



Amixicile Reduces Severity of Cryptosporidiosis but Does Not Have *In Vitro* Activity against *Cryptosporidium*

Luther A. Bartelt,^{a,b} David T. Bolick,^c Glynis L. Kolling,^c Erin Stebbins,^d Christopher D. Huston,^d Richard L. Guerrant,^c Paul S. Hoffman^c

^aDivision of Infectious Diseases, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

^bCenter for Gastrointestinal Biology and Disease, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

^cDivision of Infectious Diseases and International Health, Department of Medicine, University of Virginia, Charlottesville, Virginia, USA

^dDivision of Infectious Diseases, Department of Medicine, University of Vermont, Burlington, Vermont, USA

ABSTRACT *Cryptosporidium* species cause significant morbidity in malnourished children. Nitazoxanide (NTZ) is the only approved treatment for cryptosporidiosis, but NTZ has diminished effectiveness during malnutrition. Here, we show that amixicile, a highly selective water-soluble derivative of NTZ diminishes *Cryptosporidium* infection severity in a malnourished mouse model despite a lack of direct anticryptosporidial activity. We suggest that amixicile, by tamping down anaerobes associated with intestinal inflammation, reverses weight loss and indirectly mitigates infection-associated pathology.

KEYWORDS *Cryptosporidium*, amixicile, environmental enteropathy, malnutrition, nitazoxanide

Cryptosporidium species are intestinal apicomplexan protozoa that cause significant global morbidity and mortality in young children (1). Even when asymptomatic, childhood *Cryptosporidium* infections are associated with impaired growth attainment (2). Syndromic-based diarrhea interventions may be ineffective for cryptosporidiosis, and targeted anticryptosporidial therapeutics are unavailable, especially in resource-limited settings (2). Nitazoxanide (NTZ) is currently the only FDA-approved drug for treating cryptosporidiosis. Early clinical studies of NTZ in immunocompetent children demonstrated decreased duration of diarrhea and 75% parasitological cure (3). However, parasitological cure occurred in only 52% of malnourished children (4), and even prolonged durations of NTZ were ineffective during HIV coinfection (5). Similarly, NTZ does not consistently treat *Cryptosporidium* infection in several murine models, including immunodeficient knockout mice that develop relapsing disease (6) and malnourished mice that spontaneously clear infection (7, 8). Finally, despite its *in vitro* activity, NTZ only partially clears *Cryptosporidium* infections in piglet models (9).

It is unusual for an antibiotic to work in humans but not in established preclinical animal models, which has raised questions about its mechanism of action. NTZ is a broad-spectrum antiparasitic drug that inhibits pyruvate:ferredoxin oxidoreductase (PFOR) through targeting the thiamine pyrophosphate vitamin cofactor of PFOR (10). Anaerobic bacteria and parasites that rely on PFOR as an essential enzyme for central metabolism are highly susceptible to nitazoxanide. In the development of NTZ, the PFOR of *Cryptosporidium* was never validated by a direct enzyme assay (10). The *Cryptosporidium* PFOR is a hybrid enzyme containing a C-terminal cytochrome P450 protein, composed of flavodoxin and NADPH oxidase (Fig. 1) (11). This atypical arrangement appears to connect the oxidative decarboxylation of pyruvate with the reduction

Received 10 April 2018 Returned for
modification 8 June 2018 Accepted 28
September 2018

Accepted manuscript posted online 8
October 2018

Citation Bartelt LA, Bolick DT, Kolling GL, Stebbins E, Huston CD, Guerrant RL, Hoffman PS. 2018. Amixicile reduces severity of cryptosporidiosis but does not have *in vitro* activity against *Cryptosporidium*. *Antimicrob Agents Chemother* 62:e00718-18. <https://doi.org/10.1128/AAC.00718-18>.

Copyright © 2018 Bartelt et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Luther A. Bartelt, luther_bartelt@med.unc.edu.

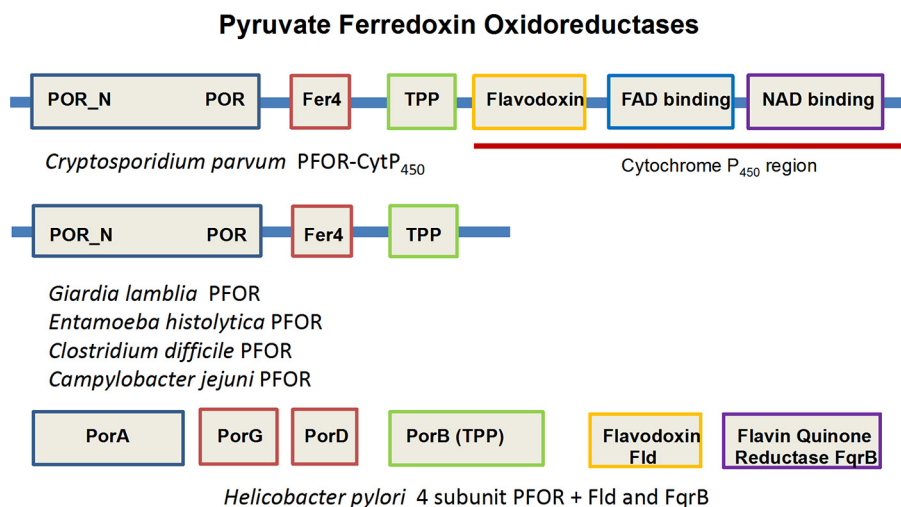


FIG 1 The *C. parvum* PFOR (GenBank accession number [AF208233](https://www.ncbi.nlm.nih.gov/nuccore/AF208233)) is a hybrid enzyme with C-terminal cytochrome P450 that oxidizes pyruvate with reducing equivalents internally transferred through flavodoxin to NADPH oxidase that produces NADPH (11). Other PFORs transfer reducing equivalents through ferredoxin to electron acceptors to produce hydrogen. The 4-subunit *H. pylori* PFOR and the single-unit PFOR of *C. jejuni* also produce NADPH via flavodoxin (Fld) and NADPH oxidase FqrB (33) with functional evolutionary similarities with the *C. parvum* enzyme, including an inability to reduce the redox-active prodrug metronidazole. Not to scale.

of NADP, thus bypassing the typical ferredoxin/hydrogenase redox route. NTZ impairs the invasion of *Cryptosporidium* species in epithelial cell monolayers (12); however, given the off-target effects of NTZ, such as anticancer (13), antiviral (14), and non-PFOR antiparasitic activities (15, 16), one cannot rule out the possibility that the hybrid PFOR of *Cryptosporidium* is not the target of NTZ. Furthermore, with the exception of the apicomplexan (*Cryptosporidium*) group, most pathogens that are susceptible to NTZ are also susceptible to metronidazole. Since PFOR is associated with the mode of action of both drugs, we sought to explain the basis for the exception.

Amoxicile (VPC162134) is a recently developed low-toxicity water-soluble derivative of NTZ, with high systemic bioavailability (17). Amoxicile was developed by replacing the 2-acetoxy group with propylamine, leading to a more water-soluble and noncytotoxic drug (18). Compared with only 30% absorption of the active NTZ metabolite tizoxanide, nearly 100% of amoxicile is absorbed (17). Amoxicile is a highly selective inhibitor of PFOR in anaerobes (19) and *Helicobacter pylori* (17, 20), and it is much more active than NTZ against other PFOR-producing pathogens, including *Clostridium difficile*, *H. pylori*, *Campylobacter jejuni*, and periodontal disease-promoting anaerobes (18–20). Amoxicile is effective against *C. difficile* and *H. pylori* infections in mice (17), but its effect on *Cryptosporidium* species and malnutrition is unknown.

In order to determine the effectiveness of amoxicile against *Cryptosporidium* species, we used previously published models of *in vivo* challenge during murine protein malnutrition (PM) (8, 21) and *in vitro* epithelial cell monolayer inhibition assays (6, 22).

All animal studies were performed at the University of Virginia with a protocol approved in accordance with the Institutional Animal Care and Use Committee (IACUC) policies of the University of Virginia (protocol number 3315). Briefly, weaned C57BL/6 mice (Jackson Laboratories) fed a 2% protein deficient diet (Research Diets) that recapitulates features of cryptosporidiosis in malnourished children, including dose-dependent disease severity (8), were challenged with 5×10^7 *Cryptosporidium parvum* oocysts (Iowa isolate; Waterborne, Inc., New Orleans, LA). Similar to other *Cryptosporidium* murine models recently developed for preclinical anticryptosporidial drug testing (6), *C. parvum* fecal shedding was determined by quantitative PCR (qPCR; 18S rRNA target) (21) that was present only after challenge with viable, but not heat-inactivated oocysts (8). Shedding in this model is greatest during the first 3 days postchallenge,

coincident with rapid weight loss. Parasites are detectable for 7- to 11-days postchallenge, but unlike immunodeficient murine knockout models, there is no relapse phase (6). Statistical analyses (two-way analysis of variance [ANOVA], including Bonferroni posttest analysis of repeated measures for growth where appropriate) were performed with GraphPad Prism 7.0 software.

Amixicile (diluted in deionized water) or NTZ (as a pediatric solution [Alinia; Romark Pharmaceuticals, Tampa, FL] diluted in deionized water) was given at equivalent doses of 100 mg/kg/day of body weight (7, 18) by orogastric gavage once daily beginning 1-day postinfection (1 dpi) and continued through 3 dpi. We performed four independent experiments investigating the effectiveness of amixicile against *C. parvum* challenge *in vivo*. First, in direct comparison, amixicile, but not NTZ, partially rescued mice from early weight loss (0.69% versus 4.5% weight loss at 4 dpi; $P < 0.05$) (Fig. 2A). *C. parvum* stool shedding on 1 (collected prior to treatment), 5, or 7 dpi was similar in all challenged mice regardless of treatment (see Fig. S1A in the supplemental material). In one of two separate follow-up experiments (Fig. 2B and C), amixicile partially attenuated severity of weight loss (Fig. 2C). *C. parvum* shedding was not conclusively reduced in amixicile-treated animals (see Fig. S1B and C in the supplemental material).

Since neither PFOR inhibitor eliminated *C. parvum*, we hypothesized that the primary effect of amixicile was to benefit the malnourished host. Whereas uninfected mice treated for 3 days with NTZ exhibited weight loss, mice treated with amixicile did not (see Fig. S2 in the supplemental material). In a fourth experiment, extending amixicile treatment to 5 days promoted weight gain by malnourished uninfected animals (Fig. 2D). Composite data for untreated and amixicile-treated infected groups compared with untreated uninfected controls for all *in vivo* experiments demonstrated a modest growth benefit in amixicile-treated animals ($P < 0.05$ for untreated *C. parvum*-challenged mice) (Fig. 2E) that was most apparent as a rescue from weight loss upon initiation of amixicile (see Fig. S3 in the supplemental material).

For *in vitro* testing, we compared both PFOR inhibitors using an assay which involves inoculating HCT-8 cells (ATCC) grown to confluence with 5.5×10^3 excystation-primed *C. parvum* oocysts (Bunchgrass Farms, Deary, ID) (12), staining for epifluorescence microscopy after a 48-h incubation, and counting parasites and host cells using automated microscopy (12). The 50% effective concentration (EC_{50}) of NTZ was 1.55 (range, 1.31 to 1.84) μM , but across repeated experiments, amixicile had no anticryptosporidial activity, even at $\geq 100 \mu\text{M}$ (Fig. 3A and B). Since NTZ and amixicile are identical in docking simulations with PFOR and amixicile is highly selective for PFOR, it is likely that the PFOR-CytP450 is not a target for either drug (20). Our suggestion that PFOR/CytP450 is an unlikely target of NTZ is consistent with a growing view that other inhibitory mechanisms are involved (16). Although off-target activities attributed to NTZ against *Cryptosporidium* species are not known, amixicile is a more selective PFOR inhibitor which might explain why nitazoxanide, but not amixicile, is active against *C. parvum in vitro*. Off-target promiscuous activities of NTZ against *Cryptosporidium* species may contribute to the variable outcomes observed clinically with this drug.

Promising new anticryptosporidial therapeutics rapidly eliminate parasites in other animal models (6, 23, 24). In contrast, amixicile mitigated weight loss, a very important disease feature of cryptosporidiosis, but did so without significantly accelerating parasite elimination, which suggests that its effect on malnutrition in cryptosporidiosis is indirect. *Cryptosporidium* infections (25) and other intestinal microbial disruptions (26) are increasingly linked to malnutrition in both children (27) and murine models (28–30). Together, these microbial exposures may result in a multimicrobial condition of chronic intestinal inflammation and injury termed environmental enteric dysfunction (EED) (25, 26, 31). In murine models of EED, there is an overabundance of anaerobic *Bacteroidales* members in the upper small intestine during malnutrition, and inclusion of *Bacteroides* spp. with a cocktail of *Escherichia coli* isolates was necessary to enhance intestinal inflammation (26).

Given the spectrum of amixicile activity (17), its absence in stools, and its apparent

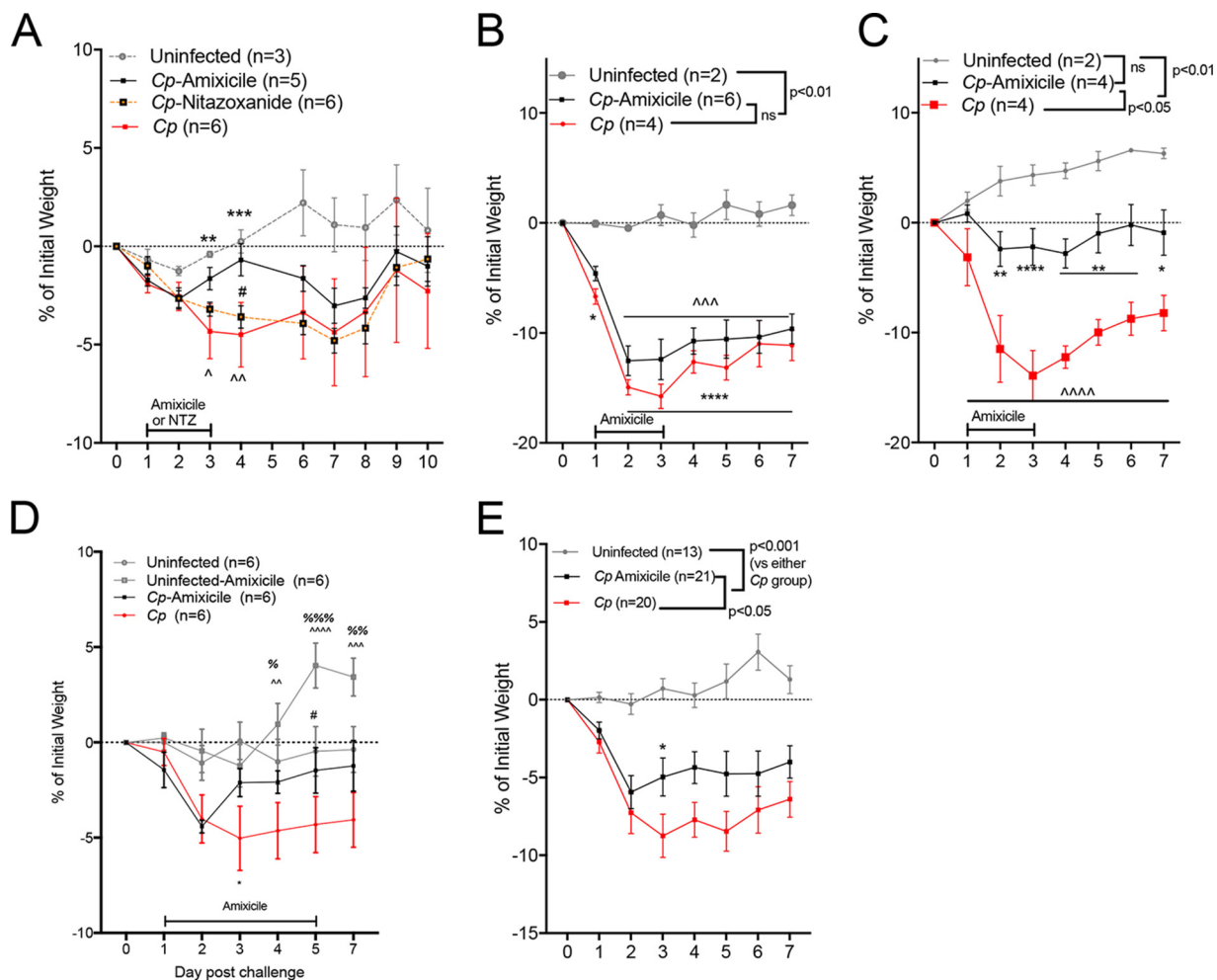


FIG 2 Amoxicillin partially reduces the severity of cryptosporidiosis during malnutrition. (A) Experiment 1. Amoxicillin or nitazoxanide (NTZ) was administered by orogastric gavage at equivalent doses (100 mg/kg/day) to protein-malnourished weaned mice beginning 1 day after *C. parvum* oocyst challenge (5×10^7 oocysts in all experiments) and continued through 3-day postchallenge. Growth is shown as percent initial weight beginning on the day of *C. parvum* challenge (**, $P < 0.01$ and ***, $P < 0.001$ for Uninfected versus Cp; \wedge , $P < 0.05$ and $\wedge\wedge$, $P < 0.01$ for Cp versus Cp-Amoxicillin; #, $P < 0.05$ for Cp-Amoxicillin versus Cp-NTZ). (B, C) Experiments 2 and 3. Growth is shown as percentage of weight on the day of *C. parvum* challenge in two separate experiments. Amoxicillin was administered orally at 100 mg/kg/day for 3 days beginning 1 day after challenge with *C. parvum* oocysts. (B) *, $P < 0.05$ and ****, $P < 0.0001$ for Cp versus Uninfected; $\wedge\wedge\wedge$, $P < 0.001$ for Cp-Amoxicillin versus Uninfected. (C) *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ for Cp-Amoxicillin versus Cp. $\wedge\wedge\wedge\wedge$, $P < 0.0001$ for Cp versus Uninfected. (D) Experiment 4. Growth in weaned mice fed a protein deficient diet as percent initial change beginning on the day of *C. parvum* challenge. Amoxicillin was administered orally at 100 mg/kg/day on day 1 to 5 postchallenge. *, $P < 0.05$ for Cp versus Uninfected; $\wedge\wedge$, $P < 0.01$; $\wedge\wedge\wedge$, $P < 0.001$ for Uninfected-Amoxicillin versus Uninfected. %, $P < 0.05$; %%, $P < 0.01$; and %%%, $P < 0.001$ for Uninfected-Amoxicillin versus Uninfected. (E) Growth curves for Uninfected, Cp, and Cp-Amoxicillin groups combined across all four experiments (*, $P < 0.05$ for Cp versus Cp-Amoxicillin; 3 dpi). For growth curves B, C, and E, brackets designate comparisons of growth curves throughout the time course (two-way ANOVA).

ability to concentrate in regions of local intestinal inflammation due to serum leakage (17, 18), we suggest amoxicillin likely targets the offending mucosal-associated anaerobes, and, thus, reduces inflammation and improves barrier function and absorption that collectively leads to weight gain and elimination of the parasite. NTZ, which concentrates in the gut lumen, does not reverse weight loss in this model. Supporting this multimicrobial pathogenesis hypothesis, *Cryptosporidium* infection as well as other enteric infections in this model of PM can exacerbate diet-dependent disruptions in gut-bacterium-derived metabolites, such as trimethylamine (TMA) and trimethylamine oxide (TMAO) (28, 30), that are also altered in some malnourished children (29, 32). These observations are consistent with previous findings that amoxicillin, which does not alter the gut microbiome, eliminates *C. difficile* and *H. pylori* infections in murine models (17, 18).

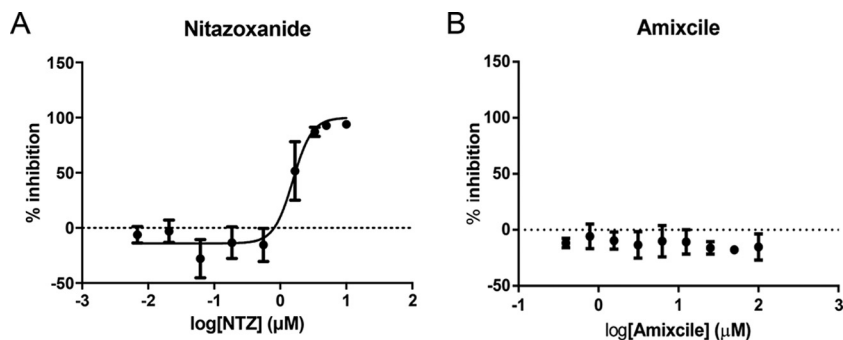


FIG 3 Amixicile, a selective PFOR inhibitor, has no *in vitro* activity against *Cryptosporidium* parasites. Nitazoxanide or amixicile was administered at concentrations ranging from $\log -2.16$ to $1 \mu\text{M}$ or $\log -0.216$ to $2.0 \mu\text{M}$, respectively. Inhibition was determined in a cell-based high-throughput screening assay using confluent HCT-8 monolayers inoculated with 5.5×10^3 *C. parvum* oocysts. (A) Nitazoxanide inhibition curve EC_{50} , $1.55 \mu\text{M}$ (range, 1.31 to $1.84 \mu\text{M}$); EC_{90} , $3.23 \mu\text{M}$ (range, 2.32 to $4.50 \mu\text{M}$). (B) Amixicile inhibition curve. Each graph shows representative results from one of two experiments, with multiple technical replicates for each data point (NTZ, $n = 5$; Amixicile, $n = 4$).

In conclusion, the NTZ derivative amixicile, a highly selective water-soluble PFOR inhibitor that appears to concentrate in sites of local inflammation (18), reversed weight loss in the malnourished host, despite no evidence of *in vitro* anticryptosporidial activity. Furthermore, neither NTZ nor amixicile resulted in a definitive reduction of parasite shedding *in vivo*. Unlike currently available antibiotic regimens for treating malnutrition/EED (31) that could cause collateral loss of potentially beneficial microbiota, amixicile spares the intestinal microbiota of mice (17), and may, therefore, preserve healthy luminal microbial density while selectively targeting subpopulations of anaerobes causing active infection. Thus, demonstrating that *Cryptosporidium* parasites are resilient to PFOR inhibition *in vitro*, but that infection can be attenuated by the PFOR inhibitor amixicile, led us to propose a model of mixed-microbial pathogenesis during cryptosporidiosis and malnutrition (30) and the potential role for both direct and indirect therapies to address the management of this emerging global pathogen.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.00718-18>.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

ACKNOWLEDGMENTS

This study was supported in part by the National Institutes of Health, National Institute of Allergy and Infectious Diseases grants 1R21 AI111604 (PSH), K08AI108730 (LAB), and 1R21 AI30807 (CDH), and the Bill and Melinda Gates Foundation grant OPP1066140 (RLG).

L.A.B. served as a temporary consultant for Lupin Pharmaceuticals.

REFERENCES

- Sow SO, Muhsen K, Nasrin D, Blackwelder WC, Wu Y, Farag TH, Pan-chalingam S, Sur D, Zaidi AK, Faruque AS, Saha D, Adegbola R, Alonso PL, Breiman RF, Bassat Q, Tamboura B, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ahmed S, Qureshi S, Quadri F, Hossain A, Das SK, Antonio M, Hossain MJ, Mandomando I, Nhampossa T, Acacio S, Omoro R, Oundo JO, Ochieng JB, Mintz ED, O'Reilly CE, Berkeley LY, Livio S, Tennant SM, Sommerfelt H, Nataro JP, Ziv-Baran T, Robins-Browne RM, Mishcherkin V, Zhang J, Liu J, Houpt ER, Kotloff KL, Levine MM. 2016. The burden of *Cryptosporidium* diarrheal disease among children < 24 months of age in moderate/high mortality regions of sub-Saharan Africa and South Asia, utilizing data from the Global Enteric Multicenter Study (GEMS). *PLoS Negl Trop Dis* 10:e0004729. <https://doi.org/10.1371/journal.pntd.0004729>.
- Checkley W, White AC, Jr, Jaganath D, Arrowood MJ, Chalmers RM, Chen XM, Fayer R, Griffiths JK, Guerrant RL, Hedstrom L, Huston CD, Kotloff KL, Kang G, Mead JR, Miller M, Petri WA, Jr, Priest JW, Roos DS, Striepen B, Thompson RC, Ward HD, Van Voorhis WA, Xiao L, Zhu G, Houpt ER. 2015. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for cryptosporidium. *Lancet Infect Dis* 15:85–94. [https://doi.org/10.1016/S1473-3099\(14\)70772-8](https://doi.org/10.1016/S1473-3099(14)70772-8).
- Rosignol JF, Ayoub A, Ayers MS. 2001. Treatment of diarrhea caused by *Cryptosporidium parvum*: a prospective randomized, double-blind, placebo-controlled study of nitazoxanide. *J Infect Dis* 184:103–106. <https://doi.org/10.1086/321008>.
- Amadi B, Mwiya M, Musuku J, Watuka A, Sianongo S, Ayoub A, Kelly P. 2002. Effect of nitazoxanide on morbidity and mortality in Zambian

- children with cryptosporidiosis: a randomised controlled trial. *Lancet* 360:1375–1380. [https://doi.org/10.1016/S0140-6736\(02\)11401-2](https://doi.org/10.1016/S0140-6736(02)11401-2).
5. Amadi B, Mwiya M, Sianongo S, Payne L, Watuka A, Katubulushi M, Kelly P. 2009. High dose prolonged treatment with nitazoxanide is not effective for cryptosporidiosis in HIV positive Zambian children: a randomised controlled trial. *BMC Infect Dis* 9:195. <https://doi.org/10.1186/1471-2334-9-195>.
 6. Jumani RS, Bessoff K, Love MS, Miller P, Stebbins EE, Teixeira JE, Campbell MA, Meyers MJ, Zambriski JA, Nunez V, Woods AK, McNamara CW, Huston CD. 2018. A novel piperazine-based drug lead for Cryptosporidiosis from the Medicines for Malaria Venture Open Access Malaria Box. *Antimicrob Agents Chemother* 62:e01505-17. <https://doi.org/10.1128/AAC.01505-17>.
 7. Costa LB, JohnBull EA, Reeves JT, Sevilleja JE, Freire RS, Hoffman PS, Lima AA, Oria RB, Roche JK, Guerrant RL, Warren CA. 2011. Cryptosporidium-malnutrition interactions: mucosal disruption, cytokines, and TLR signaling in a weaned murine model. *J Parasitol* 97:1113–1120. <https://doi.org/10.1645/GE-2848.1>.
 8. Bartelt LA, Bolick DT, Kolling GL, Roche JK, Zaenker EI, Lara AM, Noronha FJ, Cowardin CA, Moore JH, Turner JR, Warren CA, Buck GA, Guerrant RL. 2016. Cryptosporidium priming is more effective than vaccine for protection against Cryptosporidiosis in a murine protein malnutrition model. *PLoS Negl Trop Dis* 10:e0004820. <https://doi.org/10.1371/journal.pntd.0004820>.
 9. Theodos CM, Griffiths JK, D'Onfro J, Fairfield A, Tzipori S. 1998. Efficacy of nitazoxanide against *Cryptosporidium parvum* in cell culture and in animal models. *Antimicrob Agents Chemother* 42:1959–1965. <https://doi.org/10.1128/AAC.42.8.1959>.
 10. Hoffman PS, Sisson G, Croxen MA, Welch K, Harman WD, Cremades N, Morash MG. 2007. Antiparasitic drug nitazoxanide inhibits the pyruvate oxidoreductases of *Helicobacter pylori*, selected anaerobic bacteria and parasites, and *Campylobacter jejuni*. *Antimicrob Agents Chemother* 51:868–876. <https://doi.org/10.1128/AAC.01159-06>.
 11. Rotte C, Stejskal F, Zhu G, Keithly JS, Martin W. 2001. Pyruvate: NADP+ oxidoreductase from the mitochondrion of *Euglena gracilis* and from the apicomplexan *Cryptosporidium parvum*: a biochemical relic linking pyruvate metabolism in mitochondrion and amitochondrion. *Mol Biol Evol* 18:710–720. <https://doi.org/10.1093/oxfordjournals.molbev.a003853>.
 12. Bessoff K, Sateriale A, Lee KK, Huston CD. 2013. Drug repurposing screen reveals FDA-approved inhibitors of human HMG-CoA reductase and isoprenoid synthesis that block *Cryptosporidium parvum* growth. *Antimicrob Agents Chemother* 57:1804–1814. <https://doi.org/10.1128/AAC.02460-12>.
 13. Di Santo N, Ehrisman J. 2013. Research perspective: potential role of nitazoxanide in ovarian cancer treatment. Old drug, new purpose? *Cancers (Basel)* 5:1163–1176. <https://doi.org/10.3390/cancers5031163>.
 14. Dang W, Yin Y, Peppelenbosch MP, Pan Q. 2017. Opposing effects of nitazoxanide on murine and human norovirus. *J Infect Dis* 216:780–782. <https://doi.org/10.1093/infdis/jix377>.
 15. Esposito M, Stettler R, Moores SL, Pidathala C, Muller N, Stachulski A, Berry NG, Rossignol JF, Hemphill A. 2005. In vitro efficacies of nitazoxanide and other thiazolides against *Neospora caninum* tachyzoites reveal antiparasitic activity independent of the nitro group. *Antimicrob Agents Chemother* 49:3715–3723. <https://doi.org/10.1128/AAC.49.9.3715-3723.2005>.
 16. Navarrete-Vazquez G, Chavez-Silva F, Colín-Lozano B, Estrada-Soto S, Hidalgo-Figueroa S, Guerrero-Alvarez J, Mendez ST, Reyes-Vivas H, Oriá-Hernandez J, Canul-Canche J, Ortiz-Andrade R, Moo-Puc R. 2015. Synthesis of nitro(benzo)thiazole acetamides and in vitro antiprotozoal effect against amitochondrion parasites *Giardia intestinalis* and *Trichomonas vaginalis*. *Bioorg Med Chem* 23:2204–2210. <https://doi.org/10.1016/j.bmc.2015.02.059>.
 17. Hoffman PS, Bruce AM, Olekhovich I, Warren CA, Burgess SL, Hontecillas R, Viladomiu M, Bassaganya-Riera J, Guerrant RL, Macdonald TL. 2014. Preclinical studies of amoxicillin, a systemic therapeutic developed for treatment of *Clostridium difficile* infections that also shows efficacy against *Helicobacter pylori*. *Antimicrob Agents Chemother* 58:4703–4712. <https://doi.org/10.1128/AAC.03112-14>.
 18. Warren CA, van Opstal E, Ballard TE, Kennedy A, Wang X, Riggins M, Olekhovich I, Warthan M, Kolling GL, Guerrant RL, Macdonald TL, Hoffman PS. 2012. Amoxicillin, a novel inhibitor of pyruvate: ferredoxin oxidoreductase, shows efficacy against *Clostridium difficile* in a mouse infection model. *Antimicrob Agents Chemother* 56:4103–4111. <https://doi.org/10.1128/AAC.00360-12>.
 19. Hutcherson JA, Sinclair KM, Belvin BR, Gui Q, Hoffman PS, Lewis JP. 2017. Amoxicillin, a novel strategy for targeting oral anaerobic pathogens. *Sci Rep* 7:10474. <https://doi.org/10.1038/s41598-017-09616-0>.
 20. Kennedy AJ, Bruce AM, Gineste C, Ballard TE, Olekhovich IN, Macdonald TL, Hoffman PS. 2016. Synthesis and antimicrobial evaluation of amoxicillin-based inhibitors of the pyruvate-ferredoxin oxidoreductases of anaerobic bacteria and Epsilonproteobacteria. *Antimicrob Agents Chemother* 60:3980–3987. <https://doi.org/10.1128/AAC.00670-16>.
 21. Costa LB, Noronha FJ, Roche JK, Sevilleja JE, Warren CA, Oria R, Lima A, Guerrant RL. 2012. Novel in vitro and in vivo models and potential new therapeutics to break the vicious cycle of *Cryptosporidium* infection and malnutrition. *J Infect Dis* 205:1464–1471. <https://doi.org/10.1093/infdis/jis216>.
 22. Bessoff K, Spangenberg T, Foderaro JE, Jumani RS, Ward GE, Huston CD. 2014. Identification of *Cryptosporidium parvum* active chemical series by repurposing the Open Access Malaria Box. *Antimicrob Agents Chemother* 58:2731–2739. <https://doi.org/10.1128/AAC.02641-13>.
 23. Hulverson MA, Vinayak S, Choi R, Schaefer DA, Castellanos-Gonzalez A, Vidadala RSR, Brooks CF, Herbert GT, Betzer DP, Whitman GR, Sparks HN, Arnold SLM, Rivas KL, Barrett LK, White AC, Jr, Maly DJ, Riggs MW, Striepen B, Van Voorhis WC, Ojo KK. 2017. Bumped-kinase inhibitors for *Cryptosporidiosis* therapy. *J Infect Dis* 215:1275–1284. <https://doi.org/10.1093/infdis/jix120>.
 24. Manjunatha UH, Vinayak S, Zambriski JA, Chao AT, Sy T, Noble CG, Bonamy GMC, Kondreddi RR, Zou B, Geddeck P, Brooks CF, Herbert GT, Sateriale A, Tandel J, Noh S, Lakshminarayana SB, Lim SH, Goodman LB, Bodenreider C, Feng G, Zhang L, Blasco F, Wagner J, Leong FJ, Striepen B, Diagona TT. 2017. A *Cryptosporidium* PI(4)K inhibitor is a drug candidate for cryptosporidiosis. *Nature* 546:376–380. <https://doi.org/10.1038/nature22337>.
 25. Mondal D, Minak J, Alam M, Liu Y, Dai J, Korpe P, Liu L, Haque R, Petri WA, Jr. 2012. Contribution of enteric infection, altered intestinal barrier function, and maternal malnutrition to infant malnutrition in Bangladesh. *Clin Infect Dis* 54:185–192. <https://doi.org/10.1093/cid/cir807>.
 26. Brown EM, Wlodarska M, Willing BP, Vonaesch P, Han J, Reynolds LA, Arrieta MC, Uhrig M, Scholz R, Partida O, Borchers CH, Sansonetti PJ, Finlay BB. 2015. Diet and specific microbial exposure trigger features of environmental enteropathy in a novel murine model. *Nat Commun* 6:7806. <https://doi.org/10.1038/ncomms8806>.
 27. Naylor C, Lu M, Haque R, Mondal D, Buonomo E, Nayak U, Mychaleckyj JC, Kirkpatrick B, Colgate R, Carmolli M, Dickson D, van der Klis F, Weldon W, Steven Oberste M, Teams P, Ma JZ, Petri WA, Jr. 2015. Environmental enteropathy, oral vaccine failure and growth faltering in infants in Bangladesh. *EBioMedicine* 2:1759–1766. <https://doi.org/10.1016/j.ebiom.2015.09.036>.
 28. Bartelt LA, Bolick DT, Mayneris-Perxachs J, Kolling GL, Medlock GL, Zaenker EI, Donowitz J, Thomas-Beckett RV, Rogala A, Carroll IM, Singer SM, Papin J, Swann JR, Guerrant RL. 2017. Cross-modulation of pathogen-specific pathways enhances malnutrition during enteric co-infection with *Giardia lamblia* and enteroaggregative *Escherichia coli*. *PLoS Pathog* 13:e1006471. <https://doi.org/10.1371/journal.ppat.1006471>.
 29. Mayneris-Perxachs J, Bolick DT, Leng J, Medlock GL, Kolling GL, Papin JA, Swann JR, Guerrant RL. 2016. Protein- and zinc-deficient diets modulate the murine microbiome and metabolic phenotype. *Am J Clin Nutr* 104:1253–1262. <https://doi.org/10.3945/ajcn.116.131797>.
 30. Bolick DT, Mayneris-Perxachs J, Medlock GL, Kolling GL, Papin JA, Swann JR, Guerrant RL. 2017. Increased urinary trimethylamine N-oxide following *Cryptosporidium* infection and protein malnutrition independent of microbiome effects. *J Infect Dis* 216:64–71. <https://doi.org/10.1093/infdis/jix234>.
 31. Trehan I, Goldbach HS, LaGrone LN, Meuli GJ, Wang RJ, Maleta KM, Manary MJ. 2013. Antibiotics as part of the management of severe acute malnutrition. *N Engl J Med* 368:425–435. <https://doi.org/10.1056/NEJMoa1202851>.
 32. Mayneris-Perxachs J, Lima AA, Guerrant RL, Leite AM, Moura AF, Lima NL, Soares AM, Hant A, Moore SR, Pinkerton R, Swann JR. 2016. Urinary N-methylinicotinamide and beta-aminobutyric acid predict catch-up growth in undernourished Brazilian children. *Sci Rep* 6:19780. <https://doi.org/10.1038/srep19780>.
 33. St Maurice M, Cremades N, Croxen MA, Sisson G, Sancho J, Hoffman PS. 2007. Flavodoxin:quinone reductase (FqrB): a redox partner of pyruvate: ferredoxin oxidoreductase that reversibly couples pyruvate oxidation to NADPH production in *Helicobacter pylori* and *Campylobacter jejuni*. *J Bacteriol* 189:4764–4773. <https://doi.org/10.1128/JB.00287-07>.