To begin, could you briefly outline your academic background?

I started my scientific life at the University of Natal, South Africa, where I received an excellent grounding in zoology and chemistry before finally graduating with BSc (Hons) in Zoology. The breadth of this education was so accommodating that it enabled me to move on to Rhodes University, South Africa, to get an MSc in Entomology. I chose to study this because I had an interest in tsetse flies, and also because trypanosomiasis is a significant problem in my home of Rhodesia (now Zimbabwe).

What initially led you to develop an interest in tropical parasitic diseases?

Essentially, my interest in public health matters was a natural consequence of my understanding of the biology and ecology of important local diseases, and I therefore went on to join the Ministry of Health in their Malaria and Bilharzia Research Laboratory, which later became the internationally renowned Blair Research Laboratory. At this time I obtained my PhD in Aquatic Biology from Rhodes. Together with a team of local scientists at Blair, I spent 25 years working on the control of both bilharzia (schistosomiasis) and malaria.

When you live in an environment where the people around you are consistently afflicted with tropical diseases, there is a strong urge to help. From the Blair Research Laboratory we visited schools and farms all over the country and saw the ravages of schistosomiasis first-hand. Working on malaria treatment was hazardous during the early stages of my career; there were no rapid diagnostic tests, and blood film examination was often delayed. However, indoor residual spraying was increasingly used in the country to slowly bring malaria under control and, when the issue arose of how to sustain the programmes, the Government provided funds to expand a control programme which was sustained in Zimbabwe for the remainder of the 20th Century.

How did you come to develop polymerase chain reaction (PCR) urine testing for the detection of parasitic infection?

The advent of PCR opened up great opportunities to expand genetics and population science and, as the genomes of various parasites were sequenced, opportunities to expand and exploit these new technologies arose. My colleagues in Zambia postulated (and subsequently demonstrated that) Plasmodium DNA could be identified in both urine and saliva. Fortunately a colleague in Israel, Joseph Hamburger, had shown that with schistosomes, the genome contains a high proportion of small repeat fragments. Studying Schistosoma haematobium, he identified a repeat fragment he called DRA-1, which constitutes more than 16 per cent of the genome. It occurred to me that this might be a suitable diagnostic target, because the parasite has an active tegument that sloughs off material continuously to help avoid any immune response: if this is sloughed into the blood, perhaps it is also excreted in urine? We therefore obtained samples of filtered urine from a study in Nigeria and were able to extract and detect the fragment by PCR amplification.

Next, we investigated the use of coarse filter paper as a sampling method to see if this would retain the DNA fragments. Our studies showed that the DNA trapped on filter paper is stable and can easily be reconstituted and amplified. This discovery introduced the opportunity for a major change in the diagnosis of this infection.

What are the advantages of PCR testing over current diagnostic methods?

Currently, schistosomiasis diagnoses rely on taking stool samples. This means that those in the field are required to visit the patient, leave a cup to contain the stool and revisit the patient to retrieve the sample at a later time. This is time-consuming and expensive, due to transport and packaging requirements for stool specimens. With PCR testing, however, the urine can be filtered in the field and the filter papers then dried and packed in plastic sleeves that same visit, meaning that transport costs and time are both greatly reduced.

Which of your scientific achievements are you proudest of?

I have had a long career, but the success of the malaria control operations that we developed and undertook in Rhodesia from the late 1950s until the end of the 20th Century really stands out. Not only did we eliminate the disease from about two-thirds of the entire country for most of that time, but we also showed just what could be achieved in Africa through the implementation of a well-managed local programme.
Schistosomiasis affects millions of people around the world, but inaccurate diagnosis is often a barrier to effective treatment. This could be set to change, however, due to the development of a novel diagnostic technique by researchers at Johns Hopkins Bloomberg School of Public Health.

Schistosomiasis, a chronic disease caused by parasites or 'flukes' of the genus Schistosoma, is a serious illness that can, at its worst, be fatal. Carried by freshwater snails, the larval form of the parasite penetrates the human body through the skin, where it then develops in the blood vessels into an adult Schistosoma. Symptoms of schistosomiasis are precipitated by the immune response and organ damage caused by the Schistosoma's release of eggs.

There are five different species of Schistosoma spread across Africa, the Middle East, the Caribbean, South America and East Asia, yet schistosomiasis infection falls into just two categories: intestinal and urogenital. Intestinal schistosomiasis can cause abdominal pain, blood in the stool and eventually enlargement of the liver and spleen, whilst urogenital schistosomiasis can damage the urinary system and cause genital disorders that can lead to infertility and even bladder cancer. In both cases, schistosomiasis can lead to death if untreated.

The statistics look bleak. According to the World Health Organization (WHO), in 2011 schistosomiasis affected at least 243 million people globally, yet only 28.1 million were reported to have been treated. Moreover, schistosomiasis claims the lives of more than 200,000 people in sub-Saharan Africa annually.

Whilst treatment for schistosomiasis has developed significantly over past decades, one of the inhibiting factors for its eradication has been diagnosis. Schistosomes are traditionally detected through their eggs by analysing stool and urine samples using the Kato-Katz method or direct filtration. Yet these techniques are unreliable: in positive cases of infection the eggs can often be absent or only present in small amounts, meaning that no infection is detected, while in cases where the infection has already been treated, eggs may remain present in faeces and urine samples for some time afterwards, resulting in false positives and over-diagnosis. In addition, Kato-Katz relies on the collection, transport and analysis of stool samples, making the work labour- and cost-intensive. There is therefore a great need for a more reliable diagnostic technique.

This diagnostic technique will enable practitioners to distinguish between several Schistosoma species within a single urine sample, allowing them to treat the disease more effectively.
DETECTION OF PARASITE SPECIFIC DNA FRAGMENTS IN URINE, AS A MEANS TO DIAGNOSE INFECTIONS OF NEGLECTED TROPICAL DISEASES

OBJECTIVES

- To identify specific repeat fragments of nuclear DNA from intestinal and blood parasites, and optimise the amplification procedures so that they are applicable in the field
- To demonstrate the presence of such DNA in urine by filtering the urine through coarse filter paper that is then dried in the field. This will avoid the arduous collection and transport of blood, stool and large volumes of urine

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Clive Shiff obtained a BSc (Hons) in Zoology from the University of Natal, South Africa, before continuing his postgraduate education at Rhodes University, South Africa, gaining first an MSc in Entomology then a PhD in Aquatic Biology. He has worked in Rhodesia (now Zimbabwe) as a tsetse fly biologist, aquatic biologist and medical entomologist, and is now Associate Professor in Parasitology at the Johns Hopkins Bloomberg School of Public Health, USA.

need for the development of a method of schistosomiasis screening which is both more efficient and effective.

AN EXPERT INSIGHT

Answering the call for research is Professor Clive Shiff. An expert in the field of malaria and schistosomiasis prevention, Shiff has gained a broad understanding of schistosomes from his extensive experience in both field and laboratory environments. Having studied the bionomics of intermediate host snails of African schistosomes and the mechanisms used by schistosomes to locate hosts and penetrate human skin during his work as Deputy Director of the Blair Research Laboratory in Zimbabwe, Shiff has since led research into the population biology of schistosomes and the pathological effects of Schistosoma haematobium infection in Zimbabwe and Ghana, respectively.

As well as being a driving force behind the Malaria Institute at Macha (MIAM) in Zambia and a scientific consultant to national and international health organisations such as WHO, Shiff is now Associate Professor in the Department of Molecular Microbiology and Immunology at Johns Hopkins Bloomberg School of Public Health. Dedicated to improving public health in the developing world, Shiff is ideally placed to lead research into novel diagnosis methods for schistosomiasis.

POSITIVE FINDINGS

Knowing that Schistosoma eggs provide an inadequate target for schistosomiasis screening, Shiff worked with colleagues around the world to identify an alternative target which achieves a consistently accurate diagnosis. By studying the DNA of S. haematobium using polymerase chain reaction (PCR) testing and identifying short repeats in its genome, Shiff and colleagues not only succeeded in recognising Schistosoma DNA as a possible screening target, but also found that only small amounts would be sufficient to arrive at a positive diagnosis.

Moreover, samples acquired through PCR testing can also be collected simply by funneling urine through filter paper. This circumnavigates the need for cumbersome, expensive and risky transport of urine and stool samples, improving practitioners’ ability to provide world-class medical care.

ADAPTING PCR SCREENING

With a repeat fragment (DRA-1) having been identified in the genome of S. haematobium, the task now remains to identify the existence of short repeats in the genome of other species of the fluke. Scientists have already successfully identified short repeats in S. mansoni, one of the four schistosomes which cause intestinal schistosomiasis in humans. Once these repeats have been identified in all human strains of Schistosoma, the sensitivity of PCR will enable practitioners to distinguish between several Schistosoma species within a single urine sample, allowing them to treat the disease more effectively.

Although the practices employed by Shiff’s team rely on the use of PCR under laboratory conditions as a means to amplify parasite DNA, the group is keen for the technique to work in the field, especially in remote rural locations which are often off-grid. Shiff’s team is working on a possible solution in the form of loop-mediated isothermal amplification (LAMP), an alternative to PCR that could potentially be powered by a 12 V car battery. “LAMP has huge potential to bring sophisticated analysis to rural areas,” he enthuses. However, he does acknowledge that the technique still requires careful preparation and expert technical manipulation: “There needs to be careful supervision because a positive result produces a change in colour, and this can be subtle,” he further elaborates. “Efforts to use spectrometers at this stage would make the technique too expensive.”

DISSEMINATING RESEARCH

Shiff is now keen for the full potential of this novel diagnostic technique to be realised worldwide, and hopes to share his findings with others through international cooperation on a range of projects. “We have to convince sceptics that this is an effective technique, so I am collaborating with colleagues in Zambia, and Ghana, and now also in Argentina on another parasite: Strongyloides,” he elucidates. For Shiff, this international perspective is vital: “It is essential to the research because samples must come from the field, and it is in the field that the real value of the work will be felt”. By sharing his research and expertise, Shiff hopes to alleviate other parasite-related public health issues. Ultimately, he maintains that effective diagnosis is the key to gaining control of a disease. “A highly sensitive diagnosis is essential for the elimination of the reservoir of parasites in the local community,” he concludes.

An illustration of two Schistosoma mansoni eggs in mouse liver preparation.