

International Development Fund reports



Fifth International Symposium on Typhoid Fever and other Salmonellosis and Workshop on Molecular Methods in the Epidemiology and Diagnosis of Typhoid

Karachi, Pakistan, 4–7 February 2002

■ Gordon Dougan

Typhoid is still a very common disease in many poorer countries of the world. The World Health Organization estimates that globally there are still more than 17 million cases annually and that these infections are associated with around 600,000 deaths. These figures are almost certainly an underestimate as the disease is difficult to confirm clinically or immunologically. The gold standard for typhoid diagnosis is still a positive blood culture and in the absence of good microbiological facilities the disease can be missed. A major factor affecting the treatment of typhoid is the emergence of multiple-antibiotic-resistant bacteria in many regions of the world, again illustrating the importance of good microbiological practice in handling the disease and associated outbreaks. Perhaps surprisingly, the level of research activity on *Salmonella enterica* subsp. *enterica* serovar Typhi (*Salmonella* Typhi) is comparatively low. In most Western laboratories researchers prefer to work on the convenient murine model of the disease (caused by other *S. enterica* serovars such as Typhimurium). Although the murine disease superficially resembles typhoid, it is not the human disease. This point is emphasized by examining the recently published genome sequences of Typhi versus Typhimurium, where up to 10% of the genes are different.

Against this background an international meeting on typhoid has been organized on a bi-annual basis. The meetings are normally held in a country where the disease is endemic and this year it was the turn of Pakistan. The task of pulling the meeting together was given to Professor Zulfiqar Bhutta of the Aga Khan University in Karachi and a small organizing committee. International events were going to make the task extremely difficult. The outbreak of war in neighbouring

Afghanistan immediately put a cloud over the meeting as political pressure built up in Pakistan, a near neighbour. Also, a week before the meeting was due to take place the US journalist Daniel Pearl was kidnapped in Karachi. All of these events meant that there was considerable uncertainty surrounding the meeting up until the last minute.

A team of SGM-sponsored scientists, Professor Gordon Dougan, Dr John Wain and Dr Tahir Ali (all from Imperial College) and Dr Nick Thomson (Sanger Centre), set out for Karachi on 2 February, uncertain of how events would unfold. Arrival in Karachi was uneventful and we settled in by gorging on a delicious curry smorgasbord in the hotel. The next morning we met in the hotel lobby and were thrilled to be greeted by an enthusiastic gathering of dedicated typhoid scientists who had all made it to Pakistan in spite of the problems. We climbed into a bus to head off to the workshop on molecular methods (organized by Dr Rumina Hassan of the Aga Khan University, supported by John Wain, Nick Thomson and Tahir Ali). Armed soldiers simultaneously boarded the bus and took up their protective posts with rifles pointing out of the windows as we set off through the rush hour traffic. On arrival at the Aga Khan University we went straight into the first session on PCR assays for typhoid and never looked back. The audience/participants were a mixture of international academics and clinicians along with many local (Pakistani) scientists and students. Topics covered ranged from PCR methodologies, ELISA, pathogenicity islands, diagnostics and culture methods. A special treat was provided by Nick Thomson (introduced mistakenly as Nick Sanger at the meeting), who set up a real-time demonstration of the genome browsing tool Artemis and handed out free CDs containing the full program. The participants alternated between laboratory and seminar sessions and we were all enthralled by the enthusiasm of the participants and their desire to learn.

The workshop was followed by the international meeting. The programme had been depleted as several people failed to turn up, but substitutes were quickly found and the full sessions went ahead. Many excellent



ABOVE:
Participants in the Symposium and Workshop held in Karachi, Pakistan.

RIGHT:
Workshop participants learning new laboratory techniques.

PHOTOS GORDON DOUGAN

presentations were made. Of note was the effort made by Myron Levine of the University of Maryland who against all political odds and time constraints made it to the meeting and provided a superb overview of vaccinology (a testament to his dedication). Exciting data were presented by the Chinese on the impact of Vi vaccination in China and the threatened emergence of *Salmonella* Paratyphi A to take its place. Professor Dougan summarized the meeting and chaired a discussion on future prospects.

The meeting was complemented by several dinners held in different locations and excellent food was served at all times. The SGM contingent focused their attention on sampling as many curries as possible. Eventually, the time to leave arrived and we boarded the plane to Dubai, regretfully leaving a superb meeting. In Dubai we just had sufficient time to down a pint of Guinness in the Irish Pub before taking the plane to London. We all agreed that this was one of the most stimulating scientific meetings we had attended. This was in the face of so many compounding factors in the region. Much of the credit must go to Zulfiqar Bhutta who kept his nerve where others lost theirs and still managed to be the perfect scientific host.

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Respiratory bacteriology training in Tanzania

■ **Stephen Gillespie, Tim McHugh & Bambos Charalambous**

The Department of Medical Microbiology at the Royal Free and University College Medical School, University College London has been collaborating with a hospital in Northern Tanzania, the Kilimanjaro Christian Medical Centre, for more than 13 years. A new medical school and health sciences faculty has been established there since 1997 and provides training for undergraduate, postgraduate medical and many paramedical and nursing students. This is part of the Tumaini University of Tanzania ('*tumaini*' is the Swahili word for 'hope'). There is a need to develop the teaching and research infrastructure. We were awarded a grant from the SGM International Development Fund to develop the respiratory bacteriology reference and training facilities.

Lower respiratory tract infections exact an enormous toll of death in sub-Saharan Africa, caused by *Streptococcus pneumoniae* in children and *Mycobacterium tuberculosis* in people of all ages. Yet little is known about the epidemiology, population genetics and antibiotic resistance patterns of these organisms on the continent. This is of particular importance for the formulation of vaccines and antibiotic regimens which usually depend on information obtained from studies performed in Europe and North America.

The SGM grant was used to support the development of facilities for the molecular epidemiology of respiratory bacteria. A PCR machine, gel rigs, power packs and materials to archive strains, were sent out to the clinical laboratory of Kilimanjaro Christian Medical College.

The equipment has already been put to good use in a cross-sectional and longitudinal study of *S. pneumoniae* carriage that in addition to serological and antibiotic susceptibility tests will identify the molecular basis of the changing prevalence of different serotypes and resistant clones. Related studies currently underway include the collection of invasive pneumococcal isolates to identify the nature of the link between carriage and invasive disease. These will include genotyping the organisms by multilocus sequence typing to understand the processes of natural capsule switching and multiple colonization.

Also underway is a study of the genetic heterogeneity of wild-type *M. tuberculosis* in patients on treatment. A visiting UCL research fellow has established PCR assays for a range of genes associated with resistance and used this as an opportunity for

training in molecular methodology. A Tanzanian fellow is now visiting the UK to have further training in molecular respiratory bacteriology and will return to Tanzania to start a project that links the molecular lineage of *M. tuberculosis* strains to clinical outcome.

Research is not the only objective of our collaboration and there will be increasing training of local university staff in molecular methods of diagnosis. We also anticipate local courses on diagnosis of respiratory infections and testing for antibiotic resistance in the future. Thus, the grant has already contributed to the development of research and teaching capacity of the Kilimanjaro Christian Medical College, Tumaini University, and provides a foundation for further initiatives.

● **Professor Stephen H. Gillespie, Dr Timothy D. McHugh and Dr Bambos M. Charalambous, Department of Medical Microbiology, Royal Free and University College Medical School, Rowland Hill Street, London NW3 2PF, UK. email stepheng@rfc.ucl.ac.uk; tmchugh@rfc.ucl.ac.uk**



TOP LEFT: View of the rear of Kilimanjaro Christian Medical College showing some of the paramedical training schools in the shadow of Kilimanjaro.

ABOVE: Staff training in PCR.

PHOTOS ROLY GOSLING

International Research Fellowship report

■ Tsitsi Ndowora

THIS PAGE:

Fig. 1. *N. benthamiana* infected with the TBTV-A2 isolate (left) and a healthy plant (right).

OPPOSITE PAGE:

Fig. 2. dsRNA isolated from *N. benthamiana* plants (thick lane) that were mechanically inoculated with the TBTV-A2 isolate showing four prominent dsRNA bands of about 4.6 (TBTV genome), 1.6, 1.3 and 0.75 (sat-RNA) kbp. A Promega 1 kb DNA ladder was used as a molecular mass standard (thin lane).

PHOTOS COURTESY TSITSI NDOWORA

Tobacco bushy-top virus (TBTV) is a tentative member of the Umbravirus genus. This unique genus consists of plant viruses that have ssRNA genomes that do not encode a coat protein, and thus do not form conventional virus particles in infected plants. In naturally infected plants, an umbravirus occurs in association with a helper luteovirus that facilitates the transmission of the umbravirus by aphids in a persistent manner. Definitive members of this virus genus include carrot mottle (CMoV), carrot mottle mimic (CMoMV), groundnut rosette (GRV), lettuce speckles mottle (LSMV), pea enation mosaic virus-2 (PEMV-2) and tobacco mottle (ToMV) viruses. Tentative members include sunflower crinkle, sunflower yellow blotch and tobacco yellow vein viruses, as well as TBTV.

There are two diseases of economic importance caused by umbraviruses in sub-Saharan Africa (SSA). One is the groundnut rosette disease, caused by GRV and its helper virus, groundnut rosette assistor virus (GRAV), which was first recorded on the African continent in 1907 in Tanzania and occurs throughout the SSA region. The other, tobacco bushy-top disease, was first described in Zimbabwe in 1958–59 and since then has been reported in South Africa, Malawi and Zambia. An apparently similar disease is now known to occur in China. Although it is a disease of tobacco in nature, in greenhouse studies the virus complex has been transmitted to important solanaceous crops such as tomato, pepper and paprika. Another umbravirus, TMoV, in association with its helper, tobacco vein distorting virus, causes tobacco rosette disease, first reported in Zimbabwe in 1938.

In the Plant Pathology department, at the Kutsaga Research Station, we are studying the ecology and epidemiology of TBTV with the aim of devising an effective disease control strategy. To detect the virus in infected plants and viruliferous aphids requires the development of primers for RT-PCR and this in turn requires the cloning and sequencing of the TBTV genome. To speed up the process, we requested collaboration with Dr D.J. Robinson and his team at the Scottish Crop Research Institute (SCRI) in Invergowrie, Scotland, who have cloned and sequenced the GRV genome, determined the respective functions of some of the GRV ORFs, and recently cloned and sequenced portions of the TMoV genome.

The SGM International Research Fellowship funded



our work on the cloning and sequencing of the TBTV genome at the SCRI. I arrived in Scotland on a date etched in everyone's memory, 11 September 2001, and despite the shock waves work began the following day with the mechanical inoculation of a TBTV isolate (TBTV-A2) into *Nicotiana benthamiana* seedlings maintained in a glasshouse. Symptoms were visible about 5 days after inoculation. Infected plants were generally paler than healthy plants and there was a mottle of dark green upon a light green leaf. Affected plants soon developed a bushy appearance as shoots proliferated from the axillary buds (Fig. 1).

dsRNA was isolated from the affected plants 2 weeks after inoculation, bands were separated on an agarose gel (Fig. 2), and the 4.6 kbp dsRNA corresponding to the TBTV genome was electroeluted from the gel. dsRNA was denatured using methylmercuric hydroxide, transcribed into cDNA and cloned using standard methods. Of the clones obtained, six contained candidate TBTV cDNA sequences, and the sequences of these inserts were determined. Analysis of the sequence data is continuing to identify motifs characteristic of umbravirus genomes. Meanwhile, primers were designed using the sequence of one of the clones. However, these primers amplified the expected size product by both PCR and RT-PCR, suggesting that the insert in this clone is a fragment of host-plant DNA.

This work has set the foundation for and started the ball rolling on the cloning and sequencing of TBTV. We intend to do hybridization studies using the cloned fragments to determine if these clones originated from the TBTV genome. Sequences of TBTV will be compared to those of other umbraviruses, especially GRV which causes an important disease of groundnuts in SSA. The technique of cDNA synthesis from dsRNA for virus cloning learned at the SCRI will

Further reading

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MicroShorts

The Microbiology of Drinking Water 2002

The Standing Committee of Analysts has announced the publication of *The Microbiology of Drinking Water 2002* in the series *Methods for the Examination of Waters and Associated Materials*. This is a revision of *The Microbiology of Water 1994 – Part 1 – Drinking Water*, commonly referred to as *Report 71. The Microbiology of Drinking Water 2002* comprises 10 parts covering water quality and public health, sampling, laboratory procedures and analytical methods for a range of indicator and pathogenic bacteria. The revised document addresses issues arising from European and UK legislation and includes details of significant changes over recent years with respect to laboratory procedures and practices, and the use of new methods. The 10 parts are:

- Part 1** Water quality and public health
- Part 2** Practices and procedures for sampling
- Part 3** Practices and procedures for laboratories
- Part 4** Methods for the isolation and enumeration of coliform bacteria and *Escherichia coli* (including *E. coli* O157)
- Part 5** Isolation and enumeration of enterococci by membrane filtration
- Part 6** Methods for the isolation and enumeration of sulphite-reducing clostridia and *Clostridium perfringens* by membrane filtration
- Part 7** The enumeration of heterotrophic bacteria by pour and spread plate techniques
- Part 8** Methods for the isolation and enumeration of *Aeromonas* and *Pseudomonas aeruginosa* by membrane filtration
- Part 9** Methods for the isolation and enumeration of *Salmonella* and *Shigella* by selective enrichment, membrane filtration and multiple tube most probable number technique
- Part 10** Methods for the isolation of *Yersinia*, *Vibrio* and *Campylobacter* by selective enrichment

The Microbiology of Drinking Water (2002) is available in electronic format from the Environment Agency via Dr David Westwood [Tel. +44 (0)115 921 3705; Fax +44 (0)115 921 3760; email david.westwood@environment-agency.gov.uk]. In due course it is anticipated that all parts will be available from the Agency's internet website.

Food Standards Agency research programme

The FSA has announced the names of 12 eminent scientists and academics who will make up its independent Advisory Committee on Research. The Committee will guide a £30.8 million research programme, ensuring that the FSA's research is clearly focused on providing the evidence the Agency needs to develop its policy aims and is relevant to the needs of consumers. The Committee has three experts to represent life sciences, of whom two are members of the SGM: **Professor Duncan Maskell**, the Marks and Spencer Professor of Farm Animal Health, Food Science and Food Safety at Cambridge University, and **Professor William Donachie**, Deputy Director of Moredun Research Institute.

The Royal Society Summer Science Exhibition

2–4 July 2002

The annual exhibition is a showcase for scientific innovation and provides a unique opportunity to meet leading researchers from around the UK. It is particularly suitable for post-16 students with an interest in science, or who may be considering a scientific career. One of the exhibits this year, *Surfing the woodwide web*, which aims to raise the public awareness of fungi as soil microbes, has received sponsorship from the SGM PUS Fund. The exhibition takes place at 6–9 Carlton House Terrace, London, and entry is free. See www.royalsoc.ac.uk for details of opening times and other exhibits.

The BA Festival of Science

9–13 September 2002, University of Leicester

Europe's largest science extravaganza expects to attract 3,000 visitors and 400 scientists to its packed programme of talks, exhibitions, debates, visits and social events. The theme this year is *Science and the quality of life*. Full details of the programme and booking are on the web at www.the-ba.net

Women in Science

New Athena Award Scheme

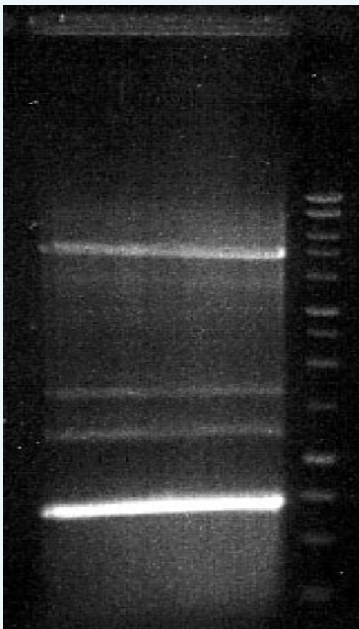
The Athena Project, which supports the advancement of women in academic science, engineering and technology (SET), is co-funding a new scheme with the Royal Society. Bids are now invited for awards which will celebrate higher education institutions that can demonstrate that they have increased the number of women working in SET, improved their career development, raised their profile or heightened awareness and understanding of the career barriers they face. A department, faculty, centre, network or whole institution can apply. The submission deadline is **19 August 2002**. Email athena@ecu.ac.uk for details.

Life Science Careers 2002

SGM is once again participating in the one-day careers conferences for undergraduate and postgraduate students of the life sciences. These include a packed programme of talks about a wide range of work options, further training opportunities, job finding and interviews, plus a CV clinic and exhibition by employers, professional bodies and universities. Attendance costs only £10 to include all refreshments and a delegate pack. This year's events are:

- 2 November *University of Sheffield*
- 16 November *University of Glasgow*
- 30 November *Kings College London*

A booking form will be published in the next issue of *Microbiology Today*. Details of the events are available on the web at www.uklsc.org/careers2002.htm



be invaluable in our work at Kutsaga Research Station. We shall continue to collaborate with Dr Robinson and his team on this, and hopefully other projects. This project also allowed me to interact with several virologists of whom I knew, but had never met, and provided excellent networking opportunities.

I wish to thank the SGM for awarding me the International Research Fellowship, Dr Robinson for welcoming me into his laboratory, all the scientists (and top-notch technical staff) at the SCRI who assisted me, and the management and plant pathologists at the Kutsaga Research Station for their support and encouragement.

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