TECHLAB®

DIARRHEA DIGEST

DIARRHEA DIGEST is an irregular publication of TECHLAB® dedicated to the etiology, diagnosis, and therapy of diarrheal diseases and related aspects of intestinal ecology

Fall 2010

The *DIARRHEA DIGEST* is now green. Just like previous paper issues, the green version will be an irregular publication and it will be available on our website. The green version may not be as easy to take to the bathroom, but by saving trees, the green version will help make sure that you don't run out of toilet paper.

ADVANCEMENTS IN GLOBAL ENTERIC HEALTH

There are approximately 2 billion cases of diarrheal disease worldwide each year. It is the third leading cause of death in developing countries, responsible for 6.9% of total deaths. In children less than five years of age, diarrheal disease is the second leading cause of mortality, killing 1.5 million children every year. Acute and chronic diarrheal illnesses also increase the risk of co-infection, malnutrition, cognitive loss and impaired immunity.

Funding efforts targeting global enteric health have increased in recent years, in part to support achievement of the eight Millennium Development Goals (MDG). The MDGs were established by the United Nations and 189 World leaders in 2000 as part of a joint effort to end poverty by 2015. "MDG #4 – Child Health" was implemented to reduce by 2/3 the under 5 mortality rate. "MDG #6 – Infectious Diseases" was implemented to reverse the impact of infectious diseases. Both support the study and intervention of diarrheal diseases worldwide.

Support for projects addressing the burden of diarrheal diseases has come from a variety of sources, with a leadership role being played by the Bill and Melinda Gates Foundation. Importantly, projects funded by the Foundation are not blindly applied intervention strategies. Instead they support rigorously designed descriptive studies that accurately portray the impact of diarrheal disease and its causative agents based on geographic region and community structure. Study sites are chosen for scientific and clinical excellence, but also in a manner to include multiple continents, urban and rural areas, and populations with high and low rates of HIV and other diseases impacting co-morbidity. The goal is to utilize information from these studies to guide the efficacious development and implementation of diagnostics, vaccines and therapeutics in a region-specific manner. Unit cost is always a determining factor in global health intervention strategies, but the impact can be lessened if targeted policies are developed on a region-by-region basis.

Two important projects describing the burden and impact of diarrheal diseases in the developing world are described below. Both are actively being conducted with support from the Bill and Melinda Gates Foundation.

World Health Organization, Fact Sheet N°330, August 2009.

http://www.globalhealth.org/infectious_diseases/

http://www.un.org/millenniumgoals/

http://www.gatesfoundation.org/topics/Pages/diarrhea.aspx

Global Enteric Multi-Center Study

The "Global Enteric Multi-Center Study", or GEMS, utilizes a case-control study design nested within demographically-defined populations to describe the burden and etiology of moderate to serve diarrhea in children from 0-59 month age at seven sites in Africa and Asia. GEMS combines a thorough clinical and population-based analysis of disease with rigorous microbiological analysis of the diarrheal cases and their matched controls. The long-term goal of the project is to efficiently and effectively guide public health interventions in the developing world that will reduce morbidity and mortality due to enteric disease.

Study Design. Together, the seven GEMS field sites in Africa and Asia represent developing countries with moderate to high infant mortality rates, urban/rural settings, and high/low HIV or malaria regions. Each site will enroll up to 660 children with moderate to severe diarrhea and 660 matched community controls in each of three age strata: 0-11 months, 12-23 months and 24-59 months. Enrollees provide epidemiological data, healthcare utilization summaries, a fecal specimen for microbiological analysis, and 60-day follow-up information. The prevalence of bacterial, viral and protozoal pathogens is compared in cases and controls, forming a pathogenicity index for the panel of screened organisms. Final microbiology and epidemiology data will be combined to provide a health-related burden of disease for each organism and the estimated healthcare costs incurred.

Collaborating Institutions. All GEMS study activities are coordinated by the lead institution – The Center for Vaccine Development (CVD) at the University of Maryland School of Medicine – and its faculty core of Dr. Myron M. Levine (Coordinating Investigator), Dr. Karen L. Kotloff (Primary Investigator Epidemiology and Clinical), and Dr. James P. Nataro (Primary Investigator Microbiology). CVD collaborates directly with the seven field sites to conduct testing; maintaining uniformity between sites with formal standard operating procedures and quality control oversight. The GEMS Field Sites:

- Center for Vaccine Development – Bamako, Mali
- Medical Research Council – Basse, The Gambia
- Manicha Health Research Center – Manhiça, Mozambique
- Kenya Medical Research Institute – Kisumu, Kenya
- National Institute for Cholera and Enteric Diseases – Kolkata, India
- International Centre for Diarrhoeal Disease Research – Dhaka, Bangladesh
- Aga Khan University – Karachi, Pakistan

Several support institutions provide critical consultation related to microbiology, data review, and downstream application of project results. They include the CDC – Foodborne and Diarrheal Disease Branch, the University of Chile, the University of Virginia School of Medicine, Goteborg University, the International Vaccine Institute and the Cooperative Studies Program Coordinating Center in Perry Point, MD. The supporting institutions meet annually with the lead institution, field sites, individual consulting scientists and representatives of the Bill and Melinda Gates Foundation to review progress and coordinate study activities.

Additional details regarding the GEMS project and its collaborative institutions can be found at the University of Maryland School of Medicine website – http://medschool.umaryland.edu/GEMS/. The GEMS research team organizes a symposium each year at the American Society of Tropical Medicine and Hygiene Annual Meeting, encouraging attendance from the global health, tropical medicine, microbiology, and vaccinology communities. Final study results will be available in the published literature by searching the coordinating investigators.

Interactions of Malnutrition and Enteric Infections

The "Interactions of Malnutrition and Enteric Infections: Consequences for Child Health and Development" project, or MAL-ED (pronounced "*mal a dee*"), is describing the complex relationship between malnutrition and enteric diseases and their impact on childhood morbidity and mortality. The study is utilizing longitudinal birth cohorts and case-control analysis to address two main areas. First, is the impact of specific entero-pathogens on intestinal inflammation and absorption; both leading to malnutrition. Second, is the combination of enteric infections and malnutrition negatively impacting growth, cognitive enhancement, and immunity. Reduced immunity may increase susceptibility to additional infections and decrease a child's ability to benefit from vaccines. Final study results will be utilized to develop targeted intervention strategies based on location ad child condition.

Study Design. Eight MAL-ED field sites in Africa, Asia and South America are conducting longitudinal birth cohorts of 200 children; children being enrolled during a two year period and followed for up to three years. Two of the sites are also conducting a case-control study of 500 children; cases being defined as moderately to severely malnourished (weight to age Z score of < -2). Criteria being evaluated are gut functional capacity, enteric infection assessment, growth and development, vaccine response and other infection assessment. Additional projects will 1) correlate human genetic susceptibility to disease and malnutrition and, 2) examine the gut microbiome as an agent of or responsive to disease and malnutrition.

Collaborating Institutions. The MAL-ED primary grantee is the Foundation for the National Institutes of Health. Administrative, scientific and financial oversight for the project is coordinated by Dr. Michael Gottlieb of FNIH. Dr. Mark Miller of Fogarty Center at NIH provides co-PI support with input on study design, protocol synchronization, quality control, and data management. Both lead organizations coordinate activities at the eight field sites. The MAL-ED field sites:

- Federal University of Ceara – Fortaleza, Brazil
- Johns Hopkins School of Public Health Satellite Laboratory – Loreto, Peru
- University of Venda – Limpopo, South Africa
- Haydom Lutheran Hospital – Haydom, Tanzania
- Aga Khan University – Karachi, Pakistan
- Christian Medical College – Vellore, India
- Walter Reed/AFRIMS Research Unit – Kathmandu, Nepal
- International Centre for Diarrhoeal Disease Research – Dhaka, Bangladesh

Several support institutions provide critical consultation related to microbiology, data review, and downstream application of project results. They include the Johns Hopkins School of Public Health, the University of Virginia, the Armed Forces Institute of Medical Sciences, The Center for Genome Sciences at the Washington University School of Medicine, and the University of Colorado. The supporting institutions meet annually with the lead institutions, field sites, individual consulting scientists and representatives of the Bill and Melinda Gates Foundation to review progress and coordinate study activities.

Additional details regarding the MAL-ED project and its collaborative institutions can be found at the FNIH website: http://mal-ed.fnih.org. The MAL-ED research team organizes a symposium each year at the American Society of Tropical Medicine and Hygiene Annual Meeting, encouraging attendance from the global health, tropical medicine, microbiology, and vaccinology communities. Final study results will be available in the published literature by searching the coordinating investigators.

STOOL NOTES

AN AVERAGE SPERM WHALE weighs about 100 tons and defecates up to about 3% of its body weight each day. In human terms, that means a 150 lb person would "shed" about 4.5 lbs each day by frequenting the toilet. Perhaps it's doable,

but it would mean a lot of fiber and heavy metals. With humans, when you have "loose" or liquid stools, it's usually an indication of a problem unless you're prepping for a colonoscopy. But with whales, it's all liquid. It can be different colors, a little bit of brown or red, but it's very loose --- even before it hits the water. Sperm whales will dive and eat other sea-dwelling creatures such as squid at lower ocean depths but then they come to the surface and defecate. If you happen to be whale watching and you see a brownish cloud in the water, then you're probably getting more than you're money's worth since you are also seeing the poop. The poop actually serves as a nutrient, allowing phytoplankton to grow to high concentrations. Plus, there often is quite a bit of iron in the "cloud" so the animal is doing a "whale-of-a-job" by recycling nutrients for others to utilize.

A GIANT FLOATING PIECE OF FECAL DEBRIS was reported in a town in Switzerland a while back. When we say giant, we mean giant --- the size of a house --- you could live in it if you really wanted to. Actually the debris was part of an art exhibit, appropriately called "Complex Sh__" and when the wind picked up, the debris was blown away from the art museum and actually broke a window of a children's center down the street. Wonder how the teachers explained that to the kids.

SLOTHS ARE VERY UNUSUAL CREATURES that live in the trees, move very slowly, and hardly ever come down to ground level unless there is something to make it worth their effort. Usually, they are contented to stay up high and eat plants. Now some researchers have recently identified something that makes the sloth want to climb down to the ground --- human toilets. Apparently, at night, the sloths have been observed to climb down the tree, make their way to the toilet, and use one hand to scoop out manure and eat it. It's a highly unusual practice for a sloth --- or for that matter, any other animal. The researchers are curious as to the type of nutrients the sloths get from this type of feeding. The problem now though, is whether certain human diseases may actually be spread to the sloths.

WHILE ATTENDING THE 2010 ANAEROBE SOCIETY OF AMERICAS CONFERENCE in Philadelphia, one of our scientists had the opportunity to tour the Mutter Museum. Although, there were many interesting specimens, including various parts and conditions of the human body, one of the most interesting and unbelievable specimens on display was the World's largest colon. This colon was so big that at first glance you wouldn't even begin

to guess it was once someone's colon. It looked more like a giant earthworm or like one of the giant worms out of the science fiction movie *Dune*. Approximately 7 feet in length, the organ weighed 47 lbs at the time of extraction. The colon had been removed from a man who died at the age of 29 due to complications of his oversized colon and Hirschsprungs disease.

WANT TO SEE WHAT YOUR TOILET might look like in a few years? Would you be interested in a toilet that can give you some ideas on how to improve your health based on your stool specimen? To check this and other nifty health-related gadgets at a recent health fair in Japan, visit http://news.bbc.co.uk/2/hi/business/9095261.stm.

DOES YOUR LOVED ONE (your pet, not your spouse) drop surprises on the floor, on mats, on carpet? You can buy Poop Freeze Aerosol Freeze Spray to freeze it and pick it up much more easily. The only catch --- you have to freeze both sides in order to pick it up more easily.

WHEN YOU BUY NEW CLOTHES, do you always assume they are new? In a recent study, "new" clothes that were on the rack included clothes that had been returned, and presumably had not been worn. And guess what? Many of the items were contaminated with bacteria --- respiratory, skin, and yes, fecal. Just what do people do with these clothes? Advice from clothes experts? You probably won't get sick by wearing these clothes, but why take chances. Go ahead and launder the garment after you buy it and before you wear it.

HMP- HEALTHY MICROBIOTA PLEASE

The Human Microbiome Project (HMP), launched in 2007, was developed by the National Institutes of Health to characterize the association of the human microbiota in health and disease. The results of this ambitious $157 million project will be far-reaching once we can begin to understand and put together the enormous amounts of data that are being generated. The project covers our microbiota at various body sites and cavities --- nasal, oral, skin, urogenital, and intestinal. Along the way, new technology will be established and/or invented to help understand what the data means.

Looking at the intestine, there are estimated to be more than a million different genes within our microbiota --- many more than the 25,000 genes we carry in our human cells. Within this "second" genome set that live in our intestines, organisms have evolved to live in very distinctive environments. So much so that most of these organisms are incapable of living outside the intestine. That is why we have only a superficial understanding of what organisms are present --- we can only grow a small fraction of these highly specialized organisms. New metagenomic technology will speed up our ability to identify new species but again, the amount of data generated will be tremendous. Trying to understand how these organisms live in specialized parts of the intestine and how they interact to protect us will be like putting together a jigsaw that consists of millions of pieces. But in the long run, it will prove beneficial to human health.

Because of TechLab's interest in *C. difficile* associated disease, we have a special interest in the HMP results on how our flora interact with each other and with our own human cells to protect us, how the flora is altered when we take antibiotics, and how it begins to be reestablished. We know that most cases of *C. difficile* arise when the normal flora is altered by antibiotics. There are other ways to alter the flora, but antibiotics do a superb job of killing the billions of diverse organisms that have evolved with us and protect us from this toxin-

producing bacterium. *Clostridium difficile* patients are treated with vancomycin or metronidazole. Unfortunately, this approach does not always work because it can leave the intestine vulnerable to infection since these antibiotics also kill off our intestinal flora. *C. difficile* is ahead of us in the game and already has been evolving ways to resist antibiotics. The outbreak strain and other ribotypes have developed resistance against fluoroquinolones by a simple missense mutation in the gyrase gene. And although *C. difficile* doesn't seem to be resistant to vancomycin and metronidazole yet, we know that some other clostridia --- healthy intestinal clostridia --- are resistant against vancomycin. A little promiscuity in the intestine --- and it happens all the time through the social networking that takes place in our gut --- and *C. difficile* strains may begin to appear that are resistant to vancomycin.

So is there a better way to treat this disease? Perhaps we should step back and see what nature has done in the first place --- provide us with a huge diversity of microbes that under healthy conditions that cover all intestinal surfaces and efficiently lap up nutrients so that *C. difficile* can't grow (there likely are other antagonistic mechanisms but how they may work isn't clear). There doesn't appear to be one particular strain that can act as a complete magic bullet for this disease and provide complete protection, but we do know that a combination of certain microbes is protective. And we can make the protection even more effective if we use a fecal enema. Fecal enema, bacteriotherapy, whatever it's called, it doesn't conjure up pleasant thoughts and that is why everyone goes "yuck" when they hear about this approach. But the bottom line (no pun intended) is that a fecal emulsion does a heck of a job replenishing our intestinal stocks and effectively treating *C. difficile* disease. It even works against relapses, which can be very challenging to treat and can go on for years.

At this stage, we don't really understand the mechanism for protection --- which microbes are best, where they really live in the intestine, what companions (i.e., other microbes) they need to survive, what functions they exert, etc. This is where the HMP will be especially exciting. It should be able to tell us what microbes are killed by treatment and what are replaced effectively by fecal enemas. This will allow us to more accurately determine the microbes on which to focus. And down the road, we should be able to develop a more defined and refined microbial mixture that can be used not only as a prophylactic but also a therapeutic. But the map is being drawn as we drive this road, and where it leads remains to be seen.

FECAL TRANSFERS- HOW EFFECTIVE?

A recent article from *The People's Pharmacy* had the right title when they were talking about *C. difficile* disease and the relapses that can and often do occur following vancomycin and metronidazole treatment … "When the cure is worse than the disease". The treatment usually works but it can be only a temporary solution and the patient may suffer through a number of relapses after treatment while hoping to eventually get on the road to recovery.

How well do fecal transplants work as an alternative treatment? Last year, there was a report by M. E. Tucker describing a home-based method developed by Dr. Thomas J. Louie that worked well in recurrent *C. difficile* disease involving patients with 4 episodes over 6 months or longer. The work was reported at the Interscience Conference on Antimicrobial Agents and Chemotherapy and the Infectious Diseases Society of America. The patients were treated with vancomycin for 14 days or longer to control the symptoms, and this was followed by a 4-day washout period or oral enema 12 hours before the procedure to remove vancomycin. Donors were healthy, had no antibiotic exposure in the preceding 6 months, were negative for the same pathogens that have to be negative for blood donors, and most donors were genetic relatives of the patient.

Donors collected stools for 3 days in pails, stored their specimens at 4-8ºC, with the final sample collected on the morning of the procedure. Stools were mixed with PBS, cysteine (a reducing agent to help protect the bacteria), and then the mixture was sieved

through a wire mesh. The preparation (800 – 1,400 mL) was administered rectally through a balloon catheter over 10 – 45 minutes. After treatment, patients were allowed to eat a light breakfast and consume only liquids to minimize the chances of a bowel movement. The results? 46 of 48 patients were effectively treated --- no more relapse or intestinal disease.

In another study performed by Silverman et al. (2010), 7 patients were effectively treated and remained totally asymptomatic throughout the 14-month follow-up period. There also were abstracts at the recent Digestive Disease Week meeting reporting on the success of the procedure. The process may not sound too appealing, but scientifically it makes sense and most importantly, it works.

Tucker, M. E. Fecal transfer effective in relapsing C. difficile. GI & Hepatology News, February, 2009.

Silverman, M. S., I. Davis, and D. R. Pillai. 2010. Success of self-administered home fecal transplantation for chronic *Clostridium difficile* infection. Lin. Gastroenterol. Hepatol. 8:471-473.

TECHLAB's Technical Support Team is available from 8:30 AM to 4:30 PM EST, Monday through Friday.

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