CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-497

21-498/S-001

MICROBIOLOGY REVIEW(S)

MICROBIOLOGY REVIEW
DIVISION OF SPECIAL PATHOGEN AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)

NDA #: 21-497 and 21-818

REVIEWER	: Kalavati Suvarna
CORRESPONDENCE DATE	: 01-28-04, 4-26-04
CDER RECEIPT DATE	: 01-29-04; 4-27-04
REVIEW ASSIGN DATE	: 02-05-04; 5-16-04
REVIEW COMPLETE DATE	: 06-18-04

SPONSOR: Romark Laboratories Inc.
6200 Courtney Campbell Causeway
Suite 880
Tampa, FL 33607

SUBMISSION REVIEWED: N-000 (AZ, BM)

DRUG CATEGORY: Anti-parasitic

INDICATION: Treatment of diarrhea caused by *Giardia lamblia* ·

DOSAGE FORM: Tablets

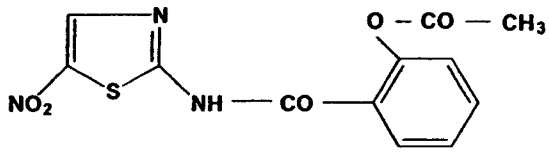
PRODUCT NAMES:

a. **PROPRIETARY:** Alinia®

b. **NONPROPRIETARY:** Nitazoxanide; CAS: 55981-09-4

c. **CHEMICAL:** 2-(acetolyloxy)-N-(5-nitro-2-thiazolyl) benzamide

STRUCTURAL FORMULA:



Molecular weight: 307.2
Empirical formula: $C_{12}H_9N_3O_5S$

SUPPORTING DOCUMENTS: NDA # 20-871; IND #
Type II DMF # , DMF DMF DMF

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1. EXECUTIVE SUMMARY:

The sponsor is seeking approval of nitazoxanide tablets (500 mg nitazoxanide tablets b.i.d. for 3 days) for the treatment of diarrhea due to *Giardia lamblia* in immunocompetent adults. In 2002, the nitazoxanide oral suspension was approved for the treatment of cryptosporidial diarrhea and giardiasis in children. However, the tablet formulation was not approved due to lack of efficacy in adults.

The clinical study (RM01-3011) was conducted in Peru and Egypt to determine the safety and efficacy of nitazoxanide in the treatment of diarrhea due to *G. lamblia* in adults. Resolution of diarrhea was observed in 85% (46/54) patients treated with nitazoxanide tablets compared to 83% (45/54) patients treated with nitazoxanide suspension, and 44% (12/27) patients treated with placebo, 4 to 7 days after discontinuation of therapy.

The sponsor used microscopic examination of unconcentrated stool stained by iodine or immunofluorescence, and concentrated stool samples stained with iodine, to assess presence of *G. lamblia* cysts in stool samples, at baseline and 4 to 7 days after discontinuation of treatment. The processing of the stool samples at the two sites appears to be similar. However, the Peru site determined the actual cyst counts and the Egypt site used a semi-quantitative grading system and approximated the counts to number of cysts per high power field. Based on absence or presence of cysts in stool sample, the percentage of patients that were parasitologically eradicated in the nitazoxanide tablet arm was 55.5% (30/54) compared to 48% (26/54) in the nitazoxanide suspension, and 18.5% (5/27) in the placebo arm. The clinical (94%) and parasitological (94%) outcome were better (94%) in patients treated with nitazoxanide tablets at the Egypt site, where cysts counts at baseline were low, compared to the Peru site (clinical outcome = 80.5%, parasitological outcome = 64%) where cyst counts at baseline were high.

Resolution of diarrhea and eradication of cysts were observed in 28 patients in the nitazoxanide tablet arm, 26 patients in the nitazoxanide suspension arm, and 4 patients in the placebo arm, at 4 to 7 days after discontinuation of treatment. These patients were followed for parasitological outcome at 12 to 14 days after discontinuation of therapy. Shedding of cysts was observed in some of these patients at 12 to 14 days after discontinuation of therapy [nitazoxanide tablet arm = 7/28 (25%), nitazoxanide suspension arm = 6/26 (23%) and placebo arm = 2/4 (50%)]. The clinical outcome was not measured at this time point. Most of these patients were from the Peru site. The sponsor has stated that Peru being a hyper-endemic area compared to Egypt, the recurrence of cysts was more likely due to re-infection rather than relapse. However, information supporting the basis for higher endemicity of *G. lamblia* in Peru compared to Egypt was not included. The differences in parasitological response in the Egypt and Peru site may also be due to host factors or differences in the virulence of the *G. lamblia* isolates at the two sites.

The effect of nitazoxanide on reduction of cysts could not be evaluated with certainty, due to limitations of the detection method, differences in methods used for quantification of cysts at the two sites, expression of the quantitative result as cysts per high power field using actual counts (Peru site) or approximated counts obtained using a semi-quantitative method (Egypt site), variability in the consistency and number of stools passed within a 24 hour period by

patients at the two sites. Several factors effect the detection of cysts in stool samples such as specimen collection and transport method, addition of stool preservatives, age of the stool (fresh versus 24 hour old), consistency, number of stools examined, presence of debris, clarity of smears prepared from concentrated stool sediments, presence of background fluorescence, and expertise of the examiner. Hanson and Cartwright (2001)[1] described the sensitivity of unconcentrated and concentrated stool for the detection of cysts to be 66-70%, when cyst counts were low. However, the stain used for testing was not specified. It is known that sensitivity of the assay can vary with the type of stain used. Because of these limitations and intermittent shedding of cysts observed in patients with giardiasis, the parasitological outcome of the patients based on reduction in cysts counts alone should be interpreted with caution.

Overall, the clinical efficacy of nitazoxanide tablets was similar to nitazoxanide suspension and greater than placebo.

2. INTRODUCTION AND BACKGROUND:

The subject of this NDA is nitazoxanide. Nitazoxanide oral suspension is approved for the treatment of diarrhea due to *Giardia lamblia* and *Cryptosporidium parvum* in immunocompetent children (NDA# 21-498). However, the tablet formulation of nitazoxanide failed to show adequate efficacy in immunocompetent adults with giardiasis (original NDA# 21-497).

The sponsor is seeking approval of nitazoxanide tablet formulation for the treatment of giardiasis diarrhea in immunocompetent adults. The sponsor has proposed 500 mg nitazoxanide tablets b.i.d. for 3 days for treatment of adults with giardiasis The efficacy of nitazoxanide tablets for giardiasis indication in adults is supported by a clinical study.

2.1. Biology of *Giardia lamblia*:

Giardia lamblia is a flagellated protozoan found in intestinal tract of humans. *Giardia duodenalis* or *Giardia intestinalis* are alternate names for *G. lamblia*. Infection is caused by ingestion of contaminated food or water containing *G. lamblia* cysts. Following ingestion, the cysts pass through the stomach to the small intestine where they excyst to give rise to trophozoites. The trophozoites attach to the epithelial cells in the duodenum and bile duct of the host, divide by longitudinal fission and encyst on reaching the colon. *G. lamblia* does not invade epithelial cells like *C. parvum*. In cases of severe infection, trophozoites are more commonly observed than cysts in diarrheic stool samples.

2.2. Pathogenesis of Giardiasis:

The major clinical manifestations of *Giardia lamblia* infection are diarrhea and malabsorption. Although changes in the villi of the intestine have been observed, the mechanism by which *Giardia* causes diarrhea is not known. The host immune response plays an important role in protection from the infection. In addition to the immune status of the host, the severity and duration of the infection can be affected by the number of cysts ingested and the virulence of the *Giardia* strain.

2.3. Biology of *Cryptosporidium parvum*:

Cryptosporidium is an intracellular parasite present in the gastrointestinal and respiratory tract. The infection is caused by ingestion of oocysts. The oocyst contains four sporozoites within a membrane. Upon ingestion, the sporozoites excyst from the oocyst and invade the epithelial cells and become enveloped in a parasitophorous vacuole. Sporozoites undergo maturation into type I meronts, which release merozoites. The merozoite stage can undergo asexual replication and reinvade the host cells or form type II meronts by sexual replication. The type II meronts release the macrogametocytes or microgametocytes that fertilize to give rise to a zygote. The zygote can develop into oocyst that may either rupture releasing sporozoites *in vivo* or shed via the feces. Therefore, the presence of oocyst(s) in the stool samples may be intermittent.

2.4. Pathogenesis of cryptosporidial infection:

The major clinical manifestation observed with cryptosporidial infection is diarrhea. The diarrheal infection can lead to malabsorption. However, the mechanism by which *Cryptosporidium* causes diarrhea is not known. The severity of the infection and ultimate pathology is influenced by the immune status of the host. Cryptosporidial diarrhea is self-limiting in immunocompetent individuals but may be life threatening in AIDS or immunocompromised patients.

3. PRECLINICAL MICROBIOLOGY:

No new information was included in this submission. Studies describing the mechanism of action, activity of nitazoxanide *in vitro* and *in vivo* against *G. lamblia* and *C. parvum* were reviewed earlier [please see microbiology reviews dated 06-01-98 (NDA 20-871, N-000), and 11-06-02 (NDA 21-497 and 21-498, N-000)]. Nitazoxanide and its metabolite, tizoxanide, were active *in vitro* in inhibiting the growth of trophozoites of *G. lamblia* and the sporozoites and oocyst stages of *C. parvum*.

4. CLINICAL MICROBIOLOGY:

4.1. Giardiasis:

The study RM01-3011 conducted to examine the safety and efficacy of nitazoxanide tablets in the treatment of giardiasis in adults from Peru and Egypt is described in detail below. In addition, study RM-NTZ-98-001 included in the original submission (NDA 21-497/21-498, N-000) provides supportive evidence for the efficacy of nitazoxanide tablets in adults [for details please see microbiology review dated 11-06-02 (NDA 21-497/21-498, N-000)].

4.2.1. Study RM01-3011:

Study RM01-3011 was a phase III, multi-center, double-blind, randomized, placebo controlled study to determine the safety and efficacy of nitazoxanide for the treatment of diarrhea due to *G. lamblia* in adults. A total of 135 immunocompetent patients (≥ 12 years of age) who showed presence of *G. lamblia* cysts in the baseline stool sample and had diarrhea (≥ 3 bowel movements/day and one or more enteric symptoms) were eligible to participate in the study. The patients were randomized (2:2:1) to receive either nitazoxanide tablets (500 mg b.i.d. for 3 days), nitazoxanide oral suspension [25 ml (500 mg) b.i.d. for 3 days], or placebo tablets (b.i.d. for 3 days) with food. Patients with diarrhea due to pathogens other than *Giardia* such as *C. parvum*, *E. histolytica* or bacteria, and those receiving drugs with anti-protozoal activity within 2 weeks of study entry were excluded. The following methods were used to exclude patients with diarrhea due to pathogens other than *G. lamblia*: bacterial culture, microscopic examination of stool for protozoa other than *G. lamblia*, acid fast staining or immunofluorescence assay for *C. parvum*, and the Baermann concentration test for *Strongyloides stercoralis*.

The *G. lamblia* cysts were detected by microscopic examination of concentrated stool samples after iodine staining and unconcentrated stool after iodine and immunofluorescence staining (— , a FDA approved immunofluorescence assay kit). The methods used at the Peru and Egypt sites are described below:

Unconcentrated stool sample: At the Peru site, approximately 2 mg of stool sample (size of a match stick head) was mixed with a drop of saline and a drop of 1% Lugol's iodine and the entire smear examined microscopically at 400 x magnification. The number of cysts per field was recorded.

The FDA approved [redacted] immunofluorescence assay (IFA) kit manufactured by [redacted] was also used to detect *G. lamblia* cysts in unconcentrated stool samples. The testing was performed at [redacted] However, the cysts in stool samples from all patients were not quantified.

At the Egypt site, the method for detecting cysts in iodine stained unconcentrated stool was identical to that at the Peru site except that the cyst count was done differently. Although, a grading system was used to quantify cysts: 1+ = one or fewer cysts per field, 2+ = 2-5 cysts per field, 3+ = 5-10 cysts per field and 4+ = >10 cysts per field, the data were expressed as number of cysts per field.

The unconcentrated stool samples were also stained by immunofluorescence using the same kit and tested at the same laboratory in [redacted], described for the Peru site.

Concentrated stool sample: At the Peru site, 1-1.5 gm of stool sample was mixed with 8 ml of 10% formalin to form a suspension. The suspension was strained through a sieve, mixed with 2 ml ether, and centrifuged at 2,500 rpm for 5 minutes. A drop of the sediment was used to prepare a smear and the entire smear examined microscopically at 400 x magnification. The number of cysts per field were recorded.

At the Egypt site, the method used for concentration was similar to the one in Peru except the exact amount of stool sample examined was not specified. The protocol called for sufficient stool sample that would result in 0.5-1.0 ml of fecal sediment. A drop of the sediment was used to prepare a smear. The entire smear was examined and the grading system used was same as the one described above for cyst quantification in unconcentrated stool sample at the Egypt site. The data were expressed as number of cysts per field.

Patients underwent clinical and parasitological evaluations at baseline and 7-10 days after initiation of therapy. For patients who responded to treatment clinically, an additional parasitological evaluation was performed at 14-17 days after initiation of therapy. However, clinical outcome was not measured.

One stool sample was examined at screening as well as baseline. Two stool samples were collected at 7 to 10 days after initiation of therapy while one stool sample was collected at follow-up (14 to 17 days after initiation of therapy).

The clinical responses were defined as:

Well: patient had no symptoms, no watery stools, ≤ 2 soft stools, and had no hematochezia within the 24 hours prior to evaluation or no symptoms and no unformed stools within the 48 hours prior to evaluation;

Continuing illness: patient passed watery stools, more than 2 soft stools per 24 hours or documentation of hematochezia or enteric symptoms plus any number of soft or watery stools during the 48 hours prior to evaluation;

Clinical treatment failure: clinical deterioration or worsening of symptoms after 24 hours of treatment.

The parasitological responses were defined as:

Eradication: Absence of cysts or trophozoites of *G. lamblia* in either of the 2 stool samples collected between days 7 and 10 after initiation of therapy;

Persistence: Presence of cysts or trophozoites of *G. lamblia* in at least 1 stool sample collected between days 7 and 10 after initiation of therapy.

The primary endpoint of the study was resolution of clinical symptoms of giardiasis. The secondary endpoints of the study were (a) eradication of *G. lamblia* cysts from 2 stool samples collected at 7 to 10 days after initiation of therapy, and (b) time to last unformed stool. Although, time to last unformed stool was one of the secondary endpoints, data for this endpoint were not collected.

The sponsor has stated that 4,278 patients were screened at the Peru site and 593 at the Egypt site. However, only 90 and 45 patients were enrolled at the Peru, and Egypt sites, respectively. The reasons for not enrolling the remaining patients were as follows:

Reason for not being enrolled at Peru	No. of Subjects
No *Giardia* cysts or trophozoites observed in stool sample at screening	4,092
Subjects declined participation in the study prior to enrollment	28
Giardia cysts or trophozoites observed in stool sample at screening, but not at baseline	28
Clinical symptoms did not satisfy inclusion criteria	25
Mixed infections (4 *Hymenolepis nana*, 1 *Strongyloides stercoralis*, 1 *Fasciola hepatica* + *Taenia*)	6
Younger than 12 years of age	5
Enrollment terminated at the study site due to completion of study	2
Concomitant therapy incompatible with study requirements	1
Pregnancy	1
TOTAL	**4,188**

Reason for not being enrolled at Egypt	No. of Subjects
No *Giardia* cysts or trophozoites observed in stool sample at screening	523
Giardia cysts or trophozoites observed in stool sample by microscopic exam but not by immunofluorescence assay	10
Giardia cysts or trophozoites observed in stool sample by immunofluorescence assay but not by microscopic exam	5
Concomitant therapy incompatible with study requirements	4
Subjects declined participation in the study prior to enrollment	4
Mixed infections with other pathogens (1 *Blastocystis hominis* + *Hymenolepis nana*, 1 *H. nana*)	2
TOTAL	**548**

All the 135 patients (90 at the Peru site and 45 at the Egypt site) that were enrolled showed presence of cysts at screening and baseline by one of the 3 methods (iodine stained unconcentrated stool, immunofluorescence stained unconcentrated stool, and iodine stained concentrated stool). However, all 3 methods were not used consistently at screening and baseline, either at the Peru or Egypt sites (Table 1).

At the **Peru site**, the unconcentrated and concentrated stools stained with iodine were used to detect and quantify cysts at screening and baseline in the 90 patients. Of the 90 patients, 89 were positive for cysts using either unconcentrated or concentrated stool stained with iodine. As expected, the cyst counts were lower using unconcentrated stool sample (median cyst count = 2.5 cysts per high power field and range = 0.5 to 20 cysts per high power field) compared to concentrated stool (median cyst count = 5 cysts per high power field and range = 0.5 to 80 cysts per high power field).

Immunofluorescence stained unconcentrated stool sample were examined at baseline but not at screening. A stool sample from 85 of the 90 patients was examined at baseline using this method. Of the 85 patients, 66 (77%) were positive for cysts. Of the 19 patients that were negative by immunofluorescence, 18 were positive by iodine staining using unconcentrated stool. The reason for such a discrepancy is unclear. As immunofluorescence testing was performed in France, it is possible that the stools were not preserved properly for transport or stools were examined after long period of storage. This could affect the morphology of the cyst, thereby effecting detection by immunofluorescence. No cyst quantification was performed in patients that were positive by immunofluorescence.

At the **Egypt site**, all 3 methods were used to detect cysts. However, quantification of cysts was performed using the unconcentrated stool stained with immunofluorescence and concentrated stool stained with iodine at screening and baseline in the 45 patients. Of the 45 patients, 30 (same patients) were positive for cysts using either unconcentrated or concentrated stool stained with iodine at baseline. Using iodine stained concentrated stool sample, the cyst counts were lower than at the Peru site (median count = 1 cysts per high power field; cyst count range = 0 to 10 cysts per high power field).

Immunofluorescence stained unconcentrated stool sample were examined at baseline and screening. All patients were positive for cysts by this method including the 15 patients that were negative using iodine stained unconcentrated or concentrated stool. Most (13/15) of the patients that were negative at baseline using iodine stained stool samples, were positive at screening using all 3 methods. The data for these 15 patients suggests that (a) immunofluorescence staining improves the detection of cysts in unconcentrated stool when counts are low, and (b) cysts are shed intermittently, therefore, examination of 2 or more samples improves the sensitivity of detection of cysts.

Table 1: Baseline characteristics of stool examinations for patients enrolled in study RM01-3011 at the Egypt and Peru sites.

Baseline characteristics of stool examination	Peru			Egypt		
	UC/I	C/I	UC/IFA	UC/I	C/I	UC/IFA
Number of patients positive for cysts	89/90	90/90	66/85	30/45	30/45	45/45
Number of patients negative for cysts	1/90	0/90	19/85	15/45[#]	15/45[#]	0/45
Number of patients in whom the cysts were quantified	81/90[a]	89/90[b]	19/85*	15/45*	40/45[c]	45/45
Median cyst counts per high power field (Range)	2.5 (0-20)	5 (0.5 – 80)	ND*	ND*	1 (0-10)	1 (1-10)

UC/I = unconcentrated stool sample stained with iodine;
C/I = concentrated stool sample stained with iodine;
UC/IFA = unconcentrated stool sample stained using an immunofluorescence assay kit;
[#] 13 of the 15 patients positive for cysts at screening by all 3 methods;
* quantification performed only on unconcentrated stool samples (stained by iodine or immunofluorescence) and did not show presence of cysts. The reason for quantifying samples which were negative is unclear.
[a] cyst quantification not performed for 9 of the 89 iodine stained unconcentrated stool samples that were positive for cysts;
[b] cyst quantification not performed for 1 of the 90 iodine stained concentrated stool samples that was positive for cysts;
[c] cyst quantification not performed for 5 of the 30 iodine stained concentrated stool samples that were positive for cysts;
Yellow highlight = number of patients in whom cysts were quantified at the Egypt and Peru sites using iodine stained unconcentrated stool sample, and immunofluorescence stained unconcentrated stool sample, respectively;
Red = number of patients in whom the baseline cyst counts were obtained using iodine stained concentrated stool sample.

In summary, the iodine stained concentrated stool sample was used consistently to detect and quantify cysts at baseline, at both sites (shown in red, Table 1).

Of the 135 patients enrolled in the study, 54 (36 in Peru and 18 in Egypt) received nitazoxanide tablets, 54 (36 in Peru and 18 in Egypt) received nitazoxanide oral suspension, and 27 (18 in Peru and 9 in Egypt) received placebo. The results of the study at the Peru and Egypt site are discussed separately because (a) there were slight differences in the method used for quantification of cysts in the stool sample obtained from patients at the two sites, and (b) differences in the baseline cyst count were noted (shown in red, Table 1). Additionally, the sponsor has stated that Peru was hyper-endemic for *G. lamblia* while Egypt was non hyper-endemic. However, the basis of determining endemicity is unclear.

The clinical and parasitological response of patients treated with nitazoxanide or placebo in **Peru** is summarized in Table 2. The individual patient data for the nitazoxanide tablet, nitazoxanide suspension, and placebo treatment groups are shown in Tables 3, 4, and 5, respectively. Overall, the clinical outcome of patients treated with nitazoxanide tablets (29/36; 80.5%) was similar to that of nitazoxanide suspension (29/36; 80.5%) and greater than placebo (9/18; 50%) at the Peru site. Based on absence of cysts in 2 unconcentrated (stained with iodine and immunofluorescence) and concentrated (iodine stained) stool samples, the parasitological outcome appears to be greater in patients treated with nitazoxanide tablets (13/36; 36%) or suspension (11/36; 30.5%) than placebo (3/18; 17%).

Resolution of diarrhea and eradication of cysts was observed in 12 patients in the nitazoxanide tablet arm, 11 patients in the nitazoxanide suspension arm, and 3 patients in the placebo arm, at 4 to 7 days after discontinuation of treatment. These patients were followed for parasitological outcome at 12 to 14 days after discontinuation of therapy. Shedding of cysts was observed in 7/12 patients in the nitazoxanide tablet arm (shaded; Table 3), 5/11 patients in nitazoxanide

suspension arm (shaded; Table 4), and 2/3 patients in the placebo arm (shaded; Table 5). However, clinical outcome was not measured. The sponsor has proposed that the parasitological response observed at 12-14 days after discontinuation of therapy at the Peru site may be due to (1) failure to eliminate all of the cysts with a single course of treatment in patients with heavy colonization, or (2) re-infection by ingestion of cysts during treatment and follow-up, as the area is hyper-endemic for *G. lamblia*. In the absence of clinical evaluation, the parasitological findings should be interpreted with caution.

Table 2: Parasitological and clinical response of patients with giardiasis at the Peru site.

Treatment group	Parasitological and clinical responses		Patients with eradication of cysts N (%)	Patients with clinical well response N (%)	Patients clinical well and showing eradication of cysts N (%)
	Cysts Eradicated (CR)	Cysts Persisted (CR)			
500 mg NTZ tablet BID 3 days (n = 36)	13 (12 well, 1 CI)	23 (17 well, 6 CI)	13 (36)	29 (80.5)	12 (33)
500 mg NTZ oral suspension BID 3 days (n = 36)	11 (11 well, 0 CI)	25 (18 well, 7 CI)	11 (30.5)	29 (80.5)	11 (30.5)
Placebo BID 3 days (n = 18)	3 (3 well, 0 CI)	15 (6 well, 9 CI)	3 (17)	9 (50)	3 (17)

NTZ = Nitazoxanide; CR = clinical response; N = number of subjects;
CI = continuing illness.

Table 3: Parasitological data for patients receiving nitazoxanide tablets for treatment of giardiasis at the Peru site.

PID	Cysts (count per high power field) in stool sample at screening			Cysts (count per high power field) in baseline stool sample			Cysts (count per high power field) in post-treatment stool samples collected 7 to 10 days after initiation of therapy						Parasitological response	Clinical response	Cysts (count per high power field) in post-treatment stool samples collected 14 to 17 days after initiation of therapy			Day 14 parasitological response
							stool sample 1			stool sample 2								
	UC/I	C/I	UC/ IFA	UC/I	C/I	UC/ IFA	UC/I	C/I	UC/ IFA	UC/I	C/I	UC/ IFA			UC/I	C/I	UC/ IFA	
NTZ tablets: 500 mg BID x 3 days																		
23	+ (ND)	+ (ND)	ND	+ (15)	+ (80)	+ (ND)	+ (3)	+ (20)	+ (ND)	+ (1)	+ (12)	+ (ND)	PERSISTENCE	WELL	+ (ND)	+ (7)	ND	PERSISTENCE
25	+ (ND)	- (0)	ND	+ (ND)	+ (1)	- (0)	+ (1)	+ (2)	- (0)	+ (ND)	+ (1)	ND	PERSISTENCE	WELL	+ (ND)	+ (1)	ND	PERSISTENCE
26	+ (1)	+ (2)	ND	+ (1)	+ (1)	+ (ND)	- (0)	- (0)	ND	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	ND	ERADICATION
30	+ (ND)	+ (2.5)	ND	+ (1)	+ (2)	+ (ND)	+ (ND)	+ (1.5)	ND	+ (ND)	+ (2.5)	+ (ND)	PERSISTENCE	CI	No follow-up			
33	+ (1.5)	+ (5)	ND	+ (ND)	+ (3.5)	+ (ND)	+ (1.5)	+ (2.5)	+ (ND)	+ (ND)	+ (3.5)	ND	PERSISTENCE	CI	ND	ND	- (0)	ERADICATION
35	+ (ND)	+ (5)	ND	+ (ND)	+ (5)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	ND	ERADICATION	WELL	+ (ND)	+ (9)	+ (ND)	PERSISTENCE
36	+ (ND)	+ (2.5)	ND	+ (ND)	+ (1.5)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	ND	ERADICATION	WELL	+ (ND)	+ (4.5)	+ (ND)	PERSISTENCE
37	+ (ND)	+ (2.5)	ND	+ (ND)	+ (1.5)	+ (ND)	- (0)	- (0)	+ (ND)	- (0)	- (0)	- (0)	PERSISTENCE	WELL	+ (1.5)	+ (3.0)	+ (ND)	PERSISTENCE
42	+ (2.5)	+ (5.5)	ND	+ (1.5)	+ (5)	+ (ND)	- (0)	- (0)	+ (ND)	- (0)	- (0)	+ (ND)	PERSISTENCE	WELL	+ (3)	+ (5.5)	+ (ND)	PERSISTENCE
45	+ (2.5)	+ (4.5)	ND	+ (1.5)	+ (2.5)	- (0)	- (0)	- (0)	+ (ND)	+ (1)	+ (2)	+ (ND)	PERSISTENCE	CI	No follow-up			
48	+ (2.5)	+ (4.5)	ND	+ (2.5)	+ (5)	+ (ND)	+ (3)	+ (7)	- (0)	+ (3.5)	+ (10)	+ (ND)	PERSISTENCE	WELL	+ (2.5)	+ (5.5)	+ (ND)	PERSISTENCE
50	+ (5)	+ (17.5)	ND	+ (9)	+ (18)	+ (ND)	- (0)	- (0)	+ (ND)	- (0)	- (0)	ND	PERSISTENCE	WELL	+ (3)	+ (4)	- (0)	PERSISTENCE
51	+ (1.5)	+ (2)	ND	+ (1)	+ (2)	+ (ND)	- (0)	- (0)	+ (ND)	- (0)	- (0)	+ (ND)	PERSISTENCE	WELL	- (0)	- (1)	+ (ND)	PERSISTENCE
55	+ (1)	+ (2)	ND	+ (1)	+ (2)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	ND	ERADICATION
58	+ (3.5)	+ (6.5)	ND	+ (4)	+ (8)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	CI	No follow-up			
60	+ (2.5)	+ (7)	ND	+ (4)	+ (8)	+ (ND)	+ (3)	+ (8)	+ (ND)	+ (2.5)	+ (7)	+ (ND)	PERSISTENCE	WELL	+ (5)	+ (12)	+ (ND)	PERSISTENCE
61	+ (1.5)	+ (5)	ND	+ (1)	+ (2)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	+ (2)	+ (4)	+ (ND)	PERSISTENCE
62	+ (6)	+ (8)	ND	+ (3)	+ (6)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	+ (4)	+ (5)	+ (ND)	PERSISTENCE
67	+ (4)	+ (6)	ND	+ (15)	+ (20)	+ (ND)	+ (3)	+ (7)	- (0)	+ (3)	+ (7)	- (0)	PERSISTENCE	WELL	+ (3)	+ (7)	- (0)	PERSISTENCE
70	+ (3)	+ (7)	ND	+ (3)	+ (5)	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (1)	+ (1)	+ (ND)	PERSISTENCE	WELL	+ (1)	+ (3)	+ (ND)	PERSISTENCE
73	+ (4)	+ (5)	ND	+ (2)	+ (4)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	+ (ND)	PERSISTENCE
75	+ (2)	+ (4)	ND	+ (1)	+ (2)	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (1)	+ (1)	+ (ND)	PERSISTENCE	CI	No follow-up			
76	+ (2)	+ (5)	ND	+ (2)	+ (4)	+ (ND)	- (0)	+ (1)	- (0)	- (0)	+ (1)	+ (ND)	PERSISTENCE	WELL	+ (1)	+ (2)	+ (ND)	PERSISTENCE
80	+ (1)	+ (1)	ND	+ (1)	+ (1)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
83	+ (2)	+ (3)	ND	+ (2)	+ (3)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
85	- (0)	+ (1)	ND	+ (3)	+ (4)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
86	+ (4)	+ (9)	ND	+ (3)	+ (8)	+ (ND)	+ (6)	+ (13)	+ (ND)	+ (7)	+ (14)	+ (ND)	PERSISTENCE	CI	ND	ND	+ (ND)	PERSISTENCE
87	+ (2)	+ (4)	ND	+ (4)	+ (6)	+ (ND)	Lost to follow-up						PERSISTENCE	CI	No follow-up			
92	+ (3)	+ (8)	ND	+ (4)	+ (10)	- (0)	+ (2)	+ (3)	+ (ND)	+ (4)	+ (9)	+ (ND)	PERSISTENCE	WELL	+ (2)	+ (7)	+ (ND)	PERSISTENCE
95	+ (5)	+ (10)	ND	+ (3)	+ (5)	+ (ND)	+ (2)	+ (3)	+ (ND)	+ (2)	+ (5)	+ (ND)	PERSISTENCE	WELL	+ (5)	+ (19)	+ (ND)	PERSISTENCE
98	+ (1)	+ (4)	ND	+ (2)	+ (4)	+ (ND)	+ (2)	+ (4)	+ (ND)	+ (3)	+ (5)	+ (ND)	PERSISTENCE	WELL	+ (2)	+ (4)	+ (ND)	PERSISTENCE
100	+ (3)	+ (16)	ND	+ (16)	+ (25)	+ (ND)	+ (4)	+ (12)	+ (ND)	+ (8)	+ (15)	+ (ND)	PERSISTENCE	WELL	+ (20)	+ (35)	+ (ND)	PERSISTENCE
101	+ (2)	+ (6)	ND	+ (3)	+ (11)	+ (ND)	+ (3)	+ (7)	+ (ND)	+ (2)	+ (5)	+ (ND)	PERSISTENCE	WELL	+ (1)	+ (2)	+ (ND)	PERSISTENCE
105	+ (1)	+ (1)	ND	+ (2)	+ (5)	ND	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	+ (ND)	PERSISTENCE
108	+ (3)	+ (10)	ND	+ (5)	+ (12)	ND	+ (1)	+ (1)	ND	+ (4)	+ (5)	+ (ND)	PERSISTENCE	WELL	+ (8)	+ (12)	+ (ND)	PERSISTENCE
110	+ (3)	+ (5)	ND	+ (4)	+ (5)	ND	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	PERSISTENCE

NTZ = nitazoxanide; UC/I = unconcentrated stool sample stained with iodine; C/I = concentrated stool sample stained with iodine;
UC/IFA = unconcentrated stool sample stained using an immunofluorescence assay kit; PID = patient identification number;
+ = presence of cysts; - = absence of cysts, ND = not done; CI = continuing illness.
Shaded rows represent patients who shed cysts after initial eradication

Table 4: Parasitological data for patients receiving nitazoxanide oral suspension for treatment of giardiasis at the Peru site.

PID	Cysts (count per high power field) in stool sample at screening			Cysts (count per high power field) in baseline stool sample			Cysts (count per high power field) in post-treatment stool samples collected 7 to 10 days after initiation of therapy						Parasitological response	Clinical response	Cysts (count per high power field) in post-treatment stool samples collected 14 to 17 days after initiation of therapy			Day 14 parasitological response
							stool sample 1			stool sample 2								
	UC/I	C/I	UC/ IFA	UC/I	C/I	UC/ IFA	UC/I	C/I	UC/ IFA	UC/I	C/I	UC/ IFA			UC/I	C/I	UC/ IFA	
NTZ suspension: 500 mg BID x 3 days																		
21	+ (ND)	+ (ND)	ND	+ (6)	+ (60)	+ (1)	+ (1)	+ (1)	ND	+ (5)	+ (18)	ND	PERSISTENCE	CI	No follow-up			
22	+ (ND)	+ (ND)	ND	+ (1)	+ (25)	+ (ND)	+ (1)	+ (1)	+ (ND)	- (0)	+ (3.5)	ND	PERSISTENCE	WELL	Lost to follow-up			
27	- (0)	+ (18)	ND	+ (1)	+ (1)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	ND	ERADICATION
29	+ (17.5)	+ (30)	ND	+ (1)	+ (10)	+ (ND)	- (0)	- (0)	ND	- (0)	- (0)	- (0)	ERADICATION	WELL	+ (8)	+ (22.5)	ND	PERSISTENCE
32	+ (ND)	+ (ND)	ND	+ (ND)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ND	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
34	+ (ND)	+ (3)	ND	+ (ND)	+ (6.5)	+ (ND)	+ (ND)	+ (7)	+ (ND)	+ (ND)	+ (2.5)	+ (ND)	PERSISTENCE	WELL	+ (ND)	+ (2.5)	+ (ND)	PERSISTENCE
38	+ (ND)	+ (2.5)	ND	+ (ND)	+ (5)	+ (ND)	- (0)	- (0)	+ (ND)	- (0)	- (0)	+ (ND)	PERSISTENCE	WELL	+ (1)	+ (2.5)	+ (ND)	PERSISTENCE
39	+ (ND)	+ (4)	ND	+ (ND)	+ (3)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
41	+ (2.5)	+ (5)	ND	+ (3.5)	+ (6.5)	+ (ND)	+ (1)	+ (1.5)	+ (ND)	+ (1.5)	+ (3.5)	+ (ND)	PERSISTENCE	WELL	Lost to follow-up			
44	- (0)	+ (1.5)	ND	+ (2.5)	+ (3.5)	- (0)	+ (1.5)	+ (2)	+ (ND)	+ (1)	+ (1)	+ (ND)	PERSISTENCE	WELL	+ (2)	+ (2)	- (0)	PERSISTENCE
46	+ (1.5)	+ (2.5)	ND	+ (0.5)	+ (0.5)	- (0)	- (0)	- (0)	- (0)	+ (1)	+ (1)	+ (ND)	PERSISTENCE	CI	ND	ND	- (0)	ERADICATION
47	+ (1)	+ (1.5)	ND	+ (4.5)	+ (5)	+ (ND)	+ (2)	+ (3)	+ (ND)	+ (1)	+ (2)	+ (ND)	PERSISTENCE	WELL	+ (2)	+ (5)	+ (ND)	PERSISTENCE
52	+ (1.5)	+ (3.5)	ND	+ (1)	+ (3)	+ (ND)	+ (3)	+ (8)	+ (ND)	+ (4)	+ (14)	+ (ND)	PERSISTENCE	WELL	+ (4)	+ (10)	+ (ND)	PERSISTENCE
54	+ (1)	+ (2)	ND	+ (3)	+ (4)	- (0)	+ (3)	+ (6)	+ (ND)	+ (2)	+ (4)	- (0)	PERSISTENCE	CI	ND	ND	- (0)	ERADICATION
57	+ (1)	+ (2)	ND	+ (2)	+ (5)	+ (ND)	+ (1)	+ (2)	+ (ND)	+ (1)	+ (1)	+ (ND)	PERSISTENCE	WELL	- (0)	- (0)	- (0)	ERADICATION
59	+ (1)	+ (2)	ND	+ (2)	+ (3)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
63	+ (1)	+ (3)	ND	- (0)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	+ (1)	+ (1)	- (0)	PERSISTENCE
64	+ (4)	+ (8)	ND	+ (5)	+ (12)	+ (ND)	+ (2)	+ (4)	- (0)	+ (4)	+ (7)	- (0)	PERSISTENCE	CI	ND	ND	+ (ND)	PERSISTENCE
66	+ (1)	+ (2)	ND	+ (1)	+ (1)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	+ (1)	- (0)	- (0)	PERSISTENCE
69	+ (3)	+ (8)	ND	+ (3)	+ (5)	+ (ND)	+ (4)	+ (5)	+ (ND)	+ (1)	+ (1)	- (0)	PERSISTENCE	WELL	- (0)	+ (1)	- (0)	PERSISTENCE
71	+ (3)	+ (7)	ND	+ (2)	+ (8)	- (0)	+ (3)	+ (5)	+ (ND)	+ (2)	+ (3)	+ (ND)	PERSISTENCE	WELL	+ (3)	+ (6)	- (0)	PERSISTENCE
72	+ (3)	+ (5)	ND	+ (1)	+ (3)	+ (ND)	- (0)	- (0)	- (0)	- (0)	+ (1)	- (0)	PERSISTENCE	WELL	+ (3)	+ (4)	+ (ND)	PERSISTENCE
77	+ (1)	+ (4)	ND	+ (2)	+ (5)	+ (ND)	- (0)	- (0)	+ (ND)	+ (1)	+ (2)	+ (ND)	PERSISTENCE	WELL	+ (1)	+ (3)	+ (ND)	PERSISTENCE
79	+ (1)	+ (2)	ND	+ (1)	+ (2)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	+ (ND)	PERSISTENCE
82	+ (2)	+ (5)	ND	+ (2)	+ (3)	- (0)	+ (1)	+ (1)	+ (ND)	- (0)	+ (1)	+ (ND)	PERSISTENCE	CI	ND	ND	- (0)	ERADICATION
84	+ (1)	+ (2)	ND	+ (2)	+ (3)	- (0)	+ (1)	+ (1)	- (0)	+ (1)	+ (1)	+ (ND)	PERSISTENCE	WELL	- (0)	- (0)	- (0)	ERADICATION
88	+ (1)	+ (3)	ND	+ (1)	+ (3)	- (0)	- (0)	+ (ND)	- (0)	- (0)	- (0)	- (0)	PERSISTENCE	WELL	- (0)	- (0)	- (0)	ERADICATION
89	+ (3)	+ (5)	ND	+ (3)	+ (7)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	+ (1)	+ (1)	+ (ND)	PERSISTENCE
91	+ (1)	+ (3)	ND	+ (4)	+ (8)	+ (ND)	No follow-up						PERSISTENCE	CI	Lost to follow-up			
94	+ (6)	+ (13)	ND	+ (1)	+ (3)	+ (ND)	- (0)	- (0)	+ (ND)	- (0)	- (0)	- (0)	PERSISTENCE	WELL	+ (8)	+ (15)	+ (ND)	PERSISTENCE
96	+ (1)	+ (2)	ND	+ (1)	+ (2)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
97	+ (1)	+ (13)	ND	+ (5)	+ (8)	+ (ND)	- (0)	- (0)	+ (ND)	- (0)	- (0)	- (0)	PERSISTENCE	WELL	- (0)	- (0)	- (0)	ERADICATION
102	+ (3)	+ (9)	ND	+ (5)	+ (14)	+ (ND)	+ (4)	+ (7)	+ (ND)	+ (3)	+ (13)	+ (ND)	PERSISTENCE	WELL	+ (8)	+ (20)	+ (ND)	PERSISTENCE
104	+ (3)	+ (5)	ND	+ (3)	+ (7)	+ (ND)	+ (2)	+ (4)	+ (ND)	+ (3)	+ (6)	+ (ND)	PERSISTENCE	WELL	+ (4)	+ (10)	ND	PERSISTENCE
107	+ (3)	+ (9)	ND	+ (2)	+ (5)	+ (ND)	+ (2)	+ (5)	+ (ND)	+ (2)	+ (5)	ND	PERSISTENCE	CI	Lost to follow-up			
109	+ (2)	+ (6)	ND	+ (6)	+ (15)	ND	- (0)	- (0)	ND	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION

NTZ = nitazoxanide; UC/I = unconcentrated stool sample stained with iodine; C/I = concentrated stool sample stained with iodine;
UC/IFA = unconcentrated stool sample stained using an immunofluorescence assay kit; PID = patient identification number;
+ = presence of cysts; - = absence of cysts, ND = not done; CI = continuing illness.
Shaded rows represent patients who shed cysts after initial eradication

Table 5: Parasitological data for patients receiving placebo for treatment of giardiasis at the Peru site.

PID	Cysts (count per high power field) in stool sample at screening			Cysts (count per high power field) in baseline stool sample			Cysts (count per high power field) in post-treatment stool samples collected 7 to 10 days after initiation of therapy						Parasitological response	Clinical response	Cysts (count per high power field) in post-treatment stool samples collected 14 to 17 days after initiation of therapy			Day 14 parasitological response
							stool sample 1			stool sample 2								
	UC/I	C/I	UC/ IFA	UC/I	C/I	UC/ IFA	UC/I	C/I	UC/ IFA	UC/I	C/I	UC/ IFA			UC/I	C/I	UC/ IFA	
Placebo tablets BID x 3 days:																		
24	+ (ND)	+ (ND)	ND	+ (1)	+ (1)	+ (ND)	+ (1)	+ (5.5)	+ (ND)	+ (ND)	+ (3.5)	ND	PERSISTENCE	CI	No follow-up			
28	+ (1)	+ (1)	ND	+ (1)	+ (1)	+ (ND)	- (0)	- (0)	ND	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	ND	ERADICATION
31	+ (ND)	+ (17)	ND	+ (5)	+ (17.5)	+ (ND)	+ (ND)	+ (4.5)	ND	+ (ND)	+ (6.5)	ND	PERSISTENCE	CI	No follow-up			
40	+ (ND)	+ (4)	ND	+ (5)	+ (6.5)	+ (ND)	+ (4.5)	+ (6.5)	+ (ND)	+ (4.5)	+ (5.5)	+ (ND)	PERSISTENCE	CI	ND	ND	+ (ND)	PERSISTENCE
43	+ (3)	+ (5.5)	ND	+ (2.5)	+ (6.5)	+ (ND)	- (0)	- (0)	ND	+ (1.5)	+ (3)	+ (ND)	PERSISTENCE	CI	ND	ND	+ (ND)	PERSISTENCE
49	+ (6.5)	+ (11)	ND	+ (7.5)	+ (16.5)	+ (ND)	+ (1.5)	+ (2)	+ (ND)	+ (1.5)	+ (3)	+ (ND)	PERSISTENCE	CI	ND	ND	+ (ND)	PERSISTENCE
53	+ (2)	+ (3.5)	ND	+ (5.5)	+ (7)	+ (ND)	+ (3)	+ (5)	- (0)	+ (2)	+ (5)	+ (ND)	PERSISTENCE	WELL	+ (4)	+ (10)	+ (ND)	PERSISTENCE
56	+ (5)	+ (12.5)	ND	+ (6)	+ (12)	+ (ND)	+ (3)	+ (7)	+ (ND)	+ (2)	+ (10)	+ (ND)	PERSISTENCE	WELL	+ (3)	+ (6)	+ (ND)	PERSISTENCE
65	+ (1)	+ (2)	ND	+ (1)	+ (1)	- (0)	- (0)	- (0)	- (0)	+ (1)	+ (1)	- (0)	PERSISTENCE	WELL	+ (1)	+ (1)	- (0)	PERSISTENCE
68	+ (2)	+ (5)	ND	+ (3)	+ (6)	- (0)	+ (1)	+ 91)	- (0)	+ (3)	+ (7)	+ (ND)	PERSISTENCE	WELL	+ (3)	+ (4)	- (0)	PERSISTENCE
74	+ (2)	+ (4)	ND	+ (2)	+ (3)	+ (ND)	+ (1)	+ (2)	+ (ND)	+ (2)	+ (3)	+ (ND)	PERSISTENCE	CI	+ (1)	+ (1)	+ (ND)	PERSISTENCE
78	+ (3)	+ (4)	ND	+ (2)	+ (5)	+ (ND)	+ (2)	+ (4)	+ (ND)	- (0)	+ (1)	+ (ND)	PERSISTENCE	CI	ND	ND	+ (ND)	PERSISTENCE
81	+ (1)	+ (2)	ND	+ (1)	+ (1)	+ (ND)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	+ (1)	+ (1)	- (0)	PERSISTENCE
90	+ (3)	+ (8)	ND	+ (4)	+ (12)	+ (ND)	+ (6)	+ (10)	+ (ND)	+ (2)	+ (5)	+ (ND)	PERSISTENCE	CI	ND	ND	+ (ND)	PERSISTENCE
93	+ (9)	+ (20)	ND	+ (2)	+ (15)	- (0)	+ (2)	+ (4)	+ (ND)	+ (3)	+ (4)	+ (ND)	PERSISTENCE	WELL	+ (2)	+ (3)	+ (ND)	PERSISTENCE
99	+ (4)	+ (5)	ND	+ (3)	+ (5)	+ (ND)	- (0)	- (0)	ND	- (0)	- (0)	ND	ERADICATION	WELL	+ (1)	+ (1)	ND	PERSISTENCE
103	+ (25)	+ (40)	ND	+ (20)	+ (38)	ND	+ (7)	+ (13)	+ (ND)	+ (14)	+ (20)	+ (ND)	PERSISTENCE	CI	+ (20)	+ (44)	ND	PERSISTENCE
106	- (ND)	+ (1)	ND	+ (3)	+ (7)	+ (ND)	+ (3)	+ (6)	+ (ND)	+ (3)	+ (5)	+ (ND)	PERSISTENCE	WELL	+ (4)	+ (9)	ND	PERSISTENCE

UC/I = unconcentrated stool sample stained with iodine; C/I = concentrated stool sample stained with iodine;
UC/IFA = unconcentrated stool sample stained using an immunofluorescence assay kit;
PID = patient identification number; ND = not done; CI = continuing illness;
+ = presence of cysts; - = absence of cysts.
Shaded rows represent patients who shed cysts after initial eradication

The clinical and parasitological response of patients treated with nitazoxanide or placebo in **Egypt** is shown in Table 6. The individual patient data for the nitazoxanide tablet or suspension, and placebo treatment groups are shown in Tables 7 and 8. Overall, the clinical outcome of patients treated with nitazoxanide tablets (17/18; 94%) was similar to that of nitazoxanide suspension (16/18; 89%) and greater than placebo (3/9; 43%) at the Egypt site. Based on absence of cysts in 2 unconcentrated (stained with iodine and immunofluorescence) and concentrated (iodine stained) stool samples, the parasitological outcome was better in patients treated with nitazoxanide tablets (17/18; 94%) or suspension (15/18; 83%) than placebo (2/9; 22%).

Resolution of diarrhea and eradication of cysts was observed in 16 patients in the nitazoxanide tablet arm, 15 patients in the nitazoxanide suspension arm, and 1 patient in the placebo arm, at 4 to 7 days after discontinuation of treatment. These patients were followed for parasitological outcome at 12 to 14 days after discontinuation of therapy. The 16 patients treated with nitazoxanide tablets and 1 treated with placebo continued to be free of cysts while shedding of cysts was observed in 1 of the 16 patients treated with nitazoxanide suspension (shaded rows, Table 7). The clinical outcome was not measured.

Table 6: Parasitological and clinical response of patients with giardiasis at the Egypt site.

Treatment group	Parasitological and clinical responses		Patients with eradication of cysts N (%)	Patients with clinical well response N (%)	Patients clinical well and showing eradication of cysts N (%)
	Cysts Eradicated (CR)	Cysts Persisted (CR)			
500 mg NTZ tablet BID 3 days (n = 18)	17 (16 well, 1 CI)	1(1 well, 0 CI)	17 (94)	17 (94)	16 (89)
500 mg NTZ oral suspension BID 3 days (n = 18)	15 (15 well, 0 CI)	3 (1 well, 2 CI)	15 (83)	16 (89)	15 (83)
Placebo BID 3 days (n = 9)	2 (1 well, 1 CI)	7 (2 well, 5 CI)	2 (22)	3 (43)	1 (11)

NTZ = Nitazoxanide; CR = clinical response; N = number of subjects;
CI = continuing illness.

Table 7: Parasitological data for patients receiving nitazoxanide tablets or suspension for treatment of giardiasis at the Egypt site.

PID	Cysts (count per high power field) in stool sample at screening			Cysts (count per high power field) in baseline stool sample			Cysts (count per high power field) in post treatment stool samples collected 7 to 10 days after initiation of therapy						Parasitological response	Clinical response	Cysts (count per high power field) in post-treatment stool samples collected 14 to 17 days after initiation of therapy			Day 14 parasitological response
							stool sample 1			stool sample 2								
	UC/I	C/I	UC/ IFA	UC/I	C/I	UC/ IFA	UC/I	C/I	UC/ IFA	UC/I	C/I	UC/ IFA			UC/I	C/I	UC/ IFA	
NTZ tablets: 500 mg BID x 3 days																		
1	+ (1)	+ (1)	+ (1)	- (0)	- (0)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
5	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	+ (3.5)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
8	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (ND)	+ (7.5)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
10	+ (ND)	+ (3.5)	+ (1)	- (0)	- (0)	+ (3.5)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
11	+ (ND)	+ (7.5)	+ (10)	- (0)	- (0)	+ (3.5)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
12	+ (ND)	+ (3.5)	+ (3.5)	- (0)	- (0)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
17	+ (ND)	+ (ND)	+ (7.5)	+ (ND)	+ (3.5)	+ (3.5)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
20	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (1)	+ (7.5)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	Lost to follow-up			
123	+ (ND)	+ (1)	- (0)	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
125	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (3.5)	+ (3.5)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
126	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (3.5)	+ (3.5)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
130	- (0)	- (0)	+ (3.5)	+ (ND)	+ (3.5)	+ (3.5)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
133	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (7.5)	+ (7.5)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	CI	- (0)	- (0)	- (0)	ERADICATION
135	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
142	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (ND)	+ (3.5)	+ (ND)	+ (3.5)	+ (1)	+ (ND)	+ (1)	+ (1)	PERSISTENCE	WELL	- (0)	- (0)	- (0)	ERADICATION
145	+ (ND)	+ (1)	+ (3.5)	+ (ND)	+ (ND)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
148	+ (ND)	+ (3.5)	+ (3.5)	+ (ND)	+ (3.5)	+ (3.5)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
150	+ (ND)	+ (3.5)	+ (3.5)	+ (ND)	+ (3.5)	+ (3.5)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
NTZ suspension: 500 mg BID x 3 days																		
2	- (0)	- (0)	+ (1)	- (0)	- (0)	+ (1)	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (3.5)	+ (3.5)	PERSISTENCE	WELL	+ (ND)	+ (10)	+ (10)	PERSISTENCE
4	+ (ND)	+ (3.5)	+ (3.5)	- (0)	- (0)	+ (3.5)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
7	+ (ND)	+ (1)	+ (ND)	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	+	+	+	PERSISTENCE
9	+ (ND)	+ (3.5)	+ (3.5)	+ (ND)	+ (3.5)	+ (3.5)	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	- (0)	PERSISTENCE	CI	- (0)	- (0)	- (0)	ERADICATION
13	- (ND)	+ (1)	+ (3.5)	- (0)	- (0)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
14	+ (ND)	+ (ND)	+ (1)	- (0)	- (0)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
16	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (3.5)	+ (1)	+ (ND)	+ (1)	+ (1)	PERSISTENCE	CI	+	+	+	PERSISTENCE
19	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
121	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
122	+ (ND)	+ (1)	- (0)	+ (ND)	+ (3.5)	+ (3.5)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
127	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
129	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
132	+ (ND)	+ (ND)	+ (1)	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
134	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
141	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (ND)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
144	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (3.5)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
146	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION
147	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION

NTZ = nitazoxanide; PID = patient identification number; UC/I = iodine stained unconcentrated stool sample; C/I = iodine stained concentrated stool sample; UC/IFA = unconcentrated stool sample stained using an immunofluorescence assay kit; + = presence of cysts; - = absence of cysts; ND = not done; CI = continuing illness; Shaded row represents the patient who shed cysts after initial eradication

Table 8: Parasitological data for patients receiving placebo for treatment of giardiasis at the Egypt site.

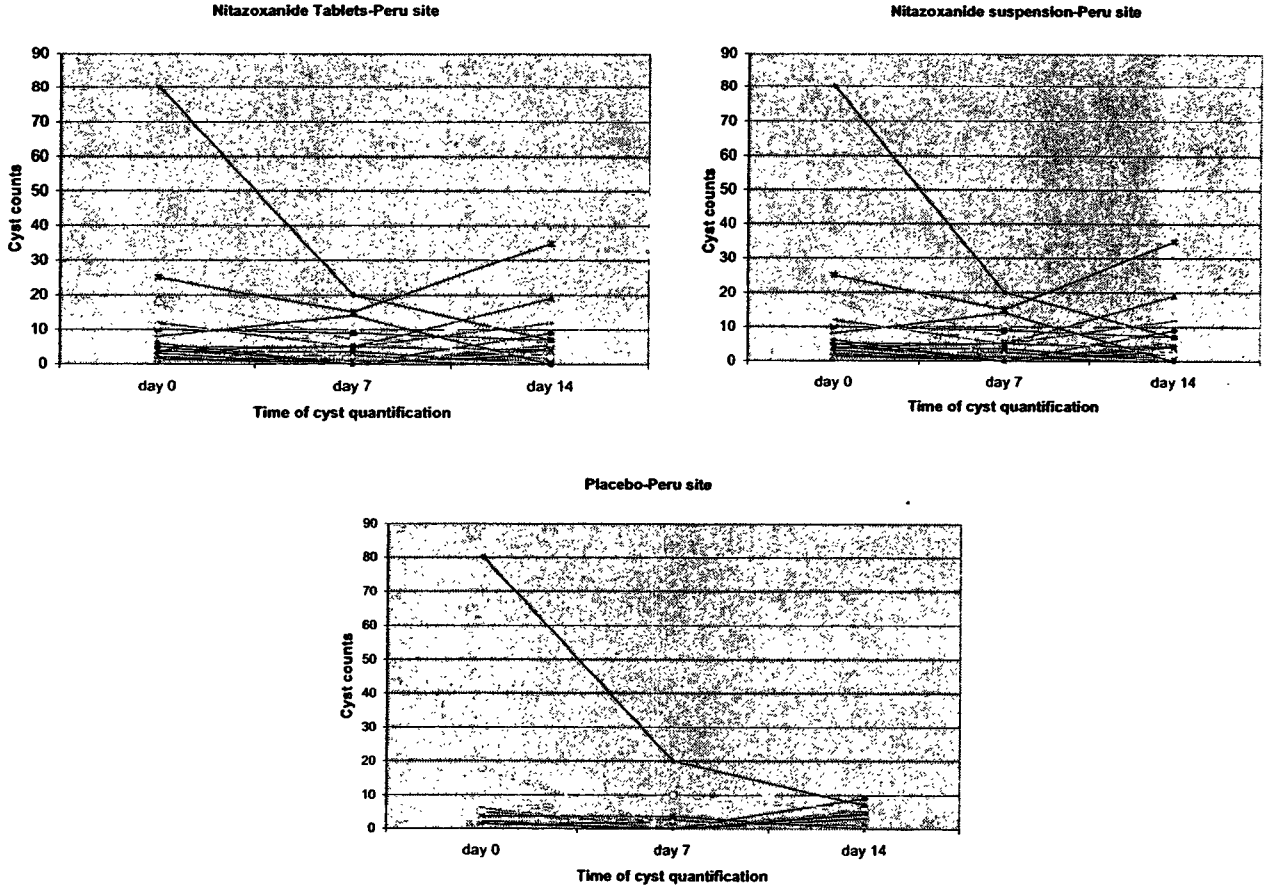
PID	Screening Cysts (count per high power field) in stool sample at screening			Baseline Cysts (count per high power field) in baseline stool sample			Cysts (count per high power field) in post-treatment stool samples collected 7 to 10 days after initiation of therapy						Parasitological response	Clinical response	Cysts (count per high power field) in post-treatment stool samples collected 14 to 17 days after initiation of therapy			Day 14 parasitological response
							stool sample 1			stool sample 2								
	UC/I	C/I	UC/ IFA	UC/I	C/I	UC/ IFA	UC/I	C/I	UC/ IFA	UC/I	C/I	UC/ IFA			UC/I	C/I	UC/ IFA	
Placebo:																		
3	+ (ND)	+ (10)	+ (10)	+ (ND)	+ (10)	+ (10)	+ (ND)	+ (3.5)	+ (3.5)	+ (ND)	+ (3.5)	+ (3.5)	PERSISTENCE	CI	+ (ND)	+ (10)	+ (10)	PERSISTENCE
6	+ (ND)	+ (1)	+ (3.5)	+ (ND)	+ (3.5)	+ (1)	+ (ND)	+ (1)	+ (ND)	- (0)	- (0)	- (0)	PERSISTENCE	CI	+ (ND)	+ (0.5)	+ (3.5)	PERSISTENCE
15	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	+ (1)	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	- (0)	PERSISTENCE	WELL	- (0)	- (0)	- (0)	ERADICATION
18	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	CI	- (0)	+ (1)	+ (1)	PERSISTENCE
124	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	- (0)	+ (ND)	+ (ND)	+ (1)	PERSISTENCE	WELL	+ (ND)	+ (ND)	+ (1)	PERSISTENCE
128	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (1)	+ (1)	PERSISTENCE	CI	- (0)	- (0)	- (0)	ERADICATION
131	+ (ND)	+ (1)	+ (3.5)	+ (ND)	+ (ND)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	+ (3.5)	PERSISTENCE	CI	- (0)	- (0)	- (0)	ERADICATION
143	+ (ND)	+ (ND)	+ (1)	+ (ND)	+ (1)	+ (1)	+ (ND)	+ (ND)	+ (1)	- (0)	- (0)	- (0)	PERSISTENCE	CI	- (0)	- (0)	- (0)	ERADICATION
149	+ (ND)	+ (3.5)	+ (3.5)	+ (ND)	+ (1)	+ (1)	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)	ERADICATION	WELL	- (0)	- (0)	- (0)	ERADICATION

UC/I = unconcentrated stool sample stained with iodine; C/I = concentrated stool sample stained with iodine;
UC/IFA = unconcentrated stool sample stained using an immunofluorescence assay kit; + = presence of cysts; - = absence of cysts,
PID = patient identification number; ND = not done; CI = continuing illness.

At the Peru and Egypt sites, quantification of cysts per high power field was done using iodine stained concentrated stool samples. The other two methods were not used consistently for all patients at both sites, at the different time points. For analysis of the reduction in cyst counts, the highest cyst count in either of the 2 concentrated stool samples stained with iodine on day 7 was used.

At the Peru site, the reduction in cyst counts in the concentrated stool samples stained with iodine in the 3 groups was similar at days 4 to 7 after discontinuation of therapy (Figure 1). Additionally, a trend towards increase in cyst counts at 12-14 days after discontinuation of therapy was observed in some patients. However, in the absence of clinical outcome, changes in the parasite counts should be interpreted with caution.

Figure 1: Reduction in *G. lamblia* cyst count (using the concentrated stool samples stained with iodine) in the nitazoxanide (tablet or suspension) and placebo treated groups at the Peru site.



At the Egypt site, the baseline cyst counts of the patients were low (median count = 1 cyst per high power field). Therefore, no meaningful conclusions could be drawn regarding reduction in cyst counts (cyst count on day 7 – cyst count on day 0) for nitazoxanide or placebo treated patients.

Various factors effect detection of cysts in stools such as specimen collection, transport, addition of stool preservatives, age of the stool (fresh versus 24 hours old), consistency, number of stool samples examined, presence of debris in the stool sample, clarity of concentrated stools sediment, and background fluorescence in fluorescent stained stool samples. The limit of detection for iodine stained unconcentrated or concentrated stool can be altered by number of factors such as quality of smears, time spent on smear examination, and expertise of the examiner in addition to the factors described above. However, the sensitivity of unconcentrated and concentrated stool (staining method not specified) for cyst detection was stated to be 66 - 70% when the cyst counts were low[1]. The limit of detection of cysts using the [redacted] IFA assay kit has not been determined using fecal samples seeded with *Giardia* cysts. However, the sensitivity and specificity of the IFA assay compared to iodine stained stool samples was stated to be 95% ([redacted] IFA kit package insert). Because of the limitation of the detection methods used to detect cysts, differences in the methods used to quantify cysts at the two sites (actual counts at the Peru site versus approximated counts at the Egypt site), consistency of the stool samples, and number of stool passed by the patient in a 24 hour period, it is difficult to estimate the actual parasite count.

The effect of nitazoxanide on cyst eradication and parasitological outcome should be interpreted with caution due to the following limitations: (a) differences in virulence of different *G. lamblia* strains, (b) resolution of diarrhea over time in an immunocompetent host, (c) inability to differentiate re-infection from relapse, (d) inability of current methods to detect cysts, when counts are low, and (e) intermittent shedding of cysts,.

In summary, nitazoxanide tablet and suspension were more effective than placebo in resolving diarrhea (Table 9).

Table 9: Parasitological and clinical outcome of all patients in study RM01-3011.

Treatment group	Parasitological and clinical responses		Patients with eradication of cysts n (%)	Patients with clinical well response n (%)	Patients clinical well and showing eradication of cysts n (%)	Patients that shed cysts after initial eradication n/N (%)
	Cysts Eradicated (CR)	Cysts Persisted (CR)				
Placebo BID 3 days (N = 27)	5 (4 well, 1 CI)	22 (8 well, 14 CI)	5 (18.5)	12 (44)	4 (14.8)	7/48(13)
500 mg NTZ tablet BID 3 days (N = 54)	30 (28 well, 2 CI)	24 (18 well, 6 CI)	30 (55.5)	46 (85)	28 (52)	6/49 (12)
500 mg NTZ oral suspension BID 3 days (N = 54)	26 (26 well, 0 CI)	28 (19 well, 9 CI)	26 (48)	45 (83)	26 (48)	3/25(12)

NTZ = Nitazoxanide; CR = clinical response; N = number of patients; n = number of patients showing response
CI = continuing illness.

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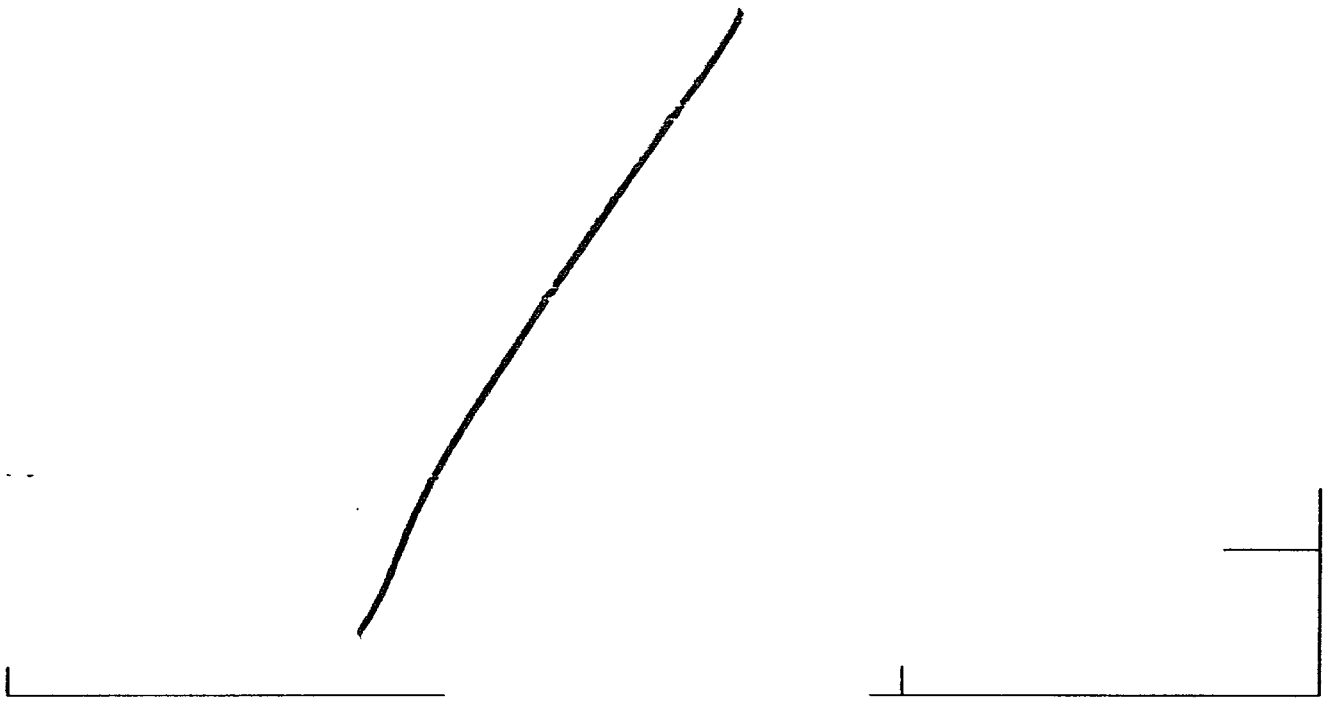


5. CONCLUSIONS:

The sponsor is seeking approval of nitazoxanide tablets for the treatment of diarrhea due to *G. lamblia* in adults. The sponsor has proposed 500 mg nitazoxanide tablets b.i.d. for 3 days for treatment of adults with giardiasis diarrhea.

Giardiasis: A total of 54 adults with giardiasis were treated with nitazoxanide tablets in a phase III, randomized, double-blind, placebo controlled study conducted in Peru and Egypt. Resolution of diarrhea was observed in 85% (46/54) patients treated with nitazoxanide tablets compared to 83% (45/54) treated with nitazoxanide suspension and 44% (12/27) treated with placebo.

The parasitological outcome was based on qualitative data, i.e., presence or absence of cysts in 2 stool samples at 4 to 7 days after discontinuation of therapy. The sponsor also quantified the cysts in the unconcentrated stool sample stained with iodine or immunofluorescence, and concentrated stool sample stained with iodine. The method for detecting the cysts at the two sites, Peru and Egypt, appear to be similar. However, the quantification of the cysts was done differently. The actual cyst counts were determined at Peru, while a semi-quantitative grading system was used and cyst counts per high power field approximated at Egypt. The cyst counts were reported as number of cysts per high power field rather than per weight or volume of stool. Based on qualitative results, the percentage of patients that showed absence of *G. lamblia* cysts in the nitazoxanide tablet arm was 55.5% (30/54) compared to 48% (26/54) in the nitazoxanide suspension arm, and 18.5% (5/27) in the placebo arm. The clinical (94%) and parasitological (94%) outcome were better (94%) in patients treated with nitazoxanide tablets

at the Egypt site, where cysts counts at baseline were low, compared to the Peru site (clinical outcome = 80.5%, parasitological outcome = 64%), where cyst counts at baseline were high.

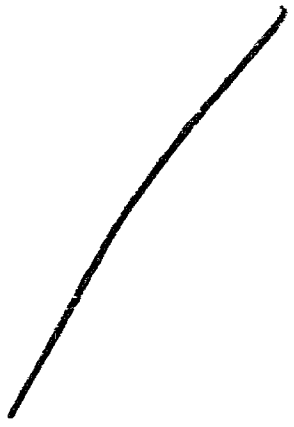
Patients (nitazoxanide tablets arm, n = 28; nitazoxanide suspension arm, n = 26; and placebo arm, n = 4) who had resolution of diarrhea and eradication of cysts at 4 to 7 days after discontinuation of therapy were followed for parasitological outcome at 12 to 14 days after discontinuation of therapy. Shedding of cysts was observed in 25% (7/28) patients in the nitazoxanide arm, 23% (6/26) patients in the nitazoxanide suspension arm, and 50% (2/4) patients in the placebo arm, at the 12 to 14 day follow-up. The clinical outcome was not measured at this time point. Most of these patients were from the Peru site. The sponsor has stated that Peru being a hyper-endemic area compared to Egypt, the recurrence of cysts was more likely due to re-infection. However, no information was included to support the basis of higher endemicity of *G. lamblia* in Peru compared to Egypt. Another explanation provided by the sponsor was that the dose of nitazoxanide was inadequate for complete elimination of cysts from patients. However, it is also possible that the patient relapsed. At the present time, there are no standardized methods to differentiating re-infection from relapse. The differences in the parasitological response may also have been influenced by differences in the strain of *G. lamblia* causing infection in these two sites, and the ability of immunocompetent host to resolve diarrhea spontaneously over time.

Quantification of cysts was done uniformly at baseline and post-treatment using only iodine stained concentrated stools at the two sites. The baseline cyst counts at the Peru site were high (median count = 5 per high power field) compared to Egypt (median count = 1 per high power filed). The reduction of cyst count at the Peru site for the nitazoxanide tablet or suspension and the placebo arms were similar. No meaningful conclusions could be made about reduction in cyst counts at the Egypt site, as the baseline cyst counts were low. The effect of nitazoxanide on reduction of cysts could not be evaluated with certainty, due to limitations of the detection method, differences in methods used for quantification of cysts at the two sites, measurement of cysts in a small volume of stool, and variability in the consistency and number of stools passed by the patients. In the absence of 24 hour collection of stool sample, the cyst count per high power field (Peru site) or approximated counts obtained using a semi-quantitative method (Egypt site) from a small aliquot of sample, irrespective of whether the stool is formed or unformed, may not be useful.

Several factors effect cyst detection in stool samples such as specimen collection, transport, addition of stool preservatives, age of the stool (fresh versus 24 hour old), consistency, number of stools examined, presence of debris, clarity of smears prepared using a concentrated stool sediment, presence of background fluorescence, when using a fluorescence assay and expertise of the slide examiner. The limit of detection of cysts using iodine or immunofluorescence stained unconcentrated, and iodine stained concentrated stool have not been determined. However, a report from the literature suggests that the sensitivity of the unconcentrated and concentrated stool for detection of cysts in patients with giardiasis is 66-70%, when cyst counts are low. The sensitivity of the [redacted] immunofluorescence assay used in this study is similar to iodine stained stool samples. Because of the inability of current diagnostic methods

to detect cysts, when the counts are low and intermittent shedding of cysts in patients with giardiasis, the parasitological outcome should be interpreted with caution.

Overall, the clinical efficacy of nitazoxanide tablets was similar to nitazoxanide suspension and greater than placebo.



6. LABEL:

6.1. Sponsor's proposed label:

MICROBIOLOGY

Mechanism of action

The antiprotozoal activity of nitazoxanide is believed to be due to interference with the pyruvate:ferredoxin oxidoreductase (PFOR) enzyme-dependent electron transfer reaction which is essential to anaerobic energy metabolism. Studies have shown that the PFOR enzyme from *Giardia lamblia* directly reduces nitazoxanide by transfer of electrons in the absence of ferredoxin. The DNA-derived PFOR protein sequence of *Cryptosporidium parvum* appears to be similar to that of *Giardia lamblia*. Interference with the PFOR enzyme-dependent electron transfer reaction may not be the only pathway by which nitazoxanide exhibits antiprotozoal activity.

Activity *in vitro*

Nitazoxanide and its metabolite, tizoxanide, are active *in vitro* in inhibiting the growth of (i) sporozoites and oocysts of *Cryptosporidium parvum* and (ii) trophozoites of *Giardia lamblia*.

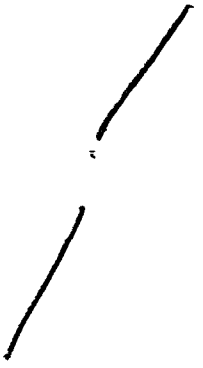
Drug Resistance

A potential for development of resistance by *Cryptosporidium parvum* or *Giardia lamblia* to nitazoxanide has not been examined.

Susceptibility Tests:

For protozoa such as *Cryptosporidium parvum* and *Giardia lamblia*, standardized tests for use in clinical microbiology laboratories are not available.

INDICATIONS AND USAGE



6.2. Comments:

The division and sponsor agreed that the label for nitazoxanide tablets and suspension could be combined. There are no changes to the Microbiology section of the label.

7. RECOMMENDATIONS:

This NDA is recommended for approval with respect to Microbiology for the treatment of giardiasis

Kalavati Suvarna
Microbiologist, HFD-590

CONCURRENCES:

HFD-590/Deputy Dir. _____Signature _____Date
HFD-590/Micro TL _____Signature _____Date
CC:
HFD-590/Original IND
HFD-590/Division File
HFD-590/MO
HFD-590/Pharm
HFD-590/Chem
HFD-590/Review Micro
HFD-590/CSO/MillerK

8. REFERENCES:

(1) Hanson KL, Cartwright CP. Use of an enzyme immunoassay does not eliminate the need to analyze multiple stool specimens for sensitive detection of *Giardia lamblia*. J Clin Microbiol. 2001;39:474-477.

(2) Ungar BLP. *Cryptosporidium*. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases. Pennsylvania: 2000:2903-2915.

(3) Hart CA. Cryptosporidiosis. In: Gilles HM, ed. Protozoal diseases. New York: Oxford University Press Inc; 1999:592-606.

(4) Hill DR. *Giardia lamblia*. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases. Pennsylvania: 2000:2888-2894.

(5) Guarino A, Canani RB, Casola A *et al.* Human intestinal cryptosporidiosis: secretory diarrhea and enterotoxic activity in Caco-2 cells. J Infect Dis. 1995;171:976-983.

(6) Buret A, Hardin JA, Olson ME, Gall DG. Pathophysiology of small intestinal malabsorption in gerbils infected with *Giardia lamblia*. Gastroenterology. 1992;103:506-513.

(7) Chen XM, Levine SA, Splinter PL *et al. Cryptosporidium parvum* activates nuclear factor kappaB in biliary epithelia preventing epithelial cell apoptosis. Gastroenterology. 2001;120:1774-1783.

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/s/

Kalavati Suvarna
7/19/04 02:27:53 PM
MICROBIOLOGIST

Shukal Bala
7/19/04 02:33:43 PM
MICROBIOLOGIST

Steve Hundley
7/19/04 03:48:54 PM
PHARMACOLOGIST

MICROBIOLOGY REVIEW
DIVISION OF SPECIAL PATHOGEN AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)

NDA #: 21-497 and 21-498

REVIEWER : Kalavati Suvarna
CORRESPONDENCE DATE : 05-29-02; 07-22-02; 08-30-02
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SPONSOR: Romark Laboratories Inc.
6200 Courtney Campbell Causeway
Suite 880
Tampa, FL 33607

SUBMISSION REVIEWED: N-000, BZ, BI

DRUG CATEGORY: Anti-parasitic

INDICATION: Treatment of diarrhea caused by *Cryptosporidium parvum* and *Giardia lamblia*

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DOSAGE FORM: Tablets and oral suspension

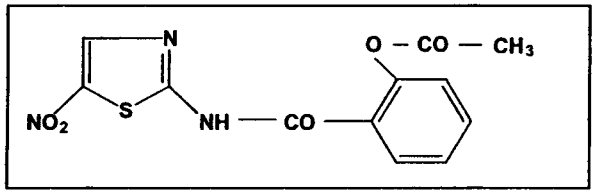
PRODUCT NAMES:

a. **PROPRIETARY:** None

b. **NONPROPRIETARY:** Nitazoxanide
CAS: 55981-09-4

c. **CHEMICAL:** 2-(acetolyloxy)-N-(5-nitro-2-thiazolyl) benzamide

STRUCTURAL FORMULA:



Molecular weight: 307.2
Empirical formula: $C_{12}H_9N_3O_5S$

SUPPORTING DOCUMENTS: NDA # 20-871; IND # —
Type II DMF # — , DMF — , DMF — , DMF —

TABLE OF CONTENTS:

1. INTRODUCTION AND BACKGROUND:

The subject of this NDA is Nitazoxanide for (a) the treatment of diarrhea due to *Cryptosporidium parvum* and (b) the treatment of diarrhea due to *Giardia lamblia*
The sponsor has proposed the following doses of nitazoxanide for the treatment of cryptosporidial diarrhea and giardiasis: 200 mg oral suspension b.i.d for 3 days in children ages 4 - 11 years, and 100 mg oral suspension b.i.d for 3 days in children ages 1 - 3 years.

Nitazoxanide is registered in Latin America for the treatment of a wide range of parasitic infections including *C. parvum* and *G. lamblia.* It is approved for veterinary use (for the treatment of helminthic infections in cats and dogs) in Switzerland and France. In the United States, the drug was the subject of NDA 20-871 (submitted in 1998) for the treatment of cryptosporidial diarrhea in AIDS patients but was not approved.

There is no approved therapy for the treatment of cryptosporidiosis in the United States. Furazolidone and Quinacrine have been approved for treatment of giardiasis in the United States. In addition to these drugs, several drugs such as metronidazole, albendazole, and paromomycin, although not approved are available in the United States for the treatment of giardiasis.

Nitazoxanide is a nitro-thiazolyl with a salicylic acid amide moiety. It is soluble in DMSO and in aqueous media at alkaline pH. The drug is highly unstable and is rapidly metabolized to various metabolites (2 major and 5 minor metabolites have been identified). The two major metabolites are tizoxanide and tizoxanide glucuronide. Following oral administration, nitazoxanide is hydrolyzed to tizoxanide (desacetyl nitazoxanide) that undergoes glucuronidation to form tizoxanide glucuronide. The time to maximum concentration (T_{max}) for both metabolites (tizoxanide and tizoxanide glucuronide) was ≤ 4.5 hours after administration of a single oral dose of nitazoxanide [500 mg to healthy adults (≥ 12 years), 100 mg to children (≤ 3 years) or 200 mg to children (4 - 11 years)]. The maximum plasma concentration (C_{max}) and the area under the concentration versus time curve (AUC) for both metabolites were about 3-fold higher in adults than children (Table 1). Both nitazoxanide and tizoxanide were shown to exhibit high protein binding (> 99%). The protein binding property of tizoxanide glucuronide was not examined.

Table 1: Pharmacokinetic parameters of tizoxanide and tizoxanide glucuronide.

Population	**Dose (mg)**	**Tizoxanide**			**Tizoxanide glucuronide**		
		C_{max} (µg/ml)	**T_{max} (hours)**	**AUC (µg.h/ml)**	**C_{max} (µg/ml)**	**T_{max} (hours)**	**AUC (µg.h/ml)**
Adults	500	10.4	3.0	41.8	10.4	4.5	64.7
12-17 years	500	91.2	4.0	39.5	7.27	4.0	46.5
4-11 years	200	3.00	2.0	13.5	2.84	4.0	16.9
12-47 months	100	3.11	3.5	11.7	3.64	4.0	19.0

The sponsor has examined the mechanism of action of nitazoxanide and its activity *in vitro* and/or in animal models against protozoa (*Cryptosporidium, Giardia,* and others), helminths (nematodes, cestodes, and trematodes), and bacteria (anaerobic and aerobic gram positive and negative bacteria). However, in this review only studies examining the activity against *Cryptosporidium* and *Giardia* (the infective agents for the indication under consideration) are discussed in detail. The activity against other parasites and bacteria are summarized briefly.

A. *Cryptosporidium parvum*:

1.1. Biology of *Cryptosporidium parvum*:

Cryptosporidium is an intracellular parasite present in the gastrointestinal and respiratory tract. The infection is caused by ingestion of oocysts. The oocyst contains four sporozoites within a membrane. Upon ingestion, the sporozoites excyst from the oocyst and invade the epithelial cells and become enveloped in a parasitophorous vacuole. Sporozoites undergo maturation into type I meronts, which release merozoites. The merozoite stage can undergo asexual replication and reinvade the host cells or form type II meronts by sexual replication. The Type II meronts release the macrogametocytes or microgametocytes that fertilize to give rise to a zygote. The zygote can develop into oocyst that may either rupture releasing sporozoites *in vivo* or is shed via the feces. Therefore, the presence of oocyst(s) in the stool samples may be intermittent.

The mechanism by which oocysts rupture allowing sporozoites to invade the mammalian cells is not known. Studies conducted *in vitro* show that oocysts can excyst spontaneously. However, the excystation of oocysts can be enhanced by exposure to acids, bile salts or enzymes. Thus, the exposure of oocysts to acids or enzymes in the gastrointestinal tract may play a role in the rupture of the oocyst cell wall.

1.2. Pathogenesis of cryptosporidial infection:

The major clinical manifestation observed with cryptosporidial infection is diarrhea. However, the mechanism by which *Cryptosporidium* causes diarrhea is not known. The severity of the infection and ultimate pathology is influenced by the immune status of the host. Cryptosporidial diarrhea is self-limiting in immunocompetent individuals but may be life threatening in AIDS or immunocompromised patients.

2. MECHANISM OF ACTION:

The survival of protozoa, that lack mitochondria, under anaerobic conditions depend on the presence of the enzyme pyruvate:ferredoxin oxidoreductase (PFOR). The enzyme PFOR is involved in carbon metabolism and oxidizes pyruvate to acetylCoA using ferredoxin as an electron acceptor *in vivo*. Nitazoxanide can act as an alternate electron acceptor for this enzyme and be activated. The activated product has not been identified, however, it is thought to play a role in the mechanism by which nitazoxanide exhibits activity against protozoa by generation of a toxic radical.

Genome analyses of *C. parvum* revealed the presence of a gene that encodes a PFOR like protein. In report RM01-0401[1], the *C. parvum* DNA derived PFOR peptide sequence was compared to peptide sequences from other organisms. The percent similarity between the peptide sequence of *C. parvum* PFOR and that of *Giardia lamblia, Entameoba histolytica, Trichomonas vaginalis* and *Clostridium pasteuranium* was 31%, 49%, 43%, and 51%, respectively. However, the effect of nitazoxanide on the activity of *C. parvum* PFOR enzyme was not measured.

3. ACTIVITY *IN VITRO*:

The *in vitro* activity of nitazoxanide against *C. parvum* was measured using different cell lines such as Madin-Darby bovine kidney (MDBK-F5D2), human adenocarcinoma ileocecal (HCT-8) or human lung carcinoma (A-549) cells infected with oocysts or sporozoites. The sponsor has submitted 3 published and 2 unpublished studies in support of the activity of nitazoxanide and its metabolites against *C. parvum in vitro*.

3.1. Activity of nitazoxanide and its metabolite against a human strain:

In study ROM-022[2], five experiments were conducted (all in the same laboratory) to determine the *in vitro* activity of nitazoxanide and/or its metabolite against the GCH1 strain of *C. parvum* [these reports were reviewed previously NDA# 20-871 (N-000), microbiology review dated 06-01-98]. All of these experiments were conducted using MDBK-F5D2 cells as the feeder layer and GCH1 oocysts at a concentration of 5×10^4 per well in DMEM with 5% fetal bovine serum. The cultures were incubated at 37°C for 24 hours and/or 48 hours with different concentrations of nitazoxanide (dissolved in DMSO; final concentration 0.025-0.5%) or paromomycin (dissolved in water/medium). The drugs were added at the time of initiation of infection in culture. The anti-cryptosporidial effect of nitazoxanide was measured by immunofluorescence using anti-*C. parvum* sporozoite rabbit serum. The anti-sporozoite antiserum raised in rabbits reacted with all developmental forms except the oocyst wall. The toxic effects of the drug on the uninfected mammalian (MDBK) cells was determined by measuring the absorbance of the supernatant at 490 nm after incubation of the cells in the presence of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) and phenazine methosulfate (PMS) for 2 hours in the dark. The results of the five experiments are shown below:

Table 2A: *C. parvum* Oocysts Assay (24 hours) - Experiment #17 (final DMSO concentration = 0.5%)

Trial 1 - 24hr

Compound	Conc.	Mean (± SD)*	Percent Toxicity	Percent Inhibition
Infected Media	0	983.5 (±128.2)	0	0
Paromomycin	2mg/ml	482 (±47.1)	23.8	51
NTZ	100µg/ml	Lost	88.1	NA**
	10µg/ml	55.5 (±13.5)	65.1	94.4
	1µg/ml	224.5 (±28.5)	8.3	77.2
	0.1µg/ml	474.5 (±29.3)	19.3	51.8

* Parasite Count/10 fields
** Not available due to toxicity

Table 2B: *C. parvum* Oocysts Assay (48 hours) - Experiment #17

Trial 2 - 48hr

Compound	Conc.	Mean (± SD)*	Percent Toxicity	Percent Inhibition
Infected Media	0	2231.25 (+90.03)	0	0
Paromomycin	2mg/ml	580 (+33.42)	40.8	74.01
NTZ	20µg/ml	68.75 (+13.77)	92.87	96.92
	2µg/ml	113.75 (+21.36)	24.93	94.90
	0.2µg/ml	1020 (+158.48)	16.56	54.29
	0.02µg/ml	1041 (+191.46)	21.23	53.33

* Parasite Count/10 fields

Table 3: *C. parvum* Oocysts Assay (24 hours)- Experiment #19

Combined Drugs vs. *C parvum* Oocysts

Paromomycin (mg/ml) (water base)	NTZ (ug/ml) (DMSO base)	Par/10	± SD	Tox/OD	± SD	%Inhib	%Tox	Score
0	0	928.50	±79.32	1.187	±.023	0	0	0
2	0	270.00	±12.65	1.023	±.006	70.92	13.82	1
1	0	373.00	±83.66	1.118	±.066	59.83	5.82	1
0.5	0	490.50	±98.36	NA*	NA	47.17	NA	NA
0.25	0	599.00	±74.13	NA	NA	35.49	NA	NA
0	0	779.50	±63.08	1.049	±.066	0	0	0
0	20	88.50	±15.86	0.329	±.074	88.65	68.62	3
0	10	110.0	±16.57	0.633	±.093	85.89	39.68	2
0	5	72.50	±22.23	1.071	±.052	90.70	0	0
0	2.5	168.50	±16.60	1.131	±.294	78.38	0	0
2	20	52.00	±18.11	0.532	±.101	93.33	49.26	2
2	10	84.50	±12.37	0.610	±.066	89.16	41.87	2
2	5	91.00	±25.32	0.901	±.152	88.33	14.07	1
2	2.5	87.50	±2.52	1.011	±.156	88.77	3.58	0
1	20	84.50	±36.93	0.601	±.041	89.16	42.68	2
1	10	75.00	±15.56	0.645	±.049	90.38	38.48	2
1	5	88.50	±25.16	0.811	±.045	88.65	22.70	1
1	2.5	135.50	±19.49	1.030	±.021	82.62	1.76	0
0.5	20	137.00	±27.25	0.350	±.034	82.42	66.62	3
0.5	10	83.33	±14.05	0.611	±.008	89.31	41.73	2
0.51	5	95.50	±22.71	0.912	±.104	87.75	13.07	1
0.5	2.5	116.50	±25.68	1.021	±.052	85.05	2.58	0
0.25	20	70.67	±12.86	0.349	±.073	90.93	66.71	3
0.25	10	65.50	±37.07	0.647	±.062	91.60	38.29	2
0.25	5	102.00	±37.63	0.896	±.007	86.91	14.56	1
0.25	2.5	126.00	±19.66	1.082	±.075	83.84	0	0

Par/10 = Parasite counts per 10 high power fields
%Inhib = Percent Inhibition of parasite infection
%Tox = Percent toxicity to cells by the drug
*NA - Information not available

Table 4A: *C. parvum* Oocysts Assay (48 hours) Experiment #28- submitted as part of Experiment #29A

Drugs	Conc.	Parasite ± SD	Tox/OD ± SD	%Inhib	%Tox	Score
Aq. Media	0	1218.4 ±210.22	1.013 ±.024	0	0	0
Paromomycin	2mg/ml	219.08 ±70.69	.873 ±.016	82.02	13.82	1
	1	279.17 ±100.80	1.061 ±.061	77.09	≤ 0	0
	0.5	309.83 ±77.92	.874 ±.158	74.57	13.72	1
	0.25	485.67 ±94.33	.697 ±.006	60.14	31.19	2
0.25% DMSO Media	0	824.92 ±173.73	.928 ±.071	0	0	0
Nitazoxanide	100ug/ml	LOST NA*	.515 ±.107	NA	NA	4
	10	43.42 ±14.69	.201 ±.023	94.74	78.34	4
	1	120.00 ±40.25	.922 ±.017	85.45	65	0
	0.1	782.75 ±251.45	.824 ±.086	5.11	11.21	1

Table 4B: *C. parvum* Oocysts Assay (48 hours)-Experiment #29A

Drugs	Conc.	Parasite ± SD	Tox/OD ± SD	% Inhib	%Tox	Score
Aqueous Media	0	895.13 ±248.28	1.753 ±.068	0	0	0
Fresh Paromomycin	*2000*	*265.00 ±63.44*	*1.527 ±.250*	*70.40*	*12.92*	*1*
0.25% DMSO Media	0	678.50 ±114.69	1.741 ±.194	0	0	0
Fresh Nitazoxanide	100	LOST NA	.243 ±.037	NA	86.04	4
	10	52.50 ±15.88	.246 ±.012	92.26	85.87	4
	1	479.67 ±94.98	1.718 ±.261	29.30	1.32	0
	0.1	549.00 ±145.22	1.834 ±.274	19.09	≤0	0

Table 4C: *C. parvum* Oocysts Assay (48 hours)-Experiment #29A

Drugs	Conc.	Parasite ± SD	Tox/OD ± SD	% Inhib	%Tox	Score
Aqueous Media	0	709.89 ±343.85	1.544 ±.066	0	0	0
Fresh Paromomycin	2000	174.50 ±58.49	1.188 ±.030	75.42	23.03	1
0.25% DMSO Med	0	535.58 ±242.96	1.479 ±.041	0	0	0
11 Day Old Nitazoxanide	100	LOST NA	.479 ±.001	NA	67.60	3
	10	46.78 ±21.66	.230 ±.016	91.27	84.41	4
	1	118.17 ±63.16	1.420 ±.013	77.94	3.99	0
	0.1	405.33 ±142.79	1.515 ±.086	24.32	≤0	0

Table 4D: *C. parvum* Oocysts Assay (48 hours)-Experiment #29A

Drugs	Conc.	Parasite	± SD	Tox/OD	± SD	% Inhib	%Tox	Score
Aqueous Media	0	406.33	±115.38	1.698	±.248	0	0	0
Fresh Paromomycin	2000	146.83	±50.71	1.455	±.130	63.86	14.32	1
0.25% DMSO Med	0	370.91	±118.02	1.474	±.064	0	0	0
17 Day Old Nitazoxanide	100	LOST	NA	.749	±.008	NA	49.20	2
	10	*32.44*	*±16.84*	*.324*	*±.008*	*91.25*	*78.05*	*4*
	1	56.00	±11.97	1.693	±.056	84.90	≤0	0
	0.1	344.67	±43.87	1.389	±.126	7.07	5.73	0

Table 4E: *C. parvum* Oocysts Assay (48 hours)-Experiment #29A

Drugs	Conc.	Parasite	± SD	Tox/OD	± SD	% Inhib	%Tox	Score
Aqueous Media	*0*	*1218.42*	*±210.22*	*1.013*	*±.024*	*0*	*0*	*0*
Fresh Paromomycin	2000	219.08	±70.69	.873	±.016	82.02	13.82	1
0.25% DMSO Media	0	824.92	±173.73	.928	±.071	0	0	0
Fresh Nitazoxanide		100	LOST	NA	.515	±.107	NA	44.56 2
	10	43.42	±14.69	.201	±.023	94.74	78.34	4
	1	120.00	±40.25	.922	±.017	85.45	0.65	0
	0.1	782.75	±251.45	.824	±.086	5.11	11.21	1

Note: It appeared that the data shown in Table #4A and #4E are the same. However, the sponsor stated that "while the results appear to be similar, they are not all the same". Given that all of the values reported in both tables were identical out to two or three decimal points it is unclear what data were new or different. Also, the results in Table 4B were stated to be anomalous.

Table 5: *C. parvum* Oocysts Assay (48 hours)-Experiment #30

***C. parvum* Oocysts Assay (48 hr.)**

Drugs	Conc.	Parasite	± SD	Tox/OD	± SD	%Inhib	%Tox	Score
Aqueous Media	0	681.58	±271.02	2.024	±.018	0	0	0
Paromomycin	2000	115.75	±44.65	1.219	±.009	83.02	39.79	2
0.025% DMSO Media	0	628.50	±171.94	1.799	±.145	0	0	0
NTZ	10	11.75	±7.33	.413	±.013	98.13	77.07	4
	1	39.67	±13.13	1.618	±.326	93.69	10.09	1
	0.1	643.42	±229.73	1.878	±.154	≤0	≤0	0
	0.01	714.33	±194.79	1.617	±.072	≤0	10.12	1
New NTZdes	10	13.75	±6.66	.337	±.005	97.81	81.27	4
	1	39.92	±13.49	1.710	±.033	93.65	4.97	0
	0.1	649.86	±152.19	1.506	±.119	≤0	16.29	1
	0.01	749.33	±139.49	1.721	±.144	≤0	4.36	0

Conc. - µg/ml
Parasite - Mean parasite count/field (12 fields analyzed)
%Inhib - Percent Inhibition of parasite infection
%Tox - Percent toxicity to cells by the drug