An email interview with Dr. Christen Rune Stensvold

TP. As an eminent member of various prestigious organizations, please share your views and experiences in parasitology.

ANSWER

I’ve been tremendously lucky to be involved in parasitology for more than 15 years. Having worked for so many years in a clinical microbiology reference laboratory setting, I’ve been extensively involved in method development, consultation, and teaching/supervision. For instance, we have a broad range of diagnostic real‑time polymerase chain reaction (PCR) assays targeting anything from normally free‑living amoebae such as *Naegleria*, and various species of *Plasmodium* to soil‑transmitted helminths such as *Ascaris* and *Trichuris*. The majority of the samples that we receive are screened for either *Toxoplasma*, *Leishmania*, *Acanthamoeba*, or intestinal protists (*Entamoeba*, *Giardia*, and *Cryptosporidium*); these are tests that we do on a daily basis. We also do quite a lot of *Toxoplasma* serology, and once a week, we do serology for the so‑called “tropical diseases,” such as malaria, leishmaniasis, trypanosomiasis, and fascioliasis.

Research-wise, I have been concentrating mostly on the role of intestinal parasites in health and disease.

I treasure my fellow parasitologist colleagues due to a lot of different reasons, but maybe especially due to the fact that they have all chosen to work with something that is not easy: Parasites are not easily isolated, let alone cultivated, and if — despite of this — you’re keen on doing whole genome sequencing, you’re not looking at those very short 13 KB genomes found in Influenza virus; with parasites, you’re looking at genomes commensurate to at least 10–20 MB. Moreover, parasitology research is not driven far as much by commercial interests as seen in, for instance, bacteriology, virology, and mycology. This makes parasitologists stand out to me as enthusiasts who are candidly interested in learning as much as possible about not only the diseases that these organisms may cause but also the benefits. And, this is also where our world differs from that of our peers in, for example, virology: We know that some parasites may actually convey health benefits, and so, working with parasites does not only mean combatting them but also understanding how we might be able to...
exploit THEM! A couple of examples: *Trichuris* is one of the soil-transmitted helminths causing a high disease burden in, for example, sub-Saharan Africa. However, at the same time, we can use *Trichuris* to modulate immune responses, which may have huge therapeutic consequences for patients with autoimmune diseases. Another example: Once infected by *Toxoplasma*, the infection usually persists throughout life. It’s possible that the parasite is constantly priming the immune system, resulting in a situation where the parasite is forced to encyst instead of propagating and cause a systemic infection. Moving from the immune system to the human microbiome, we know about member species of *Entamoeba* and *Blastocystis* that appear to be more common in gut healthy individuals than in those with functional and inflammatory bowel diseases. What does this mean? Do they select for “good microbes” or do the “good microbes” select for these parasites? What can the presence of these parasites tell us about gut ecology, physiology, and pathology? The ways parasites impact our bodies are complex! And, there is a lot of research to do for our fellow parasitologists!

When it comes to my personal career, I’m the only one in Denmark with an MSc in Parasitology who actually has a permanent position in a parasitology lab. Of course, I feel extremely privileged, but I sometimes miss to be able to liaise with people with a similar background and in a similar position. Nevertheless, I’m surrounded by passionate MDs, vets, and biologists, and cross-disciplinarity and diversity are critical to developing major advances in my opinion.

Since 2018, I’ve been the Treasurer of World Federation of Parasitologists, and together with Pikka Jokelainen, Wonderful Copenhagen, and a bunch of enthusiastic colleagues, I took part in winning the competition for hosting ICOPA 2022, which will take place in Copenhagen. We all look very much forward to hosting it and to have the opportunity to increase focus on parasitology in our part of the world.

**TP.** As *Blastocystis* is one of your specialized areas of research, please shed some light on its current trends.

**ANSWER**

So, *Blastocystis* research is really “blasting off” these years, so to say. For about 25 years, there have been studies going on regarding the genetic diversity in *Blastocystis*. This research has helped us not only to better understand the epidemiology of the parasite but also the way that humans, other primates and non-primate hosts have co-evolved with *Blastocystis*. Since *Blastocystis* is so “hyper-prevalent” and colonizing almost all sorts of animals from insects over sea snakes to wallabies, I wouldn’t be surprised if our ancestors all had *Blastocystis*. If you look at populations in some parts of the world, for example, Sub-Saharan Africa, you’ll see a 100% prevalence. In the Western World, *Blastocystis* is certainly not uncommon, but overall prevalence data indicate a much lower prevalence, which may be a sign of the eukaryotic depletion characterizing the guts of Western World populations and which has been mentioned by, for example, Dr. Laura W. Parfrey. And, although differences in hygiene play a role, this difference may also reflect differences in diets.

The major current trends in *Blastocystis* research are many, but primarily three:

1. Genetic diversity including genomics
2. Gut microbiota studies including the eukaryotic component (i.e., the gut fauna)
3. Cell biology and immunology.

My own research has been focusing on (1) and (2).

The genetic diversity seen within *Blastocystis* is like a universe. We know of at least 17 subtypes, which are arguably separate species, or even genera. Each one more or less adapted to its own host spectrum. Humans typically have ST1, ST2, ST3, and ST4; nonhuman primates ST1, ST2, ST3, and ST5; pigs, typically ST5; avian hosts typically have ST6 or ST7, and ruminants are typically colonized by ST10 and ST14. At times, we stumble on a new subtype, we discover new host for some subtypes, etc., and so, there might be much more for us to discover… information that can tell us about evolution and adaptation in both parasites and hosts. We see something similar for *Entamoeba*.

I find it thrilling that researchers across the world are now independently corroborating what we found in our lab back in 2015, where we saw an inverse relationship between Bacteroides-dominated gut microbiota and *Blastocystis*. We should explore this finding in greater detail, and we should try and find out what we can learn from the associations observed between *Blastocystis* and groups of gut bacteria. Can we use *Blastocystis* to manipulate gut microbiota and vice versa? I’m not saying that it would be a good idea, but can we eliminate *Blastocystis* by for instance changing our diets? By taking certain types of antibiotics targeting bacteria on which *Blastocystis* depends? What happens if we do? What can gut physiology, metabolism, and anatomy tell us about the likelihood of a certain species being suitable hosts for *Blastocystis* colonization? Why don’t we really see *Blastocystis* in strict carnivores? In a recent Trends in Parasitology article, my colleague Mark van der
Giezen and I mentioned some of our theories about gut ecology/physiology and the likelihood of being colonized by Blastocystis.

**TP.** What are your other areas of interest in parasitology research?

**ANSWER**

I’ve spent a lot of time on other common luminal intestinal parasitic protists (those that I refer to as CLIPPs – please see my recent paper in Parasitology). Just like Blastocystis, the genetic universe of Entamoeba appears almost just as big, so I’ve spent some time with Graham Clark to delineate novel ribosomal lineages. Just like Blastocystis, this is an ongoing process.

More than 10 years back, I started looking for Dientamoeba in Denmark. Although we didn’t use DNA-based methods, in the beginning, it wasn’t difficult to find cases. I was inspired to look for this parasite after reading some interesting papers from Dr. Damien Stark’s group in Sydney. Recently, we realized that Dientamoeba is colonizing practically all pre-school children and children in the early school classes, and so we now regard it as a commensal at least in children in Denmark. Contrary to Blastocystis, however, Dientamoeba fragilis is almost clonal, which suggests that it entered the human population relatively recently compared with Blastocystis. And what does this mean? Are we only beginning to get used to hosting it? Would it cause symptoms in some individuals? I’m not sure. Nevertheless, gastroenterologist Laura Krogsgaard helped us find out that both Blastocystis and Dientamoeba are more common in gut healthy individuals than in patients with irritable bowel syndrome. And, in patients with inflammatory bowel disease, these parasites are even rarer. So, if you’re a gut-healthy person with high microbiota diversity, chances are high that you might have either Blastocystis or Dientamoeba – or both!

Working in a parasite reference lab, of course, I’ve been involved in many interesting cases. So, part of my work and research also deals with diagnosing and documenting neglected, emerging or rare infections such as those attributable to Naegleria fowleri, Fasziola hepatica, Gnathostoma spinigerum, Toxocara spp., Plasmodium cynomolgi, Schistosoma spp., Leishmania infantum, just to mention a few: I have also been working a little with Cryptosporidium. It’s been fun to help expand the assay repertoire for gp60-based genotyping.

**TP.** Please share your work experience in evolutionary genomics of Blastocystis and their applications.

**ANSWER**

I have been involved in the sequencing of both mitochondrial and nuclear genomes of Blastocystis, but when it comes to the analysis of these, my experience draws mainly from work on mitochondrial genomes. We started to look at these during my time in London, and we set out to see whether the genetic variation existing across mitochondrial genomes reflected the genetic variation seen across nuclear ribosomal genes. And it did! We developed multilocus sequence typing assays for a couple of the subtypes (ST3 and ST4) based on some of the mitochondrial genes that we had sequenced, but although these schemes provided a little higher resolution than analysis of ribosomal genes only, we find that ribosomal gene analysis is a cost-effective and valid way to look for intra-subtype diversity. But of course, the level of resolution required depends on your research question.

Nuclear genomes have emerged, and we so have complete nuclear genomes for ST1, ST4, and ST7. Information in these genomes can tell us what “Blastocystis life” is like, what it is capable of doing – and not doing; what it’s metabolism is like for instance.

**TP.** Why is drug research in parasitology lagging when compared to the development of antibacterial or antiviral agents?

**ANSWER**

I think that this boils down to a few major issues. Vaccine development is difficult because parasites are good at evading our immune system – that’s what they have been specializing in for over millions of years.

The development of drugs to eradicate parasites is hampered by the fact that many parasitic infections are not causing severe morbidity let alone mortality. And, some of those that do are still neglected, maybe because infections tend to be chronic, and symptoms build up over a long time. We need to keep increasing awareness about these diseases, and we need to improve the methods that can diagnose these infections accurately to give better estimates of the disease burden. The increasing use of point-of-care tests and the current simplification of DNA-based methods such as LAMP shows promise for mapping diseases even in developing countries.

There may be other reasons for the lag. As mentioned previously, it’s not easy to establish in utro and in vivo experiments on parasites; you can’t just plate them out on agar and carry out drug susceptibility test. Finally,
parasites are eukaryotic organisms, like humans, and so it may sometimes be difficult to find targets specific to a given parasite, you wouldn’t want too many side effects.

**TP.** Drug resistance in protozoa has been rapidly evolving in the past decade. What is the current status of antiparasitic drug resistance in the world scenario?

**ANSWER**

According to the CDC, multi-drug resistance is a problem primarily for *Plasmodium falciparum* and *Plasmodium vivax*. Nevertheless, for vector-borne diseases such as malaria, there are a number of options to combat the disease. Since the life cycle is complex, there are many steps where intervention is possible (vector control, transmission-blocking vaccines, and the use of bednets). From this perspective, the opportunity is limited when you deal with parasites with simpler, direct life cycles, such as, for example, *Giardia*. At our laboratory, we have been involved in several cases of metronidazole-resistant giardiasis, which is seen almost exclusively in people returning from Asia. I hope that it won’t be long before we can implement screening for resistance genes in *Giardia*, but I’m not aware of any validated markers or other types of tools that can predict whether a given strain will be susceptible to metronidazole or not.

**TP.** You have been working all over the world, what is your opinion on the research approach in developing countries when compared to developed nations?

**ANSWER**

I have limited experience from work in developing countries. I’m currently involved in a project called “SOLID,” which is project funded by EDCTP (The European and Developing Countries Clinical Trials Partnership), and which aims to evaluate point-of-care testing for the diagnosis of *Taenia solium*, taeniasis, and neurocysticercosis in communities and primary care settings of highly endemic resource-poor areas in sub-Saharan Africa. The project also involves capacity building. I’m impressed by those of my colleagues who managed to get the funding for the project and who are leading it. It’s by taking part in projects such as this that you begin to understand the vast difference in opportunity, logistics, resources, culture between developing and developed countries.

One of the things that have been really rewarding for me is to supervise and in other ways assist both established and emerging researchers from, for instance, Turkey, Middle Eastern, and African countries. I have been very much involved in work carried out by PhD students from these regions, and currently, a PhD student from Algeria is working in our lab doing molecular work on *Giardia* (real-time PCR-based diagnosis, microbiota associations, and genotyping). Although time and money allow me to do this only to a limited extent, contributing to capacity building and stimulating scientific environments in, for example, the Maghreb countries is truly rewarding, and I’m thankful to have the chance.

**TP.** In your opinion, what newer perspectives can be addressed in these programs for eliminating diarrheal diseases?

**ANSWER**

If I should mention only one thing, then this would probably be it: A couple of years ago, people involved in the GEMS study identified *Cryptosporidium* as one of the most common causes of morbidity and mortality in for instance Sub-Saharan Africa. If you’re a parasitologist interested in immunology (or vice versa) and going for the Nobel Prize, I think that efforts towards developing a vaccine against *Cryptosporidium* would be time well spent.

**TP.** Please share a few words of advice for the budding scientists in the field of parasitology.

**ANSWER**

As with other professions, making a career in parasitology is mostly about passion, networking, and allocating time. I remember watching my all-time favorite movie “The Thin Red Line” over and over again at the time when I was studying Systematic Parasitology at university. The movie was set in the Solomon Islands (and Queensland, Australia), and I began fantasizing about the climate, the nature, but also the parasites and the diseases there that I was reading about, and about going to South East Asia or Oceania to study them and do something about them. The following year and thanks to my then supervisor Prof. Maria Vang Johansen, I was lucky to be able to go to Laos and Thailand for 6 months to study PCR-based diagnosis of liver flukes (*Opisthorchis*) in a WHO setting. It was one of the most interesting and rewarding experiences of my life, and it quickly spurred my interest not only in parasitology but also in research and academia in general. Two years later, in February 2006, when I was studying with Prof. Andrew Thompson in Australia, I locked myself up in a small apartment in Fremantle over a couple of sweltering weekends, trying once and for all to solve the mystery of *Blastocystis* terminology, which was all over the place at that
time. I remember I was so caught up in it that I forgot about most other things. That's when I discovered my passion for the field – I felt called upon! I just had to solve this! Later on, in 2009, I was lucky to get money for 2 years of post doc research with Prof. Graham Clark at London School of Hygiene and Tropical Medicine, who had helped me develop the new *Blastocystis* terminology…I had all the time in the world, studied genetic diversity within *Entamoeba*, *Iodamoeba*, and *Blastocystis*, and we were very productive and had a great time.

So, my advice is to get into the habit of blocking out sometime during weekends to analyze those data or write up that paper once in a while. Hook up with experienced and competent people from the beginning. When I started my career back in 2004, I quickly identified the experts in my field through articles I found using PubMed, and I managed to get in touch with them in order to share my data and ideas and to look for inspiration and areas of potential collaboration.

Moreover, if something catches your interest, pursue it! Don't be afraid that it might be too fringe; it will let your professional skills develop and your curiosity lead you to enthusiastic and experienced people who can further inspire and help you expand your work. Don’t be afraid to contact seniors with a view to exchanging hypotheses and ideas. And, train yourself in formulating research questions in a simple and accurate way.

Another thing I would recommend is to familiarize yourself with concepts of co-evolution and ecology, which will help you understand host-parasite interactions and the impact of parasitism on any given host. As a virologist of the bacteriologist, you probably don’t (need to) pay very much attention to these things, but parasitologists should.

Cross-disciplinarity is conducive to developing new thoughts and ideas, and as a member of the e-learning team in the United European Gastroenterology, I’m constantly trying to find inspiration in the intersection between microbiology and gastroenterology.

For young researchers interested in the epidemiology and clinical significance of intestinal parasites, I would recommend working with *Blastocystis* for a while. You’ll learn so much from it both in your wet and dry lab work. This parasite is so easily accessible (very common in humans, non-human primates, pigs, and ruminants), it’s one of the few parasites that can easily be cultured (for limited costs), and you can train yourself in PCR and sequencing, sequence editing and analysis, developing phylogenetic inferences, querying online databases. The *Blastocystis* research community is growing rapidly, as evidenced for instance by the fact that the number of *Blastocystis*-specific publications listed in PubMed has doubled over the past few years. And we even have a conference now every third year – specifically dealing with the latest research in *Blastocystis*. The research potential for this organism is immense!