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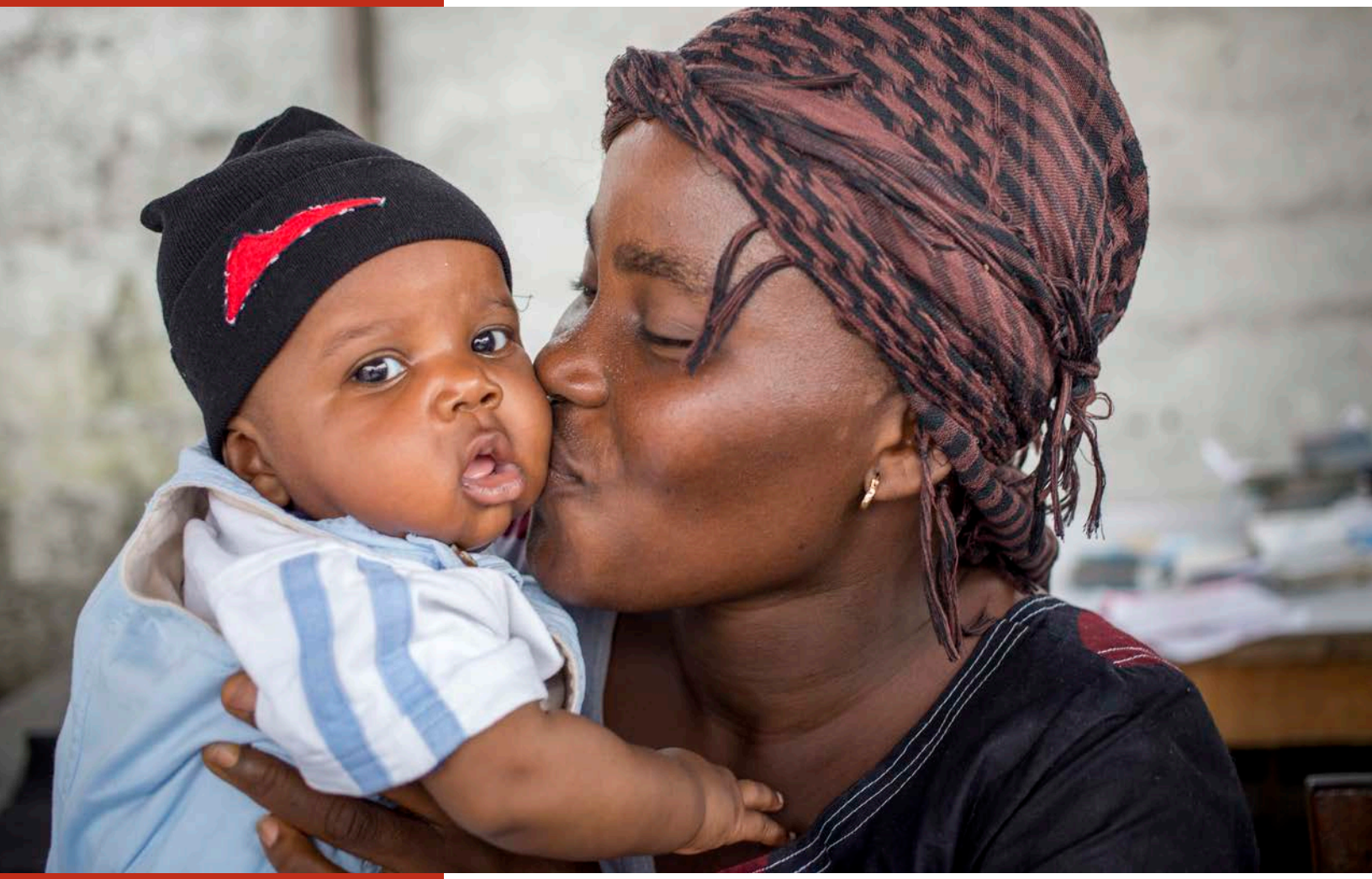

2ND SYMPOSIUM ON

Innovative Therapeutics for *Cryptosporidium*

March 7, 2018
Seattle, Washington, United States
Meeting report

June 2018

WHY *CRYPTOSPORIDIUM*? WHY NOW?

"The Global Enteric Multicenter Study findings on *Cryptosporidium* were a revelation. It was discovered that the parasite was a leading cause of moderate-to-severe diarrhea in infants and one of the deadliest diarrhea pathogens among toddlers. Still, what we do not know about *Cryptosporidium* is much more than what we know."

–Ibrahim Khalil, Institute for Health Metrics and Evaluation, University of Washington

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Why *Cryptosporidium*? Why Now?

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Cryptosporidium was first described in humans in 1976 and presumed to be a disease most commonly afflicting immunocompromised populations and those affected by outbreaks. However, recently conducted large diarrhea etiology studies have highlighted *Cryptosporidium*'s worldwide importance as a leading cause of childhood diarrhea.

In low-resource settings, where access to clean water, proper hygiene, and sanitation are often limited, children are routinely exposed to harmful enteric pathogens. These conditions make the *Cryptosporidium* parasite one of the leading causes of diarrheal disease among young children. Infection can lead to death and, even in the absence of diarrhea, can leave survivors suffering from chronic malnutrition and long-term consequences, including growth stunting and deficits in cognitive development. *Cryptosporidium* is also believed to be a leading contributor to environmental enteric dysfunction, a condition characterized by structural gut damage as a result of repeated enteric infections in childhood.

Despite the incredibly high burden, and established short- and long-term consequences, only one therapeutic is available, with suboptimal efficacy in immunocompromised populations, such as those affected by HIV and malnutrition. With no highly effective treatment currently available, millions of children remain at risk. Funding for research and development of needed drugs has lagged behind.

With increased investment and collaboration, we can accelerate progress toward controlling *Cryptosporidium*—bringing better health within reach for vulnerable children worldwide.

PRESENTATIONS

Keynote

Gagandeep Kang

ENTERIC INFECTIONS IN LOW- AND MIDDLE-INCOME COUNTRIES: BURDEN AND IMPACT

In her opening remarks, **Gagandeep Kang, Christian Medical College, Vellore**, made the connection between enteric infections and lost human potential around the world. She stated, "The epidemiology and burden of acute enteric infectious disease is changing, but is still responsible for major morbidity." She also made clear the potential for research and development, remarking, "New tools permit better understanding of infection, disease, and long-term consequences." Acknowledging the difficulties of recruiting for etiology-specific interventions, Kang said, "It's challenging, but it can be done."

Enabling Tools

Boris Striepen, University of Pennsylvania, opened the Enabling Tools session with a presentation on the biology of *Cryptosporidium*. He emphasized the lack of effective drugs and vaccines and pointed to unique roadblocks preventing meaningful study of the parasite, including its overall poor tractability, saying, "For too long, we have lacked the tools to effectively dissect this deadly parasite's complex biology." To overcome this hurdle, Striepen and team have established powerful genetic tools that have helped enhance understanding of the mechanistic underpinnings of drug sensitivity and resistance in *Cryptosporidium*. Additionally, Striepen's team is at work developing different genetic strategies aimed at better understanding *Cryptosporidium's* complex life cycle and the relationship between parasite, host, and commensal organisms as they shape susceptibility, disease, and protection. He closed by saying, "Compared to five years ago, we are now much better positioned to identify and validate promising targets for new drug candidates."

Alejandro Castellanos-Gonzalez, University of Texas Medical Branch, continued the session with a presentation on gene silencing in *Cryptosporidium* as a novel approach to identify drug targets. He highlighted the limitation of tools to genetically manipulate parasite gene expression as a major hurdle to drug development, but added, "In the last few years, considerable progress has been made on novel methods to study genes of *Cryptosporidium*." Using a rapid strategy to silence genes in *Cryptosporidium*, Castellanos-Gonzalez and his team have been able to evaluate the role of multiple genes during *Cryptosporidium* infection and identified several proteins whose silencing significantly reduced parasite burden after infection. Looking ahead, Castellanos-Gonzalez said, "Our group has identified several druggable targets—therefore our short-term goal will be to evaluate gene silencing of these targets to determine which is the best candidate for drug development."

Concluding the Enabling Tools session, **Christopher Huston, University of Vermont Larner College of Medicine**, presented his team's work, in partnership with PATH, to develop a time-kill assay and life-stage-specific assays for better identification of compounds with potential for high in vivo efficacy. Huston pointed out the shortcomings of the nascent anti-*Cryptosporidium* drug pipeline, saying, "Because most of the compounds have been identified by phenotypic screening, their molecular mechanisms of action are mostly unknown. Therefore, there is no way to ensure mechanistic diversity within the pipeline." He also emphasized the desirability of maintaining a pipeline of early-stage compounds with diverse mechanisms of actions. Huston and his colleagues hypothesize that cidal compounds may be preferable for treatment of immunocompromised individuals and that the mechanism of action will correlate with the life stage at which compounds are active. He noted, "These phenotypic assays provide a means to maintain a pipeline of mechanistically diverse compounds."


Christopher Huston

ENABLING TOOLS TAKEAWAYS

1. Innovative research is opening promising new avenues for development of genetic tools that can accelerate the development of *Cryptosporidium* therapeutics.
2. Standardized assays have been developed that can be used to benchmark different compounds and facilitate logical prioritization and maintenance of portfolio diversity.

Drug Discovery

A packed room for the biannual symposium held at PATH's headquarters in Seattle, Washington.

The Drug Discovery session started with **Melissa Love, California Institute for Biomedical Research (Calibr)**, covering Calibr's high-throughput screening campaign to identify potent inhibitors of *Cryptosporidium parvum*. Describing the process, Love said, "Calibr's state-of-the-art facilities and automation capabilities have allowed us to screen more than 1.2 million small-molecule compounds to date. We hope this large collection of molecules screened will generate new chemical space for the entire *Cryptosporidium* drug discovery field." Currently, two chemical series—aminothiazole and azaquinoline scaffolds—represent novel lead compounds undergoing medicinal chemistry optimization to improve efficacy and address liabilities to achieve the criteria set forth by the target product profile. "These series represent the first novel efficacious series to come out of the high-throughput screen, and as we continue to refine each series, we hope to identify a preclinical candidate drug with a unique mechanism of action," said Love. Additional lead compounds have also been identified in the ReFRAME library, an annotated collection of small molecules comprising clinical-stage and approved compounds to accelerate drug discovery in neglected tropical diseases.

Erkang Fan, University of Washington (UW), led the session next with a presentation on the development of methionyl-tRNA synthetase (MetRS) inhibitors for treating cryptosporidiosis, a collaboration between UW, PATH, and Takeda Pharmaceutical Company Limited. He noted, "In principle, all tRNA synthetases are potential drug targets for developing therapeutics against cryptosporidiosis, as long as selective inhibition of parasite enzymes can be achieved over host enzymes." Building on success with discovery of potent and selective inhibitors of MetRS from *Trypanosoma brucei* that benefited from multiple inhibitor-bound MetRS crystal structures, Fan and team expanded inhibitor design efforts to cover *Cryptosporidium* MetRS. Fan also discussed the factors that likely affect in vivo efficacies of MetRS inhibitors, stating, "Based on current data, it remains unclear at this stage what conventional ADME/PK [absorption, distribution, metabolism, and

excretion/pharmacokinetic] parameters to optimize for MetRS inhibitors in order to achieve good efficacy in animal models. Hopefully the upcoming detailed study of the pharmacokinetic/pharmacodynamic relationship of MetRS inhibitors can shed more light into this subject."

Wesley C. Van Voorhis, UW, concluded the Drug Discovery session by summarizing the effort to advance bumped-kinase inhibitors (BKIs) for the therapy of cryptosporidiosis in partnership with PATH as "steady progress toward the safest possible drug." Potent BKIs, targeting small gatekeeper protein kinases such as CpCDPK1, inhibit the invasion and growth of *Cryptosporidium (C.) parvum* and C. *hominis*. Van Voorhis' team has successfully advanced a late lead compound and several promising backups that are effective in vivo and have the desired safety profile to meet the target product profile for the treatment of children ages 6 to 18 months. Van Voorhis said their lead compound, BKI-1770, has "moderate to low oral bioavailability, efficacy in the mouse model, and no cardiovascular, gastrointestinal, or other toxic signals." They are awaiting compound scale-up for the calf *C. parvum* model and a dog cardiovascular safety study.

Wesley Van Voorhis asks a question of another presenter after giving his own presentation on bumped-kinase inhibitors to treat cryptosporidiosis.

DRUG DISCOVERY TAKEAWAYS

1. The *Cryptosporidium* drug discovery pipeline has rapidly filled out over the past several years with late-stage repurposed molecules from existing neglected tropical disease programs, as well as early-stage exploratory molecules from high-throughput screening.
2. The collaborative nature of the *Cryptosporidium* drug discovery field has contributed to the pace at which progress has been made in finding a preclinical candidate drug.
3. Given the high attrition of preclinical leads, continued investigation of new series for anti-*Cryptosporidium* activity is still needed.
4. More pharmacokinetic/pharmacodynamic study resources are needed to help support lead optimization efforts.

Burden and Epidemiology

Ibrahim Abdel-Messih Khalil

Opening the Burden and Epidemiology session, **Ibrahim Abdel-Messih Khalil, Institute for Health Metrics and Evaluation (IHME), UW**, gave an overview of *Cryptosporidium* burden in the Global Burden of Disease Study (GBD). While the burden of acute infections was substantial, with *Cryptosporidium* responsible for 48,000 deaths, Khalil stressed that the long-term consequences represented an even larger impact, stating, "After accounting for undernutrition-associated disability-adjusted life years (DALYs), *Cryptosporidium* was responsible for nearly 250 DALYs per 1,000 child-years among children under 1 year old in sub-Saharan Africa, the super-region with the highest burden. The majority of this burden is from the long-term outcomes associated with undernutrition. Undernutrition-associated DALYs were responsible for between 61 and 94% of all *Cryptosporidium* DALYs." Based on a meta-analysis of the impact of *Cryptosporidium* on physical growth, IHME estimates that *Cryptosporidium* is responsible for an additional 7.85 million DALYs. Regarding the importance of the IHME study, Khalil said, "Based on these burden findings, interventions designed to prevent and effectively treat infection among children under 5 will have enormous public health and social development impacts."

Poonum Korpe

Continuing the conversation, **Poonum Korpe, Johns Hopkins Bloomberg School of Public Health**, presented the results of a pilot case study of families in an urban and rural community in Bangladesh. Describing the goals of the study, Korpe said, "We set out to understand the burden of person-to-person spread of cryptosporidiosis in young children in Bangladesh." Among 48 enrolled case families, the study found high rates of secondary infection in both urban and rural settings, though the secondary attack rate was significantly higher in the 24 urban Mirpur households at 30%. Molecular genotyping of *Cryptosporidium* isolates revealed circulation of the same subspecies within families. "Person-to-person transmission is likely a major source of *Cryptosporidium* infection for children living in Mirpur, Bangladesh," Korpe stated, "and interventions aimed at interrupting anthroponotic spread of *Cryptosporidium* in children are needed to control transmission of this deleterious parasite."

The session concluded with a presentation by **Patricia Pavlinac, UW**, on caregiver and child *Cryptosporidium* infections in Western Kenya. "Given infants and toddlers spend most of their time with their caregivers, evaluating *Cryptosporidium* in this population may reveal an important source of new or recurrent *Cryptosporidium* infections in children," Pavlinac stated. Among 212 child-caregiver pairs, 33% of children and 23% of caregivers had a *Cryptosporidium* infection detected by polymerase chain reaction. Children with a *Cryptosporidium*-infected caregiver had a 1.7-fold higher prevalence of *Cryptosporidium* infection, even after adjusting for other transmission sources. No such relationship was observed for *Giardia* infection, which was found in 54% of children and 14% of caregivers. Although HIV is an established risk factor for *Cryptosporidium* acquisition, Pavlinac's team found no association between caregiver HIV and caregiver *Cryptosporidium* infection; however, children of HIV-infected caregivers, despite being HIV-uninfected themselves, were nearly two times more likely to have *Cryptosporidium*. Pavlinac closed by saying, "There appears to be a unique relationship between caregiver and child *Cryptosporidium* infection that is unexplained by shared transmission sources. For child-directed interventions to have a sustained impact, caregiver *Cryptosporidium* infection may also need to be addressed."

BURDEN AND EPIDEMIOLOGY TAKEAWAYS

1. In-depth analysis suggests that the previously reported burden of *Cryptosporidium* is underestimated, and that *Cryptosporidium* infection impacts childhood health beyond acute illness.
2. Further analysis of a proposed causal relationship between *Cryptosporidium* and childhood growth identified additional burden associated with non-fatal infection due to poor growth outcomes and increased risk of subsequent infections.
3. Consideration of transmission pathways of cryptosporidiosis will be necessary to prevent infection in young children.
4. Therapeutic targeting of children alone may have limited efficacy in light of high person-to-person spread of *Cryptosporidium*.

Translational Medicine, Clinical Development, and Beyond

Jennifer Zambriski

Jennifer Zambriski, Washington State University, kicked off the Translational Medicine, Clinical Development, and Beyond session with a presentation on refining the calf model of cryptosporidiosis to improve data integrity, quality, and translation. "A major challenge in animal models is generating data that is representative, reproducible, and can be translated to the appropriate populations,"

said Zambriski. Her team has identified four priority areas for model refinement: housing system, magnitude of the challenge inoculum, evaluation of dehydration, and measurement of gastrointestinal transit time. Zambriski reviewed recent advancements in these areas and also presented planned research to evaluate new approaches for executing the calf model, including bioelectrical impedance spectroscopy and the use of Smart Pill technology. She closed by saying, "If we continue to employ outdated approaches simply because 'that's the way we've always done it,' there is a grave risk that we will fail to identify a treatment effect where one truly does exist, and that would be a catastrophic loss."

The session continued with **Pui-Ying Iroh Tam, Malawi-Liverpool Wellcome Trust Clinical Research Programme**, reviewing an ongoing clinical trial of clofazimine for *Cryptosporidium* diarrhea in Malawi, including the trial design, objectives, endpoints, and current status. Because clofazimine is on the World Health Organization's Essental Medicines List for treatment of leprosy and was recently discovered to be effective against *Cryptosporidium* in a mouse model, it represents an excellent repurposing opportunity. She also highlighted the difficulties of conducting a clinical trial in a low-income setting, calling the work "both extremely challenging and yet highly rewarding."

Paul Kelly, Barts and The London School of Medicine, United Kingdom, closed the session with a presentation on the links between cryptosporidiosis and enteropathy. He remarked that, "Rates of *Cryptosporidium* infection in Africa have remained virtually unchanged since the AIDS epidemic, but its role in environmental enteropathy in children is only now being evaluated." Kelly continued, "Only one drug, nitazoxanide, is currently licensed to treat cryptosporidiosis, and its efficacy in HIV-negative children with diarrhea and malnutrition is only 50%. In HIV-positive children, it has no effect." He noted that with no vaccine available, the most effective preventative measures continue to be filtration or boiling of drinking water. However, development of new treatments could prove to be a gamechanger for the most vulnerable children by enabling the deconvolution of the complex etiology of enteropathy caused by polymicrobial pathogenesis.

TRANSLATIONAL MEDICINE, CLINICAL DEVELOPMENT, AND BEYOND TAKEAWAYS

1. Nitazoxanide remains the only licensed drug for cryptosporidiosis, but it has only modest efficacy at best.
2. Until we have a more efficacious therapy, we cannot dissect out cause and effect on enteropathy, growth failure, or neurocognitive development.
3. It remains unclear how much systemic exposure would be desirable in a novel drug, or if luminal activity is enough to cure the infection.
4. When using animal models of cryptosporidiosis at advanced stages of drug development, particularly in larger vertebrates, emphasis should be on attaining comparable outcomes across species, not on employing comparable methods of data collection. An approach that is appropriate for one species may confound data outcomes in another.

PANEL DISCUSSION

The symposium closed with a lively panel discussion with Dale Robinson, Ujjini Manjunatha, Wesley Van Voorhis, Karen Kotloff, and Stephen Ward.

Cryptosporidium R&D: Opportunities, challenges, and where do we go from here?

The meeting concluded with a panel of experts from the public, private, and nonprofit sectors to discuss opportunities for cross-sector partnerships and provide perspective on future directions in *Cryptosporidium* drug development. The panelists included Karen L. Kotloff, University of Maryland School of Medicine; Ujjini H. Manjunatha, Novartis Institute for Tropical Diseases; Dale Robinson, Celgene Global Health; and Stephen Ward, Bill & Melinda Gates Foundation. Wesley Van Voorhis served as the panel moderator. Key discussion points included the role of the private sector in drug discovery, new incentives to catalyze R&D, the potential role of mass drug administration for *Cryptosporidium* treatment, and lessons learned from similar public-private partnerships.

PANEL DISCUSSION TAKEAWAYS

1. Discovery of therapeutics should proceed contemporaneously with development of processes by which drugs would be implemented. Guidelines informing the clinical criteria for treatment and whether diagnostics will play a role will be required. Concurrent development of rapid, sensitive point-of-care testing could help in defining which children should be treated with anti-*Cryptosporidium* drugs.
2. Whether or not resistance will be a significant concern remains to be seen, but should be considered when developing drugs that may be used in combination.

continued

3. As the first new drug candidates move into the clinic, those studies should be positioned to establish the clinical development path for anti-*Cryptosporidium* drugs more broadly.
4. Additional data are needed to determine the value of including asymptomatic children in the target population.
5. Considerations in choosing mass drug administration (MDA) for control of cryptosporidiosis include:
   - **a.** Ease of treatment: a single-dose drug is optimal.
   - **b.** Safety: few side effects and interactions with other drugs.
   - **c.** Low potential for inducing resistance to other important antibiotics.
   - **d.** Enhanced understanding of reservoir and modes of transmission to predict the efficacy and duration of impact.
   - **e.** What are feasible short-term and long-term endpoints of MDA and how will they be measured?
6. Global health issues with neglected tropical diseases like cryptosporidiosis cannot be solved by one organization alone. Private companies, governments, nongovernmental organizations, academia, and other stakeholders need to work together to create sustainable solutions.
7. Novartis is committed to the fight against cryptosporidiosis, improving access to treatment, and helping communities deliver better health care.
8. Celgene is committed to identifying novel treatments for cryptosporidiosis by providing high-quality chemical matter for screening and research and development expertise for optimizing drug properties.
9. Adding cryptosporidiosis to the list of qualifying tropical diseases may help to incentivize development of new therapies to address unmet medical need.

Part 4

ACKNOWLEDGMENTS AND TAKE ACTION

Acknowledgments

PATH would like to thank the UK Department for International Development and the Silicon Valley Community Foundation for their support of the Symposium on Innovative Therapeutics for *Cryptosporidium*. Thanks also to speakers and attendees for the valuable information, resources, and insights shared throughout the event.

2nd Symposium on Innovative Therapeutics for *Cryptosporidium* speakers: Front row: Melissa Love, Pui-Ying Iroh Tam, Erkang Fan, Alejandro Castellanos-Gonzalez, Gagandeep Kang, Paul Kelly, Karen L. Kotloff, Ujjini H. Manjunatha, Jennifer Zambriski, Megan Cihak. Back row: Patricia Pavlinac, Poonum Korpe, Christopher Huston, Boris Striepen, Stephen Ward, Wesley C. Van Voorhis, Dale Robinson, Ibrahim Abdel-Messih Khalil.

Take Action

If you would like to learn more about PATH's work in drug research and development for diarrheal diseases, or collaborate on *Cryptosporidium*-related work, please reach out to drugdev@path.org.

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PATH is the leader in global health innovation. An international nonprofit organization, we save lives and improve health, especially among women and children. We accelerate innovation across five platforms—vaccines, drugs, diagnostics, devices, and system and service innovations—that harness our entrepreneurial insight, scientific and public health expertise, and passion for health equity. By mobilizing partners around the world, we take innovation to scale, working alongside countries primarily in Africa and Asia to tackle their greatest health needs. Together, we deliver measurable results that disrupt the cycle of poor health.

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