infections in Bachieng district, Champasack province, southern Lao PDR in February/March 2009.
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Table 1. Baseline characteristics of 171 hookworm-infected school children, Bachieng district, Champasak province, Lao PDR, in February/March 2009.

	Albendazole (*n* = 89)	***Mebendazole (n = 82)***
Boys/girls	56/33	*49/33*
Mean (SD) age, years	9.0 (2.1)	*9.0 (2.1)*
Mean (SD) weight, kg	24.0 (6.0)	*25.2 (6.0)*
Mean (SD) height, cm	124.1 (11.0)	*127.0 (11.0)*
Mean (SD) hemoglobin, mg/dl	11.9 (1.1)	*12.4 (1.3)*
Anemia (<11.5 mg/dl), n, (%)[a]	23 (57.5)	*17 (42.5)*
Latrine facility present, n, (%)	5 (5.6)	*1 (1.2)*
Parasitic infections		
Hookworm infection[b]		
Light (1–1,999 EPG)	72 (80.9)	*67 (81.7)*
Moderate (2,000–3,999 EPG)	9 (10.1)	*7 (8.6)*
Heavy (≥4,000 EPG)	8 (9.0)	*8 (9.7)*
Co-infection with		
Ascaris lumbricoides[b]		
Negative	61 (68.5)	*53 (64.6)*
Light (1–4,999 EPG)	18 (20.2)	*18 (22.0)*
Moderate (5,000–49,999 EPG)	7 (7.9)	*8 (9.8)*
Heavy (≥50,000 EPG)	3 (3.4)	*3 (3.7)*
Trichuris trichiura[b]		
Negative	51 (57.3)	*39 (47.6)*
Light (1–999 EPG)	38 (42.7)	*43 (52.4)*
Moderate (1,000–9,999 EPG)	4 (4.5)	*0*
Heavy (≥10,000 EPG)	0	*0*
Taenia spp.		
Negative	78 (87.6)	*79 (96.3)*
Positive	11 (12.4)	*3 (3.7)*
Opisthorchis viverrini[c]		
Negative	44 (49.4)	*50 (61.0)*
Light (1–999 EPG)	41 (46.1)	*25 (30.5)*
Moderate (1,000–9,999 EPG)	4 (4.5)	*7 (8.5)*
Heavy (≥10,000 EPG	0	*0*

[a]*According to guidelines put forth by WHO regarding definition of anemia* [*42*].
[b]*According to guidelines put forth by WHO* [*25*]*, based on Kato-Katz thick smear examination*.
[c]*According to Maleewong and colleagues* [*24*]*, based on Kato-Katz thick smear examination*.
Data are no; (%) of subject, otherwise indicated (95% confidence interval); EPG, eggs per gram of stool; GM, geometric mean.
doi:10.1371/journal.pntd.0001417.t001

86.7% and 76.3% (ERRR 1.0; 95%CI 0.7–1.6; $P = 0.90$. In children with moderate infection intensities (2,000–3,999 EPG), the effect of albendazole and mebendazole was significantly different ($P = 0.04$).

Effect of Albendazole and Mebendazole against *A. lumbricoides, T. trichiura*, and *O. viverrini*

Table 3 shows the effect of albendazole and mebendazole against *A. lumbricoides*, *T. trichiura*, and *O. viverrini*. At baseline, GM infection intensities of *A. lumbricoides* were 1,567 EPG in albendazole recipients and 1,584 EPG in mebendazole recipients. Both albendazole and mebendazole treatments achieved high CRs above 90% and resulted in almost complete egg elimination. The CRs of albendazole and mebendazole obtained against *T. trichiura* were 33.3% and 27.9%, respectively. The respective ERRs were 67.0% and 66.0%. No statistically significant difference was observed for CR and ERR between the two treatments (OR = 0.8; 95% CI 0.3–1.9; $P = 0.58$ and ERRR = 0.7; 95% CI 0.3–1.2, $P = 0.22$). Finally, CRs against *O. viverrini* achieved with albendazole and mebendazole were 33.3% and 24.2%, respectively (OR = 0.7; 95% CI 0.3–1.9; $P = 0.62$). The respective ERRs were 82.1% and 78.2% (ERRR = 0.8; 95% CI 0.2–3.9, $P = 0.78$).

Adverse Events

Monitoring of children within 3 hours after drug administration revealed no drug-related adverse events, neither in the albendazole nor in the mebendazole group. Hence, both treatments were well tolerated.

Discussion

This current head-to-head comparison of single-dose albendazole *vs.* mebendazole against hookworm infection in Lao school-aged children – to our knowledge the first comparative trial in this Southeast Asian country – shows sobering results. Indeed, the standard single oral doses of albendazole (400 mg) and mebendazole (500 mg) that are recommended for preventive chemotherapy targeting STHs [8,9] resulted in low CRs against hookworm infection (36.0% and 17.6%, respectively). The respective ERRs were moderate, (86.7% and 76.3%).

A sizeable number of children were co-infected with *A. lumbricoides*, *T. trichiura*, and *O. viverrini*, which allowed us to determine the effect of albendazole and mebendazole against these helminth species. With regard to *A. lumbricoides*, high efficacy of both drugs was confirmed against this helminth species [3,10]. Our study also confirms the previously reported low efficacy of both drugs against *T. trichiura* [3,10,26].

While the results obtained with mebendazole against hookworm and the efficacy observed with both drugs against *A. lumbricoides* and *T. trichiura* are in line with previous studies [20,27,28] and in agreement with overall CRs estimated through a meta-analysis [10], the low CR (36.0%) achieved with albendazole in the treatment of hookworm infection is somewhat surprising. Indeed, in the aforementioned meta-analysis, randomized controlled trials of single-dose albendazole (400 mg) revealed an overall CR against hookworm of 75% [10]. The reasons for the considerably lower efficacy of albendazole observed in our study are unclear. Quality control of drug samples performed in our laboratories revealed that disintegration, dissolution, and concentration of the albendazole tablets used in our trial were comparable to Zentel® (data not shown). The hookworm species (and strains) endemic in southern Lao PDR might be an explanation. However, there is a paucity of information on which hookworm species is predominant in Southeast Asia. Indeed, in our study setting the infection rates of the two hookworm species, *A. duodenale* and *N. americanus*, are not known. Furthermore, recent studies documented that in Southeast Asia humans are at risk of acquiring *Ancylostoma ceylanicum*, which is endemic in dogs and cats of the region and its importance in humans might be underestimated [29,30]. Hence, further analysis on the circulating parasite species is required to elucidate this issue. In addition, day-to-day variability in hookworm egg counts from individuals is a well described

Table 2. Hookworm infection at baseline and follow-up and cure rate of albendazole and mebendazole (per-protocol analysis).

	Pretreatment		***Posttreatment***	
	Albendazole (*n* = 89)	**Mebendazole (*n* = 82)**	**Albendazole (*n* = 89)**	***Mebendazole (n = 82)***
No. of hookworm-infected patients	89 (100)	82 (100)	57 (64.0)	*67 (81.7)*
No. of children cured (cure rate, %)	n.a.	n.a.	32 (36.0)	*15 (17.6)*[a]
Light infection (1–1,999 EPG)	72 (80.9)	67 (48.2)	55 (61.8)	*59 (72)*
No. of children cured (cure rate, %)	n.a.	n.a.	17 (19.1)	*8 (9.8)*[b]
Moderate infection (2,000–3,999 EPG)	9 (18.0)	7 (46.7)	2 (2.2)	*6 (7.3)*
No. of children cured (cure rate, %)	n.a.	n.a.	7 (7.9)	*1 (1.2)*[c]
Heavy infection (≥4,000 EPG)	8 (1.1)	8 (1.1)	0 (0)	*2 (2.4)*
No. of children cured (cure rate, %)	n.a.	n.a.	8 (9)	*6 (7.3)*[d]
GM fecal egg count (range), EPG	859.1 (699.0–1,057.0)	707.0 (559.0–894.3)	63.0 (34.0–116.0)	*147.3 (90.0–242.0)*
Egg reduction rate, %	n.a.	n.a.	86.7	*76.3*[e]

[a]*OR 0.4 [95% CI (0.2–0.8; P = 0.01)] comparison of treatment outcomes between mebendazole vs. albendazole;*
[b]*P = 0.13;*
[c]*P = 0.04;*
[d]*P = 0.46;*
[e]*ERRR 1.0 [95% CI (0.7–1.6; P = 0.90)] comparison of treatment outcomes between mebendazole vs. albendazole.*
Note. Data are number; (%) of children, unless otherwise indicated (95% confident interval); GM, geometric mean; EPG, eggs per gram of stool; ERRR egg reduction rate ratio; OR odds ratio; n.a. not applicable.
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phenomenon [31]. Finally, the study's sample size is rather small and therefore a few incidental effects such as failure of some children to swallow the tablet correctly, might have contributed to low efficacy of albendazole for the treatment of hookworm infection. To sum up, differences in strain and species susceptibilities, host factors, and co-infections with other

Table 3. Infection rate and cure rate of albendazole and mebendazole for hookworm co-infections.

	Pretreatment		***Posttreatment***	
	Albendazole	**Mebendazole**	**Albendazole**	***Mebendazole***
Parasitic infection				
A. lumbricoides (n = 58)	(*n* = 28)	(*n* = 30)	(*n* = 28)	*(n = 30)*
No. of *A. lumbricoides*-infected children	28 (100)	30 (100)	2 (7.1)	*2 (6.7)*
No. of patients cured (cure rate, %)	n.a.	n.a.	26 (92.9)[a]	*28 (93.3)*[a]
GM fecal egg count (range), EPG	1,567.0 (553.0–4,444.0)	1,584.0 (528.0–4,751.0)	0	*0*
ERR, %	n.a.	n.a.	100[b]	*100*[b]
T. trichiura (n = 82)	(*n* = 39)	(*n* = 43)	(*n* = 39)	*(n = 43)*
No. of *T. trichuris*-infected children	39 (100)	43 (100)	26 (66.7)	*31 (72.1)*
No. of patients cured (cure rate, %)	n.a.	n.a.	13 (33.3)	*12 (27.9)*[c]
GM fecal egg count (range), EPG	94.1 (48.3–184.0)	65.2 (39.3–108.3)	75.0 (42.2–133.2)	*48.0 (25.0–93.0)*
ERR	n.a.	n.a.	67.0[d]	*66.0*[d]
O. viverrini (n = 77)	(*n* = 45)	(*n* = 32)	(*n* = 45)	*(n = 32)*
No. of *O. viverrini*-infected children	45 (100)	32 (100)	30 (66.7)	*25 (75.8)*
No. of patients cured (cure rate, %)	n.a.	n.a.	15 (33.3)[e]	*8 (24.2)*[e]
GM fecal egg count (range), EPG	84.9 (41.8–184.0)	120.8 (48.9–297.9)	73.0 (34.3–155.7)	*114.4 (48.9–267.3)*
ERR, %	n.a.	n.a.	82.1[f]	*78.2*[f]

[a]*OR 0.8 [95% CI (0.2–2.6; P = 0.71) comparison of treatment outcomes between mebendazole vs. albendazole.*
[b]*ERRR n.a.*
[c]*OR 0.8 [95% CI (0.3–1.9; P = 0.58)] comparison of treatment outcomes between mebendazole vs. albendazole.*
[d]*ERRR 0.7 [95% CI (0.3–1.2; P = 0.22)] comparison of treatment outcomes between mebendazole vs. albendazole.*
[e]*OR 0.7 [95% CI (0.3–1.9; P = 0.62)] comparison of treatment outcomes between mebendazole vs. albendazole.*
[f]*ERRR 0.8 [95% CI (0.2–3.9; P = 0.78)] comparison of treatment outcomes between mebendazole vs. albendazole.*
Note. Data are number; (%) of children, unless otherwise indicated (95% confident interval); GM, geometric mean; EPG, eggs per gram of stool; ERRR, egg reduction rate ratio; OR odds ratio; n.a. not applicable.
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helminths are factors that might all play a role in explaining treatment failures [28,32].

Nevertheless, we cannot rule out that albendazole resistance is developing in our study setting. To date, nematode resistance in humans has not been reported. On the other hand, drug resistance is a major problem in veterinary public health [33,34]. The development of broad spectrum anthelmintic resistance, in particular resistance of nematodes to benzimidazoles, has been recognized in ruminants for decades [34,35]. Extensive studies on the underlying mechanisms of drug resistance have been carried out [36]. Further investigations on failure of the drugs to completely cure the patients are necessary in our study setting to substantiate this suspicion.

It is interesting to note that the two drugs employed, even at single oral doses, showed some effect against *O. viverrini*. Although CRs were low (24.2–33.3%), the moderate ERRs of 78.2–82.1% are encouraging. At present, praziquantel is the drug of choice against opisthorchiasis [3,18]. Studies carried out in the 1980s in *O. viverrini*-infected hamsters and patients infected with *O. viverrini* documented opisthorchicidal properties of albendazole and mebendazole [19,37]. However, long treatment courses of up to 7 days were recommended in view of these initial laboratory and clinical findings. Experiences with long treatment courses have been reported from a hospital-based randomized trial; albendazole given at dosages of 400 mg twice daily for 3 and 7 days resulted in CRs of 40% and 63%, respectively, and corresponding ERRs of 92% [19]. Furthermore, mebendazole in dosages of 30 mg/kg daily for 3 or 4 weeks resulted in CRs of 94% against *O. viverrini*. Long treatment courses compromise compliance, increase costs and are not feasible for large-scale community-based control, which might explain that albendazole and mebendazole were not further promoted for *O. viverrini* treatment [37].

It should be noted that in our study Kato-Katz thick smears served as method for helminth diagnosis. However, this diagnostic approach does not allow differentiating the eggs of *O. viverrini* from minute intestinal flukes [38,39]. In addition, since the emphasis of our research was on hookworm, the efficacy of albendazole and mebendazole against other STHs and *O. viverrini* could not be compared with the appropriate sample sizes. Finally, mostly light *O. viverrini* infections were present in our study and the sample of *O. viverrini*-infected patients was not representative of the overall community as hookworm infection was the leading selection criterion. Hence, additional clinical investigations are warranted to assess the opisthorchicidal properties of albendazole and mebendazole in comparison to praziquantel. Moreover, the anthelmintic drug tribendimidine [40] showed high CR and ERR against *O. viverrini* in a recent, open-label exploratory trial carried out in Lao PDR [41]. It would therefore be interesting to conduct a four-arm study, comparing praziquantel (treatment of choice) with tribendimidine, albendazole, and mebendazole.

In conclusion, we have assessed the efficacy of standard single-dose regimens of albendazole and mebendazole against hookworm infection in school-aged children from Lao PDR and provide further evidence of the effects these two drugs have against other helminth species concurrently harbored in the human host. Both drugs showed a similar profile, with low efficacy against hookworm and, additionally, low efficacy against *T. trichiura*, and high efficacy against *A. lumbricoides*. The low efficacy of single-dose of albendazole against hookworm should be followed-up closely and further investigated as this drug is widely used for preventive chemotherapy against STHs and in combination with ivermectin in the current global effort to eliminate lymphatic filariasis. The effects of the two drugs against *O. viverrini* warrant further investigations, in comparison with the current drug of choice praziquantel as well as tribendimidine.

Supporting Information

Checklist S1 CONSORT Checklist.
(DOC)

Protocol S1 Trial Protocol.
(PDF)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: PAS JU JK PO. Performed the experiments: PAS SS KP VX. Analyzed the data: PAS PV PO. Wrote the paper: PAS JK PO. Overall responsibility of data collection: KA. Assisted in results interpretation and manuscript writing: JU CH. Obtained funding: PO.

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Reference 8.

A COMPARATIVE STUDY OF DIFFERENT ALBENDAZOLE AND MEBENDAZOLE REGIMENS FOR THE TREATMENT OF INTESTINAL INFECTIONS IN SCHOOL CHILDREN OF USIGU DIVISION, WESTERN KENYA

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A COMPARATIVE STUDY OF DIFFERENT ALBENDAZOLE AND MEBENDAZOLE REGIMENS FOR THE TREATMENT OF INTESTINAL INFECTIONS IN SCHOOL CHILDREN OF USIGU DIVISION, WESTERN KENYA

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ABSTRACT: A clinical trial to compare the effectiveness of 4- and 6-mo repeated treatment with albendazole 600 mg (Zentel®, SmithKline Beecham) or mebendazole 600 mg (Vermox®, Janssen) on geohelminth infections was carried out on children in 6 primary schools; the study included 1,186 children, ages 4 to 19 yr. Kato–Katz examination was performed on stool samples before and after treatment. Overall, albendazole produced better cure rates and egg reduction rates for geohelminths. The cure rates for albendazole were 92.4% for hookworm infection, 83.5% for *Ascaris lumbricoides*, and 67.8% for *Trichuris trichiura*. Mebendazole given either 2 or 3 times in a year had cure rates of 50 and 55.0% (respectively) for hookworm, 79.6 and 97.5% for *A. lumbricoides*, and 60.6 and 68.3% for *T. trichiura* infection. The geometric mean intensity of hookworm eggs per gram (epg) of stool decreased by 96.7% after albendazole treatment compared with 66.3 and 85.1%, respectively, for 2 or 3 doses of mebendazole ($P < 0.05$) over the same period. Reductions in epg for *A. lumbricoides* and *T. trichiura* were comparable for both drugs. Our results indicate that treatment with albendazole at a 6-mo interval was more effective than mebendazole regimens and may be the best choice for use in the control of the 3 geohelminths.

The availability of safe, effective, broad-spectrum anthelmintics that can be administered in single doses has changed the approach to the control of intestinal helminthiases (WHO, 1992). Mass chemotherapy is viewed as a cost-effective approach to reduce intestinal helminthic infections and their transmission at the community level (Savioli et al., 1992). The effectiveness and practicability of this approach have been demonstrated by investigators working on geohelminths (hookworm, *Ascaris lumbricoides*, and *Trichuris trichiura*) in endemic areas (Albonico, Smith et al., 1994; Magnussen et al., 1997). Albendazole or mebendazole given as a single dose to treat children in areas where geohelminths are endemic significantly reduces infection and worm burden of infected individuals (Stephenson et al., 1989; Savioli et al., 1992; Albonico et al., 1995, 1997; Jackson et al., 1998; Magnussen et al., 1997).

Treatment directed at school-age children reaches a large segment of population that typically harbors heavy intestinal helminthic infections, and so reduces transmission of the worms within communities. Furthermore, it can reduce the operational costs of drug delivery for worm treatment programs. However, because transmission of helminths in endemic areas is dependent on many factors, including species of intestinal helminths prevalent in the area, seasonality of transmission, and reinfection rates, the issues of drug choice and how often treatment should be repeated are important in planning a control program. The present study describes the results of a drug trial designed to compare the efficacy of different single-dose regimens given as multiple treatments with albendazole or mebendazole in school children.

MATERIALS AND METHODS

Study area

The study was conducted in 6 primary schools from February 1996 to February 1997 in Usigu Division of Bondo District in an area known to be endemic for geohelminths (Chunge et al., 1985). The area lies approximately 20–25 km west of Bondo town, on the shores of Lake Victoria. The climate is equatorial with an average rainfall of about 1,600 mm/yr, most of which occurs from March to June. Temperatures are generally stable, ranging from 25 to 30 C, and humidity is relatively high. The majority of inhabitants are Luo fishermen, subsistence farmers, or both. The area has a problem with proper sanitation because the water table is very close to ground level; hence, the types of latrines used are usually shallow, allowing easy transmission of geohelminths.

Study population

During a previous study, a survey was conducted to map the distribution of intestinal helminthic infections in 50 primary schools of the Usigu and Bondo Divisions. The overall prevalence of intestinal helminths ranged between 10 and 80%, with a median of 30%. Of the 50 schools surveyed, 6 satisfied the following criteria, e.g., prevalence of 40% for any of the common geohelminths and no history or documented large-scale antihelminthic campaigns within the previous 5 yr. Schoolchildren in the 6 schools were recruited for the study and verbal consent was obtained from a parent or guardian.

The objectives of the study were explained to the community, to their leaders, and to school and health administrators through local meetings. A total of 1,226 schoolchildren (mean age 10.8 ± 2.6 yr; 52.1% males and 47.9% females) was registered in the participating primary schools. Each child was assigned a unique identifier coding for the school, class, and class register.

Study design

The 6 primary schools were randomized to a single dose of 600 mg of mebendazole (Vermox®, Janssen) given at a 6-mo interval (MBZ1); mebendazole (600 mg) given at a 4-mo interval (MBZ2); and albendazole (600 mg, Zentel®, SmithKline–Beecham, Nairobi, Kenya) administered at a 6-mo interval (ABZ). The impact of the repeated treatment in the 3 groups was evaluated in a sample of 40% of the schoolchildren who were reexamined at 4 wk, 3, 6, and 12 mo after the first round of treatment. The cure rates and mean egg reduction of hookworm, *A. lumbricoides*, and *T. trichiura* infections for the treatment groups were compared 4 wk and 12 mo after treatment. Drugs were provided in sealed, coded packages with uniform sizes and color, and administered en masse to children in each schools. Drug codes were not revealed to project staff until the end of the study. Treatment was administered under direct supervision of a clinician attached to the project. The study was approved by the review committee of Kenya Health Research, the Ethics committee of the Ministry of Health, Kenya. The study was also approved by the Central Danish Ethical Committee.

Parasitological examination

Each child provided a stool sample in a labeled container with the subject's unique identifier for enumeration of hookworm, *Ascaris*, and

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TABLE I. Characteristics of study groups before treatment and percentage of complete treatment schedule and coverage during the 12-mo follow-up period.

Characteristic	Treatment groups		
	Mebendazole* (N = 416)	Mebendazole† (N = 397)	Albendazole* (N = 373)
Sex (F/M)	195/221	201/196	164/209
Age (years)	10.9 ± 3.0	10.3 ± 3.1	11.2 ± 3.5
Prevalence of infection			
Hookworm	256 (61.5%)	258 (64.9%)	212 (56.8%)
Ascaris	197 (47.4%)	161 (40.5%)	139 (37.2%)
Trichuris trichiura	127 (30.5%)	155 (39.0%)	87 (23.3%)‡
Geometric mean intensity (GMI)§			
Hookworm	196	215	120‡
Ascaris lumbricoides	2,271	2,062	1,924
Trichuris trichiura	61	102	73‡
Completed treatment			
Scheduled‖	293 (70.4%)	226 (56.9%)	268 (71.8%)‡
Coverage rate#	390 (93.8%)	361 (90.9%)	352 (94.4%)

* Albendazole or mebendazole (600 mg) administered at 6-mo intervals.
† Mebendazole (600 mg) given on a 4-mo basis during a 12-mo study period.
‡ Difference between groups was significant.
§ GMI was calculated as $\text{antilog}_{10}\Sigma$ x/n and expressed as eggs/g of feces.
‖ Defined as children who completed assigned treatment schedule.
Calculated as the percentage of schoolchildren who had at least a single dose of assigned treatment during the 12-mo study period.

Trichuris ova on the same day. A modified Kato–Katz technique using 50-mg templates and cellophane coverslips soaked in a glycerin–malachite green solution was used for diagnosis of intestinal helminths (WHO, 1993). Hookworm eggs were counted within 60 min of smear preparation and the same smear read later the same day for egg counts of *Ascaris* and *Trichuris* infection. Infection intensity was expressed as eggs per gram (epg) of stool.

Data and statistical analyses

All data were entered into an IBM-compatible computer for analyses using SPSS-PC version 5.01 (SPSS, Chicago, Illinois). The computer entry files were validated by cross-checking with original record forms, and frequency distribution of variables performed before statistical analyses. Geometric mean intensity (GMI) at pre- and posttreatment surveys was calculated after logarithmic transformation of egg count by $\log_{10}(x + 1)$. Group means were compared by Student's *t*-test or analysis of variance. χ^2 test or Fisher's exact test was used for comparison of the proportions. The 5% significance level was used. Cure rates were calculated as proportion of children found negative for ova of hookworm, *Ascaris*, or *Trichuris* after receiving the assigned treatment. The percentage egg reduction was calculated as the difference in geometric means between baseline and follow-up surveys for hookworm, *Ascaris*, or *Trichuris* infections divided by geometric mean at baseline examination and expressed as a percent.

RESULTS

One-thousand one-hundred eighty-six children, ages 4 to 19 yr (47.6% females and 52.8% males), were enrolled in the study from the 6 schools. Through randomization, 373 (31.5%) children were assigned to receive albendazole, and 416 (35.1%) to mebendazole repeated twice in a year; 397 (33.4%) children were assigned to mebendazole given over a 4-mo period (once per school term). Age and sex distribution, prevalence, and mean intensity of *Ascaris* were not different between the treatment groups (Table I).

The treatment coverage between the 3 groups was comparable, with over 90% of the children in the study receiving at least a single dose of a 600-mg treatment with albendazole or mebendazole over the 12-mo study period (Table I). Mebendazole given 3 times in a year had the lowest compliance rate (defined as subjects present at first examination and completing assigned treatment schedule during the study period) of 56.9%, compared with 70.4% for mebendazole given twice yearly and 71.4% for albendazole ($\chi^2 = 23.9$, $P < 0.001$).

Prevalence and intensities of intestinal helminths

Of 1,186 children who provided a stool sample for examination before treatment, 726 (61.2%) were positive for hookworm infections, with a GMI of 176 epg of stool. The prevalence of *Ascaris* and *Trichuris* infections was 41.9 and 31.1%, respectively. GMIs for *Ascaris* and *Trichuris* infections were 2,102 and 79 epg of feces, respectively. There were significant differences between the treatment groups in prevalence and GMI (Table I).

Therapeutic effects of regimens on geohelminths

Of the 1,186 children examined before treatment, 792 (66.8%) provided a stool sample for examination 12 mo later. The parasitological cure rates (defined as persons found positive for helminths by ova at pretreatment examination and then negative for helminths by ova at posttreatment examination) and egg reduction rates were significantly different (Tables II, III). Albendazole given twice, with a 6-mo interval, appeared more effective against hookworm, *Ascaris*, and *Trichuris* compared with mebendazole given at the same interval. The cure rate of albendazole against hookworm was 92.4%, compared with 55 and 50% for 3 and 2 doses of mebendazole, respectively ($P <$

TABLE II. Posttreatment cure rates of intestinal helminths among schoolchildren treated with at least a single dose of mebendazole (600 mg) or albendazole (600 mg) 12 mo after treatment.

Helminths	Treatment	No. of children examined	No. of children positive for ova: Pre-treatment	No. of children positive for ova: Post-treatment	% Cured
Hookworm	Mebendazole†	416	256	128	50.0
	Mebendazole‡	397	258	116	55.0
	Albendazole†	373	212	16	92.4
Ascaris	Mebendazole†	416	197	40	79.6
lumbricoides	Mebendazole‡	397	161	4	97.5
	Albendazole†	373	139	23	83.5
Trichuris	Mebendazole†	416	127	50	60.6
trichiura	Mebendazole‡	397	155	49	68.3
	Albendazole†	373	87	28	67.8

* 12 mo later.
† A single 600-mg dose of mebendazole or albendazole given at a 6-mo interval over a 12-mo study period.
‡ Treatment repeated on a 4-mo basis (once every school term) over a 12-mo period.

0.0001). Three doses of mebendazole were more effective against *Ascaris,* with a cure rate of 97.5% compared with 83.5% for 2 doses of albendazole ($\chi^2 = 45.1$, $P < 0.0001$). The cure rate for *Trichuris* by mebendazole given at intervals of 4 mo was comparable to albendazole given at 6-mo intervals, but higher than that of mebendazole given at an interval of 6 mo (67.8/68.3% vs. 60.6%, $P = 0.035$). Egg reduction according to the treatment strategy (Table III) was significantly different for the 2 drugs. Albendazole decreased the GMI of hookworm by 96.7%, from 212 epg of feces before treatment to 16 epg of feces 12 mo later. This reduction was significantly higher than that obtained of 85.1% ($\chi^2 = 17.5$, $P < 0.0001$) and 66.3% ($\chi^2 = 24.9$, $P < 0.0001$) for 3 or 2 single doses of mebendazole, respectively. Before treatment, the GMI of *A. lumbricoides* for the total population was 2,101 epg of feces, and 55.9% (278) of the children were producing more than 3,000 epg of stool. Each of the 3 treatment strategies produced >99% reduction in GMI 12 mo after initial treatment. A reduction of >90% in the intensity of infection was also recorded for *Trichuris* with each of the treatment regimes. Mebendazole every 4 mo produced slightly better reduction in mean egg intensity (94.1%) versus 2 doses of albendazole (90.5%) and 2 doses of mebendazole (93.4%).

Tables IV and V provide data on cure rates and egg reduction rates in the sample of schoolchildren reexamined 4 wk after initial treatment. The albendazole regimen gave better cures for intestinal worms than either mebendazole regimen, e.g., hookworm infection (79.0 vs. 46.6%, $P < 0.0001$), *Ascaris* (90.9 vs. 89.7%, $P > 0.05$), and *Trichuris* (69.1 vs. 57.1%, $P < 0.05$). At 12 mo, albendazole continued to show superiority over 3 doses of mebendazole for hookworm infection (92 vs. 66.7%, $P < 0.0001$) and for *Trichuris* infection (80.4 vs. 71.4%, $P = 0.04$) (Table III).

The effect of the 3 different drugs regimens on infection intensity is shown in Table V. At 4 wk, the GMI of hookworm decreased by 98.5% in the albendazole regimen, compared with a decrease of 91.2% ($P < 0.05$) for the 4-mo mebendazole regimen. For *Ascaris,* there was no difference in mean intensity

TABLE III. Egg reduction rates for hookworm, *Ascaris,* and *Trichuris* infections in schoolchildren treated with 2 regimens of mebendazole (600 mg) or albendazole (600 mg).

Helminths	Treatment	Children positive for ova	Geometric mean intensity eggs/g of feces: Pre-treatment	Geometric mean intensity eggs/g of feces: Post-treatment*	% Egg reduction
Hookworm	Mebendazole†	256	196	66	66.3
	Mebendazole‡	258	215	32	85.1
	Albendazole†	212	120	4	96.7
Ascaris	Mebendazole†	197	2,271	12	99.4
lumbricoides	Mebendazole‡	161	2,062	2	99.9
	Albendazole†	139	1,924	7	99.6
Trichuris	Mebendazole†	127	61	4	93.4
trichiura	Mebendazole‡	155	101	6	94.1
	Albendazole†	87	74	7	90.5

* 12 mo.
† A single 600-mg dose of mebendazole or albendazole given at a 6-mo interval.
‡ A single 600-mg dose of mebendazole repeated on a 4-mo basis (once every school term) over a 12-mo period.

TABLE IV. Cure rates of hookworm, *Ascaris* and *Trichuris* infections in a 40% sample of schoolchildren (n = 491) examined at 4 wk and 12 mo after treatment.

Helminths	Treatment	Number of children infected: Before treatment	After treatment: 1 mo	After treatment: 12 mo	% Cured: 1 mo	% Cured: 12 mo
Hookworm	Mebendazole*	92	52	39	43.8	57.6
	Mebendazole†	120	64	40	46.6	66.7
	Albendazole*	100	21	8	79.0	92.0
Ascaris	Mebendazole*	57	5	14	91.2	75.4
lumbricoides	Mebendazole†	68	7	3	89.7	95.6
	Albendazole*	66	6	16	90.9	75.8
Trichuris	Mebendazole*	42	18	14	57.1	66.7
trichiura	Mebendazole†	77	33	22	57.1	71.4
	Albendazole*	56	17	11	69.1	80.4

* A single dose of 600 mg given at a 6-mo intervals over a 12-mo study period.
† Treatment repeated on a 4-mo basis (once every school term) over a 12-mo period.

reduction between the regimens. Albendazole reduced *Trichuris* only slightly more than mebendazole.

DISCUSSION

The choice of drug for an intestinal helminth control program and how often treatment should be repeated to give maximum impact on infection intensity and transmission needs serious consideration. The issues of efficacy, availability of the drug, cost, and the delivery system are important in determining the sustainability of a deworming program (WHO, 1987). Among the drugs available are the benzimidazoles, albendazole, and mebendazole, which are generally recommended as effective, broad-spectrum anthelmintics and appropriate for use in control programs (WHO, 1992). The present study compared the efficacy of a 600-mg single dose of albendazole and mebendazole, repeated at 4- or 6-mo intervals in a local setting to be used in the National Schistosomiasis and Intestinal Helminths Control Program of Kenya.

The results show that albendazole is more effective in clearing hookworm infections among schoolchildren compared with mebendazole. A single dose of albendazole evaluated after 4 wk produced a cure rate of 79.0% and an egg reduction of 98.5 % as compared with a 45.2% cure rate and an 88.9% egg reduction by mebendazole. The superiority of albendazole over mebendazole for hookworm infection remained even after the 2 single doses of albendazole, given at 6-mo intervals, was compared with 2 or 3 doses of mebendazole, given at 4-and 6-mo intervals, respectively, over the 12-mo study period. Our results also are in agreement with previous studies (Albonico, Smith et al., 1994) indicating that the 2 drugs are equally effective against *A. lumbricoides*. Both drugs achieved cure rates and total reduction in egg excretion of >99% in 4-wk and 12-mo posttreatment surveys. There were, however, some differences in cure rates of *T. trichiura* by both drugs given at a 6-mo interval that are in disagreement with trials in the Pemba Islands by Albonico, Smith et al. (1994). This discrepancy could have resulted from several factors that make our study and that in the Pemba Islands not strictly comparable. Because

TABLE V. Egg reduction rate of hookworm, *Ascaris*, and *Trichuris* infections in a 40% sample of school children (n = 491) treated with mebendazole or albendazole during the study period.

Helminths	Treatment	Geometric mean intensity egg per gram of feces (EPG): Pre-treatment: No. positive	Pre-treatment: Baseline EPG	Post-treatment: 1 mo EPG	Post-treatment: 12 mo EPG	% Egg reduction rate: 1 mo	% Egg reduction rate: 12 mo
Hookworm	Mebendazole*	92	212	29	20	86.3	90.6
	Mebendazole†	120	251	22	7	91.2	97.2
	Albendazole*	100	130	2	2	98.5	98.5
Ascaris	Mebendazole*	57	2,201	2	11	>99	>99
lumbricoides	Mebendazole†	68	2,736	2	2	>99	>99
	Albendazole*	66	3,891	3	8	>99	>99
Trichuris	Mebendazole*	42	51	5	5	90.1	90.1
trichiura	Mebendazole†	77	99	8	4	91.9	95.8
	Albendazole*	56	68	3	2	95.6	97.0

* A single dose (600 mg) given at a 6-mo interval over a 12-mo study period.
† Treatment repeated on a 4-mo basis (once every school term) over a 12-mo period.

of resource constraints, we followed only a 40% sample in each class (1 to 8) at 4 wk, and 3 and 6 mo, and then the entire school population at the 12-mo survey. However, in the 40% follow-up sample, we examined 3 stool samples from each subject as opposed to a single stool sample that was examined at the 12-mo survey. It is possible, therefore, that the observed differences in our results and those of Albonico, Smith et al. (1994), especially for *Trichuris,* might be due to differences in the number of stool samples that were examined. Thus, the accuracy in detecting light infections and estimating worm burdens increases with the number of stool samples examined. Because we examined more stool samples, we consider the 40% follow sample results to be more precise and, therefore, give a better assessment of the effect of the drugs.

In the present study, we used 600-mg single doses of albendazole and mebendazole regimens, which is higher than WHO-recommended doses of 400 and 500 mg, respectively. Our choice was based on poor cure rates of hookworm and *T. trichiura* infections reported in several areas, including the Pemba Islands studies and the experience of the Kwale studies (Stephenson et al., 1993; Magnussen et al., 1997). Comparison of the present study, however, with other studies that have used both drugs, irrespective of the dose differences, shows generally that albendazole is more effective than mebendazole on hookworm and *T. trichiura* infections (Bartoloni et al., 1993; Rahman, 1996; Sorensen et al., 1996; De Clercq at al., 1997). The follow-up sample size and the differences in the stool samples examined perhaps appear to limit the interpretation of our data, especially for a large control program. However, because all the regimens used in the present study produced reductions in egg excretion, but of different magnitude, we believe that albendazole had an advantage over mebendazole against hookworm and *Trichuris* infections.

Although mebendazole is effective against intestinal helminths and is relatively cheaper than albendazole, its effectiveness on hookworm and *Trichuris* infections in areas with mixed infections may not be optimal if transmission has to be interrupted, as reported by Jongsuksuntigul et al. (1993). More importantly, even with more frequent dosing with mebendazole (4-mo intervals), the cure rate and egg reductions of hookworm at 12 mo were lower than those by albendazole given at 6-mo intervals.

Several studies have shown that albendazole is an effective therapy for intestinal helminths (Bartoloni et al., 1993; Albonico, Smith et al., 1994; Sorensen et al., 1996; Rahman, 1996; Magnussen et al., 1997). Unfortunately, it is not clear how often treatment should be repeated to sustain reductions in intensity levels associated with reduced morbidity and reduced transmission. Our findings indicate that albendazole given in 6-mo cycles substantially reduced intensities of the 3 common intestinal worms. In control programs aiming at reducing morbidity, transmission, and secondary conditions such as iron-deficiency anemia and protein energy malnutrition, albendazole may be preferred over mebendazole. Moreover, mebendazole given to schoolchildren every 4 mo was not very effective on hookworm and *Trichuris* infections, which may mean that it may require more frequent treatment and aggressive follow-up of treatment. Furthermore, because school absenteeism and drop-out rates are a real problem in many schools, the requirement for more frequent mebendazole treatment may counteract the benefit of its lower cost compared with albendazole.

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REVIEW

CLINICIAN'S CORNER

Efficacy of Current Drugs Against Soil-Transmitted Helminth Infections

Systematic Review and Meta-analysis

Jennifer Keiser, PhD

Jürg Utzinger, PhD

Context More than a quarter of the human population is likely infected with soil-transmitted helminths (*Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*) in highly endemic areas. Preventive chemotherapy is the mainstay of control, but only 4 drugs are available: albendazole, mebendazole, levamisole, and pyrantel pamoate.

Objective To assess the efficacy of single-dose oral albendazole, mebendazole, levamisole, and pyrantel pamoate against *A lumbricoides*, hookworm, and *T trichiura* infections.

Data Sources A systematic search of PubMed, ISI Web of Science, ScienceDirect, the World Health Organization library database, and the Cochrane Central Register of Controlled Trials (1960 to August 2007).

Study Selection From 168 studies, 20 randomized controlled trials were included.

Data Extraction and Data Synthesis Information on study year and country, sample size, age of study population, mean infection intensity before treatment, diagnostic method used, time between evaluations before and after treatment, cure rate (the percentage of individuals who became helminth egg negative following treatment with an anthelminthic drug), egg reduction rate, adverse events, and trial quality was extracted. Relative risk, including a 95% confidence interval (CI), was used to measure the effect of the drugs on the risk of infection prevalence with a random-effects model.

Results Single-dose oral albendazole, mebendazole, and pyrantel pamoate for infection with *A lumbricoides* resulted in cure rates of 88% (95% CI, 79%-93%; 557 patients), 95% (95% CI, 91%-97%; 309 patients), and 88% (95% CI, 79%-93%; 131 patients), respectively. Cure rates for infection with *T trichiura* following treatment with single-dose oral albendazole and mebendazole were 28% (95% CI, 13%-39%; 735 patients) and 36% (95% CI, 16%-51%; 685 patients), respectively. The efficacy of single-dose oral albendazole, mebendazole, and pyrantel pamoate against hookworm infections was 72% (95% CI, 59%-81%; 742 patients), 15% (95% CI, 1%-27%; 853 patients), and 31% (95% CI, 19%-42%; 152 patients), respectively. No pooled relative risks could be calculated for pyrantel pamoate against *T trichiura* and levamisole for any of the parasites investigated.

Conclusions Single-dose oral albendazole, mebendazole, and pyrantel pamoate show high cure rates against *A lumbricoides*. For hookworm infection, albendazole was more efficacious than mebendazole and pyrantel pamoate. Treatment of *T trichiura* with single oral doses of current anthelminthics is unsatisfactory. New anthelminthics are urgently needed.

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SOIL-TRANSMITTED HELMINTHIASIS (STH) is caused by an infection with intestinal nematodes, of which *Ascaris lumbricoides*, *Trichuris trichiura*, and the hookworms (*Ancylostoma duodenale* and *Necator americanus*) are the most widespread species.[1,2] An estimated 4.5 billion individuals are at risk of STH and as many as 1.2 billion individuals might be infected with *A lumbricoides*, close to 800 million with *T trichiura*, and more than 700 million with hookworm.[1,3] Infection intensity is a key factor in understanding the morbidity of STH; although light infections are often asymptomatic, heavy infections cause an array of morbidities, including dietary deficiencies and delayed physical and cognitive development. Additionally, hookworm and *T trichiura* infections contribute to iron-deficiency anemia.[1,2,4] Estimates of the global burden due to STH range between 4.5 million and 39 million disability-adjusted life-years.[5,6] Recent findings of increased susceptibility of individuals concurrently infected with hookworm and bacterial, protozoan, or viral infections, including human immunodeficiency virus (HIV)/AIDS and tuberculosis, are of considerable public-health concern because of large geographical overlaps of STH with HIV/AIDS and tuberculosis.[1,3,6]

Despite progress made in recent years, there is still no vaccine against STH.[7] In May 2001, preventive chemo-

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therapy was endorsed by World Health Assembly resolution WHA54.19, urging member states to control morbidity due to STH through regular administration of anthelminthic drugs. The declared aim is to regularly target at least 75% of school-aged children and other high-risk groups by the year 2010.[5,8] Four anthelminthics are currently on the World Health Organization model list of essential medicines for the treatment and control of STH: albendazole, mebendazole, levamisole, and pyrantel pamoate.[5,9] The former 2 are benzimidazoles, which are widely used against STH, often in combination with other drugs to form an integrated approach targeting the so-called neglected tropical diseases.[3,6,10] However, there is considerable concern that large-scale administration of anthelminthics might result in the development and spread of drug-resistant nematodes, which is already a significant problem in veterinary medicine. Recent studies point to another growing problem in public health; administration of a single dose of mebendazole lacked efficacy against hookworm infections among schoolchildren in Zanzibar[11] and Vietnam.[12] Comparisons among these 4 anthelminthics in terms of efficacy are not available, but this kind of information is crucial for guiding national STH control programs.

We conducted a systematic review and meta-analyses to assess the efficacy of currently recommended single-dose, oral regimens of albendazole, mebendazole, levamisole, and pyrantel pamoate for treating infections with *A lumbricoides*, *T trichiura*, and hookworm. We examined randomized, placebo-controlled trials and compared the efficacy of the different anthelminthics against placebo. Additionally, we extracted data on safety whenever possible.

METHODS

We adhered to the Quality of Reporting of Meta-analyses (QUOROM) guidelines.[13] We searched PubMed (http://www.ncbi.nlm.nih.gov) (1966 to August 2007), ISI Web of Science (http://www.isiknowledge.com) (1960 to August 2007), ScienceDirect (http://www.sciencedirect.com) (1960 to August 2007), the Cochrane Central Register of Controlled Trials (http://www.mrw.interscience.wiley.com/cochrane/cochrane_clcentral_articles_fs.html) (1960 to August 2007), and the World Health Organization library database (1960 to August 2007) to identify clinical trials, studies, and case reports pertaining to the use of albendazole, mebendazole, levamisole, and pyrantel pamoate for treating infections with *A lumbricoides*, hookworm, and *T trichiura*. No restrictions were set on year or language of publication. We used the terms *albendazole*, *mebendazole*, *levamisole*, and *pyrantel pamoate* in combination with *trial* or *study* or *case report* and *ascariasis*, *Ascaris lumbricoides*, *hookworm*, *Ancylostoma duodenale*, *Necator americanus*, *trichuriasis*, *Trichuris trichiura*, and *soil-transmitted helminths*. Bibliographies of identified articles were screened for additional relevant studies.

Selection Criteria

We selected studies and trials that reported single-dose drug administration with albendazole, mebendazole, levamisole, and pyrantel pamoate for treating infections with *A lumbricoides*, hookworm, and *T trichiura*. Studies and trials were stratified by parasite and drug, and the following information was retrieved: year and country where the study was implemented, sample size, age of study population, mean infection intensity before treatment, diagnostic method used, and time period between evaluations before and after treatment.

We were interested in both cure rate and egg reduction rate as primary outcomes. Whenever possible, we extracted data on reported adverse events as measure of safety. Within each of the 12 subanalyses (ie, 3 parasites and 4 drugs), we assessed the effect of dosage with an emphasis on the current recommended single-dose regimens, ie, albendazole (400 mg), mebendazole (500 mg), pyrantel pamoate (10 mg/kg), and levamisole (80 mg or 2.5 mg/kg).[1,5,8,9,14]

We assessed all randomized controlled trials for the following quality criteria: randomization methods, description of withdrawals and dropouts, and blinding. A numerical score between 0 and 5 was assigned as a measure of study design and reporting quality with 0 being the weakest and 5 designated the strongest, based on the validated scale put forward by Jadad and colleagues.[15]

Only those trials that were randomized and placebo-controlled were included in our meta-analyses. We allowed nonblinded trials to be included in our analysis by acknowledging that such studies are of poorer quality and hence might overestimate treatment efficacy.

Our goal was to use both cure rate and egg reduction rate as primary outcome measures for anthelminthic drug efficacy. However, calculating the treatment and control groups' mean weighted differences in egg count change before and after treatment was not possible due to an insufficient number of studies reporting egg counts in the same format (arithmetic or geometric mean, including standard deviation). Hence, cure rate, defined as the percentage of individuals who became helminth egg negative after treatment with an anthelminthic drug, served as the sole primary outcome measure in our meta-analyses. To gauge safety, we compiled adverse events in the few trials that reported such measures.

Statistical Analysis

We used StatsDirect version 2.4.5 statistical software for meta-analyses (StatsDirect Ltd, Cheshire, England). If data from more than 2 randomized controlled trials were available, we combined data from trials within a class (eg, albendazole for treating hookworm infections) and calculated the relative risk (RR), including 95% confidence interval (CI) (significance level of $P < .05$). Because of large variations in study populations, sample sizes, designs, diagnostic methods, and duration be-

tween appraisals before and after treatment, we applied random-effects models to compute the pooled relative effectiveness of the studies according to the method described by DerSimonian and Laird.[16] Between-study heterogeneity was examined with Cochran *Q* statistics (significance level of $P \leq .10$) and I^2, whereas potential publication bias was measured using an Egger test and Begg test where a small-study bias is evident when $P \leq .10$.

RESULTS

Studies Identified and Characteristics

FIGURE 1 summarizes the search results of our systematic review. We identified 168 studies carried out in 54 countries using albendazole, mebendazole, pyrantel pamoate, and levamisole against *A lumbricoides, T trichiura*, and hookworm infections. TABLE 1 summarizes for each of the 4 drugs and the 3 parasites investigated the number of patients treated and overall cure rates achieved in non–randomized controlled trials.

There were 20 randomized trials published between 1974 and August 2007 that compared an anthelminthic drug with a placebo[11,12,17-34] (TABLES 2, 3, and 4). The efficacy of single oral doses of albendazole (400 mg), mebendazole (500 mg), and pyrantel pamoate (10 mg/kg) was assessed in 14, 6, and 4 randomized studies, respectively. We could not identify a single study that evaluated the efficacy of levamisole in a randomized placebo-controlled trial at current recommended doses. Anthelminthic drug efficacy was assessed by different diagnostic methods and at different time points after treatment (usually between 2 and 7 weeks following drug administration). Although some studies focused on school-aged children, others administered drugs to adults; hence, different age groups were involved. Infection intensities before treatment showed large variations from one trial to another.

Methodological Quality

Tables 2, 3, and 4 summarize methodological quality issues of the 20 trials included in our meta-analyses. According to our inclusion criteria, all studies included a placebo group. The design of the trials were double-blind (n=9), single-blind (n=2), or nonblinded (n=2), whereas no information was available regarding the blinding procedure in the remaining 7 studies. Concealment allocation and withdrawal from studies was clearly described in 5 (25%) and 12 studies (60%), respectively. According to the quality criteria set forth by Jadad and colleagues,[15] the studies included in the current meta-analyses had scores ranging from 1 to 5.

Albendazole

For the treatment of *A lumbricoides* infection, there were 10 placebo-controlled trials including 557 individuals (Table 2).[19,20,22,24,26-29,31,32] Four trials used Zentel (GlaxoSmithKline, London, England) whereas the source of albendazole was not given in the remaining 6 trials. Egg reduction rates of 86.5% to 100% were reported. Heterogeneity between the studies was pronounced ($Q=25.9$; $P=.003$, $I^2=65.3\%$). The pooled random RR for albendazole treatment against *A lumbricoides* infection relative to placebo was 0.12 (95% CI, 0.07-0.21; $P<.001$) (FIGURE 2). The results indicated the presence of a publication bias when an Egger test (intercept –3.34, $P=.001$) and a Begg test were used ($P=.03$).

For the treatment of *T trichiura* infection, we used results from 9 randomized placebo-controlled trials, including 1 multicenter trial and 735 patients, for our meta-analysis (Table 2).[19,22,24,26-29,31,32] Cochran *Q* statistics revealed heterogeneity ($Q=76.8$; $P<.001$, $I^2=89.5\%$). Relative to placebo, the pooled random RR for albendazole against *T trichiura* infection was 0.72 (95% CI, 0.61-0.87; $P=.001$) (Figure 2). There was an indication of a publication bias (Egger test, intercept –1.48, $P=.03$; Begg test,

Figure 1. Decision Tree Showing Inclusion and Exclusion of Studies Identified

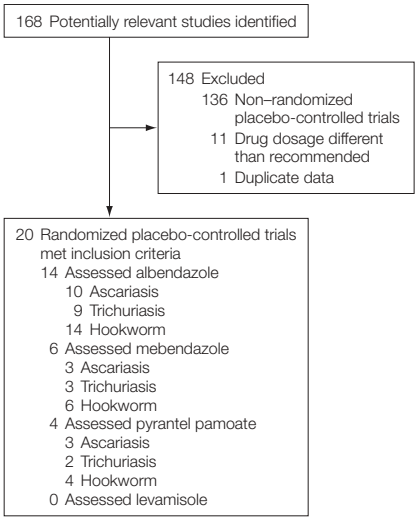


Table 1. Summary of Observational and Case Studies Reporting the Use of Single-Dose Oral Albendazole, Mebendazole, Pyrantel Pamoate, and Levamisole Against *Ascaris lumbricoides*, *Trichuris trichiura*, and Hookworm Infection

Drug	Parasite	Studies Identified and Included, No.	Individuals, No.	Overall Cure Rate, %
Albendazole (400 mg)	*A lumbricoides*	65	5126	93.9
	T trichiura	64	5147	43.6
	Hookworm	64	6334	78.4
Mebendazole (500 mg)	*A lumbricoides*	12	2036	96.5
	T trichiura	12	3112	23.0
	Hookworm	14	3192	22.9
Pyrantel pamoate (10 mg/kg)	*A lumbricoides*	17	1208	87.9
	T trichiura	11	458	28.1
	Hookworm	21	1208	87.9
Levamisole (2.5 mg/kg)	*A lumbricoides*	3	202	91.5
	T trichiura	2	186	8.6
	Hookworm	4	178	38.2

Table 2. Randomized Placebo-Controlled Studies Reporting the Use of Single-Dose Oral Albendazole (400 mg) Against *Ascaris lumbricoides*, *Trichuris trichiura*, and Hookworm Infection

Source (Location, Year Trial Was Implemented)	Age, y	Diagnostic Approach	Treatment Evaluation	Study Design[a]	Quality Assessment[b]	Product Used	Parasite	Active Treatment Group			
								Individuals, No.	Mean Pretreatment Infection Intensity (Eggs/g)	Efficacy, %	
										Cure Rate	Egg Reduction Rate
Ovedoff[24] (Philippines, 1984)	NA	NA	NA	Double-blind; follow-up and withdrawal not described	2	NA	*A lumbricoides*	16	NA	100	100
							T trichiura	29	NA	68.9	NA
							Hookworm (*N americanus*)	15	NA	93.3	NA
Sinniah et al[28] (Malaysia, 1990)	6-13	Brine flotation technique and Beavers technique	3 wk after treatment	Blinding not known; follow-up and withdrawal not described	1	NA	*A lumbricoides*	56	80 553[c]	91.1	99.2
							T trichiura	52	21 635[c]	42.3	71.2
							Hookworm	16	2614[c]	100	100
Beach et al[31] (Haiti, 1999)	7.4 (Mean)	Formalin ethyl acetate concentration technique	5 wk after treatment	Double-blind; follow-up and withdrawal described	4	Zentel[d]	*A lumbricoides*	62	284[e]	98.4	100
							T trichiura	93	120[e]	52.7	42.2
							Hookworm	12	74[e]	100	100
Stephenson et al[29] (Kenya, 1990)	6-12	Modified Kato-Katz technique	7 wk after treatment	Blinding not known; follow-up and withdrawal not described	2	Zentel	*A lumbricoides*	7	69[e]	100	100
							T trichiura	17	2112[e]	0	0
							Hookworm	16	1027[e]	40.0	96.6
Olds et al[32] (Africa, Asia, 1999)	10.4 (Mean)	Kato-Katz technique (2 samples)	45 d after treatment	Double-blind; follow-up and withdrawal described	5	NA	*A lumbricoides*	219	NA	81.7	NA
							T trichiura	297	NA	33.3	NA
							Hookworm	172	NA	77.4	NA
Bwibo and Pamba[22] (Kenya, 1984)	13.2 (Mean)	Kato-Katz technique (2 samples)	21 d after treatment	Blinding not known; follow-up and withdrawal described	3	NA	*A lumbricoides*	40	NA	90.0	93.1
							T trichiura	31	NA	83.9	89.7
							Hookworm (*N americanus*)	34	NA	88.2	NA
El-Masry et al[20] (Egypt, 1983)	25.7 (Mean)	Stool egg counts and merthiolate-iodine-formaldehyde concentration for 5 d	2 wk after treatment	Double-blind; follow-up and withdrawal not described	2	Zentel	*A lumbricoides*	11	515[e]	100	100
							Hookworm (*Ancylostoma duodenale*)	19	404[e]	89.0	NA
Oyediran and Oyejide[19] (Nigeria, 1983)	8-17	Concentration and Kato-Katz technique	14 d after treatment	Double-blind; follow-up and withdrawal not described	4	NA	*A lumbricoides*	27	NA	85.2	99.6
							T trichiura	29	NA	37.9	69.3
							Hookworm (*N americanus*)	26	NA	53.8	82.8
Upatham et al[27] (Thailand, 1989)	Adults	Kato-Katz technique (up to 3 samples)	1 mo after treatment	Double-blind; follow-up and withdrawal not described	2	Zentel	*A lumbricoides*	78	9311[c]	94.9	99.3
							T trichiura	146	655[c]	33.6	59.4
							Hookworm	260	1516[c]	45.8	90.5

(continued)

Table 2. Randomized Placebo-Controlled Studies Reporting the Use of Single-Dose Oral Albendazole (400 mg) Against *Ascaris lumbricoides*, *Trichuris trichiura*, and Hookworm Infection (cont)

Source (Location, Year Trial Was Implemented)	Age, y	Diagnostic Approach	Treatment Evaluation	Study Design[a]	Quality Assessment[b]	Product Used	Parasite	Active Treatment Group: Individuals, No.	Active Treatment Group: Mean Pretreatment Infection Intensity (Eggs/g)	Active Treatment Group: Efficacy, %: Cure Rate	Active Treatment Group: Efficacy, %: Egg Reduction Rate
Chien et al[26] (Malaysia, 1989)	8-9	Direct fecal smear	4 wk after treatment	Blinding not known; follow-up and withdrawal not described	1	NA	*A lumbricoides*	41	NA	90.2	86.5
							T trichiura	41	NA	4.9	52.3
							Hookworm (*N americanus*)	41	NA	82.9	64.2
Flohr et al[12] (Vietnam, 2007)	≥16	Salt flotation technique (1 sample)	2 wk after treatment	Double-blind; follow-up and withdrawal described	5	Mekozetel[f]	Hookworm	47	1120[c]	45.0	79.0
Sacko et al[33] (Mali, 1999)	3-70	Kato-Katz technique (2 samples)	10 d after treatment	Single-blind; follow-up and withdrawal described	2	Zentel	Hookworm (*N americanus*)	37	174.5[c]	83.8	97.7
Farid et al[23] (Egypt, 1984)	NA	Kato-Katz technique	NA	Blinding not known; follow-up and withdrawal not described	1	NA	Hookworm (*A duodenale*)	19	NA	89.4	NA
Morgan et al[21] (Malawi, 1983)	6-19	Kato-Katz technique	21 d after treatment	Double-blind; follow-up and withdrawal described	3	Zentel	Hookworm (*N americanus*)	28	564[c]	85.0	94.9

Abbreviation: NA, not available.
[a] All studies were randomized, placebo-controlled trials.
[b] A numerical score between 0 and 5 was assigned as a measure of study design and reporting quality (0 being the weakest, 5 the strongest), based on the validated scale put forward by Jadad and colleagues.[15]
[c] Arithmetic mean.
[d] Manufactured by GlaxoSmithKline, London, England.
[e] Geometric mean.
[f] Manufactured by Mekophar Chemical Pharmaceutical Joint Stock Co, Ho Chi Minh City, Vietnam.

$P=.02$). Egg reduction rates in these 9 trials ranged from 0% to 89.7%.

For the treatment of hookworm infection, we included 14 randomized placebo-controlled trials with 742 patients in our meta-analysis (Table 2).[12,19-24,26-29,31-33] The effect of albendazole on *N americanus* and *A duodenale* was assessed in 6 and 2 trials, respectively. In the remaining 6 trials, hookworms were not identified at species level. Egg reduction rates varied from 64.2% to 100%. The random RR for albendazole treatment for hookworm infection (both species) was 0.28 (95% CI, 0.19-0.41; $P<.001$) (Figure 2). There was considerable heterogeneity between trials ($Q=85.6$; $P<.001$, $I^2=84.8\%$). According to the Egger test, there was a publication bias ($P=.003$). However, the Begg test showed no statistical significance ($P=.12$).

Albendazole was well tolerated. In 11 studies included in our meta-analysis, no significant adverse events were reported following albendazole administration.[12,19-23,26-28,31,32] One trial carried out in the Philippines reported nausea and diarrhea in 2 and 1 individuals, respectively.[24] There was no indication whether or not adverse events were assessed in the remaining 2 randomized placebo-controlled trials included in our meta-analysis.[29,33]

Mebendazole

For the treatment of *A lumbricoides* infection, only 3 studies including 309 individuals were placebo-controlled trials and hence were included in our meta-analysis (Table 3).[11,25,34] Egg reduction rates ranged between 96.1% and 99.0%. A pooled random RR of 0.05 (95% CI, 0.03-0.09; $P<.001$) was calculated (FIGURE 3). Heterogeneity was low ($Q=1.7$; $P=.42$, $I^2=0\%$). Because there were only 3 studies included, it was not possible to investigate whether publication bias was an issue.

For the treatment of *T trichiura* infection, only 3 studies (685 patients) fulfilled the selection criteria and were included in our meta-analysis (Table 3).[11,25,34] Egg reduction rates were 81.0% to 92.8%. The pooled random RR was 0.64 (95% CI, 0.49-0.84; $P=.001$). Heterogeneity was pronounced ($Q=35.4$; $P<.001$, $I^2=94.5\%$) (Figure 3). Given the low number of studies entering our meta-analysis, we could not determine whether publication bias was an issue.

For the treatment of hookworm infection, 6 placebo-controlled trials (853 patients) met our inclusion criteria and were used for our meta-analysis (Table 3).[11,12,25,30,33,34] The overall random RR was 0.85 (95% CI, 0.73-0.99; $P=.01$). Heterogeneity was high ($Q=49.3$; $P<.001$, $I^2=89.6\%$) (Figure 3). Although 1 trial found no reduction in

hookworm egg burden following mebendazole treatment,[30] 1 trial found a high egg reduction rate of 98.3%.[25] According to an Egger test, there was no indication of a publication bias ($P=.15$).

Mebendazole was well tolerated. In 3 trials, no adverse events were observed.[11,12,34] One study reported abdominal discomfort in 6 of 45 children who were treated with 500-mg mebendazole.[25] No information on adverse events was given in the remaining 2 studies.[30,33]

Pyrantel Pamoate

For the treatment of *A lumbricoides* infection, there were 3 randomized placebo-controlled trials including 131 patients (Table 4),[17,18,28] and the pooled random RR was 0.12 (95% CI, 0.07-0.21; $P<.001$). There was a low level of heterogeneity ($Q=2.3$; $P=.32$, $I^2=11.5\%$) (FIGURE 4). One of the trials reported an egg reduction rate of 87.9%.[28] Because of the small number of trials included in our meta-analysis, it was not possible to assess whether there was a publication bias.

For the treatment of *T trichiura* infection, only 2 trials were randomized and placebo-controlled (Table 4), and calculating random RR was not feasible. The cure rates in these 2 trials were 11.5%[28] and 38.1%.[17] In one of the trials, an egg reduction rate was also reported; it was 52.0%.[28]

For the treatment of hookworm infection, there were 4 randomized placebo-controlled trials (152 patients) (Table 4),[17,18,28,30] resulting in a random RR of 0.69 (95% CI, 0.58-0.81; $P<.001$) (Figure 4). Heterogeneity was low ($Q=3.9$; $P=.26$, $I^2=24.3\%$). Egg reduction rates ranged from 56.4% to 75.0%. Based on an Egger test, there was no indication of a publication bias ($P=.93$).

Almost half of the patients (47.8%) treated with pyrantel pamoate in a study in Nigeria experienced adverse events, mainly abdominal pain, nausea, and

Table 3. Randomized Placebo-Controlled Studies Reporting the Use of Single-Dose Oral Mebendazole (500 mg) Against *Ascaris lumbricoides*, *Trichuris trichiura*, and Hookworm Infection

Source (Location, Year Trial Was Implemented)	Age, y	Diagnostic Approach	Treatment Evaluation	Study Design[a]	Quality Assessment[b]	Product Used	Parasite	Active Treatment Group: Individuals, No.	Active Treatment Group: Mean Pretreatment Infection Intensity (Eggs/g)	Efficacy, %: Cure Rate	Efficacy, %: Egg Reduction Rate
Albonico et al[11] (Tanzania, 2003)	7-18	Kato-Katz technique (1 sample)	21 d after treatment	Not blinded; follow-up and withdrawal described	3	Vermox[c]	*A lumbricoides*	141	114[d]	96.5	99.0
							T trichiura	214	302[d]	22.9	81.0
							Hookworm	224	447[d]	7.6	52.1
Albonico et al[34] (Tanzania [Pemba], 2002)	9.5 (Mean)	Kato-Katz technique (1 sample)	21 d after treatment	Not blinded; follow-up and withdrawal described	3	NA	*A lumbricoides*	107	5[d]	98.0	96.1
							T trichiura	404	257[d]	25.2	83.6
							Hookworm	424	588[d]	13.2	67.0
Abadi[25] (Indonesia, 1985)	2-70	Kato-Katz technique (1 sample) and Harada Mori	2-4 wk after treatment	Double-blind; follow-up and withdrawal not described	3	NA	*A lumbricoides*	61	37 653[e]	93.4	99.0
							T trichiura	67	6434[e]	77.6	92.8
							Hookworm (*Necator americanus*, *Ancylostoma duodenale*)	45	1928[e]	91.1	98.3
De Clercq et al[30] (Mali, 1997)	5-54	Kato-Katz technique (2 samples)	4 wk after treatment	Single blinded; follow-up and withdrawal described	2	Vermox	Hookworm (*N americanus*)	35	264.2[e]	22.9	0
Flohr et al[12] (Vietnam, 2007)	6-11	Salt flotation technique (1 sample)	2 wk after treatment	Double-blind; follow-up and withdrawal described	5	Phardazone[f]	Hookworm	90	263[e]	38	52
Sacko et al[33] (Mali, 1999)	3-70	Kato Katz technique (2 samples)	10 d after treatment	Single-blind; follow-up and withdrawal described	2	Vermox	Hookworm (*N americanus*)	35	185.3[e]	51.4	68.5

Abbreviation: NA, not available.
[a] All studies were randomized, placebo-controlled trials.
[b] A numerical score between 0 and 5 was assigned as a measure of study design and reporting quality (0 being the weakest, 5 the strongest), based on the validated scale put forward by Jadad and colleagues.[15]
[c] Manufactured by Janssen, Beerse, Belgium.
[d] Geometric mean.
[e] Arithmetic mean.
[f] Manufactured by Central Pharmaceutical Company No. 1, Hanoi, Vietnam.

dizziness.[18] Two studies did not describe the occurrence of adverse events,[17,30] and 1 trial found that pyrantel pamoate was well tolerated.[28]

Levamisole

For the treatment of *A lumbricoides* infection, 2 levamisole dosages are currently recommended: a single oral dose of 80 mg[35] or 2.5 mg/kg (http://www.who.int/wormcontrol/statistics/useful_info/en/index3.html).[1,14] For the latter dosage, which had been applied in 3 studies,[36-38] an overall cure rate of 91.5% was obtained (Table 1). Two of these studies were placebo-controlled, but none was randomized,[36,37] so calculating a random RR was not possible.

For the treatment of *T trichiura* infection, we identified only 1 randomized placebo-controlled trial. It was carried out in Tanzania, and children infected with *T trichiura* received either 40- or 80-mg levamisole, depending on weight (equivalent to 1.25-2.5 mg/kg). A low cure rate (9.6%) and a low egg reduction rate (41.5%) were found.[11] The overall cure rate of 2 non–randomized placebo-controlled trials-[36,37] was 8.6% (Table 1).

For the treatment of hookworm infection, none of the studies identified fulfilled our inclusion criteria for meta-analysis, so calculating a random RR was not possible. One randomized placebo-controlled trial carried out in Tanzania[11] and another one in Malawi,[39] administering levamisole at 40 or 80 mg and 80 or 120 mg, depending on the individual's weight or age, achieved cure rates of 11.9% and 10%, respectively. We calculated an overall cure rate of 38.2% in 4 non–randomized placebo-controlled trials (Table 1).[36,37]

COMMENT

Hundreds of millions of people are affected by STH the world over, with a global burden that might be as high as 39 million disability-adjusted life-years,[1,5] which is similar to the global burden owing to malaria.[40] Nonetheless, STH and other helminth, protozoan, and bacterial infections have been called neglected tropical diseases (NTDs) because these diseases are particularly rampant in developing countries and inflict a disproportionate burden on the global poor.[3,6,41] There is growing awareness of the public-health significance of NTDs, and concerted advocacy for their control has resulted in increased political will and financial means to combat NTDs. Preventive chemotherapy plays a seminal role.[6,8] In 2006, for example, millions of school-aged children were given albendazole or mebendazole (http://www.who.int/wormcontrol/newsletter/PPC8_eng.pdf). However, to achieve the 2010 global target to regularly treat at least 75% of all school-aged chil-

Table 4. Randomized Placebo-Controlled Studies Reporting the Use of Single-Dose Oral Pyrantel Pamoate (10 mg/kg) Against *Ascaris lumbricoides*, *Trichuris trichiura*, and Hookworm Infection

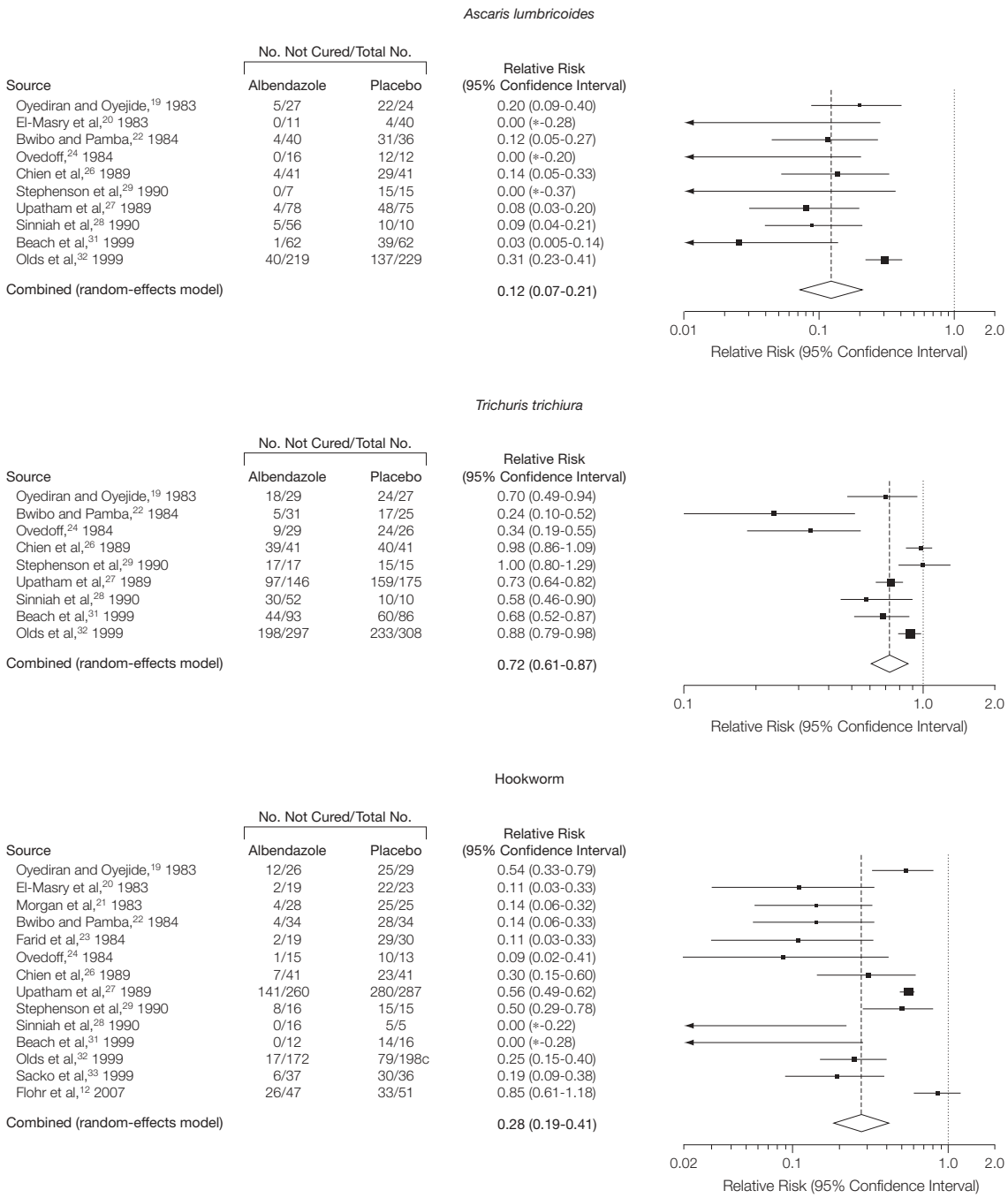
Source (Location, Year Trial Was Implemented)	Age, y	Diagnostic Approach	Treatment Evaluation	Study Design[a]	Quality Assessment[b]	Product Used	Parasite	Active Treatment Group			
								Individuals, No.	Mean Pretreatment Infection Intensity (Eggs/g)[c]	Efficacy, %	
										Cure Rate	Egg Reduction Rate
Kale[18] (Nigeria, 1977)	6-17	Quantitative egg count	42 d after treatment	Blinding not known; follow-up and withdrawal not described	1	Combantrin[d]	*A lumbricoides*	64	NA	93.8	NA
							T trichiura	63	NA	38.1	NA
							Hookworm	55	NA	29.1	56.4
Chege et al[17] (Kenya, 1974)	Children	Formol ether technique (1 sample)	2 mo after treatment	Blinding not known; follow-up and withdrawal described	3	NA	*A lumbricoides*	20	NA	90.0	NA
							Hookworm (*Necator americanus*)	60	NA	42.0	NA
Sinniah et al[28] (Malaysia, 1990)	6-13	Brine flotation technique and Beaver technique	3 wk after treatment	Blinding not known	1	NA	*A lumbricoides*	47	107 958	85.1	87.9
							T trichiura	52	3271	11.5	52.0
							Hookworm	8	3150	37.5	71.4
De Clercq et al[30] (Mali, 1997)	5-54	Kato-Katz technique (2 samples)	4 wk after treatment	Single-blind; follow-up and withdrawal described	2	Combantrin	Hookworm (*N americanus*)	29	472.1	44.8	75.0

Abbreviation: NA, not available.
[a] All studies were randomized, placebo-controlled trials.
[b] A numerical score between 0 and 5 was assigned as a measure of study design and reporting quality (0 being the weakest, 5 the strongest), based on the validated scale put forward by Jadad and colleagues.[15]
[c] All means were arithmetic.
[d] Manufactured by Pfizer, New York, New York.

dren and other populations at risk of STH, the frequency of benzimidazole administration will increase further. Knowledge on the safety and efficacy of anthelminthics is therefore crucial to guide clinicians and control program officers in selecting the appropriate drug against specific STH infections.[12]

To our knowledge, we present the first systematic review and meta-analysis of the comparative efficacy of the 4 anthelminthic drugs that are currently on the

Figure 2. Risk Ratio Estimates and Pooled Random Risk Ratios of Randomized, Placebo-Controlled Trials of Albendazole Against *Ascaris lumbricoides*, *Trichuris trichiura*, and Hookworm Infections



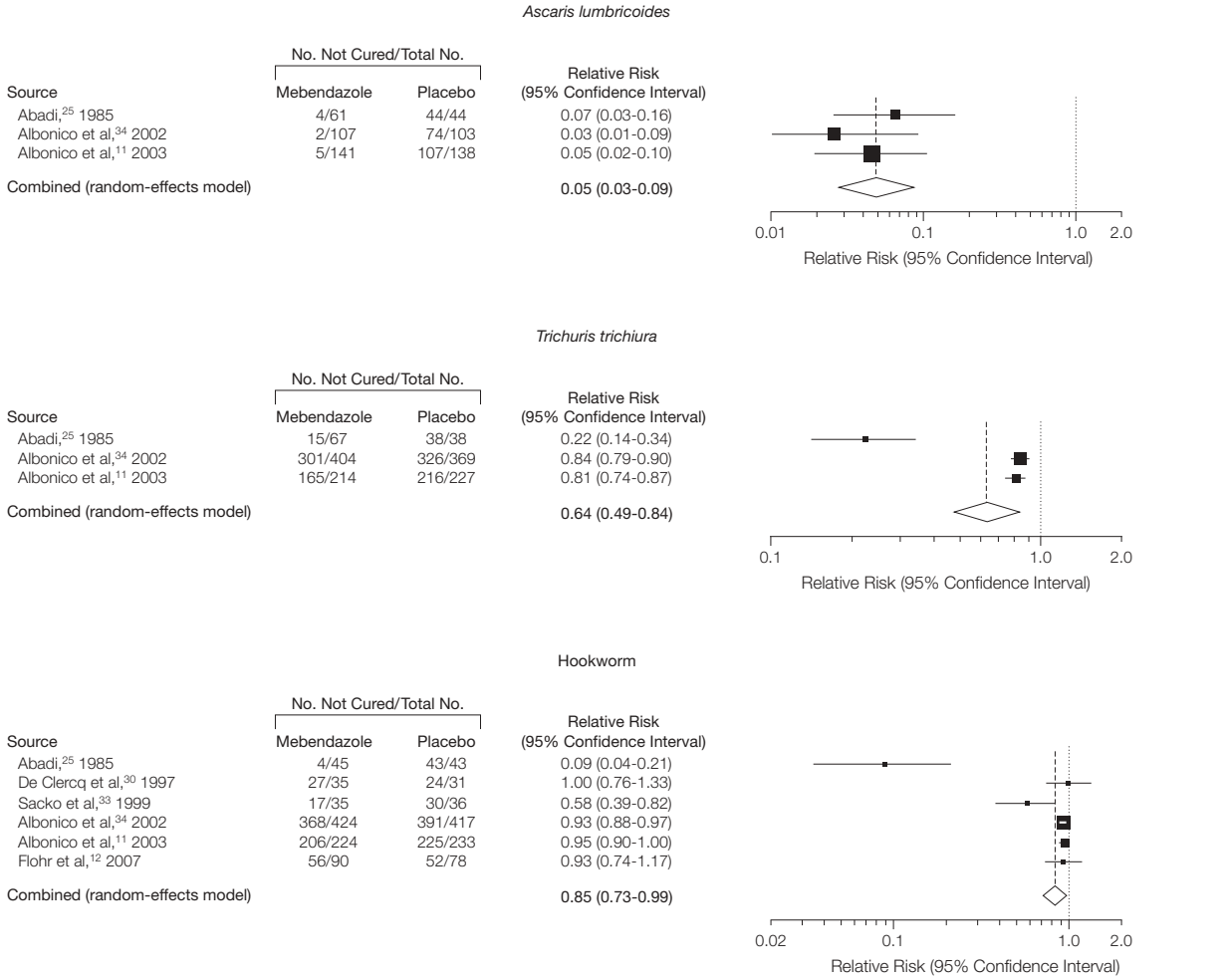
Rectangles indicate risk ratios (RRs), and sizes of the rectangles represent the weight given to each study in the meta-analysis. Diamond and vertical dashed line indicate combined RR; horizontal lines indicate 95% confidence intervals.

World Health Organization model list of essential medicines. The anthelminthic efficacy of albendazole has been reviewed before (although the review made no attempt to distinguish between randomized, nonrandomized, and placebo-controlled trials),[42] and recently, a meta-analysis of randomized controlled trials was presented regarding the effect of simultaneous treatment targeting 2 or more NTDs.[43]

An important observation of our systematic review is the paucity of high-quality studies, which are crucial to guide clinical decisions about which anthelminthic drug to use. This issue is underscored by the following considerations. First, only a few studies met our inclusion criteria; ie, they were randomized and placebo-controlled and used the currently recommended single oral dose regimen. Examining the effect of anthelminthics compared with placebo by means of meta-analysis would not have been possible at all if we would have included only double-blind studies. The lack of high-quality trials might be explained, at least partially, by the fact that the majority of trials were carried out more than 20 years ago. It is noteworthy that not a single randomized, placebo-controlled trial using levamisole at the recommended dose (ie, 80 mg or 2.5 mg/kg) could be identified in the peer-reviewed literature according to our selection criteria.

Second, results on both cure and egg reduction rates should be reported as primary outcome measures regarding the efficacy of anthelminthic drugs. The latter measure is of particular relevance because infection intensity correlates with worm burden and hence morbidity due to helminth infections.[1,2,5,44] However, calculation of the combined mean difference of egg counts between treatment and placebo groups was not possible because some trials re-

Figure 3. Risk Ratio Estimates and Pooled Random Risk Ratios of Randomized, Placebo-Controlled Trials of Mebendazole Against *Ascaris lumbricoides*, *Trichuris trichiura*, and Hookworm Infections



Rectangles indicate risk ratios (RRs), and sizes of the rectangles represent the weight given to each study in the meta-analysis. Diamond and vertical dashed line indicate combined RR; horizontal lines indicate 95% confidence intervals.

ported no data on egg counts and others reported either arithmetic or geometric means, often in the absence of the standard deviation.

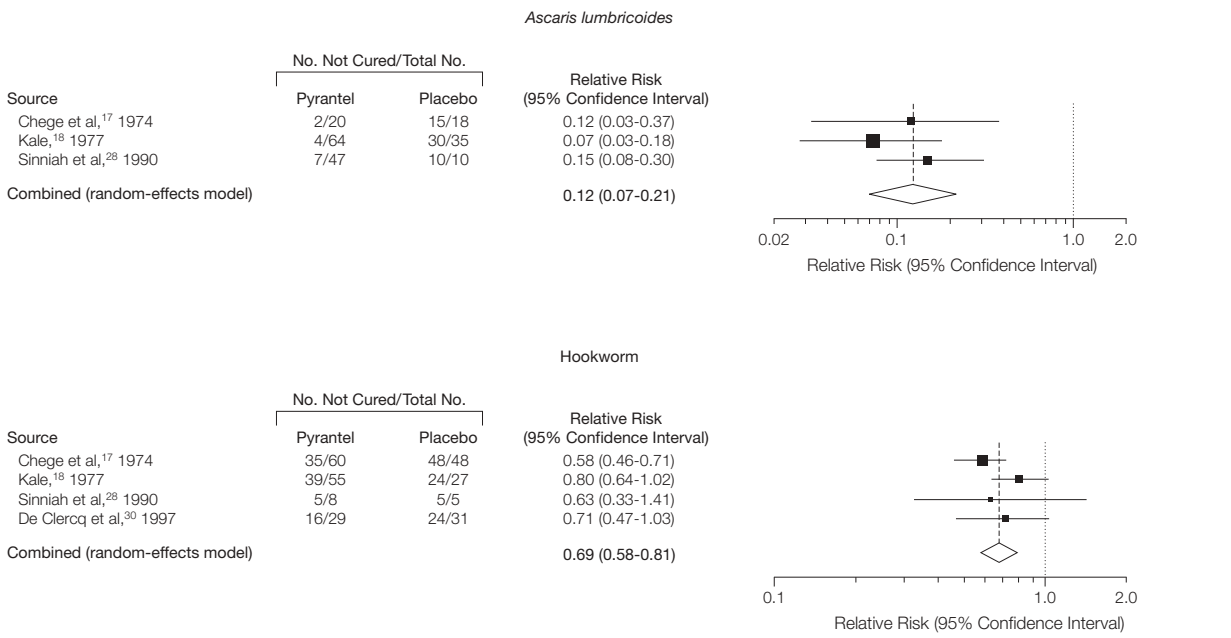
Third, a number of additional methodological issues need to be considered because they might have influenced our findings; therefore, caution must precede efforts to make policy recommendations. For example, the sample sizes in several of the trials included in our meta-analyses were small (eg, <50 individuals infected with a specific STH and treated with an anthelminthic drug), so these trials were likely underpowered. With regard to the diagnostic approach taken, most trials evaluated drug efficacy based on a single stool sample per individual examined before and after treatment, employing only 1 diagnostic test. It is widely acknowledged that there is significant day-to-day and intraspecimen variation in helminth egg output and that diagnostic tests lack sensitivity, particularly for low infection intensities.[45,46]

Fourth, our results point to a publication bias as evidenced by a considerable number of our subanalyses reporting significant *P* values according to either an Egger test or Begg test. It appears that anthelminthic drug trials resulting in significant cure rates were more likely to be reported in the peer-reviewed literature than those lacking efficacy. Finally, some trials failed to report whether adverse events were monitored at all, and safety measures overall lacked quality.

Although all 4 anthelminthics are considered to exhibit a broad spectrum of activity, we identified significant therapeutic differences when they were administered at single-dose oral regimens. Differences in helminth species–specific susceptibilities are multifactorial, including drug- and batch-related variations, differences between individual parasite strains, differences between infections with *N americanus* and *A duodenale* (in the case of hookworm), infection intensities before treatment, host-specific factors (eg, coinfections), and the emergence of drug resistance.[12,30,47] All drugs were highly efficacious against *A lumbricoides* in a single dose. With regard to *T trichiura*, our results indicated that current anthelminthics were unsatisfactory as shown by low cure rates revealed by our meta-analyses. Indeed, the risk of still being infected with *T trichiura* after a single 400-mg oral dose of albendazole was only reduced by 28%. A similarly low risk reduction was found after a single 500-mg oral dose of mebendazole (36%). Low overall cure rates of 28.1% and 8.6% were calculated from non–randomized placebo-controlled trials for pyrantel pamoate and levamisole, respectively.

No conclusion on the effect on infection intensities can be made, although this outcome measure is of key importance from the point of view of morbidity control. It should be noted that clinical manifestations can be serious for *T trichiura* infection, such as chronic dysentery or rectal prolapse.[1] Higher cure and egg reduction rates were reported when 3-day dose schedules of albendazole (400 mg for 3 days) and mebendazole (100 mg twice daily for 3 days) were administered.[1] However, such treatment schemes are not

Figure 4. Risk Ratio Estimates and Pooled Random Risk Ratios of Randomized, Placebo-Controlled Trials of Pyrantel Pamoate Against *Ascaris lumbricoides* and Hookworm Infections



Rectangles indicate risk ratios (RRs), and sizes of the rectangles represent the weight given to each study in the meta-analysis. Diamond and vertical dashed line indicate combined RR; horizontal lines indicate 95% confidence intervals.

feasible for large-scale preventive chemotherapy because they are likely to result in reduced compliance rates.

With regard to hookworms, our data suggest that, when administered as single-dose therapy, albendazole was the most efficacious drug reducing the prevalence of hookworm infection. At the recommended single dose of 400 mg, albendazole cured hookworm infections by 72%. The efficacy of mebendazole and pyrantel against hookworm infections was 15% and 32%, respectively. Cure rates from nonrandomized, placebo-controlled trials following levamisole treatment were low (10%-38%). Pyrantel pamoate and levamisole are currently regarded as alternative drugs for the treatment of hookworms.[1] Although the low efficacy of single-dose mebendazole against hookworm infection has been described and thus a 3-day mebendazole therapy (100 mg twice daily for 3 days) has been recommended,[1,48] single-dose mebendazole treatment is widely used. For example, recently in Ghana, an estimated 4 to 5 million children aged 3 to 15 years were treated with single 500-mg mebendazole.[49] Nonetheless, we do not disavow that single-dose mebendazole might have a significant impact on infection intensity and hence morbidity reduction.

CONCLUSION

Our systematic review and meta-analysis identified a number of gaps regarding the evidence base of current anthelminthic drugs. Well-designed, adequately powered, and rigorously implemented trials should address these gaps, not only providing new data regarding the efficacy (considering both cure and egg reduction rates) of anthelminthic drugs against the main species of STH, but also aiding in establishing benchmarks for subsequent monitoring of drug resistance. In turn, these findings will be crucial to enhance the effectiveness of national control programs targeting STH that might be implemented in an integrated fashion addressing multiple NTDs.

Our results showed that the efficacy of single-dose oral albendazole for curing hookworm infections was higher than that of mebendazole, levamisole, and pyrantel pamoate, although few studies compared the drugs head-to-head. Finally, our findings stress the pressing need for discovery and development of novel anthelminthic drugs, ideally with different mechanisms of action to complement the current therapeutic arsenal.[50,51] To our knowledge, tribendimidine is the only anthelminthic drug for STH in late-stage development and registration.[52] Compared with albendazole, tribendimidine achieved superior cure rates against hookworm, particularly *N americanus*, and is similarly effective against *A lumbricoides*, but also resulted in disappointing cure rates against *T trichiura* infection when used in a single oral dose. Phase 4 trials in China involving more than 2000 individuals have been completed recently and confirmed the safety of tribendimidine also in school-aged children.[53]

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Study concept and design: Keiser, Utzinger.
Acquisition of data: Keiser.
Analysis and interpretation of data: Keiser, Utzinger.
Drafting of the manuscript: Keiser, Utzinger.
Critical revision of the manuscript for important intellectual content: Keiser, Utzinger.
Statistical analysis: Keiser.
Obtained funding: Keiser, Utzinger.
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