

Microbiologist

The magazine of the Society for Applied Microbiology ■ March 2009 ■ Vol 10 No 1

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ZOONOSES

past, present and future

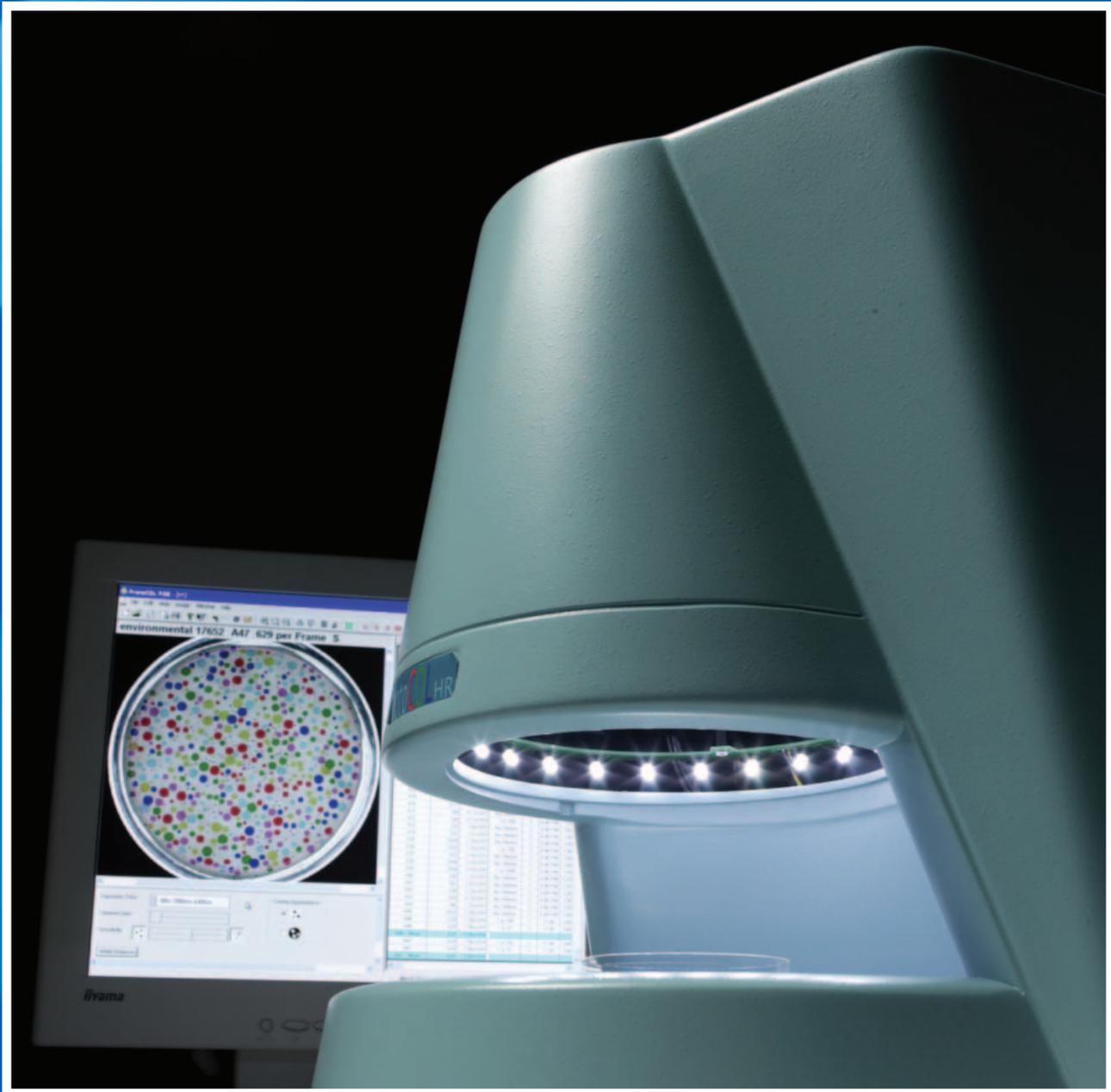
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- Careers: medical microbiology
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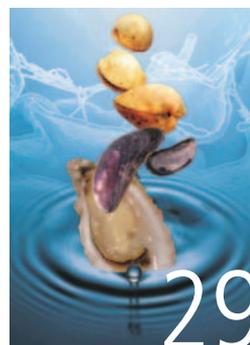
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ZOONOSES past, present and future

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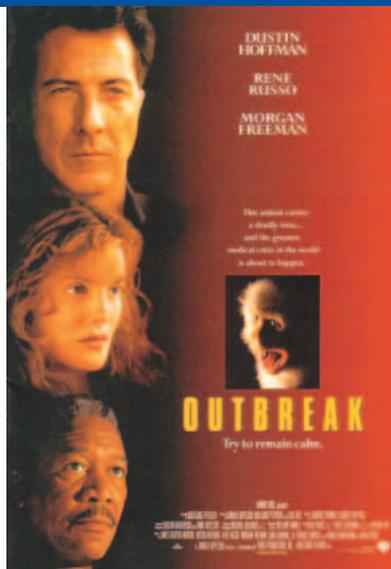
Cover image: zoonosis interaction — Stephen Pollard

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Did you know that around 60% of new emerging pathogens are zoonotic? I didn't until I began researching this editorial and a quick search online for news items on zoonotic diseases (disease that can be transmitted between animals and humans), has reinforced the importance of this vital area of microbiology. A recent report from the European Food Safety Authority/European Centre for Disease Prevention and Control (EFSA/ECDC) who are actively tracking zoonotic diseases across the EU, stated that in EU member states, campylobacteriosis was the most reported zoonotic disease. On a more positive front, reported cases of salmonellosis have fallen for the fourth year in a row. Outside Europe, in Texas, there are plans to air-drop a rabies vaccine and also in the USA, mice are spreading hantavirus. The importance of zoonotic disease has been recognised by the EU through many projects including Med-Vet-Net,



for which SfAM have formed the Communications Unit (see page 14) and through numerous global initiatives including, with its headquarters in California, the Global Viral Forecasting Initiative (GVFI), which aims to monitor the interface between animals and humans to identify viruses while it is still possible to contain their spread.

It is zoonotic disease which forms the theme of this issue of *Microbiologist*. We have a fascinating overview of Zoonoses from Professor

Peter Boriello and Dr Mark Fielder (page 24) and a focus on diseases that can be caught (pardon the pun) from shellfish by Rachel Rangdale of the Centre for Environment, Fisheries & Aquaculture Science (CEFAS, see page 29). While we're talking about zoonoses, this is probably an appropriate time for me to remind you all that the topic of our forthcoming summer conference is *fur, feather and fever — zoonotic challenges of the 21st century* (see page 21 for details and booking form). Don't forget to book early to take advantage of our early bird discounted booking fee.

As a way of highlighting the conference, we will be involved in National Science and Engineering Week engaging the public with infectious disease through an event to be held in at Manchester Museum. Stimulated by watching the film *Outbreak* (and a little food and drink), we will be asking invited members of the public to voice their concerns about infectious diseases. There'll be a full report of the event in the next issue of the magazine, but in the meantime, don't forget, that our innovative project / public engagement grant is a way for you to get involved in similar initiatives — visit www.sfam.org.uk/grants for details of how you can apply for funding to engage and communicate with the public about applied microbiology.

While we're talking about the communication of applied microbiology, and science in general, you will find with this issue of *Microbiologist*, a leaflet entitled: Standing up for Science 2. If you want to know how to effectively communicate your research through the multitude of media available, then read on — there are some fascinating case studies, hints and tips. But if you know of someone who's already a brilliant science communicator, don't forget there's still time to make a nomination for the SfAM Communications award — see www.sfam.org.uk/grants. Get your nominations in soon, and you could see your nominee tell us all about their communications work at the SfAM summer conference.

editorial

Lucy Harper discusses zoonotic diseases and the communication of applied microbiology

contribute

We are always looking for enthusiastic writers who wish to contribute articles to the magazine on their chosen microbiological subject.

For further information please email the editor, Lucy Harper at: lucy@sfam.org.uk



Lucy Harper

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Subscriptions:

A subscription to *Microbiologist* is included in the annual SfAM membership fee. For further information about the many benefits of membership please see page 6.

Advertising:

Information about advertising in *Microbiologist* and how to submit advertisements can be found on the Society website.

Website: our website (www.sfam.org.uk) is a timely source of up-to-date information on all Society matters and maintains a comprehensive archive of articles and reports on a variety of microbiological topics.

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Erratum

In the 2009 *SfAM* calendar, the image used for May 2009 entitled "Blondes and microbiologists have more fun" was supplied by Pattie Hendrie and Martin Adams. The incorrect spelling of Pattie Hendrie's name appears on the calendar and the Editor would like to extend apologies to Pattie Hendrie for this error.

benefits

The Society for Applied Microbiology is the voice of applied microbiology within the UK and was founded in 1931. Society members play a leading role in shaping the future of applied microbiology, and enjoy many benefits, including:

- The opportunity to apply for one of our many grants or funds
- Eligibility to win any of our awards or nominate a candidate for the SfAM Communications Award
- Access to our five peer-reviewed Journals: *Journal of Applied Microbiology*, *Letters in Applied Microbiology*, *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*
- Free access to the entire collection of digitised back files for *JAM* and *LAM* dating back to 1938
- A topical quarterly magazine, *Microbiologist*
- Substantially reduced rates for attendance at SfAM meetings and conferences
- Networking with worldwide professionals in over 80 countries
- Access to private members area of the SfAM website
- Monthly email bulletins with the latest news from SfAM
- Invitation to the annual *Environmental Microbiology* lecture
- Fostering cross disciplinary research
- A 25% discount on the extensive Wiley-Blackwell collection of titles

Detailed information about all these benefits and more can be found on the Society website at: www.sfam.org.uk

GRANTS & AWARDS: Many grants, awards and prizes are available to members including the W H Pierce Memorial Prize and prizes for student oral presentations and posters at the Summer conference. In addition to these substantial awards, the Society has funds to assist members in their careers as microbiologists. These include the President's Fund, Conference Studentships, Sponsored Lecture Grants and the popular Students into Work Scheme.

Full details of all the Society's grants and awards can be found on the website together with PDF downloadable application forms.

JOURNALS: The Society publishes two monthly journals: *Journal of Applied Microbiology* and *Letters in Applied Microbiology*. We also produce this quarterly colour magazine, *Microbiologist*, which contains features, topical news stories and full details of our meetings. The Society is also a partner with Wiley-Blackwell in the monthly journals *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*.

All Full and Student members receive free access to the online versions of the Society's journals, and can also submit papers to our journals via an online submission service.

MEETINGS: We hold three annual meetings; the winter meeting is a one-day meeting with parallel sessions on topical subjects. The spring meeting is a one-day meeting tailored for personnel in clinical microbiology. The summer conference is held every July and comprises a main symposium, a poster session, the AGM and a lively social programme. All members are invited to our prestigious annual lecture held to commemorate the success of our *Environmental Microbiology* journal. We also hold joint ventures with other organisations on topics of mutual interest.

WEBSITE: The website is the best source of detailed information on the Society and its many activities. It has fully interactive membership areas where you can find archive issues of *Microbiologist*, exclusive SfAM documentation and much more.

membership options

■ **Full ordinary membership** gives access to our many grants and awards, online access to the *Journal of Applied Microbiology*, *Letters in Applied Microbiology*, *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*, copies of *Microbiologist*, preferential registration rates at Society meetings and access to the members areas of the website.

■ **Full student membership** confers the same benefits as Full membership at a specially reduced rate for full time students not in receipt of a taxable salary.

■ **Associate membership** is only open to those with an interest in applied microbiology without it being a prime aspect of their job. For example, school teachers and those taking a career break; on maternity leave, or working temporarily in other areas. It does not provide access to any journals or Society grants and awards.

■ **Honorary membership** of the Society is by election only and this honour is conferred on persons of distinction in the field of applied microbiology. Honorary members have access to our online journals.

■ **Retirement membership** is available to Full members once they have retired from their employment. Retired members are entitled to all the benefits of Full membership except grants and access to the Society's journals.

■ **Corporate membership** is open to all companies with an interest in microbiology.

Corporate members benefits include:

- Quarter page advertisement in each issue of *Microbiologist* (which can be upgraded to a larger size at discounted rates)
- the opportunity to publish press releases, company news, etc., in each issue of *Microbiologist*
- FREE banner advert on the Society Website with a direct link to your company site.
- Up to three members of company staff attending Society meetings at members' rate (this means a 50% discount on non member registration rate).

JOIN US!

You can apply for membership on, or offline. To apply offline, please contact the Membership Co-ordinator, Julie Wright on +44 (0)1234 326846, or email julie@sfam.org.uk. Alternatively, write to her at:

The Society for Applied Microbiology, Bedford Heights, Brickhill Drive, Bedford MK41 7PH, UK

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In December last year my first President's Dinner was held at the headquarters of the Royal Pharmaceutical Society of Great Britain (RPSGB) in London. The reason for holding it there was because I was trained as a pharmacist and have been a member of the RPSGB for over 30 years so I wanted to give guests an insight into my professional body. Over the years I have been involved with a number of its organisational and scientific committees where my microbiological expertise was utilised. Latterly, however, I found that microbiology was increasingly regarded as a fringe subject, which is odd because if you ask NHS workers what the biggest single issue is facing them today they will nearly all say healthcare associated infections. Despite this, the amount of microbiology being taught in medical schools and schools of pharmacy is decreasing.

president's column

Geoff Hanlon goes in search of microbiological patron saints

However, the pharmacy profession and the RPSGB in particular is currently undergoing a period of change as it adapts to the Government's 2007 White Paper dealing with the regulation of the healthcare professions. Since its inception in 1841 the RPSGB has carried out the dual functions of regulation and professional leadership but that now has to change. By 2010 a new General Pharmaceutical Council will be formed to carry out the regulatory role and a new professional body will perform the remaining functions including leadership, advocacy, professional development and education. This new professional body has made the decision to open its doors to pharmacists and non-pharmacists alike and so from 2010 we will be seeing a quite different landscape and one which provides opportunities for us.

As a longstanding member of RPSGB I have to say that the benefits one gets from being a member of SfAM are far greater and at a fraction of the cost. Registered pharmacists receive a weekly copy of a paper called the *Pharmaceutical Journal* which is directed primarily at the hospital and community sectors and in my office mainly gets filed in the bin unread. For a reason not immediately clear to me, I opened one issue recently and read an intriguing article concerning the patron saint of pharmacists. His name was St John Leonardi and he worked as a pharmacist's apprentice before forming the Confraternity of Christian Doctrine in 1579. As well as writing a number of influential texts he spent time tending the sick and in fact died of the plague in 1609 contracted whilst working with his patients. When I say that the article was about the patron saint of pharmacists I should say one of the patron saints, because it appears that, although St John is very important, pharmacists are patronised by no fewer than eight saints.

This fact led me to wonder: who is the patron saint of microbiologists? In an effort to find out, my first port of call was, of course, *Google* which led me to a website with over

5,000 saints and a listing of their patronage. Looking up the topics under the letter M, I came across a myriad of professions and causes (many of which I didn't understand) but there was no mention of microbiologists. Miners, midwives and even murderers were represented but not us. Maybe I was looking at the wrong section. An examination of the letter B for bacteriologists yielded bachelors, bailiffs and even bald people but still no joy for us. I thought that maybe microbiology was too new a science to be eligible, but why then were astronauts, air crews/pilots and television presenters included? It appears that other health professions have been recognised since doctors, nurses, medical technologists, hospital workers, dieticians, radiologists and of course pharmacists are just a few of those included.

This experience left me feeling a little depressed; not that being without a patron saint is of particular significance in itself, but it reinforces the fact that we as microbiologists are under-recognised for what we do. Individually there is a limited amount we can do to address this. It is therefore all the more important that as individuals we engage with learned societies such as SfAM and that one of the main functions of this society should then be to promote the subject of microbiology to a wider audience. In order to do this SfAM must itself engage with bodies such as the newly formed professional body emerging from RPSGB to ensure microbiology is pushed up the agenda in the education of pharmacists and doctors. In addition, we must become more closely involved with the Biosciences Federation as it goes through its merger with the Institute of Biology to form the new Royal Society of Biology. This new organisation will be acting as a voice piece for the biosciences at a political level and we should be helping to influence its formation and future direction.

We are facing a challenging future both economically and organisationally but thankfully we now have a robust office structure and an enthusiastic and talented committee to help take things forward. We have to be aware of opportunities as they present themselves and not be afraid to take advantage of them.

In closing, it may be worth saying that as a university professor of pharmaceutical microbiology I have the patronage of no fewer than 20 saints; two covering lecturers; five specifically looking after professors; four overseeing scholars, two for scientists and of course eight for pharmacists. In case you think I can't add up there is one saint common to two of those categories.



Professor Geoff Hanlon
President of the Society

Erratum

The article by Dr Bernard Dixon which appeared in the December 2008 issue of *Microbiologist*, contained information regarding his latest book, *Animalicules*. This information wrongly stated that the book will be published in December 2009. This should have read "His latest book *Animalicules* will be conventionally published by the American Society for Microbiology in December 2008". The Editor would like to extend apologies to Dr Dixon for this error.

ceo's column

Philip Wheat reports on the latest developments within the Society

Once again in 2009 we are holding the 3rd in the series of meetings entitled: Broadening microbiology horizons in biomedical science (22nd April, Aston University, see page 19 for full details).

We have introduced a new feature for this meeting. The inaugural Procter and Gamble lecture will take place at the start of the meeting. The Procter and Gamble award is a new initiative for this year. We were approached by representatives of Procter and Gamble

because they wanted to present an award in Europe, to an individual who had made a significant contribution in the field of applied microbiology. They chose the Society to administer and make such an award in Europe as a counterpart to a similar award they present in the United States, which is administered by the American Society for Microbiology. I am pleased to announce the first winner of Procter

and Gamble award is Professor Sally Bloomfield.

Professor Bloomfield has been a long standing Society member and has an impressive record of research in the field of applied microbiology. In recent years this has mainly focused on areas associated with home hygiene. One of the conditions of the award is that Professor Bloomfield will present the opening address at the forthcoming meeting, which will be entitled: *"The fall and rise of home hygiene."*

Following the success of the inaugural *Environmental Microbiology* lecture in 2008 I am delighted to announce that the Society will once again be holding a similar event in 2009.

This year's event will take place at the Royal Society of Medicine, London on 12th October 2009 and will be presented by Professor Edward de Long. All members will find their formal invitation in this issue (see opposite). Numbers are restricted so please indicate whether you are able to attend as soon as possible. For members unable to attend, I can also confirm that once again the lecture will be available online immediately after the event.

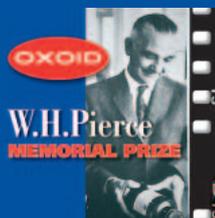
Another new benefit for 2009 has been the introduction of *Environmental Microbiology Reports*. Member of the Society are now entitled members to access **five journals** online at no extra cost.

Again in 2009 we will be exhibiting at several international conferences. We will be attending the American Society for Microbiology meeting in Philadelphia in May. This will be followed by the International Food Technology meeting which is in Anaheim in June. Finally, we will also be attending the International Association of Food Protection meeting in Dallas during July. As well as enrolling new members we always appreciate meeting existing members at these events. Also do not forget if you would like to attend these meetings and you have insufficient funding why not apply for one of Society's grants such as the President's Fund? Or if you are student and you wish to attend the Society's summer conference (Manchester, 6 - 9 July — see page 21 for details) why not apply for a studentship which will cover registration, travel and subsistence costs. If you are planning to attend any of the meetings mentioned please stop by the stand and collect your member's lapel badge.



Philip Wheat
Chief Executive Officer

Call for nominations for W H Pierce Prize



Do you know a young microbiologist (under 40 years of age) who has made a substantial contribution to microbiology? If so, why not nominate them for this prestigious and substantial award which is now worth £3,000. The award was instituted in 1984 by Oxoid to commemorate the life and works of the late W H (Bill) Pierce, former chief bacteriologist at Oxoid Ltd and a long time member of the Society. The prize is presented annually at the summer conference. Full members wishing to make a nomination for the 2009 prize should write in

confidence to the Hon. General Secretary, Dr Mark Fielder, at the Society Office in Bedford, including a full CV of the person nominated and a letter of support. Please note there are no official forms for this award.

Closing date for nominations is 27 April 2009.

Please note that application is through nomination by Full members of SfAM only.

Call for Nominations to Committee

There will be up to five vacancies on the SfAM Committee in **July 2009**. Nominations are invited from all Full members of the Society for these vacancies.

Nominations must be made in writing and received by the Society Office by **8 May 2009**. Should nominations exceed vacancies, election will be by a system of postal voting arranged by committee.

Membership Changes

NEW MEMBERS

We would like to warmly welcome the following new members and hope that you will participate fully in the activities of the Society.

Algeria

B. Deraa

Uganda

C. Muyanja

Greece

F. Parlapani

India

S. V. K. Chaganti

Ireland

C. Hill; S. O'Brien

Italy

P. Carnevali

Japan

Y. Magae

Nigeria

S. O. Ajado; A. R. Ezeh

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United Arab Emirates

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W. Abdelrahman; E. Adukwu; S. Allen; A. Benson;
A. Bulsiewicz; L. Cavill; R. Fernandes; D. Field;
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B. Halliday; P. Jenkins; L. P. Joseph; C. Kim; J. Landau;
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S. Mohammed; E. L. Moynihan; C. O. Oladoye;
D. Purchase; E. Rashid; M. F. Scott; J. C. R. Shinwell;
H. Siani; H. Simpson; D. C. Spencer; F. Varga;
D. I. Walker; C. Walker; D. P. White

USA

S. Adhya; P. Fedorka-Cray; S. Shannon

UK NEW CORPORATE

AES-Chemunex Ltd

SfAM Environmental Microbiology Lecture — invitations in this issue!



Following the success of the inaugural *Environmental Microbiology* lecture in 2008 we are delighted to announce that this year's event will take place at the Royal Society of Medicine, London on 12th October 2009. The *Environmental Microbiology* lecture will be presented by Professor Edward de Long of Massachusetts Institute of Technology (MIT), USA. He will present a lecture entitled '*Deciphering microbial community dynamics, from genomes to biomes*'. You will find your formal invitation in this issue of *Microbiologist*. Don't forget to RSVP to be in with a chance of booking a place. For members unable to attend, the lecture will be available online immediately after the event.

membership matters

SfAM President tells us why evidence matters

To mark the 40th anniversary of the Medicines Act (1968) and the start of a two-year season of activities by Sense About Science on why evidence-based medicine matters, SfAM President, Professor Geoff Hanlon was asked to comment on why he thinks evidence matters. To see what he had to say and to browse through others' opinions as to why evidence is important, visit:

<http://www.evidencebasedmedicinematters.org/indy.php?id=79>

SfAM welcomes new Publications Subcommittee

To ensure the *Microbiologist* remains a top quality publication brimming with topical, interesting and accurate articles for our members, SfAM has launched a new **Publications Subcommittee** who will provide invaluable input into sourcing authors, researching themes and helping to generate content for the magazine. Here, we introduce each of the new Editors:



Features Editor

Claire Cassar

I graduated in microbiology from King's College, University of London in 1992. After completing a Masters degree in Aquatic Resource Management I went on to obtain my Ph.D in 1998 working in the field of food microbiology and food safety. Following my Ph.D, I joined the Cellular and Molecular Sciences group at St. Georges Hospital Medical School, London as a postdoctoral researcher where I worked on multi drug resistance mechanisms in *Plasmodium falciparum* and then characterisation of factors involved in the suppression of HIV replication.

I joined the Food & Environmental Safety Department at the Veterinary Laboratories Agency (VLA) in 2001 where I undertook a role as the enteric diseases detection and diagnostics manager, leading a team of scientists to deliver work under zoonoses surveillance and endemic disease portfolios. In 2005 I joined Med-Vet-Net, an EU network of scientific excellence working for the prevention and control of zoonoses and food borne diseases, to provide project management support to the Med-Vet-Net Project Manager. Subsequently I moved to the biotechnology department at VLA where I am currently part of a team assuring test validation for detection and

diagnostic tests developed or adapted for use across VLA. Additionally my responsibilities include providing support to the Transmissible Spongiform Encephalopathy (TSE) Central Reference Laboratory (CRL) and National Reference Laboratory (NRL) based at VLA.

I was very interested in taking up an editorial position as I have an interest in the communication of microbiology and science in general. Additionally and as importantly I want to give something back to the society and this role provides such an opportunity.



Features Editor

Louise Fielding

I am the Director of Enterprise in the Cardiff School of Health Sciences at University of Wales Institute, Cardiff (UWIC). I graduated from the University of Reading with a B.Sc. in food technology and a Ph.D in food microbiology. I am a member of the Welsh Food Advisory Committee and the UK Foodborne Disease Strategy Consultative Group of the Food Standards Agency and I teach basic and applied microbiology for a variety of courses at UWIC. My research interests include: the effect of novel biocides and detergents for use in the food industry and hospital environments; the application of Hazard

Analysis and Critical Control Points (HACCP) by the food industry; the control of biofilms in the food industry and the development of novel cleaning systems to remove biofilms from narrow-bore tubing systems; detection and identification of *Listeria monocytogenes* in manufacturing plants and implementation of intervention strategies to control the spread of the organism. I have been a member of SfAM since 1990, a member of the SfAM Committee since 2007 and I'm also a Fellow of the Institute of Food Science and Technology (IFST) and a member of the International Association for Food Protection (IAFP). The publication process has always interested me and I'm looking forward to further contributing to the work of the Society through my role as Features Editor.



Grants Editor

Louise Hill-King

I am currently a Team Leader in the Microbiology Department at Frimley Park Hospital, Surrey. I graduated from the University of Sheffield in 1987 with a B.Sc. in chemistry and astronomy. This raised the eyebrows of my tutor (a certain Philip Wheat) when I enrolled onto the M.Sc. course in Medical Microbiology at Sheffield. I haven't escaped from Medical Microbiology since. Apart from a year as a Research Scientist for Cambridge Life Sciences in Ely, I have spent all of my working life in NHS laboratories in Sheffield, Leeds, Morley and now Frimley.

I run lunchtime Continuing Professional Development (CPD) meetings in my own laboratory and am involved with Wessex Applied Microbiologists (WAM), organising evening meetings across central, southern England.

I was volunteered onto the Publications Subcommittee by a grammatically challenged SfAM committee member. They were tired of having their spelling corrected so thought they'd direct my attention elsewhere! My role on the Subcommittee is Grants Editor, editing reports submitted by those who've been awarded SfAM grants to attend meetings, courses and conferences they wouldn't otherwise have been able to attend.



Regular Content Editor

Andrew Fox

I have worked for the Health Protection Agency and former Public Health Laboratory Service in the North West of England as a microbiologist and clinical scientist for many years. Communication is a fundamental part of a scientist's role and good channels of communication are particularly valuable in science and specifically microbiology.

Throughout my association with SfAM I have always valued the Society's contribution in communicating the breadth of issues in microbiology. The *Microbiologist* is an excellent platform for communicating and when the request for editorial support was made I was glad to contribute in whatever way I could. In my opinion the *Microbiologist* appeals to young scientists and provides invaluable educational material with which I am proud to be associated.



Journal of Applied Microbiology

The following articles published in 2008 were the most downloaded articles from Journal of Applied Microbiology between October – December 2008:

Control of enteric pathogens in ready-to-eat vegetable crops in organic and 'low input' production systems: a HACCP-based approach. C. Leifert, K. Ball, N. Volakakis and J.M. Cooper. **Vol. 105**, No. 4, October 2008.

Novel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides. A. Parisien, B. Allain, J. Zhang, R. Mandeville and C.Q. Lan. **Vol. 104**, No. 1, January 2008.

Salmonella must be viable in order to attach to the surface of prepared vegetable tissues. E.J. Siggers, C.R. Waspe, M.L. Parker, K.W. Waldron and T.F. Brocklehurst. **Vol. 105**, No. 5, November 2008.

Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. G.T. Macfarlane, H. Steed and S. Macfarlane. **Vol. 104**, No. 2, February 2008.

Investigation into the effect of detergents on disinfectant susceptibility of attached *Escherichia coli* and *Listeria monocytogenes*. J.T. Walton, D.J. Hill, R.G. Protheroe, A. Nevill and H. Gibson. **Vol. 105**, No. 1, July 2008.

Letters in Applied Microbiology

The following articles published in 2008 were the most downloaded articles from Letters in Applied Microbiology between October – December 2008:

Metagenomics. R.D. Sleator, C. Shortall and C. Hill. **Vol. 47**, No. 5, November 2008.

Preliminary characterization of exopolysaccharides produced by a marine biofilm-forming bacterium *Pseudoalteromonas ruthenica* (SBT 033). P. Saravanan and S. Jayachandran. **Vol. 46**, No. 1, January 2008.

A simple and fast method for determining colony forming units. S. Sieuwerts, F.A.M. de Bok, E. Mols, W.M. de Vos and J.E.T. van Hylckama Vlieg. **Vol. 47**, No. 4, October 2008.

Real-time subtyping via PFGE reveals potential epidemiological relatedness among human salmonellosis cases in Northern Italy. S. Lomonaco, D. Nucera, B. Griglio, L. Pezzoli, V. Marotta I. Casonato and T. Civera. **Vol. 47**, No. 4, October 2008.

Disruption of fungal and bacterial biofilms by lauroyl glucose. D.H. Dusane, J.K. Rajput, A.R. Kumar, Y.V. Nancharaiah, V.P. Venugopalan and S.S. Zinjarde. **Vol. 47**, No. 5, November 2008.

Environmental Microbiology

The following articles published in 2008 were the most downloaded articles from Environmental Microbiology between October –

December 2008:

The microbial nitrogen cycle. M. S. M. Jetten. **Vol. 10**, No. 11, November 2008.

Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. J. I. Prosser and G. W. Nicol. **Vol. 10**, No. 11, November 2008.

Sorting out the mix in microbial genomics. M. Y. Galperin. **Vol. 10**, No. 12, December 2008.

The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. G. W. Nicol, S. Leininger, C. Schleper and J. I. Prosser. **Vol. 10**, No. 11, November 2008.

Denitrifying bacteria anaerobically oxidize methane in the absence of Archaea. K. F. Ettwig, S. Shima, K. T. van de Pas-Schoonen, J. Kahnt, M. H. Medema, H. J. M. op den Camp, M. S. M. Jetten and M. Strous. **Vol. 10**, No. 11, November 2008.

Microbial Biotechnology

The following articles published in 2008 were the most downloaded articles from Microbial Biotechnology between October – December 2008:

Microbial-based motor fuels: science and technology. L. P. Wackett. **Vol. 1**, No. 3, May 2008.

Environmental Microbiology meets Microbial Biotechnology. J. L. Ramos, M. Fernández, J. Solano, P. Pizarro-Tobias and C. Daniels. **Vol. 1**, No. 6, November 2008.

Biofuels from microbes: a comprehensive view. F. Rojo. **Vol. 1**, No. 3, May 2008.

Metabolic engineering to enhance bacterial hydrogen production. T. Maeda, V. Sanchez-Torres and T. K. Wood. **Vol. 1**, No. 1, January 2008.

Genomics of dairy fermentations. R. J. Siezen and H. Bachmann. **Vol. 1**, No. 6, November 2008.

Environmental Microbiology Reports — first articles now online!

Read the first three Brief Reports published in *Environmental Microbiology Reports* – a new journal published jointly by SfAM and Wiley-Blackwell:

Synthesis of glycine betaine from choline in the moderate halophile *Halobacillus halophilus*: co-regulation of two divergent, polycistronic operons. Janin Burkhardt, Xaver Sewald, Bettina Bauer, Stephan H. Saum and Volker Müller.

Physiological role and regulation of glutamate dehydrogenase in *Prochlorococcus sp.* strain MIT9313. Oriol Alberto Rangel, Guadalupe Gómez-Baena, Antonio López-Lozano, Jesús Diez and Jose Manuel García-Fernández.

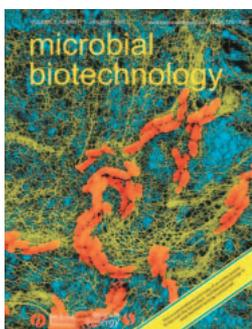
The gut microbiota of toll-like receptor 2-deficient mice exhibits lineage-specific modifications. Eric J. Albert, Katrin Sommerfeld, Sharon Gophna, Jean S. Marshall and Uri Gophna



Lucy Collister
Wiley-Blackwell

journalWatch

News about the Society's journals



Hugh Lamont, North West Communications Manager at the Health Protection Agency (HPA) describes his work and the importance of maintaining a two-way relationship with the news media

I am responsible for external and internal communications in the HPA's North West Regional Office. Dealing with the news media is a significant part of that job. I treat the media as partners who will help us get across important health protection messages to mass audiences and I try to be proactive, working to a calendar of events. We promote seasonal flu jabs in the autumn, issue advice on how to avoid and/or deal with norovirus infection as winter approaches, use World AIDS day in December to issue safer sex messages, seize on local outbreaks of measles or mumps to promote MMR vaccine and issue food safety tips as Christmas approaches, to name but a small number.

mediawatch

microbiology in the news

If you have any views on science in the media which you think should feature in this column, please send them to the Editor at: lucy@sfam.org.uk

In between these events we have publication of data on healthcare associated infections (HCAs), vaccination cover data and the latest quarterly figures for sexually transmitted infections (STIs). All of these are of interest to the press and there's often a good story to tell. It's my job to interpret the data for the media and that's where I draw upon the expertise of colleagues. I work closely with the HPA leads for such specialisms as tuberculosis (TB), influenza, immunisation, gastrointestinal infections, chemical hazards and radiation, to name just some. I also draw on the expert knowledge of colleagues in the HPA Regional Laboratory in Manchester. These experts provide the ammunition and I pull the trigger, hopefully at the right time and with an accurate aim.

These things we plan in advance. Other developments we hear about in the course of our travels or are advised about by local teams or partner agencies. A presentation to the HPA board on the North West Regional Laboratory's contribution to the development of a new meningococcal A vaccine for use in the meningitis belt of sub-Saharan Africa, for example, led to the production of a press release that generated useful publicity for this invaluable work. The same unit's contribution to the development of a new meningococcal B vaccine for use in the UK received international attention.

It is my role to give honest and accurate information to the public, raise the profile of the HPA and protect its reputation. I am usually the first point of contact for the media in the North West Region and a gateway to our expertise.

I attend incident and outbreak group meetings, advising on communications strategy. It may be a case of meningitis in a school, a cluster of salmonella cases associated with a restaurant, an outbreak of *E. Coli* O157 associated with a nursery school or farm visit, a healthcare worker with TB or hepatitis B or C or HIV infection, listeria associated with sandwiches, norovirus on board a cruise ship or an investigation into a cluster of Legionnaires Disease cases in a particular community.

In planning a communications strategy in these circumstances, I ask myself: is there a public health requirement to go public in this instance? Sometimes the answer is yes. If the risk is ongoing,

we need to alert people to it and recommend actions that people can take to protect themselves. An example of this would be the need to do a food recall if we suspected that people may still have contaminated products in their refrigerators and the fastest way to alert the public to the risk would be through local radio announcements.

It is critical when making public announcements to ensure that our advice is sound, scientifically-based and not at odds with pronouncements by other recognised national authorities on the subject, or that of our local partners. Consistency of message is essential. When we believe that there is a case for issuing new advice or changing the accepted advice on any given subject, we must persuade our partners at local and national level of the validity of our case. If the scientific evidence is strong, that will not usually be a problem.

Often when dealing with an incident or outbreak, it isn't necessary to go public, although we are always ready to react to press calls with honest and accurate information should the story "leak." Being open means taking appropriate action at the right time and being prepared to justify our decisions if challenged. It does not always mean giving a story to the press. If there is no on-going public health risk, but a real risk that in going public someone's well-run and safe business (say a restaurant or nursery school) would be severely damaged or even ruined, I would usually advise that our responses to the press should be reactive. However, one should always be ready for the press call with lines to take that fully explain the situation, leave no room for speculation and have been agreed with all partners on the incident group.

From time-to-time we involve the media as partners in campaigns. I have worked with regional television, newspapers and local radio to promote MMR vaccine uptake and safer sex and to educate the public about norovirus infection. A safer sex campaign with the radio station *Key103* and three universities in Manchester secured a prestigious Sony Award for the radio station. A campaign on norovirus infection in the winter of 2007 was said by GPs to have helped ease pressure on their surgeries.

It's important to trust the media. The majority of reporters and their editors work hard to ensure that reports are accurate and substantiated. I've made several references to press releases in this article, but press releases by themselves aren't enough. It's important to have personal contact with journalists so that you can inform their reports by giving them a real understanding of issues and "sensitivities" and such background information as they will need. I also try to make life easy for them by respecting their deadlines, finding "experts" who can explain complex subjects in simple lay terms and interpret data. It is a partnership that in most instances works.



Hugh Lamont

North West Communications Manager, Health Protection Agency

our policy on the media

We will:

- always do our best to provide facts, information and explanation.
- if speculation is required, explain the rationale behind that speculation.
- desist from hyping a story—whether it is the journalist or the scientist doing the hyping.



MED • VET • NET

Geographic information systems as an integral part of epidemiology

med-vet-net

Med-Vet-Net is a European Network of Excellence that aims to improve research on the prevention and control of zoonoses by integrating veterinary, medical and food science research. Comprising 15 European partners and over 300 scientists, Med-Vet-Net will enable these scientists to share and enhance their knowledge and skills, and develop collaborative research projects.

information

For more information about Med-Vet-Net, visit:
www.medvetnet.org/
 or contact Teresa Belcher on:
 +44 (0)1908 698810

A geographic information system (GIS) captures, stores, analyses, manages, and presents data that refer to or are linked to geographic location. A GIS can be used to construct maps and analyse spatial data.

Geographic information system applications are tools that allow users to create interactive queries (user created searches), analyse spatial information, edit data and maps, and present the results of all these operations.

Geographic information system technology can be used for scientific investigations, resource management, asset management, archaeology, environmental impact assessment, urban planning, cartography, criminology, geographic history, marketing, logistics, and other purposes. For example, GIS might allow emergency planners to calculate emergency response times in the event of a natural disaster. Geographic information systems might also be used to find wetlands that need protection from pollution, or a GIS can be used by a company to site a new business location taking advantage of a previously under-served market.

Using GIS to track disease

There has always been an integral geographical element to epidemiology in accounting for the location of disease outbreaks. A GIS may be used to analyse the distribution of zoonotic agents and identify unknown risk factors for zoonotic diseases. This methodology can also be helpful for basic surveillance and outbreak investigations, or to analyse how contaminated foods are distributed from one country to another.

“When maps start to become used more, they are really good tools. In particular, they are really good for non-scientists to look at because they immediately give you a lot of information,” said Steen Ethelberg, leader of the Med-Vet-Net Workpackage 6, based at the Statens Serum Institut (SSI) in Denmark.

One of the key factors influencing the spread of zoonotic diseases is the movement of livestock and their products. The variability of movement in time and space can be considerable, and is influenced by environmental variation in animal production systems as well as economic factors. An understanding of both animal movement and product dynamics is essential for control and prevention of animal diseases such as foot-and-mouth disease (FMD), bovine spongiform encephalopathy (BSE), avian

influenza and bluetongue. Therefore, integration of trade dynamics into a geographical surveillance system is vital to enhance understanding and make better policy decisions which support a reduction in cases of zoonoses. At present, information regarding the movement of animals is collated at a national level. This means that very different types of information and levels of detail are available in each of the Member and Associated States.

With recent advances in spatial analyses, new possibilities have emerged with potential for the study of zoonotic and food borne diseases. It is essential for data from both the public health and veterinary sectors to be integrated to contribute to the greater understanding and control of the epidemiology of zoonoses.

Med-Vet-Net Workpackage established

When Med-Vet-Net began in 2004, it was recognized that there was a need for the development of tools and skills for the geographical surveillance of zoonoses.

“GIS has been around for 40 years in different scientific communities, but only recently has Med-Vet-Net taken up an interest,” said Martin Rudbeck Jepsen, Deputy Workpackage Leader, also at SSI. *“Even though it is an old discipline to map cases of different diseases, the use of GIS software has not been widespread.”*

Thus, Workpackage 6 (WP6), entitled: ‘Development and application of geographical information systems and spatio-temporal methods in the epidemiology of food borne bacterial zoonoses’ was implemented to establish a European network for the use of GIS within Med-Vet-Net Partner Institutes. This WP has aimed to build capacity among the participants primarily through the conduction of scientific projects.

There are currently three projects in Workpackage 6: the first project concerns the pathogen verocytotoxin-producing *Escherichia coli* (VTEC) which, as well as causing diarrhoea may lead to haemolytic uraemic syndrome (HUS) a serious condition that can be fatal, particularly in children. VTEC has a natural reservoir in ruminants, but the routes of transmission to humans have not been fully elucidated. Workpackage 6 is examining whether the spatial pattern of human VTEC cases is related to the spatial location of cattle farms, suggesting direct transmission to humans from live animals or their faeces. The project relies on existing recorded data on demographics, VTEC patients and cattle farms. These data are analysed using GIS and spatial statistical analysis such as *buffer analysis*, and *Moran’s I* in combination with statistical regression models. The project involves five countries: England and Wales, the Netherlands, Italy, Sweden and

Denmark and, in addition, Germany and Scotland are part of the project group. Because geography and the types of data available differ strongly between participating countries, each country is conducting individual analyses using different methodology. Results are now available from all participating countries.

The second project aims to construct a dynamic, interactive web atlas of the incidence of human salmonellosis in Europe. This project builds on the previous *Salmonella* Atlas project (www.epigis.dk) and will, if successful, deliver a new version of this atlas which is more user-friendly. Through a new interface, it will be possible to create maps with a finer temporal detail (including 'zoomable' maps) and the data that is displayed will be more detailed, adding many more serotypes and other parameters, such as phage types, and information about travel-related cases (for more information on the *Salmonella* Atlas see: www.eurosurveillance.org/ViewArticle.aspx?ArticleId=8016).

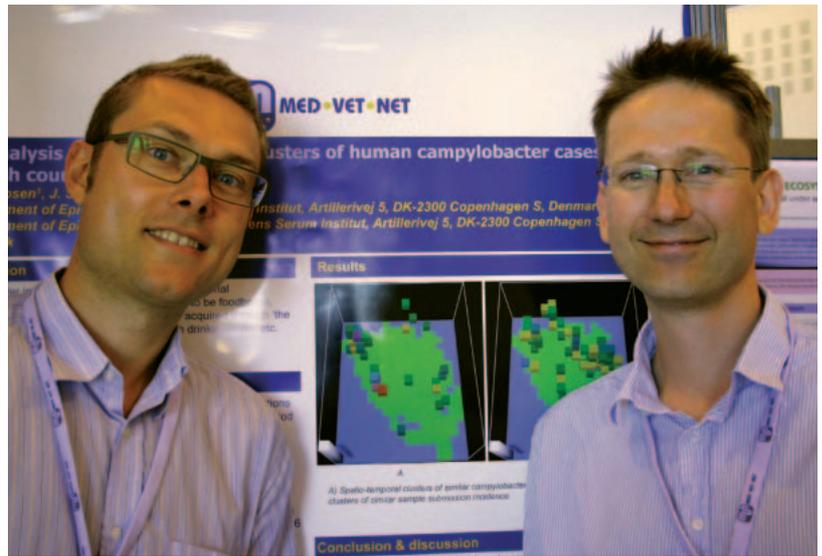
In the third project a series of descriptive spatial and temporal analyses of selected types of salmonella in Europe are being performed. The aim is to gain a better understanding of the spatio-temporal dynamics of the distribution of the selected salmonella types together with the epidemiology of these types. This project uses so-called Enternet data consisting of surveillance data on laboratory confirmed salmonella cases collected by a number of European countries. A series of ten different sero- or phage types of salmonella, which are known to have caused outbreaks or are of importance for other reasons, have been selected and their distribution is being analysed using various statistical methods including the SatScan™ technique. Because surveillance data are generally very difficult to compare between European countries, the data are adjusted by analysis of the proportion of each salmonella subtype among all reported salmonellas, rather than by analysis of the actual reported number of cases.

In addition to these core projects, work was also carried out on the *Salmonella* Atlas project (www.epigis.dk), where additional data were added, producing a new set of maps.

To build up epidemiological GIS capacity in Europe, a GIS course was taught by members of Workpackage 6 to provide the participants with skills in epidemiological mapping procedures and understanding of the concepts in advanced spatial statistical analysis procedures.

Achievements to date

Workpackage 6 has initiated preliminary studies demonstrating that spatio-temporal data can contribute to understanding disease dynamics within the European Union (EU). It is evident that the tools, expertise and datasets are



now available to build on these preliminary studies.

There are a variety of surveillance, movement and spatial datasets collected by each Member State. These differ in quality, completeness, spatial scale and format. Thus, harmonisation of the spatial data infrastructure is vital to ensure optimal integration at a European level (e.g. in storage, access, organization, manipulation, compatibility and dissemination of spatial data). Harmonisation of these data will be the next challenge facing scientists.

"We are hoping that GIS will be a focal point for some of the new EC agencies such as EFSA and ECDC. We hope these agencies will be interested in using GIS more, and in collecting a geographical data set so that they are able to show geographical patterns in the spread of diseases," said Ethelberg.

"I believe that GIS will become an integral part of the work of European agencies like ECDC to produce maps of incidences across Europe," added Rudbeck Jepsen. *"Maps are so visually appealing and this type of information will give you an overview much faster than looking at incident rates in a table."*

A major achievement of this Workpackage is that GIS is now on the agenda within the zoonotic disease community in Europe. *"GIS has great potential within epidemiology but it hasn't been used much,"* Ethelberg said. *"Within this Workpackage we have created a network across the Med-Vet-Net institutions and we hope that GIS will continue to be promoted even more."*

▲ *Martin Rudbeck Jepsen (left) and Steen Ethelberg (right) presented a number of posters at the Med-Vet-Net Annual General Meeting in St Malo in June 2008*

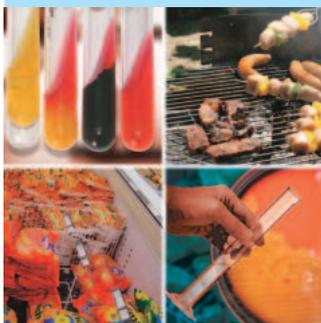


Teresa Belcher
Communications Director
Med-Vet-Net

information

For more information about the Society's meetings please visit the website at: www.sfam.org.uk

You can also find details of this year's summer conference on page 21 of this issue of *Microbiologist*



Winter Meeting 2009 Report

Enterobacteriaceae in foods and rapid methods in microbiology

Royal Society, Carlton House Terrace, London, Wednesday 14 January 2009

After a warm welcome from Geoff Hanlon, SfAM President and Chair of the morning session, he introduced the first presentation, the Denver Russell memorial lecture. This lecture was established in 2006 in the memory of Denver Russell, a leading world authority on biocide usage and its possible association with antibiotic resistance. Denver spent almost his entire career at the Cardiff School of Pharmacy, during which time some 45 of his research students obtained PhDs and he produced over 450 publications and authored or edited 16 books. Denver joined the Society for Applied Microbiology in 1971 and had always been closely associated until his death in 2004. Very appropriately, this year's memorial lecture was presented by Professor Stephen Denyer (Head of the Welsh School of Pharmacy, Cardiff University) who spoke on a topic dear to Denver Russell: the identification and design of effective preservatives for control of spoilage in pharmaceuticals and cosmetic products. He spent a little time on biocide use in disinfection, taking the audience through the complexity of preservation for pharmaceutical and cosmetic products. He accounted for the many factors influencing the impact of a preservative, including the chemical and physical properties of the product as well as the biocide, and defence and recovery systems on the site of the targeted micro-organisms. Having provided an appreciation of the mechanistic background of the way in which preservatives target specific sites in a micro-organism, he presented the physico-chemical

behaviour of biocides related to their inhibitory activity and delivery. Professor Denyer then went on to discuss the "Holy Grail" in the design of successful preservation systems, the quest to find a combination of biocides or other factors that exert a synergistic effect, which he described as being like Don Quixote fighting imaginary windmills. In many cases, the antimicrobial impact enhancing effects noted in experiments are not truly synergistic, rather they are a result of the nonlinear behaviour between the concentration of a biocide and its antimicrobial impact. Due to the latter, the dilution of a biocide may have a disproportional effect the likes of which need to be duly accounted for in practice. Professor Denyer closed his interesting presentation with a look into the future, where optimization of preservation by design is an important strategy in view of the strong pressures not to introduce new chemicals. Jean-Yves Maillard presented Prof. Denyer with a token of the memorial lecture, this year sponsored by Ciba and Steris.

The second lecture was presented by Dr. Christopher Baylis (Campden Brewery Research International) who spoke about Enterobacteriaceae in food. He began by discussing the (ever changing) taxonomy of this family of microorganisms. In his lecture, Dr. Baylis accounted for the important role Enterobacteriaceae play in food microbiology. Many of these organisms are (opportunistic) pathogens for humans or animals and among them are some important emerging human pathogens. He stressed the importance of these

bacteria in food spoilage and as prime contributors to food wastage, aspects which have a major impact in everyday practice of the food industry, but are not always appreciated by food microbiology professionals and researchers. Dr. Baylis discussed the use of *E.coli*, coliforms or Enterobacteriaceae as indicators of hygiene or of food or water contamination, providing a perspective on the appropriateness of such use. He closed his presentation with a thorough review of detection and enumeration methods for specific representatives of the Enterobacteriaceae, including classic and rapid methodologies.

The programme then continued with a presentation by Dr. Peter Wilson (University College London Hospitals) who discussed findings of clinical trials on the reduction in methicillin-resistant *Staphylococcus aureus* (MRSA) acquisition and bacteraemia through rapid detection methods. MRSA in hospitals leads to increased public health costs as it is the cause of surgical site infections. Dr. Wilson and colleagues found that hospitals may be able to significantly reduce the occurrence and spread of MRSA, when they are able to identify and treat MRSA positive patients immediately after admission to the hospital. Key to successful identification is the use of an adequate method to screen and identify MRSA infected patients. However, critically important to the success of screening methods is the discipline of staff in applying the screen and complying to hygiene measures. Dr. Wilson used molecular methods to rapidly screen patients, and this contributed to the hospital successfully meeting government targets in MRSA reduction.

Leon Gorris

Afternoon – Session A - Rapid detection and identification in microbiology

One of the afternoon sessions was chaired by Andrew Sails who introduced the first lecture: microbial DNA sequence profiling of human pathogens by mass spectroscopy, which was given by Cath Arnold (HPA, Colindale). Cath explained the different levels of resolution that are achieved by different molecular techniques, which can range from genus all the way to clonal complexes. She then explained how the *Neisseria gonorrhoeae* multi-antigen sequence typing (NG-MAST) scheme, which in combination with multilocus sequence typing (MLST) and single nucleotide polymorphism (SNPs) conferring antibiotic resistance, had been used to differentiate/link *Neisseria gonorrhoeae* strains from a seaside town, using 30 housekeeping genes. Looking at 300 strains, 85 different sequence types (ST) including 28 new STs were found.

The second lecture was given by Dr Pradip Patel (Alaska Food Diagnostics) and detailed a new commercial phage-mediated adenylate kinase (AK-Phage™) assay for the rapid detection of salmonellae in food and swab samples. Dr Patel went on to describe the costs of food borne illnesses and how the most recent regulation for micro-organisms in foodstuffs was in 2005 (EC2073/2005). He then went on to discuss some of the considerations necessary when designing new technologies within the food testing market. As food testing can often resemble the proverbial “looking for a needle in a haystack”, Dr Patel then went on to describe some of the enrichment and purification techniques available. In particular, he concentrated on the phage-based methodology

that increased the limit of detection when compared to conventional ATP detection by 100-fold. The methodology proved to be 100% specific, with a sensitivity of 94% in the 50 poultry products tested for salmonellae. It also gave next day results.

The third lecture was given by Nigel Silman (Porton Down) and was entitled: Development of multi-pathogen microarrays for diagnosis of infectious disease. Nigel went on to describe why microarrays were an appealing methodology to develop, especially due to the poor clinical details and lack of travel history that are often provided when samples are sent to the reference laboratory. Nigel then went on to list the diseases that have emerged over the last 35 years and how a new arenavirus that was detected in South Africa and Zambia in 2008 is the most recently described.



Professor Stephen Denyer presenting the Denver Russell Memorial lecture

Random PCRs often give negative results and consequently microarrays are preferable, as they offer the possibility of a sensitive, rapid, point-of-care testing, multiplexed assay format, that can be used as ‘Syndromic sets’, based on limited clinical information available. Nigel explained that their microarray does not rely on mRNA but uses genomic DNA or RNA and due to gene-variability it is necessary, in some cases, to use 16 probes per virus. Using DNA from *Mycobacterium tuberculosis*, *Bacillus anthracis* and *Yersinia pestis*, he described how they had devised a reproducible 40 minute assay which detects both bacterial and viral pathogens by simply looking for the presence or absence of genes.

Tea and coffee was followed by the penultimate lecture by Dr Paul Dark, (Salford Hospital), who described rapid diagnosis in sepsis. Dr Dark highlighted the difference between sepsis and Systemic Inflammatory Response Syndrome (SIRS) and how clinically they can be difficult to differentiate, enhancing the need for the rapid detection of sepsis. He then went on to describe a study using the 6 hr Septifast technique (Roche Diagnostics) directly from EDTA blood samples. The study demonstrated a 89% specificity and a 50% predictive value of a positive result. As the prevalence rate of a positive result was only 12%, Dr Dark felt this could help prevent unnecessary antimicrobial therapy, even though the methodology only detects 24 target organisms.

The final talk was given by Martin van der Kaap (Kiestra Laboratory Automation) and was entitled Automation in clinical microbiology. Martin went on to describe the company and how they are trying to automate microbiology departments. Interspaced with several short videos demonstrating current capabilities, Martin highlighted some of the benefits of automation. In addition, he showed a video of the magnetic bead approach which is being developed to streak out culture plates. This appeared both impressive and very rapid and was a great way to end the session.

Steve Davies



Session B The Enterobacteriaceae in foods

This session expanded on the topic introduced by Christopher Baylis in the morning session. Professor Mieke Uyttendaele (Ghent University, Belgium) discussed the ecology of Enterobacteriaceae in foods, presenting the idea of foods as a specific habitat with variation in important growth parameters/controlling factors, including the importance of considering microbial interactions. She provided a useful reminder that Enterobacteriaceae, although generally regarded as of faecal origin, are more habitat specific, with organisms like *Erwinia*, *Klebsiella* and others being plant/environmental inhabitants. She discussed various food types and how the pertaining parameters would influence the presence/numbers of Enterobacteriaceae genera present. Sarah O'Brien, (University of Manchester) was the second speaker and she discussed the epidemiology of foodborne illness caused by *Salmonella*, *Yersinia* and *E. coli* O157:H7. Professor O'Brien outlined the changing causes of salmonellosis, from the 1981 rise in cases due to *S. Enteritidis* PT4, its association with eggs and poultry and its decline through the introduction of vaccination of hens for Lion branded eggs in 2001. She then went on to talk about the subsequent problems with imported eggs and the association of *Salmonella* with fresh produce, such as the Saintpaul serovar in the USA associated with jalapeno and serrano peppers from Mexico. She stressed the need for 'biological plausibility' of a suspect food source and the importance of 'thinking outside the box' when looking at an outbreak. She then discussed the role of food in *E. coli* O157:H7 transmission, recognising the significance of the environment and direct contact with animal faeces as primary transmission

routes. The final organism she considered was *Yersinia* and she questioned whether the recent decline in yersiniosis case numbers is real or simply due to the fact that until recently we have not been looking for the organism.

Professor Steve Forsythe (Nottingham Trent University) then discussed the emergence since 2001 of *Cronobacter* (formerly *Enterobacter*) *sakazaki* as a pathogen. Initially recognised for causing necrotising enterocolitis in babies through the consumption of infant formula, the association of this pathogen with adult infections has become more apparent. The development of a selective medium, DFI agar, has facilitated isolation studies which demonstrate this is a relatively common organism in some dried foods. For example, one study showed 5/49 samples of weaning foods carried the organism. The severity of the disease in neonates was graphically brought home by the description that when infecting the brain, the organism does not cause meningitis but liquifaction causing vacuolation of the brain. Another shocking fact was that, although clear guidelines for rehydration of infant formula have been produced by the WHO which eliminate the risk through the use of water at 50-70°C, some baby formula manufacturers recommend rehydration at 40°C. Professor Forsythe finished his talk by discussing taxonomically related organisms which are also associated with disease such as *Enterobacter hormaechei*.

The next talk was given by Nick Johnson (Unilever) who presented quite a different perspective, that of the use of Enterobacteriaceae for hygiene monitoring in frozen foods. Nick discussed the approach Unilever have used in their ice-cream production, using trends in Enterobacteriaceae counts as a monitoring system for factory performance. Factories producing a poorer performance than the average are subjected to investigation and improvement. Using a series of examples, Nick showed that poor performance has usually resulted from poor equipment design/maintenance, complexity or inappropriateness of procedure, failures in cleaning leading to reservoirs of contamination and unhygienic practices. He informed us that presence of *Listeria* was twice as likely in factories with poor Enterobacteriaceae records and hence poor hygiene, even though there is no direct count correlation between the two. This demonstrates that Enterobacteriaceae are a good reflection of total quality system management. Overall, this approach has produced cost savings through increased efficiencies for producing quality products.

The final talk was given by Geraldine Smith (Health Protection Agency) who focused on the pathogenic *E. coli*. After defining the pathogenic types (EPEC, ETEC, EIEC, VTEC, EAaggEC) and their pathogenicity mechanisms, the limitations of detecting most of these organisms through a lack of routine culture media was discussed. The O157 VTEC is the only group where genetic homogeneity has allowed a selective and diagnostic culture medium to be developed and thus allows its association with food to be investigated. Examples of typical food types and processing conditions where it survives were covered, together with the rare examples of national and international outbreaks. Finally detection of non-O157 VTEC by DNA/ELISA based methods suggest they may in fact be more important than O157.

Christine Dodd

Spring meeting 2009

■ Including the the Procter and Gamble Lecture — *The fall and rise of home hygiene* given by Professor Sally Broomfield

CPD
ACCREDITATION

7 IBMS CPD
Points awarded

A one day meeting on

3rd broadening microbiology horizons in biomedical science

Lakeside Conference Centre, Aston University, Birmingham, UK
Wednesday 22nd April 2009

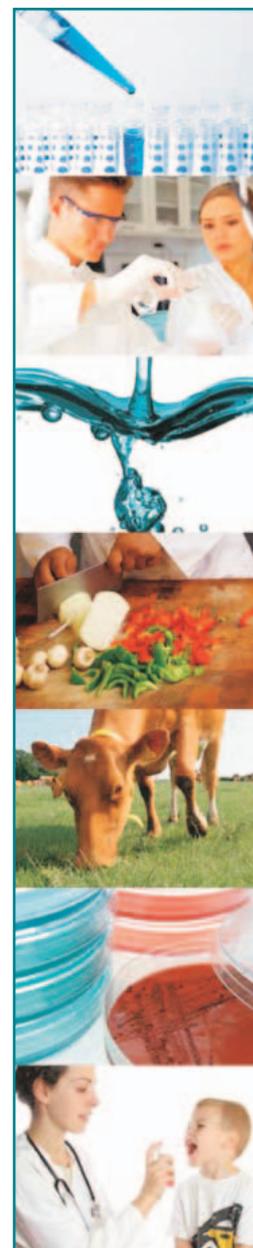
Including talks on:

- Bioterrorism
- Emerging respiratory viruses
- Device related infections

Programme

09.15-10.15	Coffee, Trade Exhibition and Registration	12.35-14:00	Lunch and Trade Exhibition
10.15-10.20	Chairman's Welcome	14.00-14.30	Device related infections Professor Peter Lambert, Birmingham, UK
10.20-11.00	Procter and Gamble Lecture <i>The fall and rise of home hygiene</i> Professor Sally Broomfield, London	14.30-15.00	<i>Clostridium difficile</i> and the germination theory Dr Tony Worthington, Birmingham, UK
11.05-11.35	Update on ESBLs Professor Peter Hawkey, Birmingham, UK	15.00-15.30	Changing epidemiology of viral hepatitis Laura Ryall, Cambridge, UK
11.35-12.05	Bioterrorism Professor Les Baillie, Cardiff, UK	15.30-16.00	Q fever Dr Phillipa Moore, Gloucester
12.05-12.35	Emerging respiratory viruses Dr Kate Templeton, Edinburgh, UK	16:00	Finish and Tea

The programme for this meeting was correct at the time of going to press.
For the latest information please visit: www.sfam.org.uk/spring_meetings.php



BOOKING FORM and INVOICE

SfAM SPRING MEETING WEDNESDAY 22 APRIL 2009

Only ONE person per form please. CLOSING DATE FOR REGISTRATIONS: Wednesday 8 April 2009
EARLY BIRD DISCOUNT of £30.00 is applied to all bookings made before Friday 27 March 2009

Cancellation policy: Up to 30 days prior to the event all cancellations will be subject to a 10% cancellation fee, up to 14 days prior to the event there will be a 50% cancellation fee, and no refunds will be given on cancellations made within 7 days of the event.

***Non members please note:** You can add 1 year's membership to your event booking using this form, then register at the member rate and spend the same amount of money or less!

F E E S	Before 27/03/2009	Between 28/03/2009 and 8/04/2009
Full member	£50 <input type="checkbox"/>	£80 <input type="checkbox"/>
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Honorary member	£30 <input type="checkbox"/>	£60 <input type="checkbox"/>
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Retired member	£30 <input type="checkbox"/>	£60 <input type="checkbox"/>
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Non member	£100 <input type="checkbox"/>	£130 <input type="checkbox"/>

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Cheque enclosed Please charge my *Mastercard/Visa card /Debit card* (please delete inapplicable items)

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Signature: _____ Date: _____

Please return the completed form by fax (post if you are enclosing a cheque) to: **The Society for Applied Microbiology, Bedford Heights, Brickhill Drive, Bedford MK41 7PH, UK. Tel: 01234 761752 Fax: 01234 328330. Email: meetings@sfam.org.uk**

Summer conference 2009

■ Including the Lewis B Perry Memorial Lecture — *Prion zoonoses: past, present and future* given by John Collinge



Fur, feather and fever — zoonotic challenges of the 21st century

Manchester Metropolitan University, Manchester
Monday 6 to Thursday 9 July 2009

Including sessions on:

- Arthropod borne zoonoses ■ Wildlife and companion animals
- Livestock and foodborne zoonoses ■ Emerging and re-emerging zoonoses

There will be a packed social programme including:

- Drinks reception and lecture on Monday 6 July followed by Quiz Night
- Trade exhibition on Tuesday 7 July with wine and prizes
- Conference dinner at the URBIS Centre on Wednesday 8 July

studentships

SfAM offers **Studentships** to enable student members to attend Society meetings. These grants cover registration, accommodation, meals (where appropriate) and modest travel expenses. Preference is given to students offering a paper or poster and who have not previously received this award. To be considered for a Studentship grant, please download an application form by visiting: www.sfam.org.uk/grants.php?2
Application forms must be returned to the Society **no later than 6 weeks** before the date of the summer conference (22 May 2009). **Applicants MUST hold / have access to a legitimate UK bank account.**

CPD
ACCREDITATION
APPLIED FOR

Summer Conference Short Course for PECS and student members

A short course in 'Basic statistics for applied microbiology' will be offered from 11.00 to 17.00 on Monday 6 July 2009, at Manchester Metropolitan University, primarily for PECS and student members

The course, restricted to a maximum of 20 delegates, will be presented by Professor Basil Jarvis and is free of charge to SfAM student members and members of PECS. Delegates will be provided with course notes and exercises on a SfAM Memory Stick and the course will be held in a computer laboratory on the university campus. Lunch, morning and afternoon refreshments will be provided.

The objective is to provide an introduction to the terminology and concepts of analytical statistics to enable participants to understand their practical applications. The meaning and derivation of statistical probability will be considered and discussed in relation to frequency distributions (including Poisson, Binomial, Normal, Lognormal and Negative Binomial distributions) of importance in analytical microbiology. Practical exercises will be used to derive, and to discuss, key distribution parameters in different scenarios.

Sources of 'error' associated with microbiological procedures will be discussed in the context of understanding statistical aspects of sampling and measurement. Practical exercises will be used to estimate parameters of variability and to illustrate the concepts of measurement uncertainty. Finally, some of the "dos and don'ts" of experimental planning will be discussed and illustrated using examples of both good and bad practice.

As limited spaces are available for this course student members and PECS members need to pre-register to reserve their place by emailing Sally Cryer at: sally@sfam.org.uk

Programme

Monday 6th July

- 14.00 onwards Arrive and Register
- 11.00–17.00 **Basic statistics for applied microbiology**
Basil Jarvis (**A short course primarily for PECS members and student members**)
- 18.00–18.50 **Lewis B Perry Memorial Lecture: prion zoonoses: past, present and future**
John Collinge
- 19.00–20.00 Drinks reception
- 20.00 Evening at leisure
- 21.30 Quiz night — Jury's Inn Hotel (to be confirmed)

Tuesday 7th July

Arthropod-borne zoonoses

- 09.00–09.35 **What is a zoonosis?**
Malcolm Bennett, University of Liverpool, UK
- 09.35–10.00 **Plague — historical perspectives to modern infection trends**
Nils Chr Stenseth, University of Oslo, Norway
- 10.10–10.45 **Lyme borreliosis — facts & fantasy**
Sue O'Connell, Health Protection Agency, Southampton
- 10.45–11.15 **Coffee/posters**
- 11.15–11.50 **Bartonellosis an increasingly recognised zoonosis**
Bruno Chomel, School of Veterinary Medicine, University of California, USA
- 11.50–12.25 **Rickettsiosis — the unwanted holiday souvenir**
Philippe Parola, University of Marseilles, France
- 12.25–13.00 **Relapsing fever — forgotten but not gone**
Sally Cutler, University of East London, UK
- 13.00–14.00 Lunch

Wildlife and companion animals

- 14.00–14.35 **Bats, bites and fury — can we control rabies?**
Tiziana Lembo, University of Glasgow, UK.
- 14.35–15.10 **Controlling wildlife reservoirs for bovine TB**
Glyn Hewinson, Veterinary Laboratories Agency, UK
- 15.10–15.45 **Zoonoses in UK wildlife and their detection through sentinels**
Anna Meridith, Edinburgh
- 15.45–16.15 **Tea/posters**
- 16.15–16.50 **Exotic pets — what are the zoonotic risks?**
F. Meslin, World Health Organisation, Switzerland
- 16.50–18.00 **Student Session**
- 17.30–19.30 **Trade Show**

Wednesday 8th July

- 09.00–09.35 **Controlling wildlife zoonoses without eliminating wildlife**
Marc Artois, Ecole Nationale Veterinaire de, Lyon, France
- 09.35–10.10 **Nature bites back!**
Marina Morgan
- Livestock and foodborne zoonoses**
- 10.10–10.45 **Anthrax — wool-sorters disease in Belgium!**
Pierre Wattiau, VAR-CODA-CERVA, Brussels, Belgium
- 10.45–11.15 **Coffee/posters**
- 11.15–11.50 **New challenges and perspectives on brucellosis**
Phil Elzer, LSU, USA
- 11.50–12.25 **Cryptosporidiosis — challenges for control**
Rachel Chalmers
- 12.25–13.25 **Lunch**
- 13.25–14.00 **Unravelling the mysteries of Q Fever**
Didier Raoult, Marseilles, France

- 14.00–14.35 **Farm to fork — emerging trends in foodborne zoonoses**
Tom Humphrey, University of Bristol, UK
- 14.35–15.00 **Tea/posters**
- 15.00–16.00 **Student presentations**
- 16.00–16.30 **W H Pierce Memorial Prize**
- 16.30–17.00 **AGM**
- 19.30–20.00 **Drinks reception, Dinner**
URBIS Centre, Manchester.

Thursday 9th July

- 09.00–09.35 **New trends in toxoplasmosis**
Author to be confirmed

Emerging and re-emerging zoonoses

- 09.35–10.10 **Modelling zoonotic disease — challenges & successes**
Nigel French Massey Uni, NZ
- 10.10–10.45 **Drivers of zoonotic disease emergence in general, using Nipah virus as a case study**
Jonathan Epstein, The Consortium for Conservation Medicine, New York and University of Kingston, UK
- 10.45–11.15 **Coffee/posters**
- 11.15–11.50 **Predicting pandemics or scare mongering?**
Dilys Morgan, HPA, UK
- 11.50–12.25 **Emerging/re-emerging viral zoonoses**
Ernie Gould, France
- 12.25–13.00 **Leishmaniasis and pet travel**
Sue Shaw, Bristol
- 13.00–14.00 **Lunch & Close**

This programme was correct at the time of going to press. For the latest programme please visit us online at www.sfam.org.uk

BOOKING FORM and INVOICE

S F A M S U M M E R C O N F E R E N C E 6 – 9 J u l y 2 0 0 9

CLOSING DATE FOR REGISTRATIONS: Friday 26 June 2009

EARLY BIRD DISCOUNT of £30.00 is applied to all bookings made before **Friday 5 June 2009**

Cancellation policy: Up to 30 days prior to the event all cancellations will be subject to a 10% cancellation fee, up to 14 days prior to the event there will be a 50% cancellation fee, and no refunds will be given on cancellations made within 7 days of the event.

***Non members please note:** You can add 1 year's membership to your event booking using this form, then register at the member rate and spend the same amount of money or less!

FEES BEFORE 5 JUNE 2009	Full Member	Student, Honorary, Associate & Retired Member	Student Non -Member	Non - Member
Full Conference Rate: (inc accommodation)	£450.00 <input type="checkbox"/>	£200.00 <input type="checkbox"/>	£450.00 <input type="checkbox"/>	£650.00 <input type="checkbox"/>
Conference Rate: (no accommodation)	£145.00 <input type="checkbox"/>	£50.00 <input type="checkbox"/>	£145.00 <input type="checkbox"/>	£300.00 <input type="checkbox"/>
Conference Day Rate:	£100.00 <input type="checkbox"/>	£50.00 <input type="checkbox"/>	£100.00 <input type="checkbox"/>	£150.00 <input type="checkbox"/>
FEES BETWEEN 6 JUNE and 26 JUNE 2009	Full Member	Student, Honorary, Associate & Retired Member	Student Non -Member	Non - Member
Full Conference Rate: (inc accommodation)	£480.00 <input type="checkbox"/>	£230.00 <input type="checkbox"/>	£480.00 <input type="checkbox"/>	£680.00 <input type="checkbox"/>
Conference Rate: (no accommodation)	£175.00 <input type="checkbox"/>	£80.00 <input type="checkbox"/>	£175.00 <input type="checkbox"/>	£330.00 <input type="checkbox"/>
Conference Day Rate:	£130.00 <input type="checkbox"/>	£80.00 <input type="checkbox"/>	£130.00 <input type="checkbox"/>	£180.00 <input type="checkbox"/>

Conference Day Rate delegates please tick the day you wish to attend: Mon 6th Tue 7th Wed 8th Thur 9th

CONFERENCE DINNER: £50.00

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ZOOZOSES

Past, present and future



Illustration: Stephen Pollard

Throughout history man and animals have been intrinsically linked, from man as hunter-gatherer through to the domestication of animal species. As a result of our close contact with various animals we have been exposed to a range of pathogens that sometimes infect us, and disease may develop as a consequence. The development of an infection as a result of contact between humans and a vertebrate animal is described as a zoonosis or zoonotic infection. Some of the defining moments in human history have arisen as a result of zoonotic infection, such as the Bubonic plague pandemic ('black death') in the 1300s through to the Influenza pandemic from 1918 to 1919 that resulted in the estimated death of 21 million people worldwide. This, coupled with the recent evolution of the avian Influenza (H5N1) virus, West Nile virus, Ebola and severe acute respiratory syndrome (SARS) coronavirus demonstrates the dynamism and complexity of this area of infectious disease and microbiology.

It is suggested that the emergence of new zoonotic infections is likely to result from increased interaction between man and wild and domestic animals (Daszak, Cunningham & Hyatt 2001). There is some evidence that certain occupations may have a greater association with zoonotic diseases (Thomas *et al.*, 1994, Weese *et al.*, 2006, Armand-Lefevre *et al.*, 2005, Voss *et al.*, 2005). Indeed anecdotal information from the mid 1980s reported that in parts of Sudan cutaneous anthrax was not uncommon in the local tribal huntsmen following the skin to skin contact involved in the movement of a carcass (Personal Communication). This was also the case in the leather industry in the UK, and was highlighted following cases of human infection from drum skins. However, it is notable that the transmission of zoonotic pathogens between humans is not always easy, this is especially true of viral zoonoses where man is often the dead end host (Weiss & McMichael, 2004). This is not always the case, with an estimated 61% of all human diseases (Taylor *et al.*, 2001) and up to 75% of emerging infectious disease (EID) being of zoonotic origin (Taylor *et al.*, 2001, John *et al.*, 2008). More recently Jones *et al.*, (2008) reported that just over

60% of EIDs are zoonotic and almost 72% of these originate in wildlife.

There is a global and social aspect to the movement and development of zoonotic disease, with many endemic areas showing lower health and socioeconomic status (Blancou *et al.*, 2005, Pappas *et al.*, 2006). Pappas *et al.*, (2008) extend this argument suggesting that due recognition is only given to these diseases once they appear in what might be termed as developed or industrialised parts of the world. Although the emergence of disease that affects companion animals or world travel is likely to stimulate research investment against zoonotic diseases, this may not be the case in less developed countries or populations where the same diseases are more likely to be endemic (Chomel *et al.*, 2007). We are also seeing the impact of globalisation on the pattern of zoonoses worldwide. There is a significant legal market in moving live animals in a global context as food items, zoo exhibits, part of breeding programs and conservation drives, as well as trade in exotic pets. Other legal movements are also based around human migration and companion animals and there are indirect contacts in the form of tourism (Marano *et al.*, 2007).

This level of live animal movement should not be underestimated. For example, Marano *et al.*, (2007) found that between 2000 and 2004 there were 37.8 million live animal movements (including amphibians, birds, mammals and reptiles) into the USA from approximately 160 different countries. This of course represents only legal movements into one country. It is difficult to assess the global level of legal movements, and impossible to assess the level of illegal movements with any confidence. These illegal movements are likely to be the source of some disease outbreaks and this risk is highlighted by the smuggling of H5N1 infected birds into Europe (Van Borm *et al.*, 2005). It is important to note that reports are scarce, demonstrating the difficulties involved in tracing and detecting such practices. This is of concern as it has been stated that legal commercial importation of animals represents a significant human health risk (Marano *et al.*, 2007). Cases to support this claim include the tracing of a human monkeypox infection that emanated from the importation of

Gambian giant rats housed with prairie dogs for the US pet trade (Anon 1 2003, cited by Marano *et al.*, 2007). Infections such as tularaemia and salmonellosis have been reported following contact with both prairie dogs and hedgehogs in the US (Marano *et al.*, 2007) and in Europe a similar scenario is observed. The increase in ownership of exotic companion animals and reptiles has also provided infectious risk to humans. Organisms such as *Salmonella chameleon* and *S. arizonae* caused infections in children in a household with snakes as companion animals (Sanyal *et al.*, 1997). Similarly, Berendes *et al.*, (2007) reported a case of a 17 year old woman who presented with gastrointestinal symptoms caused by a splenic abscess as a result of an infection by *Salmonella* type Telelkebir. The infection and subsequent pathology led to a splenectomy. The authors are robust in their warnings of the risk involved in keeping exotic reptiles as pets, stating that these pets can act as a source of infection and cause a "catastrophic course of the disease even in healthy people" (Berendes *et al.*, 2007). However, bacterial infections are not the only zoonoses that are causing concern in relation to companion animals: protozoa and viruses are also well represented (Harrus & Baneth, 2005, Patel & Heldens, 2009).

It is interesting to speculate what may happen when the influence of climate change combines with infectious zoonotic disease. For influenza, it has been suggested that environmental change is likely to alter avian migratory pathways and the survival of viruses outside the host (Gilbert *et al.*, 2008). Little is currently known about the effects of environmental factors on avian influenza transmission and persistence in relation to domestic poultry. Therefore it is clear that the potential influence of environmental changes cannot be ignored in the context of zoonotic disease. A number of ways in which a change in climate may influence the ecology and biologic spectrum of zoonotic disease have been proposed. If temperatures rise as predicted, natural animal and arthropod movement may facilitate the movement of infectious agents into new environments or a recolonisation of old

Table 1. A representative list of zoonotic diseases, their aetiological agents and their occurrence globally

Agents	Disease	Causative agent	Implicated animal(s)	Global occurrence
Bacterial	Anthrax	<i>Bacillus anthracis</i>	Domestic and wild animals, particularly cattle, sheep, horses, mules, goats	Worldwide
Bacterial	Bovine tuberculosis	<i>Mycobacterium bovis</i>	Cattle, seals	Worldwide
Bacterial	Brucellosis	<i>Brucella</i> spp.	Cattle, Bison, Sheep, Goats, pigs, Dogs, Elk, Caribou, seals	Worldwide
Bacterial	Campylobacteriosis	<i>Campylobacter</i> spp., <i>C. jejuni</i>	Poultry, ruminants, Pigs, Dogs, Cats	Worldwide
Bacterial	Bloody diarrhoea	Vero toxigenic <i>Escherichia coli</i>	Cattle, Deer, Dogs, Pigs, Horses, sheep	Worldwide
Bacterial	Glanders farcy	<i>Burkholderia mallei</i>	Horses, Donkeys, Mules, Guinea pigs, Dogs, Cats	Asia, Mediterranean
Bacterial	Lyme disease	<i>Borrelia burgdorferi</i> (US), <i>B-afzelii</i> and <i>B-garinii</i> (Europe)	Sheep tick (<i>Ixodes ricinus</i>)	Asia, Europe, USA.
Bacterial	Leptospirosis	<i>Leptospira</i> spp.	Rats, Dogs, Pigs, Cattle	Worldwide
Bacterial	Listeriosis	<i>Listeria monocytogenes</i>	Many wild and domestic animals	Worldwide
Bacterial	Pasteurellosis	<i>Pasteurella multocida</i> , <i>P. septica</i>	Many wild and domestic animals	Worldwide
Bacterial	Q fever	<i>Coxiella burnetii</i>	Sheep, Goats, Cattle as primary reservoirs	Worldwide
Bacterial	Salmonellosis	<i>Salmonella</i> spp.	Many wild and domestic animals, poultry and exotic pets	Worldwide
Bacterial	Tularaemia	<i>Francisella tularensis</i>	Wide range of wild and domestic animals and birds and some non human primates	Western Hemisphere
Bacterial	Yersiniosis	<i>Yersinia enterocolitica</i>	Rodents and Domestic animals	Worldwide
Fungal	Ringworm	<i>Tinea capitis</i> , <i>Tinea corporis</i> , <i>Tinea cruris</i> , <i>Tinea pedis</i>	Cats, Dogs, Horses	Europe
Viral	Hantavirus pulmonary syndrome	Hantaviruses	Rodents	Worldwide
Viral	Hendra virus disease	Hendra virus (equine morbillivirus)	Pteropid fruit bats, infected horses	Australia
Viral	Influenza	Orthomyxoviridae	Birds, Pigs	Worldwide
Viral	Japanese encephalitis	Japanese Encephalitis virus	Pigs, wading birds	Asia, Pacific
Viral	Nipah virus encephalitis	Nipah virus (Bunyavirus)	Pteropid fruit bats, infected pigs	South East Asia
Viral	Orf	Poxvirus	Sheep	Worldwide
Viral	Rabies	Rhabdovirus	Mammals	Worldwide
Viral	West Nile	Flavivirus	Birds	Africa, India, USA
Protozoa	Cryptosporidiosis	<i>Cryptosporidium</i> spp.	Numerous animal species	Worldwide
Protozoa	Giardiasis	<i>Giardia duodenalis</i>	Birds, Mammals	Worldwide
Protozoa	Hydatid disease	<i>Echinococcus vogeli</i> , <i>E. canids</i> , <i>E. multilocularis</i>	Dogs, Canids, foxes respectively	Central/South America, Widespread, Europe respectively
Protozoa	Leishmaniasis	<i>Leishmania</i> spp.	Rodents, Hyraxes, dogs	Worldwide
Protozoa	Toxocariasis	<i>Toxocara</i> spp.	Dogs, Cats	Worldwide
Protozoa	Toxoplasmosis	<i>Toxoplasma gondii</i>	Cats, meat mammals	Worldwide

environments, thereby increasing their range and abundance of potential reservoirs. There may be an extension to cycles of transmission and an increased risk of pathogen importation, possibly as a consequence of changes in migration routes (Greer *et al.*, 2008, Gilbert *et al.*, 2008). It is clear from the knowledge we currently have on zoonoses, potential climate change and the potential for infectious agents to cross the species barrier, that zoonoses will remain a significant problem. In particular, infectious agents transcending the species barrier have

given rise to some of the most dramatic and possibly devastating infectious events in human history (Stein, 2008) such as pandemic influenza, SARs and BSE.

Aetiological agents of Zoonotic disease.

There is a large and ever-expanding number of zoonotic organisms. A representative but not exhaustive list is presented in table 1 and it is striking how many of these diseases have been long standing problems. This may well be due to poor treatment, sub-optimal

implementation of public health measures, changes in human/animal behaviour, and sustainability of the ‘pathological fitness’ of the particular organism in question. Looking at table 1 in a broader context, over 60% of the diseases listed currently occur worldwide. It would be interesting to monitor changes in the geographical score of each zoonotic disease, particularly in the context of climatic changes and transmission chains.

There is merit in considering zoonoses from a microbial ecosystem perspective, where humans are simply a

component of a total mammalian habitat. This then raises questions about the fundamental basis of determinants of host-species specificity, where certain microbes colonise and/or infect only certain mammals. It also raises questions about host determinants of resistance to, or amelioration of, virulence factors, where microbes express differing pathogenicities in different hosts; and about determinants of chains of transmissibility. A better understanding of these factors would enable an improved risk assessment of emergence of new, or alterations in current, zoonoses based on molecular and associated antigenic drift in microorganisms.

Some existing work on the identification of determinants of host specificity highlight some of the issues raised above. *Neisseria meningitidis* is a human-specific pathogen. This host specificity appears to be dictated by the fact that it acquires essential iron by binding human transferrin, and is unable to bind and acquire iron from the transferrin of other species (Gray-Owen & Schryvers, 1996). A strain of *N. meningitidis* was engineered whereby the receptors for human transferrin were replaced by the transferrin receptors from a pig pathogen, *Actinobacillus pleuropneumoniae*. The engineered strain could no longer grow in the presence of iron bound to human transferrin, but could grow in the presence of porcine transferrin (Litt, Palmer & Borriello, 2000). In theory, if both transferrin receptor types had been engineered into *N. meningitidis* this pathogen could potentially become a new zoonotic pathogen, causing infection in both pigs and humans, with pigs acting as a reservoir of transmissible infection.

Emerging exotic agents

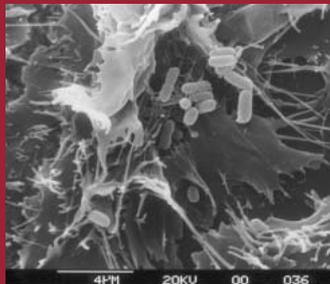
In comparatively recent times we have experienced changes in the classical group of organisms defined as zoonotic. *Salmonella* species are not of course newly recognised as zoonotic, but some of the vehicles of transmission are reflective of changes. The recent interest in home herpetology has led to the public coming into close contact with some exotic animals, some exotic faeces and some exotic salmonellas! Consequently, we are not

just examining a change in natural history of the organisms, both microbial and host, but also changes in human behaviour.

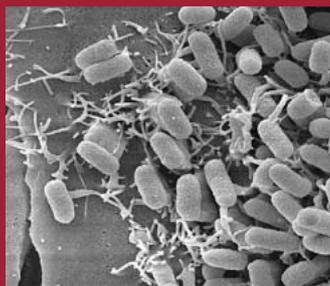
Some rarer cases of unusual, potentially zoonotic infections have also arisen in recent times. One such example has been in the form of isolated cases of seal bite finger purportedly caused by a seal mycoplasma, *Mycoplasma phocacerebrale*. Seal bite finger is a rare condition and might well not fit a strict definition of a zoonosis but it is indicative of how novel or emerging infections are coming to the fore. Other evidence suggests that laboratory technicians in animal units are likely to become colonised with the rodent

Staphylococcus aureus, specifically methicillin resistant *S. aureus* (MRSA). The role of *S. aureus* in human disease is clear (Stryjewski & Chambers, 2008); the organism is also well recognised as a cause of bovine mastitis with small colony variants linked to more persistent infections (Devriese *et al.*, 1972, Atalla *et al.*, 2008). Other new variants of *S. aureus* have emerged with the recent appearance of community acquired MRSA (caMRSA) in humans and the ST398 clone in the animal population (Wulf *et al.*, 2008). Whilst reports of MRSA in animals have been relatively infrequent, recent evidence indicates that MRSA is increasingly isolated from animal sources (Cuny *et al.*, 2008; Morgan, 2008; van Belkum *et al.*, 2008).

A recent review extends the list of animals in which MRSA has now been identified, inclusive of psittacine birds (those of the parrot family) and seals, a bat, a turtle, guinea pigs and chinchillas (Morgan, 2008). The emergence of ST398, non PFGE SmaI typable strains from pigs (Morgan, 2008; Van Duijkeren *et al.*, 2007, Huijsdens *et al.*, 2006), is the first MRSA with a so called 'zoonotic lineage' (Van Duijkeren *et al.*, 2007, Huijsdens *et al.*, 2006). Prior to the recognition of ST398 it was assumed that the infection of animals with strains of MRSA was actually a form of 'humanosis' (Morgan, 2008). However, doubt may now be cast on this assumption. Indeed, the evolution of ST398 in pigs might actually represent the emergence of a potentially novel zoonosis, with some evidence of colonisation or infection of humans in contact with such herds (Van Belkum *et al.*, 2008). There have been few reports of Panton Valentine Leukocidin (PVL) positive caMRSA strains in animals but nonetheless they are reported in companion animals, rabbits, birds, bats (Van Duijkeren *et al.*, 2005, Rankin *et al.*, 2005) and importantly pigs and cattle (Walther *et al.*, 2008, Voss *et al.*, 2005, Kwon *et al.*, 2005). Morgan (2008) cites recent studies comparing MRSA and *S. aureus* carriage by clinicians and veterinarians attending conferences, where carriage rates of MRSA in clinicians was low at less than 1% (Nulens *et al.*, 2005). Contrastingly a study of veterinary staff and students at a Dutch veterinary conference showed almost 5% to be nasal carriers (Wulf *et al.*, 2006). At a



Ruffle formation by *S. Enteritidis* (SEM)



E. Coil O157 (SEM)

Images by kind courtesy of the EM Unit at the Veterinary Laboratories Agency, Weybridge, UK

mycoplasma *M. pulmonis* (Ferreira *et al.*, 2008). These reported observations are interesting on two counts, primarily, under conventional thinking mycoplasmas are not deemed as zoonotic and secondly, there is evidence of mycoplasmas colonising and indeed infecting outside of their normal host range (Pitcher & Nicholas, 2005). As Pitcher and Nicholas attest, there is increasing evidence to suggest that mycoplasmas can infect outside their 'normal' host range and indeed these 'crossings' might result in pathological consequences.

Another area where the emergence of a potentially new zoonosis has recently courted controversy is with

Danish pig conference in Denmark over 30 delegates from nine different countries were positive for MRSA carriage and from this study approximately 90% of the isolates identified corresponded to ST398 (Hanselman *et al.*, 2006). As studies progress it is becoming clear that MRSA and *S. aureus* are dynamically 'fit' pathogens capable of further niche exploitation.

Concluding remarks

The fact that zoonoses span animals and humans, and in many instances contamination of food-stuffs serves as the means of transmission, has implications for their control. In most parts of the world different policy and operational areas of Government deal with animals, food and humans, and similarly, the detection of problems is also common to a number of bodies. The importance of considering zoonoses in a more horizontal and integrated way is increasingly recognised. In the UK, the previously separate Surveillance Group on Diseases and Infections of Animals, and the UK Zoonoses Group have been re-organised to form the UK Zoonoses and Animal Diseases and Infections Group, with representation from Government Departments and Agencies responsible for human health, animal health, and food, and the Devolved Administrations. The already existing Human and Animal Infections Risk Surveillance (HAIRS) group now reports to this committee.

It is clear that we have a vast amount of knowledge about zoonotic infections, their aetiology and their potential and real effects. What is also clear is that we do not know enough about zoonoses. Infectious disease, both human and veterinary is an ever-evolving science and the zoonosis aspect is no different. We should not ignore the prediction purported to have been made by Pasteur, that it is the organisms that will have the last word.

authors note

A review of the current situation of the recently recognised emerging zoonosis: reptile associated salmonellosis, is provided by Bertrand *et al.*, (2008) and is available online at:

www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18902

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Sewage borne pathogens associated with bivalve shellfish

In 43 AD when Emperor Claudius successfully invaded Britain his armies brought with them an enthusiasm for eating bivalve shellfish. Oysters particularly, were considered a delicacy amongst the higher echelons of Romano-British society. In the

years after the Romans left Britain the status of this protein rich food source diminished and it wasn't until the 14th century that their popularity, this time amongst rich and poor alike, was revived. ▶

This was due in part to their widespread availability, numerous, dense beds of many indigenous species such as cockles (*Cerastoderma edule*), mussels (*Mytilus edulis*) and native oyster (*Ostrea edulis*) and the large number of Christian ‘fish days’ observed during medieval times, where consumption of meat or fowl was prohibited. Until Victorian times, fresh and pickled oysters continued to form a very important part of the diet, principally amongst the urban poor in the fast growing estuarine cities of England. In Dickens’ popular serial of the day, ‘The Pickwick Papers’ (1837), the fictional Sam Weller observed, “Poverty and oysters always seem to go together”. However, in the 1850s over-fishing and industrial pollution caused native populations of oysters and scallops to crash. Prices skyrocketed and once again, along with other shellfish species, oysters became the preserve of the wealthier classes.

The sedentary lifestyle of bivalve molluscs make them suitable for mariculture (Figure 1, 2), and since the late 19th century the cultivation of both indigenous and introduced species has become widespread. Today, the commercial oyster fishery in the U.K. is estimated to be worth £23.5 million, and thousands of tonnes of bivalve shellfish species are traded annually throughout the European Union and elsewhere. Bivalve species vary greatly in their characteristics and habitats, but two features are common to all; they possess two shell halves connected laterally with a hinge, and they obtain food by filtering small particles, such as plankton, from their surrounding water. Commercial shellfish production areas tend to be located in shallow, sheltered, in-shore estuaries where nutrient levels are usually highest and the proximity to shore enables farming with relative ease.

In our crowded continent such areas are often close to coastal populations, or downstream of rivers draining urban populations, and can frequently be contaminated with human sewage. In the process of filter-feeding shellfish can accumulate human pathogens derived from sewage. Filtering large quantities of water also makes shellfish susceptible to concentration and retention of autochthonous toxic algae and pathogenic bacteria. The risks posed by bioaccumulation of harmful

micro-organisms are compounded by the traditional consumption of many bivalve species, either raw or lightly cooked, and by the consumption of the whole animal including the viscera.

Human health problems associated with the ingestion of sewage polluted bivalve molluscs have been chronicled since the early 17th century when oysters were implicated in a gastrointestinal disorder of King Henry IV of France, a prolific and enthusiastic consumer. Historically, shellfish transmitted diseases were largely of bacterial aetiology, the most significant being *Salmonella* Typhi. Today

Escherichia coli. Across the EU *E. coli* levels measured in shellfish flesh are used to assess the degree of faecal contamination and inform decisions on implementation of control measures. In parallel an end-product standard requiring absence of *Salmonella* spp. in 25g of shellfish flesh adopted in Regulation (EC) No. 2073/2005 has largely eliminated salmonellosis as a hazard for the shellfish consumer. Whilst, a number of other faecal borne bacterial pathogens have been sporadically implicated in shellfish associated illness (e.g. *Vibrio cholerae* O1, non-O1 *V. cholerae*, *Campylobacter*



Figure 1. Commercial mariculture of Pacific oysters (*Crassostrea gigas*)

however, the general improvements in community sanitation have largely eradicated this as an endemic pathogen in developed countries and thus *S. Typhi* is not considered a significant shellfish-associated hazard in the industrialised world. Food borne non-typhoidal *Salmonella* spp. is estimated to be responsible for 1.3 million illnesses in the U.S annually, although only a small percentage of these cases have been associated with seafood. Across the EU the sanitary quality of bivalve molluscs is controlled through the Food Hygiene Regulations. Regulation (EC) No. 854/2004 requires classification, monitoring and purification of bivalve mollusc production areas based upon levels of the faecal indicator bacteria

species), it is widely accepted that the incidence of bacterial infections has reduced dramatically and that today the most common bivalve shellfish vectored illnesses are acute viral gastroenteritis caused by human noroviruses and infectious hepatitis caused by the hepatitis A virus.

Norovirus, one of four viruses comprising the *Caliciviridae* family, has a single stranded, non-segmented RNA genome with three open reading frames. It shows positive polarity, is small (30-35 nm diameter) and exhibits characteristic fuzzy or indistinct capsid edges (Figure 3). Human noroviruses can be further subdivided into two genogroups. Norovirus has become recognised as the most frequent cause

of non-bacterial intestinal disease in the U.K. and United States, accounting for almost 50% of all cases. The illness exhibits a strong seasonality with the majority of recorded outbreaks occurring during the colder winter months. As norovirus infections are self-limiting, it is likely that they are under-reported in the community. An epidemiological association between consumption of sewage contaminated bivalve shellfish (oysters) and norovirus was first made in the late 1970s in Australia following a prolonged period of extreme rainfall. Since human noroviruses cannot be routinely grown

RNA genome surrounded by a small protein capsid (approximately 30nm in diameter). Strains recovered from temporally and spatially diverse human sources are antigenically indistinguishable and it is thought that a single serotype exists amongst human hosts. Whilst attenuated cell culture adapted strains have been used to develop vaccines, wild type hepatitis A remains very difficult to grow and for diagnostic purposes the virus is considered uncultivable. The virus was only recognised in 1973 as the aetiological agent of hepatitis A from immune electron microscopic

Japan and several European countries. However, without comprehensive epidemiological investigation, definitive linkage between food vector and disease transmission is often problematic due to the extended incubation period (3-6 weeks). One notable exception occurred in Spain in 1999, where imported coquina clams (*Donax* spp.) compliant with EU shellfish standards were identified as the highest risk factor in a large hepatitis A outbreak. This was confirmed by detection by reverse transcription (RT)-PCR of hepatitis A virus RNA in shellfish samples. Unlike norovirus, where the high mutation rate and high antigenic diversity help the virus to evade host immunity, hepatitis A infection confers a long-term resistance. As a consequence, in world regions with poor sanitation, the incidence of subclinical childhood infection is high and retrospective seroepidemiological investigation has shown that in excess of 90% of the population is immune. However, in industrialized countries the majority of individuals are not exposed to the virus in childhood and most of the population is highly susceptible to infection resulting in the potential for rapid evolution of common source epidemics.

Electron microscopy is a relatively insensitive method requiring the region of a million viral particles for effective visualisation. This makes it impractical as an epidemiological tool in the examination of food borne viruses where as few as 10-100 virions have been reported to cause infection. The potential for identification of noroviruses and hepatitis A virus in shellfish and other foodstuffs, was not generally realised until the advent of the reverse transcriptase polymerase chain reaction (RT-PCR) in the mid 1990's. Both viruses comprise RNA genomes and thus reverse transcription is necessary to convert the target sequence to complementary DNA that can then be amplified to detectable levels using PCR. First applied within a clinical setting, these powerful techniques have quickly been adopted by food microbiologists and numerous reports of positive detection of genomic signatures, indicative of both viruses in many species of shellfish, have been published. Studies carried out in a number of European countries have shown that norovirus may be prevalent



Figure 2. Common mussels (*Mytilus edulis*) commonly farmed on vertical ropes suspended from buoys in estuarine areas

in conventional tissue culture models, electron microscopy (EM) of patients' stools and serology were used to confirm norovirus infection. During the 1980s and early 1990s, using EM and serodiagnostics, a clear association between the consumption of sewage-contaminated shellfish and norovirus infection emerged throughout the developed world.

Another important viral pathogen that has been associated with the consumption of sewage polluted bivalve shellfish is hepatitis A virus, also known as infectious hepatitis. Hepatitis A is a spherical, non-enveloped virus classified as an enterovirus within the *Picornaviridae* family. Hepatitis A virus, like norovirus, consists of an

examination of faecal specimens from human volunteers.

Hepatitis A is considered the most serious viral infection epidemiologically related to the consumption of shellfish. It causes an acute infectious disease of the liver in adults, which can result in a severe debilitating illness, and occasionally a fatal fulminant hepatitis; although the disease is asymptomatic in children. The largest recorded epidemic of shellfish-vectored hepatitis A occurred in 1988 in Shanghai where ingestion of sewage-polluted clams resulted in almost three hundred thousand cases. Relatively large outbreaks and sporadic cases of shellfish associated outbreaks have also been reported in the United States,

in shellfish harvesting areas especially where elevated levels of faecal indicators indicate chronic or localised sewage contamination. Hepatitis A is more readily detected in Southern Europe than in colder Northern European climates, but again positive correlation with severe-to-moderate pollution of the harvesting areas has been shown. It has however generally proved much more difficult to establish reliable detection of either virus in products on the market or in batches associated with outbreaks.

The reasons for this are probably two-fold. Firstly, in many countries throughout the EU depuration is commonly practised. Depuration is a purification method where shellfish are retained in tanks of clean seawater with ultra-violet disinfection under controlled conditions. Depuration relies upon the natural filter feeding mechanisms employed by shellfish to flush out and remove incurred pathogens. Whilst this process may not result in the complete removal of all viruses, studies with cultivable viral surrogates such as the FRNA bacteriophage have shown that if carried out properly viral levels can be reduced. Secondly, since the introduction of RT-PCR based detection in the context of food microbiology, a very wide range of individual laboratory developed methodologies have been used, potentially with very different efficacies in terms of limits of detection and quantitation. Thus, direct comparisons between data generated by different laboratories and from different countries may not always be legitimate. From a legislative perspective in food microbiology there is a requirement for quality assurance of test results and standardisation of methods to ensure inter-laboratory equivalence. This is particularly important if the test results are used to inform important public health or trade decisions.

With the goal of virus method standardisation in mind, the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) (designated by the European Commission as the Community Reference Laboratory for monitoring viral and bacteriological contamination of bivalve molluscs), has been working with a network of expert virology laboratories throughout Europe via the European Committee for

Standardisation (CEN), to establish an internationally recognised method for the quantitation of norovirus and hepatitis A in shellfish and other foodstuffs. The methodology under development is known as real-time RT-PCR, which follows the general principles of RT-PCR but in addition incorporates a fluorescent reporter probe. This enables the measurement of specific target amplification throughout the PCR cycle by determining the excitation of fluorescently labelled

to allow for detection of the wide variety of circulating norovirus strains of both genogroups I and II. The method, which will be the first internationally recognised standard for the detection of viruses in foods using molecular techniques, will allow for simultaneous quantification of the pathogens in a variety of food matrices and is due to be published in 2012. Application of novel molecular based testing in food microbiology will bring substantial public health benefits, and

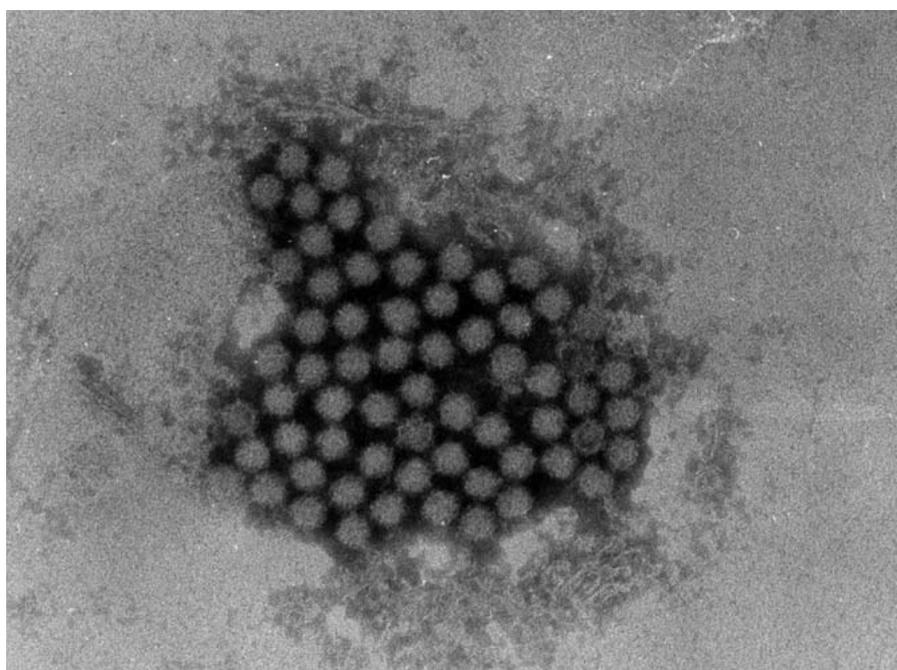


Figure 3. Electron micrograph showing characteristic fuzzy or indistinct capsid edges of human norovirus

molecules. Fluorescence is measured at each reaction throughout the cycle. As the point in the PCR cycle at which amplification can first be detected is proportional to the quantity of template, analysis of the fluorescence plots enables determination of the quantity of target sequence in the sample. In the 5' fluorogenic nuclease (TaqMan) real-time RT-PCR assay the fluorescent labels are attached to sequence-specific nucleotide probes that allow simultaneous confirmation of the target sequence. In the CEN methodology under development primers and probes are designed so as to be complementary to the 5' non-coding region of the hepatitis A virus and the ORF1/ORF2 junction of the norovirus genomes. The ORF1/ORF2 junction region is sufficiently conserved

help to ensure that we continue to enjoy dining on bivalve shellfish for the next two thousand years.

further information

For further information see:
www.crlcefas.org

D.N. Lees (2000) Viruses and bivalve shellfish. *International Journal of Food Microbiology* 59 81-116.

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How irreplaceable are microbes?

In November 2008, millions of avid fans were tuning in to watch their favourite celebrities battle it out on *Strictly Come Dancing*. Meanwhile, the Earthwatch Institute was holding its own *Strictly Come Species* event, “Irreplaceable — The World’s Most Invaluable Species.”

Irreplaceable is a complex concept in terms of terrestrial organisms. It is both subjective and time-sensitive; ask a *Tyrannosaurus rex* about irreplaceable species and it would tell you the world could not do without its favourite snack, an herbivorous hadrosaurid. We must also appreciate that the judgement is from our very human perspective — not from that of a cow, an oak tree or a mamavirus, to which very different organisms would be essential. Just like *Strictly Come Dancing*, the ‘Irreplaceable’ debate is part-popularity contest, but with an added measure of scientific argument.

There are between 90 and 225kg of microbes per acre of good agricultural soil and, according to estimates, the total mass of microbial life on Earth is so large it is almost incalculable. In fact, microbes account for an estimated 5-25 times the total mass of all animal life and almost the mass of plant life (animals account for a measly one thousandth of the Earth’s biomass). It comes as little surprise, then, that two of the five contenders were microbes — fungi and plankton.

The creatures themselves were unable to self-represent due to insurmountable communication difficulties (the waggle-dance is an excellent way for honeybees to exchange messages, but most humans would find it difficult to understand. Chemical signals are perfect for plankton but don’t project so well in a room at the Royal Geographical Society). So scientists were asked to debate on behalf of their preferred species using a language that we can all understand.

The debate

Dr Kate Jones of the Zoological Society of London thinks one of the reasons bats should be protected is their diet — some bats consume tonnes of insect pests, quashing the need for pesticides. Dr Jones was keen to draw attention to the “extraordinary uniqueness” and variety exhibited by bats, which range from the size of a bumblebee to a flying fox. They are the only mammals capable of powered flight and account for one in five mammalian species. Bats communicate via a sonar system using the loudest (ultrasonic) noises in nature. They are also used as headline indicator species; ecologists monitor them closely as they give an early warning if other species are in danger.

Some 20,000 species of bee have evolved extremely close symbiosis with 250,000 species of flowering plants, started Dr. George McGavin of Oxford University Museum of Natural History. Three quarters of our agricultural crops are also pollinated by bees. In 2005 the total economic value of bee pollination was estimated to be £130 billion, representing 10% of the world’s food value. Despite their economic and ecological value, “*Bee populations all around the world are in freefall... this trend is expected to continue.*” ▶

Dr McGavin ended with a warning: *"In the next 50 years you will find out which of those species tonight are truly irreplaceable."*

Professor Lynne Boddy of Cardiff School of Biosciences began her argument: *"Most people think of fungi as things that rot their food, their homes, kill their plants, or even have the audacity to grow on them... I'm here to show you different."* Mushrooms, Quorn, beer and bread are all made of or by fungi, which are also responsible for the flavour of blue cheese and the texture of Camembert. In fact, most cheeses are made using fungal enzymes. Fungi also produce the active compounds in many so-called wonder drugs, such as cyclosporin, statins, penicillin, steroid contraceptives and anti-inflammatories. Fungi work away behind the scenes to bring us the everyday items we take for granted, including *"The most important of all — chocolate!"*

Professor Boddy then turned her attention to the environment. Over 600 million years ago, fungi allowed plants first to colonise land. Now over 90% of plants live with fungi called mycorrhizas associated with their roots. In a symbiotic relationship such as this, the fungus takes up water and mineral nutrients and protects the plant from pathogens. *"Whenever you look at a plant now outside,"* said Professor Boddy, *"you've got to think not 'that's a plant, a lovely plant', but 'that's a wonderful plant plus fungus'. It wouldn't be there without the fungus."* Some fungi have become very well adapted to extreme ecosystems, including lichen. In all, there are 1.5 million fungal species, half of which are still waiting to be discovered. And the final question: *"Would the biosphere function without fungi? No."*

According to Professor David Thomas of the School of Ocean Sciences, University of Bangor, if all the other contenders were wiped out, plankton would still be here. But if plankton were removed, none of the others would survive for very long. *"We live on Planet Water and, of course, water covers 70% of the Earth's biosphere. That's where the plankton live."* Plankton can be everything from bacteria to large jellyfish that drift in the water, everywhere from a birdbath to the Pacific Ocean. There is so much we can even see it from space!

Professor Thomas chose to focus on phytoplankton, the main players in the world's biosphere; *"Phytoplankton... are the gas regulators of the planet."* These microscopic ocean-dwellers keep the level of oxygen stable for organisms that need it for respiration, including us. The balance of oxygen and carbon dioxide is very closely regulated and if plankton were taken out of the equation, that balance would collapse.

In addition to this, phytoplankton can control the climate: they contain dimethylsulphoniopropionate (DMSP) which, when broken down, releases dimethyl sulphide (DMS), the 'seaside smell'. When this gas is oxidised in the atmosphere, it promotes the formation of clouds, which then cool the Earth's surface. Plankton are also the producers in the marine food web, providing many animals, including the world's largest mammal, the Blue Whale, with food. When these animals die, it is planktonic bacteria that break down their remains. In fact, these microbes decompose a greater mass of organic matter than all the sea animals combined! Professor Thomas concluded: *"My case is simple. Vote for plankton — your life depends on it, my life depends on it... the Earth would cease if plankton was not there."*

Although alluding to the fact that we are all primates, Ian Redmond OBE, Chairman of the Ape Alliance, decided to focus on the non-human species. Economically, primates are very important — Chimpanzee tourism is helping to improve the lives of people in the local community. Ian also refers to primates as the *"gardeners of the forest"*; they build nests in the trees, gathering foliage into tight balls to leave gaps through which the sun's rays can penetrate, germinating seeds on the forest floor below. Some seeds even have to pass through the digestive system of a fruit-eating mammal in order to grow — primates and plants have evolved together.

After the first round of voting, plankton were in the lead with bees in second place.

Dr McGavin reviewed his argument for the bees, calling for more research to avert a disastrous situation involving hunger and potentially the loss of thousands of species. *"Einstein is supposed to have said that if the world was to lose its bees, humans*

would have less than four years... he knew a thing about bees. I hope you will vote the only way you can vote, and that is to save the bees."

"Life started with the plankton, that's where it all began, and believe me, life is going to end with the plankton." David Thomas recapped his argument, then turned to diatoms, the beauty queens of the microbial world. *"They have been an inspiration for lightweight design and architecture throughout the ages... even today, there are people that are looking at the structure of the plankton and the way they are built,"* said David. *"There are car companies out there building wheel hubs out of plankton-inspired structures... Plankton have been the inspiration for many, many people."*

The results

The final vote brought a surprise twist — bees swarmed past the plankton and Dr George McGavin took the lead, winning the debate.

Although bees are very deserving winners, my vote went to plankton, which have a significant impact on the Earth's biosphere. But where, I hear you ask, were the bacteria? Without bacteria, millions of animals would be unable to digest their food. Cyanobacteria were (arguably) the first species on Earth and their relatives have colonised unimaginably harsh environments, including the International Space Station. Like fungi, bacteria are used in many industrial processes. They protect us as well as making us ill — even bees have gut bacteria that keep them healthy!

Of course, a similar argument could be put forward for any number of different species — the key to the debate was to make us think about different organisms and to consider life without them. The last word goes to Dr Kate Jones: *"We are all going to lose if we don't find a way of working together sustainably. And we shouldn't have to have this debate and we shouldn't have to make a choice. And that, speaking as a scientist and as a conservation biologist, is the critical challenge of our generation."*

Lucy Goodchild

Imperial College London

Standing up for Science



It is easy to find examples of sensational headlines and even misinformation in the public domain around subjects such as MRSA and avian influenza. Scientists are increasingly encouraged to engage with the public around their subject area, but it can be hard to see what scientists can do to counteract so-called “pseudoscience” or to publicly set misinformation straight particularly when they are at the beginning of their career.

Over five years ago, the charity *Sense About Science* noticed that the voice of early career scientists was missing from public debates. When we asked them why they weren't involved, we found most were very passionate but didn't know how to get started, or weren't aware of the opportunities available. To respond to this problem we set up the Voice of Young Science programme, consisting initially of a series of workshops to help bridge the gap between scientists and the media. Some of the concerns early career scientists raise at the workshops include apprehension about talking to the media because their work is sensitive in nature or even confidential, or that their supervisors may not be supportive. These concerns may seem like a barrier, but early career researchers know more than the public and can get involved in debates about science that stretch beyond their own research.

The VoYS network have since taken on an active role to combat pseudoscience, exploring different ways they can stand up for science in public and make an impact on public debates.

In 2007 a few of them shared their frustration of pseudoscientific product claims — from herbal mixtures that “can rid you of over 100 types of parasites” to yoghurt that “optimises the release of energy from our diet”. They decided to contact the manufacturers of these products and find out what evidence they had for these claims. In October 2007 VoYS released a dossier of these experiences, *There Goes the Science Bit...* which was picked up by both national and international media. By questioning these claims, the VoYS network made the companies aware that someone was watching and holding them to account.

Since the project was launched, we have had a huge response with people joining our campaign and more and more examples being sent to us. We have also had a lot of requests from schools who find these products are something students can relate to, helping them to question and evaluate claims they come across in the media. In the new examples one thing we noticed was the misuse of scientific terminology to sell products. In particular we kept coming across the word ‘detox’. When we looked further we found a huge variety of ‘detox’ products ranging from teas to hair straighteners. VoYS decided once more to investigate and find out what companies meant by ‘detox’ and what evidence they had for their claims.

They found that companies had different definitions of what ‘detox’ meant but in reality ‘detox’ doesn't exist outside drug addiction or poisoning treatments. The companies

had little or no evidence to back up their claims and worryingly they were making misleading claims about how the body actually works. VoYS responded by putting together an ‘anti detox’ leaflet explaining how the body is capable of dealing with most chemicals we encounter. Some of the VoYS network even took to the streets to distribute the leaflet outside chemists explaining that the best thing after Christmas is to get a good night's sleep and have a glass of water. This ongoing campaign, has been picked up by a range of media, from local radio to international media, creating a public debate about why ‘detox’ products don't work.

It doesn't always have to be about big actions — there are plenty of things that can make an impact on public debates about science. The VoYS network have shared their experiences in a new publication, *Standing up for Science 2 – the nuts and bolts* in which they recount what works and what doesn't. For example, writing a letter to a newspaper may not seem like it can have a big impact but it gives you a chance to get across the science and evidence around a subject. Sometimes a letter will lead to journalists following up the story and *Sense About Science* has often found scientists to speak out on issues through this route. As Chris Benfield, *Yorkshire Post*, said “*Writing a letter usually gets you more column inches that giving a reporter a quote and the letters page is probably the best read page in any publication*”.

It is frustrating to see bad science reported but no matter who you are, what your background is or how much experience you have, there are always ways you can raise awareness of good science and evidence in public debates.

further information

You'll find a copy of *Standing up for Science 2 – the nuts and bolts*, with this issue of *Microbiologist*. To find out more about joining VoYS, or to order copies of *There Goes the Science Bit*, contact Alice at voys@senseaboutscience.org. You can also visit our website at: www.senseaboutscience.org

Alice Tuff

VoYS Co-ordinator, *Sense About Science*



Historical perspectives:

Familiarity with the disease known colloquially as “Blight” or “Late Blight” of potatoes, caused by the fungus *Phytophthora infestans*, will furnish a microbiologist with knowledge of an historical and socio-biological nature, potentially well beyond that obtained through the microscope objective or PCR gel. The biology, pathology and demands placed upon present day growers by *Phytophthora*, whether subsistence or large-scale, requires continuous monitoring. However, greater horizons than that of the necrotrophic relationship of fungal pathogen and potato host demand foremost attention. Particularly as there are lessons of human fallibility and the consequences of ignorance or reliance on false premise to be learnt, since ignorance and prejudice compounded the miseries resulting from outbreak of fungal disease alone.

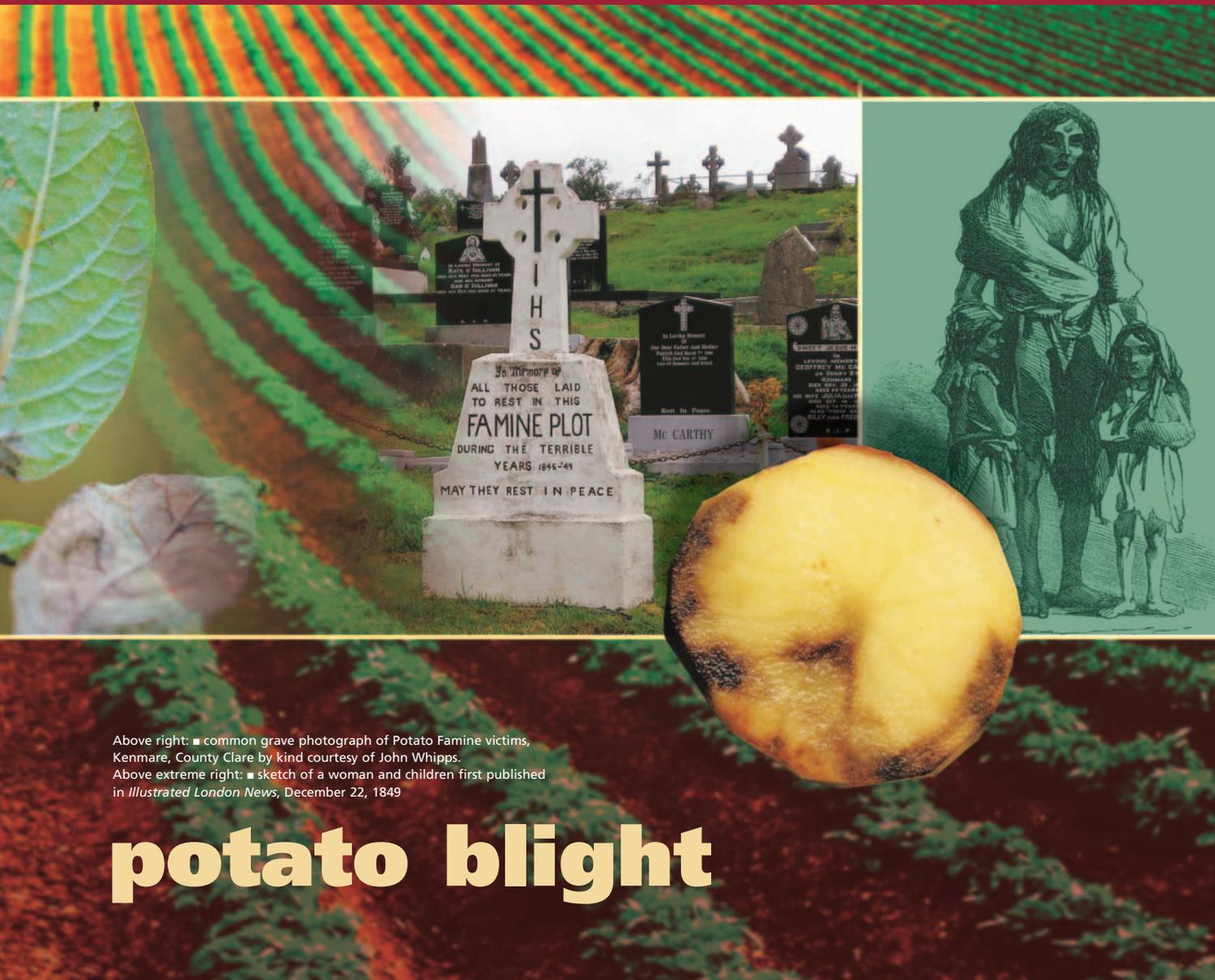
Potato blight made its first well documented or large scale occurrence

in 1845, when potato crops of Belgium, Holland, Poland, UK and particularly Ireland, were devastated. Symptoms were black necrotic areas, developing rapidly from the edges of initially a few plants (Figure 1), quickly spreading throughout a plants foliage, into tubers and on to other plants. Leaf material or haulm rapidly decayed in such a widespread nature that reports of the period indicate even the air became tainted with a smell of putrefaction. In turn, any part developed tubers were destroyed in the ground, as were those harvested and stored before obvious signs of blight. Reports suggest that the whole potato crop of a large county or its equivalent was destroyed in a matter of days.

Such a dramatic and shocking phenomena left individuals and authorities powerless. Nothing similar had been experienced on such a grand scale, even though some past local scale potato crop failure had been

Figure 1. Early blight manifestation with characteristic black necrotic leaf edges





Above right: ■ common grave photograph of Potato Famine victims, Kenmare, County Clare by kind courtesy of John Whipps.
 Above extreme right: ■ sketch of a woman and children first published in *Illustrated London News*, December 22, 1849

potato blight

experienced in Ireland. Learned and with hindsight not so learned individuals or groups were tasked with determining the reasons for such a catastrophic occurrence, particularly as authorities of the time were aware of the dire consequences which could unfold.

A number of explanations were put forward, which are well worth consideration in the light of hindsight and development of scientific method: a particularly popular explanation was that affected plants were suffering from a surfeit of water. Weather preceding onset of symptoms had been “balmy” and excellent for potato growing. So much so, that governmental authorities in the likes of Ireland were confident of an abundant crop as reported by Irish constabulary - itself indicative of the importance of the potato crop. In turn, explanation of the widespread destruction was that plants had grown rapidly during the sunny warm weather,

but with the onset of rain and protracted overcast conditions, plants had taken up too much water. Without the drying effects of the sun it was thought the plants had become surcharged with water and in effect exploded. Other suggestions of the period included:

- Potatoes blighted by static electricity generated in the atmosphere by smoke issuing from newly developed steam engines.
- Poisonous mortiferous vapours arising from underground, hence unseen volcanoes.
- Poisonous guano manure particularly from sea birds
- Clothes/naked plants — an explanation apparently originating in County Clare, where the practice was to dry and air washing by spreading such over plants. In turn the poor weather precluded such practice hence the plants were naked and presumably shriveled up in shame.

Such suggestions effectively indicate that in the absence of structured scientific method, such explanations had little foundation and effective response was thereby compromised. In contrast, the observations of Rev. M.J. Berkeley and his associated deduction that plants were succumbing to an infectious entity, has stood the test of time. Detractors of such an explanation, while accepting the presence of fungal structures, claimed they developed by spontaneous generation from rotting plants and as such were merely a manifestation of plant stress. Rev. M.J. Berkeley had in effect anticipated the concepts of Louis Pasteur by nearly a quarter of a century.

In contrast to other European countries, Ireland and the Irish were particularly stricken. An understanding of the circumstances, which gave rise to such a state of affairs provides historical as well as significant continuing socio-biological and

geopolitical insights. Some indication of the nature of said insights is given by a past colloquial term for the potato, that of the “Irish Root”, a manifestation of the potato crops importance to Irish peoples of the period.

A number of contributing factors account for the near exclusive contribution that the potato made to the diet, hence nutrition of many Irish people:

1) Potatoes are relatively nutritious, comparatively easy to grow, productive in short and damp periods or growing conditions (6 tons/acre) with any waste readily consumed by livestock.

2) Small parcels of land for growing became commonplace due to the nature of inheritance patterns. Sub-division of agricultural land, poor growing conditions and soils precluded growth of cereal staples such as wheat.

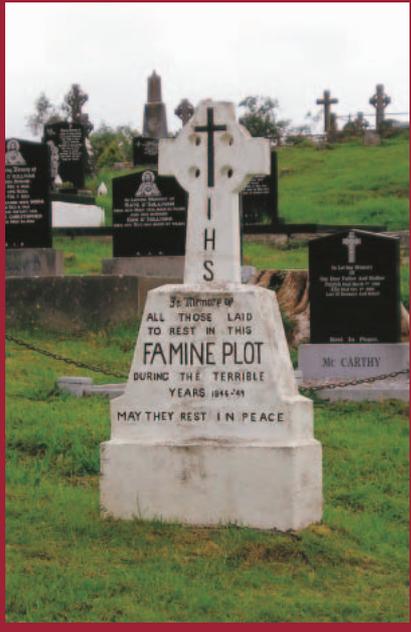
3) An expanding population — Irish females were considered very fertile as over a twelve year period nineteen out of twenty women produced a child every other year, leading to an expanding population, on the edge of famine and reliant on a limited diet.

4) Religious bigotry, economic, political indifference and dogmatism: weak or non-existent administrative structures to acquire and distribute alternative staple foods

5) Poor infrastructure as relates to roads and harbours, compromising supply and delivery of alternatives should that have been envisaged.

These factors and the near subsistence level of existence experienced by much of the Irish population, with a typical balance of a couple of pounds per annum per family precluded purchase of alternatives. Hence, the collapse of the potato harvest and even destruction of what seemed healthy tubers put into storage, mediated by *Phytophthora infestans*, resulted in near immediate famine. The Irish population collapsed, from a population of approximately eight million, estimates suggests that close to a million died of famine (Figure 2). Also, close to two million were driven off the land, or emigrated in great poverty, distress and extreme poor health, resulting in their death during passage, or in quarantine. Their plight and suffering is still widely remembered through the likes of the Famine Museum, Co. Roscommon (www.strokestownpark.ie/museum.html),

Figure 2. Common grave of Potato Famine victims, Kenmare, County Clare (Courtesy John Whipps)



housed in the former classical property of Thomas Mahon, a notorious landlord of the period.

Circumstances were nothing like as desperate in mainland Europe or much of the remainder of the British Isles, as there was not the reliance of the Irish poor on potatoes. Other staple crops such as wheat and rye were available and through the rapidly developing industrial revolution, monies were forthcoming to buy the likes of bread. However, in addition to famine, poignant memories remain deeply entrenched and consequences far reaching. These include a perpetuated historical enmity to the English, who according to common sentiments had acted brutally from Cromwellian times if not before. Furthermore, a large and influential American community and the likes of “Repeal of the Corn Laws” in the UK, with associated importation of American and Canadian wheat and a marked shift of Britain away from agriculture to an industrial trading nation is quite a sobering legacy for a simple fungus.

In the light of such occurrences the biology of *Phytophthora infestans* deserves at least some consideration and understanding, as it would be arrogant to assume this destructive pathogen has been consigned to the annals of history and museum displays. Furthermore as highlighted by a recent

BBC series of “factoids” (June 2008) potatoes are the world’s third staple food, readily processed into many forms or products. Hence even though modern day producers invest heavily in sophisticated production regimes (Table 1), can call upon extensive and diverse equipment, the health of potato crops worldwide is of continuing great importance, with the related threat of blight never far removed.

The epidemiology of the 1845 outbreak of blight and the arrival of *Phytophthora infestans* on European shores remains something of a mystery (Table 2). However transportation and movement of materials as relates to human activity, remain central to the biology and spread of blight.

Phytophthora in the form and strain which arrived in Europe in the 1840s, cannot survive through a winter period in the absence of a living host. Blight therefore initially spreads from tubers leftover (Figure 3) from a previous harvest or discarded substandard and poorly disposed of tubers. With the onset of a fresh growing season, such tubers sprout producing new plants or volunteers (Figure 4), which may also carry the pathogen inside them. Although this is a well recognized occurrence, it makes eradication near impossible to this very day. Should volunteers carry *Phytophthora* they act as disease loci from which the pathogen may rapidly spread. The fungus erupts from leaf surfaces through stomata, a natural point of weakness to produce zoosporangia, which are rather like enclosed miniature swimming pools, in turn containing potentially motile biflagellate zoospores.

Zoosporangia dispersed on the wind

Figure 4. Typical “volunteer” potato plant, developing from a tuber left from the preceding seasons harvest



Table 1. Summary of potato growing costs and potential profit (Courtesy of John Davison — grower for Walkers Snackfoods)

Land and cultivation costs Land rental/purchase, irrigation, 'seed', cultivation practices (ploughing/stoning/bed formation/planting etc.)	£750
Chemical inputs Fertilisers, herbicides, fungicides, insecticides, growth regulators, adjuvants, and dessicants	£300
Harvest and storage costs Harvesting, grading procedures, equipment hire/building depreciation, storage chemicals.	£700
Management costs Agronomy consultants, office administration, IT, whisky, Range Rover etc	£100
Returns/Income Yield approx 18 tons/acre, value £135.00/ton	Profit £250.00/acre

Table 2. Possible routes by which *Phytophthora* arrived in Europe

● First reported around the ports of Philadelphia and New York in 1843 - 1845 reported/found in Europe
● Mexico - United States - Europe ● *Andean region/Peru - United States - Europe
● Mexico - Peru - United States - Europe
*Andean countries exported guano as fertiliser and seed potatoes on steamships to USA and Europe in the 1840s

or more locally by rain splash, release on impaction actively swimming zoospores, which can move through a water film and across a leaf surface to locate an open stomata by chemotaxis. Encystment rapidly occurs over the stomata and a filament penetrates through the stomatal pore to ramify and exploit underlying tissue. Within three to five days characteristic dark necrotic lesions develop accompanied by rapid widespread tissue degeneration, a sequence of events repeated many times over.

Weather conditions are central to such a life cycle, particularly water availability and its role in supporting zoosporangium dispersal and zoospore activity. Weather periods when conditions are conducive to increase disease incidence / spread have been termed Beaumont or Smith periods. In addition, modern day growers with the greatest acumen have developed local level integrated sensors linked to international central prediction facilities to detect humidity and leaf surface moisture amongst other parameters, which in turn warn of blight and requirement for prophylactic action.

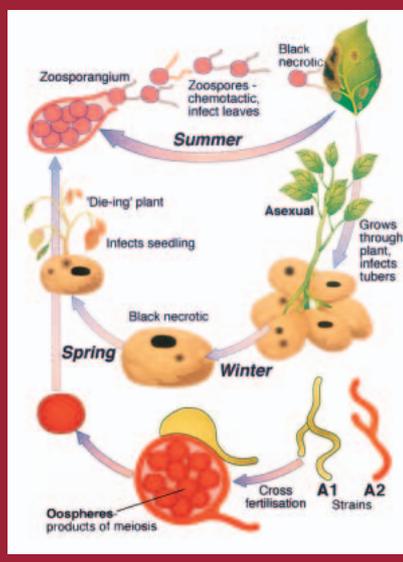
Such expensive and careful monitoring confirms that the threat of blight remains of great concern, furthermore a history of blight and an understanding of biology suggests this disease will continue to present itself in

challenging forms. As already noted, human practice, particularly transportation patterns, contributed to the spread of blight in the 1840s. In turn, transportation has further contributed to blight concerns as until the 1980s only a single strain, designated A1, of *Phytophthora infestans* appeared to exist in Europe (Figure 3), hence sexual reproduction was precluded. However, restriction fragment length polymorphism (RFLP)

studies of the mid 1990s (Govers, *et al.*, 1997), demonstrated that new strains were appearing in Europe, for example in Scotland amongst both commercial and private concerns. These were potentially derived through the importation of another strain (A2) originating and probably imported with a single consignment of potatoes from Mexico. Marked consequences potentially result as gene shuffling and selection of strains can occur, including resistance to commonly employed fungicides and a capacity to exploit established resistant potato varieties. However, greater concern is manifest in the life cycle of *Phytophthora*. Figure 3 shows that sexual reproduction gives rise to typical structures termed oogonia containing oospores. These entities are relatively robust and able to survive periods of drought, cold and nutrient deprivation, such as winter or fallow periods, without the requirement for a living host. Such dormant entities, buried in soils, are not amenable to prophylactic treatments and measures of crop sanitation or rotation to reduce blight incidence could be greatly compromised.

In conclusion potato blight as mediated by *Phytophthora infestans* presents us with many lessons of an historical and biological nature, as well as those related to human character, practice and specifically the consequences of poor, inadequate or even the abandonment of scientific method when facing disease incidence.

Figure 3. *Phytophthora* asexual life cycle and putative sexual life cycle, a consequence of further strain advent



references

- Govers F, Drenth A & Peiterse CMJ, 1997. The Potato Late Blight Pathogen *Phytophthora infestans* and Other Pathogenic Oomycota. In "The Mycota V" ed. K. Esser & P. A. Lemke, Springer-Verlag Berlin Heidelberg New York.

acknowledgment

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In the sixteenth of a series of articles about statistics for biologists, **Anthony Hilton & Richard Armstrong** discuss:

Fitting a regression line to data

Stat Note 16

In Statnotes 14 (Hilton & Armstrong, 2008a) and 15 (Hilton & Armstrong 2008b), the use of correlation methods to analyse the relationship between two variables was described. One of the most widely used statistics is Pearson's correlation coefficient r which tests whether there is a linear correlation between two variables X and Y . Once a *linear* correlation between two variables has been established, however, a regression line can be fitted to the data to describe the relationship between the two variables in more detail.

Regression analysis is one of the most useful techniques for the analysis of data in microbiology and has many uses. For example, the objective may be to determine simply whether a relationship exists between Y and X , to study the shape of the relationship (whether linear or curved), to establish a mathematical equation linking Y and X , or to predict the value of Y for a new value of X . This statnote describes the methods used to fit a regression line to data and how to test the statistical significance of the line. Subsequent statnotes will describe how to use the line for prediction and calibration, and how to compare two or more regression lines. As in statnotes 14 and 15, upper case letters are used to label variables and lower case letters to indicate individual observations.

Theory

To illustrate the theory of regression, a study was carried out to investigate whether bacterial biomass in a fermentation flask (Y) was linearly related to the concentration of media supplement supplied (X). Eight flasks with different concentrations of media were inoculated with a bacterium. As in Statnotes 14 and 15, Y is the 'dependent', 'outcome', or 'response' variable and X the 'independent', 'predictor', or

'explanatory' variable. Bacterial biomass is considered to be 'dependent' upon media concentration and hence, biomass is the 'dependent variable' and concentration the 'independent variable'.

The first stage in a regression analysis is to plot the values of Y against X on a graph (Figure 1) from which it is apparent that there is an increase in biomass with increasing concentration of media. The straight line that provides the best fit to these data is called the *sample regression* of Y on X or the *fitted line*. This regression line has the equation:

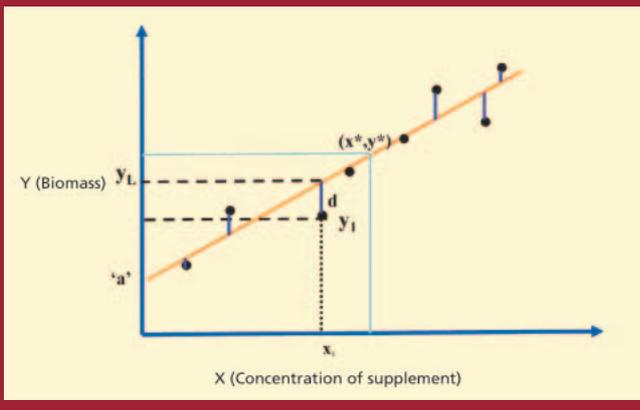
$$Y = a + bX \quad (1)$$

where a is the point at which the line cuts or 'intercepts' the Y axis and b is the slope of the line, i.e., the rate of change in Y per unit of X . This equation may look familiar and is indeed derived in the same way as the straight line $Y = mx + C$ more frequently encountered in mathematical situations.

How to fit the line of 'best fit'

The line of best fit is calculated according to the '*least squares method*'. First, the line passes through the centre of the cluster of points, i.e., the point with co-ordinates that are the means of the X and Y values (x^*, y^*). The distance d measures the difference on the Y scale between the actual value of Y at that point (y_i) and the corresponding value of Y on the fitted line (y_L), i.e., $d = y_i - y_L$. Hence, the distances d , calculated for all data points, measure the deviations of the points from the fitted line. The fitted regression line satisfies two further conditions: 1) that the sum of the distances d from the line is zero ($\Sigma d = 0$), i.e., approximately half the data points will be above and half below the line and 2) that the sums of squares (SS) of the distances (Σd^2) will be as

Figure 1. Fitting a regression line to data.



small as possible, i.e., the *SS* of the deviations from the line (hence, *least squares*) is minimised. It can be shown for n pairs of observations that there is only one straight line that can be drawn that fulfils all of these conditions and this line has a slope b given by the formula:

$$b = \frac{\{\sum xy - (\sum x \sum y / n)\}}{\{\sum x^2 - (\sum x)^2 / n\}} \quad (2)$$

The equation for the slope of the line b is similar to that of Pearson's r (Statnote 14). The numerator is the sum of products, i.e., the sum of the x and y values multiplied together and the denominator is the *SS* of the x values alone. The slope b , also known as the *regression coefficient*, estimates the average change in Y associated with a unit increase in X . It is not intuitively obvious why equation (2) should estimate the slope of the regression line 'b'. However, this equation is based on two independent estimates of the increase in Y per unit of X and is the weighted mean of these estimates (Snedecor & Cochran, 1980).

Scenario

Background

To see regression analysis in practice, we return to the scenario described in Statnote 14. Essentially, adequate skin antiseptics prior to invasive procedures is important in preventing infections. Nevertheless, skin antiseptics permeate poorly into the deeper layers of the skin and into hair follicles, which may harbour microorganisms and cause infection when the protective skin barrier is broken. One potential mechanism of delivering antiseptics deeper into the skin is to co-administer a 'carrier' compound to facilitate movement of the biocide through the various skin layers. Hence, the aim of the study was to evaluate the permeation of a commonly used biocide into the full thickness of human skin when applied alone, or in combination with a 'carrier' compound.

Method

Full thickness human skin samples were obtained from patients undergoing breast reduction surgery. The skin permeation studies were performed with vertical diffusion cells with the stratum corneum of the skin sample uppermost. One ml of antiseptic solution in the presence or absence of the carrier compound was aliquoted onto the skin, and incubated for 2 minutes, 30 minutes, or 24 hours. The assay was performed in triplicate. Following the exposure to the antiseptic solution (+/- carrier) the skin was washed with PBS

and three 7mm punch biopsies taken from each sample. The biopsies were cut with a microtome into 20µm slices from the skin surface to a depth of 600µm and 30µm slices from 600µm to 1500µm. The weight of the skin samples was determined and each analysed by HPLC to determine the concentration of antiseptic present as µg antiseptic per mg of tissue. A number of mathematical models might describe the pattern of penetration of the antiseptic into the skin. In the correlation analysis described in Statnote 14, we wished initially to describe and test the model by which the antiseptic penetrated into the thickness of the skin. The data suggested that a diffusional model poorly described the penetration profile observed and further models were tested specifically whether the antiseptic alone, i.e., without carrier and after 30 minutes, would have penetrated the skin according to a 'power-law' model. A variable Y is distributed as a power-law function of X if the dependent variable has an exponent ' a ', i.e., a function of the form $Y = CX^a$. If penetration of the antiseptic does follow such a law, then a log-log plot of the data should be linear.

Regression analysis

The relationship between log concentration of the biocide (Y) and log skin depth (X) is shown in Figure 2. A linear regression line has been fitted to these data by the method of least squares and has the equation:

$$\text{Log}Y = 0.0114 - 0.811\text{log}X \quad (3)$$

The regression analysis confirms the negative relationship between concentration of biocide and skin depth and provides a mathematical equation linking these two variables.

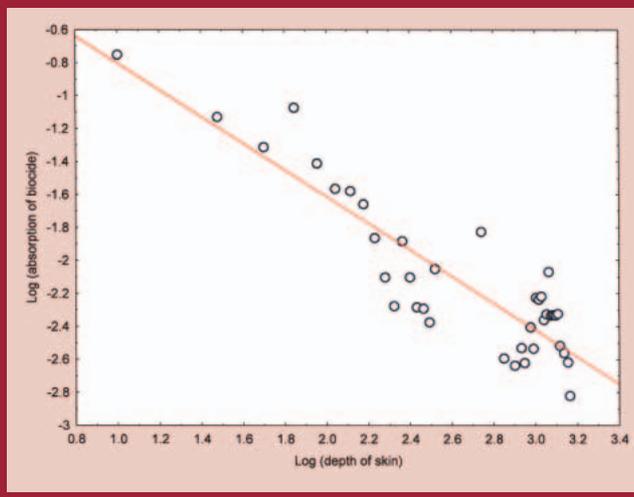
Goodness of fit of the points to the line

It is important to test how well the line fits the data points. Three methods are commonly used.

The coefficient of determination (r^2)

First, the square of the correlation coefficient r^2 (also known as the 'coefficient of determination') represents the proportion of the variance of the Y values attributable to the linear regression on X (Statnote 14). Hence, r^2 provides an

Figure 2. Relationship between the degree of penetration of the biocide (without carrier) and depth of skin (Pearson's correlation coefficient $r = -0.89$, $r^2 = 0.79$; $F = 144.72$, $P < 0.001$; $t = 12.03$, $P < 0.001$).



estimate of the *strength* of the relationship between Y and X . In the present example, $r = -0.89$ and $r^2 = 0.79$, suggesting that 79% of the variance in log concentration of biocide was attributable to log skin depth. Hence, the line ‘accounts for’ or ‘explains’ 79% of the variation in concentration of biocide with depth, a highly significant proportion of the total variation. Note that there is no established ‘cut-off’ in r^2 below which the line would be regarded as a poor fit. Nevertheless, a line accounting for less than 50% of the variance would be regarded as a poor fit to the data.

Analysis of variance

Second, goodness of fit of the line can be tested using analysis of variance (ANOVA) (Armstrong & Hilton, 2004; Statnotes 9-12). ANOVA determines the statistical significance of the line rather than the strength of the relationship between the two variables. In an ANOVA, the total variation of the Y values is divided into a ‘linear effect’, i.e., that portion of the variance accounted for by the line, and the ‘error variance’ associated with deviations from the line, the two sources of variation being compared using a variance ratio (F) test. The total variation is the SS of the deviations of the Y values (Y_{ss}) from their mean (y^*):

$$Y_{ss} = \sum y^2 - (\sum y)^2/n \tag{4}$$

The total variation can then be broken down into the linear effect and error. The linear effect is the variation accounted for by the points that lie along the fitted regression line (y_L) from their mean (y^*):

$$\text{Linear effect} = \{ \sum xy - (\sum x)(\sum y)/n \}^2 / \{ \sum x^2 - (\sum x)^2/n \} \tag{5}$$

This equation closely resembles that for the slope of the line b (Equation 2) except that the numerator, the sum of products, is squared. The residual or error variation is based on the deviation of the actual y values from the regression line (i.e., the d values) and can be determined by subtraction:

$$\text{Error Variation} = SS \text{ of } Y \text{ values} - \text{Linear effect} \tag{6}$$

The ANOVA is shown in Table 1. The F ratio is a comparison of the magnitude of the linear effect with the degree of deviation of the points from the line. Hence, for there to be a statistically significant linear effect, the variation accounted for by the line must be greater than the variation of the data points from the line. To obtain a P value, the value of F can either be taken to the table of the F ratio (Fisher & Yates, 1963; Snedecor & Cochran, 1980) entering the table for 1 and $n - 2$ DF (where n is the number of pairs of observations) or the P value can be obtained from statistical software. If F is greater than the critical value at $P = 0.05$, there is a statistically significant fit to the data points. In the present example, $F = 144.72$ giving a P value considerably less than 0.001. The probability of obtaining an F value of this magnitude by chance is less than one in a thousand and hence the fitted regression line is highly significant.

***t*’ test of the slope of a regression line**

Third, we can test the significance of a regression by testing whether the slope of the line (b) is significantly different from zero. The ratio of b to its standard error (SE) (s_b) converts b so that it is a member of the t distribution. The SE of the slope of the line is given by the following equation:

$$s_b = \sqrt{(\text{Mean square error}) / \sum x^2 - (\sum x)^2/n} \tag{7}$$

Table 1. Analysis of variance (ANOVA) of the linear regression of log concentration of biocide (Y) in relation to log skin depth (X)

Effect	SS	DF	MS	F
Linear	7.0902	1	7.0902	144.72 (P < 0.001)
Error	1.76378	36	0.048994	

SS = Sums of squares, DF = Degrees of freedom, MS = Mean square, F = variance ratio

The mean square error is taken from the ANOVA (Table 1). The value of t is taken to the t table with $n - 1$ DF where n is the number of pairs of observations. In this example, a highly significant value of t was obtained ($t = 12.03$, $P < 0.001$) indicating that the slope of the line is highly significantly different from zero.

To decide which of the three methods of testing a regression line is appropriate depends on the precise hypothesis posed by the study. Hence, r^2 estimates the ‘strength’ of the relationship between Y and X , ANOVA whether the regression line is ‘statistically significant’, and the t test whether the ‘slope of the line’ is significantly different from zero.

A further use of regression analysis is the use of fitted lines in the prediction of one quantity from another and in calibration studies, e.g., predicting bacterial cell numbers from optical density readings and this use of regression will be described in Statnote 17.

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acknowledgement

We thank **Tarja Karpanen** and **Tony Worthington** (both of Aston University) for the use of data to illustrate this Statnote.



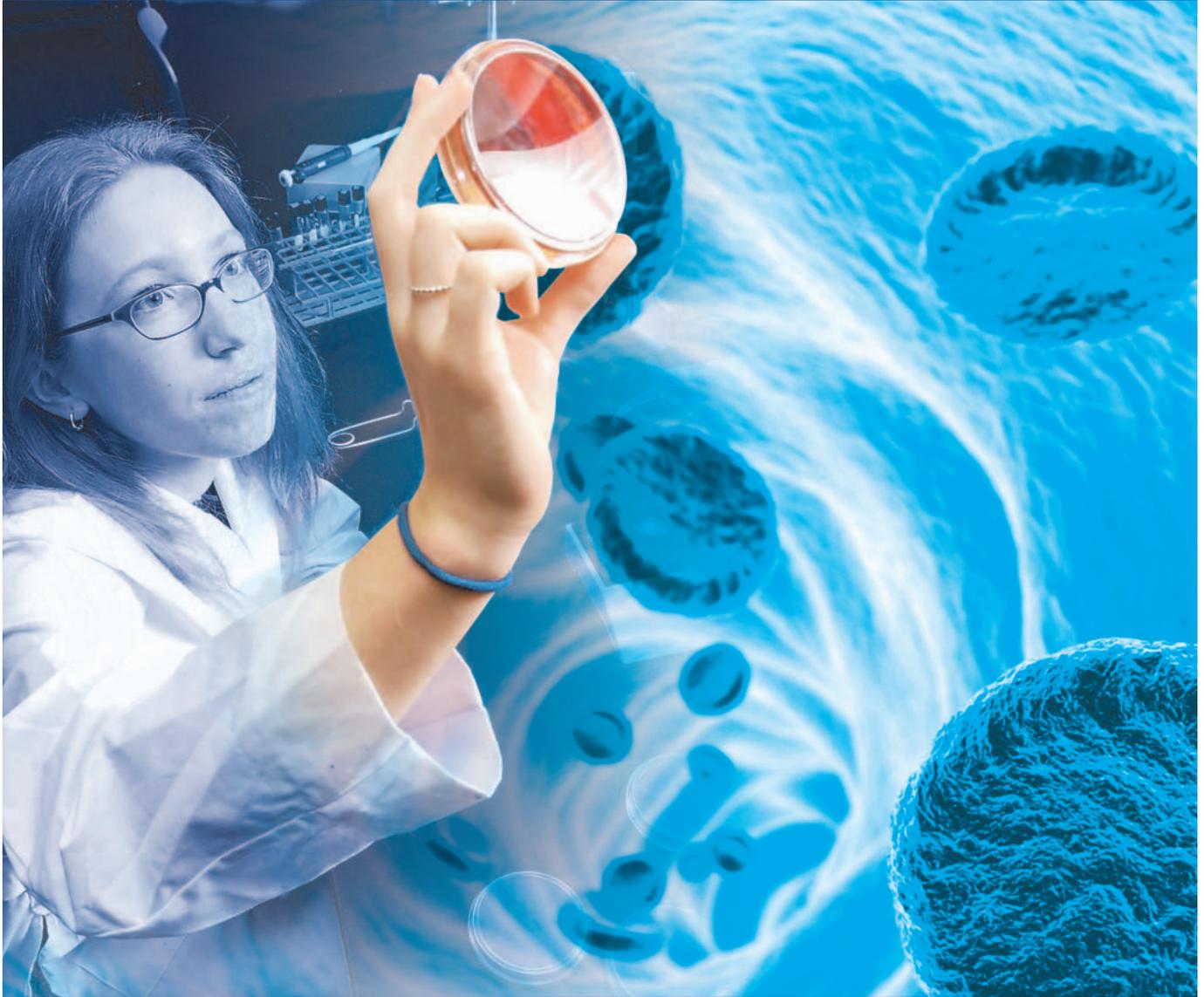
Anthony Hilton



Richard Armstrong

Dr Anthony’ Hilton and Dr Richard Armstrong²

¹Biology & Biomedical Sciences and ²Vision Sciences, Aston University, Birmingham, UK



Medical Microbiology

Bernard Wood describes the routes into Medical Microbiology and his work as a Biomedical Scientist

The role and title of a Biomedical Scientist has gone through many changes since I began my working career in Medical Microbiology. When I was first employed within microbiology I was given the title: Junior B Medical Laboratory Scientific Officer (MLSO). Since then our title has gone through another name change to that of Biomedical Scientist (BMS) and we are

sometimes referred to as laboratory technicians.

At present I am employed as the Lead Biomedical Scientist for Microbiology at Central Manchester University Hospitals NHS Foundation Trust. I am based in a department that is referred to as the Manchester Medical Microbiology Partnership (MMMP) which is composed of: two

Microbiology laboratories (one based at Manchester Royal Infirmary (MRI) the other at Wythenshawe Hospital), the Virology Department (including Electron Microscopy and Molecular Diagnostics), the Health Protection Agency (HPA) Meningococcal Reference Unit, and the Vaccine Evaluation Unit also based at Manchester Royal Infirmary. As a

Biomedical Scientist in Microbiology I am involved in the diagnosis, management and control of infection (viral, bacterial, fungal, and parasitic) in both hospital and general practice. Medical Microbiologists play a major part in finding ways to identify, prevent and treat infectious diseases such as TB, and control outbreaks of infections such as MRSA.

Having been employed as a Junior B MLSO, I completed an HNC in Medical Microbiology to become a registered MLSO. Career progression to senior grade was based upon becoming a Fellow of our professional body the then Institute of Medical Laboratory Science, by passing the Microbiology Fellowship Examination. Further progression through the ranks was achieved by obtaining a Diploma in Medical Laboratory Medicine and more recently a Masters in Management. This route of progression has been partially superseded by the move towards a policy of graduate entry into the profession. More changes are likely with the publication of The Future of the Healthcare Science Workforce which sets out proposals for Modernising Scientific Careers, as published by UK Health departments.

The route to becoming a Biomedical Scientist

The Institute of Biomedical Science (IBMS), as the professional body for biomedical scientists, verifies professional competence against the Health Professions Council's Standards of Proficiency. The Institute then awards a Certificate of Competence that can be presented as part of an application to the Health Professions Council (HPC) to register as a Biomedical Scientist.

The preferred route to enter into biomedical sciences is to enrol at a University offering a degree accredited by the IBMS. A number of Universities offer the option of a four year sandwich course or co-terminus degree that includes clinical laboratory placements with the opportunity to complete the IBMS Certificate of Competency Registration Portfolio. On graduation a student can therefore apply directly for registration with the Health Professions Council (HPC) emerging from their degree as a fully registered Biomedical Scientist.

There are also a number of

alternative routes of entry. For example it is possible to obtain a suitably accredited degree and complete the portfolio whilst employed within a pathology department. Other honours degrees which include as principal subjects one or more of the following subjects may also be accepted: animal physiology, biochemistry, biology, chemistry, microbiology, physics and zoology. Partially acceptable honours science degrees may need to be 'topped up' with an Institute accredited postgraduate certificate or diploma (PgC or PgD). Potential job applicants need to be aware of these requirements for registration and should approach the IBMS to obtain validation and details of any necessary 'top ups' required.

The first step is to submit an application to the IBMS to have your non-accredited degree programme and educational qualifications assessed by the Institute - unfortunately there is an assessment fee of £95 for this service. Once you have received details of your assessment it is advisable to then approach a University offering accredited top-up degree courses to ascertain what is required, this is usually a free service, but you may need to check this with the individual University. The University will then tell you what and how many modules you will be required to complete, and how many terms or years you will need to attend the University to complete the course.

Based upon the information provided by the Institute, a University is able to determine the number of units required to complete the Institute study requirements. This can be as many as six units which could potentially involve two years of part time study. With the recent Government decision to withdraw support for this type of study, Universities have been placed in a position where they may have to charge full cost for each extra unit. This may amount to as much as £1,500 each, potentially resulting in a fee in the region of £9,000 for somebody requiring six units of study. The removal of funding for these top-up degrees and the prospect of large bills could potentially make such candidates undesirable to potential employers, not only in terms of cost but also in the amount of laboratory work time lost in attending University courses.

In summary there are four entry routes into the profession:

1. Entry with an approved Biomedical Sciences degree programme plus one year of in-service training, having completed the IBMS Certificate of Competency Registration Portfolio whilst employed within a pathology department.
2. Entry after completion of a part-time approved Biomedical Sciences degree, plus completion of IBMS Certificate of Competency Registration Portfolio.
3. Entry with non-accredited degree which usually involves PgC or PgD plus completion of the IBMS Certificate of Competency Registration Portfolio.
4. Entry from a co-terminus Biomedical Sciences degree where professional practice is integrated.

The complexity of entry is further highlighted by the terms of conditions of employment as outlined in Annex U of NHS Terms and Conditions. Salaries are based around a percentage of Band 5 for options 1-3 depending upon the number of years training required to gain the necessary qualifications. Students having completed the Certificate of Competency as part of their degree (option 4) are appointed directly into Band 5.

The future may be uncertain for all healthcare scientists including biomedical scientists. The publication of *The Future of the Healthcare Science Workforce Modernising Scientific Careers: the next steps* aims to develop a common training and career pathway, education and training standards for all healthcare scientists. At present the education and training pathways are somewhat confusing, especially with respect to pre-and post-registration training. It is hoped that this new initiative will bring some clarity to the present situation. The prospects may be uncertain, yet the challenge of adapting to these changes may bring greater opportunities to an undervalued workforce in the future.



Bernard Wood

Manchester Medical
Microbiology Partnership



News from the SfAM Post-Graduate and Early-Career Scientist Committee

Call for Nominations!

The PECS committee invites nominations for Chair and Secretary. The posts will be available from July 2009 (for one year's term) and are open to all student or early career scientist members of SfAM. Descriptions of the key responsibilities and benefits of each of these roles can be found on the 'in the loop' page of the December issue of *Microbiologist*. So if you are keen to get involved with PECS and think either of these roles could be for you, then please forward your nomination along with a supporting statement to George Aboagye (PECS Chair) at: gkaboagye@yahoo.com



Vicki McCune
PECS Communications
Officer
Newcastle University

Congratulations!

Congratulations go to **Katie Fisher** a SfAM student member who was awarded her PhD in November at the University of Northampton. PECS wishes Katie all the best in her future career.

If you know a SfAM student or early career scientist who has been awarded their PhD or a prize/award then please get in touch and we will publish their achievements on this page. v.l.mccune@newcastle.ac.uk

Microbiology product development and sales



When my undergraduate degree ended in 2006, my ambition was to pursue a career in academic research. However, after I graduated I had an offer to join an R & D product development team at Invitrogen. Over the next two years I was placed on laboratory based product development projects, which aimed to improve current methods and develop alternative methods in food and environmental microbiology. In 2008 I transitioned into a new role, as a Field Applications Specialist, which involves research and development, marketing and sales. It requires me to understand all aspects of the product development process, as well as the market in which the new product is to be launched, how the product can be sold, what the customer's needs and problems are, the accepted criteria for a product/method, and technical support and troubleshooting procedures.

Customer focus is paramount to the role and it's necessary that the needs of the customer are addressed and actions are in place to meet these needs. It is not just laboratory research and scientific understanding that is needed to develop new products/methods, there is a complex system in place which brings about many hurdles to overcome before your 'wonder idea' is the next big thing.

The aim of developing new products and methods is usually to meet certain design goals and is started because of issues/problems which have been highlighted, usually from customer feedback. Examples of design goals are

to improve sample throughput, reduce time to result, reduce the complexity of a method and to reduce the price per test. Method improvements can include automating a method or introducing a new system to barcode samples or media; this can increase throughput and reduce labour. Developing alternative methods may be focused on reducing time to result, for example using PCR for the detection of *Legionella* as an alternative to culture, to reduce turnaround time from weeks to days.

The industrial food and environmental microbiology market is very competitive and highly regulated, meaning improvements or new methods must meet strict guidelines, a challenge when working in product development. For every method in this area there are standards or guidelines which laboratories follow, which have been rigorously tested to establish the optimised method and method performance characteristics. The International Standards Organisation (ISO) are responsible for co-ordinating the standards for each method and most methods recognised worldwide have an ISO standard produced, for example the standard for *Legionella* is ISO 11731. There are also method validation bodies such as AFNOR, AOAC and NordVal who follow the ISO guidelines and approve and validate new methods. Some countries only accept certain validation body approvals, so multiple validations may be needed during product development to increase the potential market size of your product.

Product development in food and environmental microbiology is complex, challenging, and requires a variety of skills. I have thoroughly enjoyed my time at Invitrogen and have learnt that a career in microbiology doesn't have to be based solely in a laboratory, but could be in other areas such as sales, marketing, laboratory auditing, production and bioinformatics.



Dean Barton
Invitrogen - part of Life
Technologies

Students into Work Grant reports

information

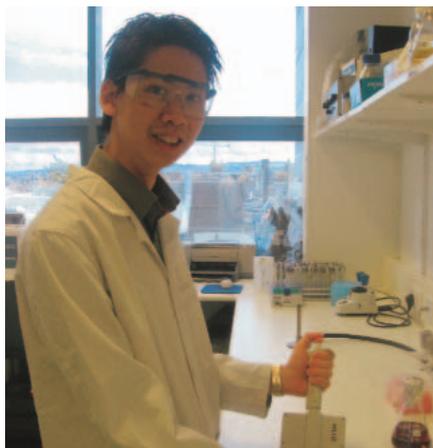
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Yes — if you are FULL member who can offer an undergraduate microbiology student the chance to obtain work experience. If you would like to read about the experiences of Students who have benefited from this Grant, you can do so in each issue of *Microbiologist*.

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Synthesis and evaluation of ultra-short antimicrobial lipopeptides



The growing number of bacterial strains resistant to currently available antibiotics has become a major concern and has led to research into the development of new antibiotics. One of the promising classes of new drugs that meet these criteria is that of the antimicrobial peptides. The repertoire of antimicrobial peptides has increased to more than 800 compounds in the last decade.

Antimicrobial lipopeptides are a series of small molecules which exert amphipathic and cationic properties. They act by incorporating into the microbial cell surface membrane and disrupting the membrane integrity, ultimately resulting in cell death. Some of these antimicrobial lipopeptides are found naturally as an important component of mammalian immune defence, as they function as the effector molecules for the innate immune system. Therefore, these lipopeptides have the additional advantage of inducing inflammation which will further enhance the elimination of bacteria.

The ultrashort lipopeptides share the same mechanism of action with many of the longer chains of antimicrobial lipopeptides. Shorter chains of antimicrobial lipopeptides enable them to be synthesised at relatively low cost, making them more economically viable.

I was fascinated by current work in the development of ultrashort antimicrobial lipopeptides and was lucky enough to be given the opportunity to spend 10 weeks during

the summer working with Dr. Brendan Gilmore to complete a project, the main objective of which was to determine the efficacy of these ultrashort antimicrobial lipopeptides. The project was supported by the SfAM Students into Work scheme and took place in the School of Pharmacy in Queen's University of Belfast. The initial phase of the project mainly involved synthesising a series of ultrashort antimicrobial lipopeptides (based on those described by Makovitzki *et al.*, 2006) using the solid-phase synthesis process. Rink amide resin was used as the solid phase and four amino acids were added to the resin sequentially. All lipopeptides synthesised have the sequence of lysine-X-X-lysine, where X represents lysine, leucine, alanine or glycine, and one of the amino acids in the sequence was replaced with its D-enantiomer. Lipid chains were subsequently attached to the N-terminus of these tetrapeptides. Mass spectroscopy was then employed to verify the structure of these ultrashort antimicrobial lipopeptides after synthesis.

In the following weeks, the lipopeptides were tested on various bacterial cultures and their potency measured in terms of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). These lipopeptides were highly effective when tested against many bacterial strains, including *S. epidermidis*, *E. coli*, *P. aeruginosa*, *C. tropicalis* and methicillin-resistant *S. aureus*, with MICs as low as $10\mu\text{mol dm}^{-3}$ to $42\mu\text{mol dm}^{-3}$. However, they were found to be ineffective when used against both normal strains and metalloprotease ZapA deficient strains of *P. mirabilis*.

Kill kinetics of the ultrashort antimicrobial lipopeptides were also determined to obtain the rate of kill of the bacteria. The concentrations of the ultrashort lipopeptides employed in the initial kill kinetics tests were the MBC of the lipopeptides, and the tests were repeated with a higher lipopeptide concentration if viable bacterial cells were still present in the solution 24

hours after initiating the kill kinetics test. Concentration of the lipopeptides used ranged from about $40\mu\text{mol dm}^{-3}$ to $160\mu\text{mol dm}^{-3}$, and most lipopeptides tested were capable of reducing the viable bacterial cell population for approximately 4 log cycles within 45 minutes of initiating the test.

The minimum biofilms eradication concentration (MBEC) of some lipopeptide compounds were also determined at the end phase of the 10 week project. Biofilms were initially allowed to grow on the pegs in the MBEC™ plate overnight before immersing the pegs into the solution containing antimicrobial lipopeptides. Following a period of incubation, the pegs were rinsed and bacterial cells in the biofilms were sonicated into the recovery medium containing universal neutraliser made from L-histidine, L-cysteine, reduced glutathione and Tween. Universal neutraliser acts by deactivating any antimicrobial lipopeptide introduced into the recovery medium during the transfer of pegs from the MBEC™ plate containing antimicrobial lipopeptide solution. The recovery medium was then further incubated for another 24 hours before determining MBEC by measuring the turbidity of the recovery medium.

This work experience provided me with a precious learning opportunity in further understanding the characteristics of ultrashort antimicrobial lipopeptides. It also provided an insight into the daily life of a microbiology researcher as well as a great range of laboratory skills. Although 10 weeks may be a little too short to collect all the data required, I hope that our work contributes to a more detailed understanding of these lipopeptides and places us one step closer to the employment of these ultrashort antimicrobial lipopeptides in a clinical setting. I would like to thank Dr. Brendan Gilmore and every member of the Biomolecular Science department in the School of Pharmacy for all their help and making the summer project enjoyable.

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Jing Huai Foong

Queen's University, Belfast



Prevalence of polyomaviruses and norovirus detection

Following completion of my Biomedical Sciences degree programme, I was provided with the exciting opportunity to conduct a Research Project at the Health Protection Agency (HPA), based in Newcastle General Hospital. This was made possible by the SfAM Students into Work scheme. In addition, reagents were provided for part of the project by the multinational biotechnology company, Invitrogen Ltd.

The 10-week placement was divided up to allow two projects to be carried out. The aim of the first was to determine the prevalence of two recently discovered polyomaviruses; Washington University (WU) and Karolinska Institut (KI), in respiratory samples from patients in the Newcastle area. KI polyomavirus and WU polyomavirus were documented in 2007 (Allander *et al.*, 2007 & Gaynor *et al.*, 2007) as two novel polyomaviruses. The role of these viruses in respiratory disease has not yet been clearly established, although studies in other countries indicate that they may have a role in acute respiratory tract infection.

Total nucleic acid was extracted from respiratory specimens from the Newcastle HPA archive. These specimens were previously submitted to the laboratory for respiratory virus investigations and had been tested using conventional laboratory methods and screened for an extended set of viral respiratory pathogens using real-time PCR. The total nucleic acid extracts were investigated for the presence of the WU and KI viruses using two previously described real-time PCR

assays (Bialasiewicz *et al.*, 2007). Plasmid positive control material was kindly sent by Seweryn Bialasiewicz (Australia). This was used to optimise the assays. Two separate assays were required for detection of WU virus, owing to the fact that the WU-B and WU-C assays target different genes.

The optimised assays were applied to 178 previously tested respiratory samples. Eight samples were positive in the KI assay and four samples were positive in each of the WU assays. Some of these were co-infections of WU and KI. In the majority of cases the presence of WU and KI demonstrated an association with other viruses, e.g. adenovirus, rhinovirus. However, two samples had no association with any other viruses and were taken from post-bone marrow transplant patients. This prompted the testing of 50 samples from immunocompromised patients. This revealed the presence of KI virus in 15 of the samples and seven and ten samples were positive in the WU-B and WU-C assays respectively. Prior to the discovery of WU and KI, only two other viruses of the *Polyomaviridae* were known to commonly infect humans. These are JC virus (JCV) and BK virus (BKV), which may cause human disease, particularly in immunocompromised patients. JCV is the causative agent of the neurological disease progressive multifocal leukoencephalopathy (PML), which occurs primarily in AIDS affected patients. Disease associated with BKV includes haemorrhagic cystitis and other urinary tract diseases in transplant patients undergoing immunosuppressive therapy. The findings of this study reaffirmed the consideration that there may be an association between the immunocompromised and the presence of WU and KI and also an analogy with JCV and BKV.

Bialasiewicz found a prevalence of 2.5% for KI virus and 10.5% for WU virus. My study revealed slightly higher rates of prevalence in the Newcastle region, with 10% for KI and 11% for WU.

The second half of the project involved the evaluation of a new one-step reverse transcriptase PCR (RT-PCR) assay for the detection of norovirus in human faecal samples. Norovirus is widely regarded as the most common cause of gastrointestinal

disease in the UK. Although the number of confirmed laboratory reports are low (2787) the Food Standards Agency (FSA) estimate the true incidence in the UK to be between 600,000 and 1,000,000 cases per year. The FSA also estimate that norovirus infection results in nearly 200,000 GP consultations, approximately 800 hospital admissions, and nearly 200 deaths per annum. Therefore, rapid and sensitive methods for the detection of the virus in faecal samples are required to aid outbreak investigations, facilitate implementation of infection control measures and appropriate patient management.

The 1-step RT-PCR assay for the detection of genogroup I and genogroup II norovirus was developed based on previously described primer and probe sets and incorporated a new real-time RT-PCR amplification kit (Ultrasense, Invitrogen Ltd). It was envisaged that this would increase the sensitivity of detection when compared with the conventional 2-step real-time PCR assay. The optimised assay was applied to 100 human faecal samples from patients with confirmed norovirus infection and 20 samples from norovirus negative patients.

The novel 1-step method produced lower Ct values, indicating a more sensitive method of detection. The 1-step method was a much less laborious process and has the potential to replace the current 2-step method. Further validation of the new method requires testing of additional routine samples.

During my time at the HPA, in addition to my projects, I had the opportunity to spend several days in each section of the routine diagnostic laboratory to gain experience and understanding of the technology and services offered. This placement has been an invaluable experience and will contribute significantly to my future career path.

I would like to take this opportunity to thank all of the employees at the Newcastle HPA, in particular Dr Andrew Sails, Mr Gary Eltringham and Mr Jeff Taylor. Further to this I would like to thank Dr David Wareing of Invitrogen Ltd for providing the Ultrasense kits and Dr Seweryn Bialasiewicz for providing the plasmid positive control material.

Clare Foreman

President's Fund reports

information

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Biodegradation of recalcitrant compounds

Many anthropogenic (synthetic) and biogenic (natural) compounds are resistant to microbial attack. They include synthetic compounds such as aldrin, dieldrin, heptachlor, mirex, dibenzo-p-dioxins and polychlorinated biphenyls (PCBs). Recalcitrant natural products include lignin, terpenes, and polycyclic aromatic hydrocarbons (PAHs).

Aromatic hydrocarbons formed naturally by combustion and diagenesis include anthracene, pyrene, coronene and ovalene. Most natural products of plant origin do not accumulate in the environment since microbes have been exposed to them for millions of years and thus have evolved the required enzymes for their biodegradation. That is why leaves which have a high percentage of lignin and phenolics are readily biodegradable. Many man-made compounds, however, present a great challenge to microorganisms. Their recalcitrance is due, in part, to their non-availability because they are sparingly soluble in water at part per million or part per billion levels. Also, microorganisms lack the necessary enzymes for their metabolism, they adhere to particulate matters and they can be adsorbed and absorbed. Furthermore, many anthropogenic compounds have functional groups such as methyl, alkyl, chloride and bromide which impede biodegradation.

Recalcitrant compounds are capable of long-range transport, bioaccumulate in human and animal tissues, biomagnify in food chains and have potentially significant impacts on human health and the environment. Interestingly however, there exist for many, an array of microorganisms with the potential ability to transform and metabolise the compounds. Prominent recalcitrant compounds worthy of mention are crude oil, PAHs and organo-chlorine compounds.

Oil pollution is a problem of note globally because the major oil-producing countries are not the major consumers. As a result, massive movements of oil have to be made from areas of high production to those of high consumption. Inevitably, either by accident or during normal operations, a certain amount of the oil is released

into the environment. At times, as seen in the Niger Delta, oil is released into the environment by sabotage. Microbial degradation appears to be the natural process by which the bulk of polluting oil is eliminated. Bacteria and fungi are the most important groups of organisms involved in oil degradation. Prominent genera with such ability include *Pseudomonas*, *Achromobacter*, *Burkholderia*, *Flavobacterium*, *Mycobacterium*, *Acinetobacter*, *Candida* and *Aspergillus*.

Hydrocarbons are completely reduced organic substrates that can be metabolised mainly in an oxidative manner. Anaerobic metabolism has been described but is highly inefficient. As a result, the initial steps in the biodegradation of hydrocarbons by bacteria and fungi involve the oxidation of the substrates by oxygenases. Complete oxidation of 1mg of a hydrocarbon may require 3-4mg of oxygen.

PAHs are organic molecules with two or more fused benzene rings in a linear, angular or cluster arrangement. PAH contamination of the environment is a major problem worldwide. It is due mainly to oil pollution, utilisation of fossil fuels for power generation, combustion in cars, trucks etc. and the use of creosote for wood preservation. It has generally been established that while two- and three-ringed PAHs are readily biodegradable, four- and five-ringed PAHs are more difficult to biodegrade (Cerniglia, 1992). Nocardioform actinomycetes of the genera *Mycobacterium*, *Rhodococcus* and *Gordonia* are reputable complete utilisers of PAHs with up to four aromatic rings. Microorganisms with the potential to degrade high molecular weight PAHs such as phenanthrene and pyrene can be readily isolated from polluted soils. Such microorganisms degrade these PAHs by producing dioxygenases which are extremely labile enzymes. Catechol, an intermediate of 2-3 ringed PAHs, is oxidised via the *ortho* pathway which involves cleavage of the bond between carbon atoms attached to the two hydroxyl groups to yield *cis*, *cis*-muconic acid or via the *meta* pathway which involves cleavage of the bond between a carbon atom with a hydroxyl group and the adjacent atom with a hydroxyl group.

Organo-chlorine compounds are

among the most problematic chemicals made by man. They have either been produced intentionally or formed as by-products of industrial processes. Synthetic halogenated aromatic compounds have been used extensively as biocides, disinfectants, solvents, hydraulic fluids and fire retardants. They are also used in the manufacture of rubbers, plastics, dyes, lubricants and electrical goods. Aliphatic halogenated compounds such as chlorinated ethanes and ethenes are used as solvents for dry-cleaning agents, in semiconductor manufacturing, as extractants of caffeine from coffee, general anaesthetics in medicine and dentistry, and as reagents in the production of plastics and industrial degreasers.

Polychlorinated biphenyls (PCBs) are a class of 209 structurally related congeners with a biphenyl nucleus and chlorine atom substitution possibilities of 1 to 10. PCBs were mainly synthesised between the early 1920s and 1978 by manufacturers in Europe, Japan and the US under trade names including Aroclor and Kanechlors. The PCBs were appreciated for their excellent thermal and electrical properties and were used in a wide variety of commercial applications such as dielectric fluids in transformers and capacitors, adhesives, plastics, paints, textiles, carbon papers and printing inks.

These widespread applications led to their worldwide distribution that made them become widespread pollutants throughout the world. The persistence of biphenyl molecules increases with increasing chlorine substitution. Although PCBs, as a group, are generally regarded as recalcitrant compounds, their biodegradation is known to occur in three ways: aerobically as a growth substrate, by cometabolism or anaerobically by reductive dehalogenation.

Ahmed and Focht (1973) reported the first evidence for PCB biodegradation. Bacterial isolates have been described that can cometabolise specific congeners in the presence of biphenyl using the same suite of enzymes employed in biphenyl metabolism. Furthermore, isolates have also been reported which can utilise monochlorobiphenyls and dichlorobiphenyls as sole growth substrates (Adebusoye *et al.*, 2007). Aerobic transformation of PCBs often

results in the accumulation of dead-end metabolites, usually chlorobenzoates. The chlorobenzoates formed, however, not only mirror the degradation of the parent compounds with a diversity of monochlorinated, dichlorinated and trichlorinated congeners possible, but are of paramount importance because the problems from the parent individual congeners are magnified and presented in much more water-soluble forms than the PCBs (Robinson & Lenn, 1994).

The chlorobenzoates could, thereafter, enter the ground water and may consequently be biomagnified. The biodegradation of a halogenated aromatic compound is considered complete only when its carbon skeleton is converted to intermediary intermediates that can be mineralised and its organic halogen returned to the mineral state.

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Matthew Olusoji Ilori

The Systematics of *Rhodococcus equi*

Rhodococcus equi is a Gram-positive pathogen which is recognised as the most common cause of respiratory infections in foals. Until the late 1980s it resulted in high mortality rates worldwide. Improvements in diagnosis and treatment regimes have improved prospects but the organism remains a significant cause of mortality in foals. In more recent decades, *R. equi* has emerged as an opportunistic pathogen of humans that generally manifests itself as a respiratory infection leading to bronchopneumonia.

Traditionally, *R. equi* has been detected and identified using standard culture, biochemical tests or serological

assays. More recently, work has been carried out to enable detection using PCR based methods (Bell *et al.*, 1998). Despite such advances, accurate identification can be problematic and routine screening of patient samples for *R. equi* is not routinely performed. This can lead to it being overlooked as a potential causal agent of disease. *R. equi* is an important pathogen in both foals and humans and therefore the need for an accurate and stable classification system is imperative in order that further improvements can occur in identification, prevention and treatment of disease.

The current species *R. equi* and the genus *Rhodococcus* have had a complex taxonomic history. Initially, members of this genus were assigned to *Nocardia* before being associated with *Mycobacterium rhodochrous*, later forming what became known as the 'rhodochrous' complex. Studies showed that members of this complex formed a heterogeneous group which could not be assigned to one particular genus. It was not until the work of Goodfellow & Alderson (1977) that the new genus *Rhodococcus* was proposed, containing several new species including *R. equi*. Rhodococci belong to a group known as nocardioform actinomycetes which are characterised by the presence of a special group of fatty acids known as mycolic acids. This has led to the group being referred to as the mycolata. Other members of the mycolata include the genera *Nocardia*, *Corynebacterium*, *Mycobacterium*, *Gordonia*, *Dietzia*, *Tsakamurella*, *Williamsia*, *Skermania* and *Segniliparus* which all belong to the sub-order Corynebacterineae.

The classification of existing and new species has improved greatly with the advent of molecular techniques, in particular the direct sequencing of genes such as 16S rDNA. The genus *Rhodococcus* has benefited significantly through the use of 16S rDNA sequence data and has led to the genus becoming taxonomically more stable. However, the taxonomic position of *R. equi* within the genus remains unstable. Previous studies have confirmed the heterogeneity of *R. equi* and shown the species to form a distinct phyletic clade (McMinn *et al.*, 2000; McMinn, 2001; Butler *et al.*, 2005). This has led to the supposition that *R. equi* should, in fact, be reclassified as a genus containing several species (McMinn, 2001) but as

yet there is insufficient evidence to make a formal proposal.

In my recent thesis work, a polyphasic approach involving the use of numerical taxonomy, 16S rDNA sequence analysis and DNA:DNA hybridisation was used to investigate the current taxonomic status of *R. equi*. Analysis of 16S rDNA sequence data for eleven *R. equi* strains confirmed that the *R. equi* group formed a distinct phyletic line. Recent changes have been suggested for the 16S rDNA sequence similarity threshold level, above which strains should be investigated further to determine membership of the same genomic species. It has been proposed that the level should be raised from 97% to 98.9 - 99.1% (Stackebrandt & Ebers, 2006). Sequence similarities between strains below this new suggested threshold indicate that the strains belong to separate genomic species.

16S rDNA sequence data from *R. equi* strains from my investigation showed significant sequence variation (98.3-99.1%) suggesting that the *R. equi* group represented two species. DNA:DNA hybridisation data for ten of these strains reinforced the division of the *R. equi* clade into at least two groups warranting species status. In addition, the genotypic evidence for raising *R. equi* to generic status was supported by phenotypic data provided by my own numerical taxonomic study using 105 rhodococcal strains and investigating 119 different characters. Again, the *R. equi* clade could be divided into the same two distinct groups. These groups were easily differentiated from other validly described rhodococcal species using a chemical inhibition test and utilisation of novel substrates such as acrylamide and phenol. These would be valuable for use in the identification of the new species in diagnostic laboratories.

Overall, the data suggest that there are at least two species within a potential new genus '*R. equi*' which substantiates and significantly extends the findings from previous studies. The requirements for the formal proposal of new species have become more stringent and it is clear that further data is required for the current investigation. Nevertheless, the data confirms that the current *R. equi* taxon requires reclassification. This could significantly benefit the diagnosis of disease in foals and humans by

enabling more specific and sensitive identification methods to be devised for the one or more new '*R. equi*' species responsible for causing infection.

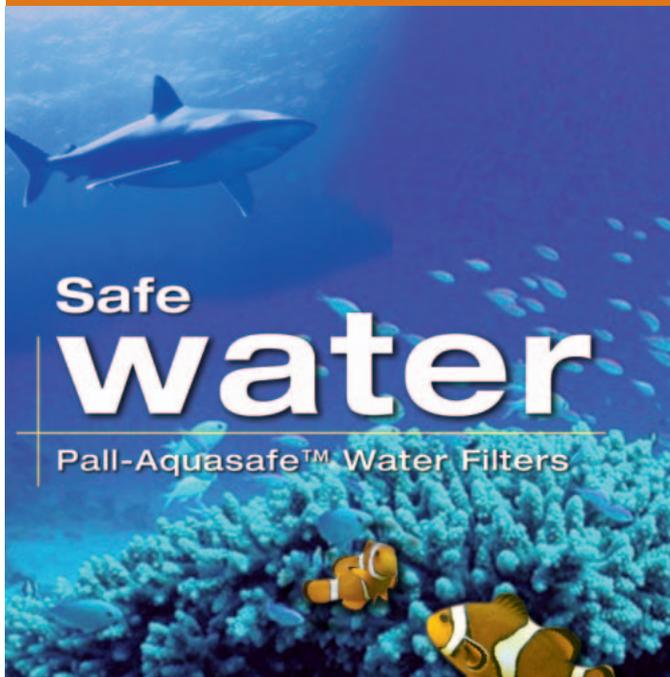
I applied to the President's Fund for help towards attending the 14th *International Symposium on the Biology of the Actinomycetes* taking place in Gateshead in 2007. This is a specialised area of microbiology and since the conferences within this area only happen every four years I was keen to attend in order to present and discuss my findings on *R. equi*, an important pathogenic actinomycete.

I wish to thank the Society for Applied Microbiology for the President's Fund award enabling me to attend this important conference as part of my career development.

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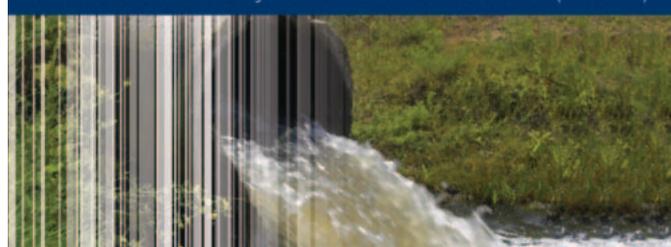
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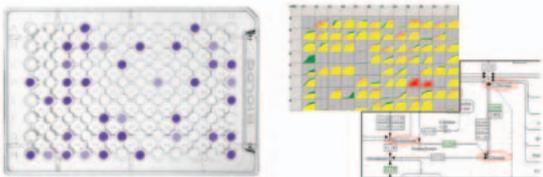
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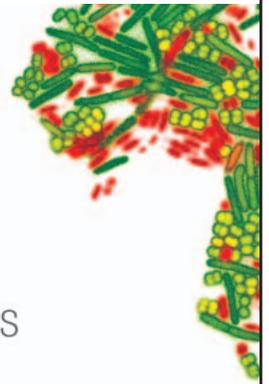
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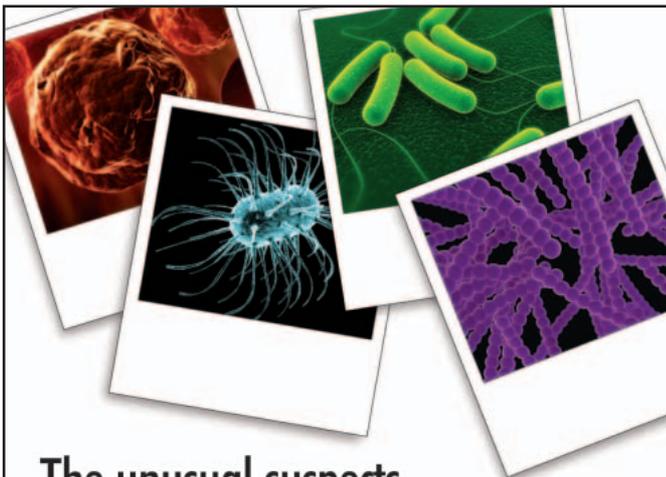
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Three new product innovations from Pro-Lab

The Ruskin Bug Box Workstation available from Pro-Lab Diagnostics has recently undergone a number of innovative improvements, enhancing the already widely successful unit. Whilst occupying a minimal amount of bench space the unit offers flexibility for Anaerobic and Microaerophilic work with a capacity up to 300 plates, the unit is very simply adaptable for *Campylobacter* work at no additional cost. Single side plate entry systems and variable interlock capacity also enhances economical running by saving on gas usage. Full details and demonstrations are available from Pro-Lab Diagnostics, email uksupport@pro-lab.com.

The IUL Polystainer automated staining unit from Pro-Lab Diagnostics has also recently been improved with additional program features and capability to handle up to 20 slides for up to 10 different staining methods. The Polystainer offers

convenience, consistency and time saving for Gram stains, AFB stains, Cold ZN stains, *Cryptosporidium* stains and *Trichomonas* stains. Demonstration units are available. The Prolex® range of Rapid Latex agglutination systems also expanded with the addition of the Prolex® Staph Xtra kit for rapid

identification of *Staphylococcus aureus* including MRSA, using Carboxylate Modified Microparticles used with all Prolex® Latex Agglutinations systems. Samples available on request.

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Campden BRI appoints new Director-General

Dr Steven Walker has been appointed Director-General Designate of Campden BRI. Dr Walker will assume the role of Director General on the retirement of Professor Colin Dennis CBE later this year.

Bob Clarke, Chairman of Campden BRI commented, "I am very much looking forward to working with Steven in the further development and strengthening of Campden BRI. These are exciting times as we begin to deliver the benefits of the recent merger between the former

Campden & Chorleywood Food Research Association (CCFRA) and Brewing Research International (BRI), including the increasingly international outlook of the business."

"Steven knows the business extremely well having 22 years service with 14 of those at Director level. He has played a major role in both the scientific and commercial aspects of the business, has worked closely with our members, Government and trade bodies on many issues, and has been actively involved in the evaluation of other research organisations in the UK and overseas. He is ideally placed to take the business forward."

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NCIMB Introduces the MARA Ecotoxicity Test to SfAM Readers

NCIMB Ltd has developed a Microbial Assay for Risk Assessment (MARA) from the public collection of environmental isolates it maintains. This new, novel test comprises an array of 11 genetically diverse microorganisms which are freeze dried *in situ* in a 96 well microtitre plate.

Test plates are resuscitated and inoculated with dilutions of the unknown sample (e.g. potable water, discharge waters, industrial effluents, etc.) After a short incubation, growth responses are measured using proprietary software and a simple flatbed scanner, from which a microbial toxic concentration is calculated for each species. The result is a numerical value (MTC) and a pictorial fingerprint for each strain in the array as a measure of the sample toxicity.

A more rapid all luminous version of the array is at an advanced stage of production and NCIMB intends to develop a family of MARA products tailored to client needs. The advantage of this new MARA product over other microbe-based toxicity test kits lies in the multi-species nature of the test, the diverse data set produced and the flexibility of the concept to adaptation for different markets.

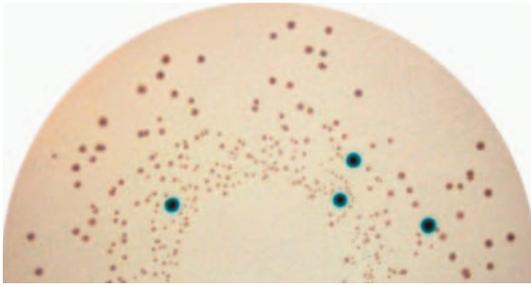
We welcome the opportunity to work with partners to design arrays for their requirements and to participate in funded research programmes.

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Lab M launches new media for *Cronobacter sakazakii* isolation

Microbiology specialist Lab M is launching the first product in its new range of dehydrated culture media for the isolation of *Cronobacter sakazakii*. Formerly known as *Enterobacter sakazakii*, this largely environmental organism has been associated with serious infections in newborns fed on infant formula milk. Lab M's range will include both broth and chromogenic media, and is designed to meet the various testing regimes of different laboratories.

The first in what will soon be a series of three new media is HAL012 Harlequin™ CSIM (Harlequin™ *Cronobacter sakazakii* Isolation Medium). A chromogenic medium, this product uses the formulation currently recommended as part of the isolation protocol under ISO/TS 22964:2006(E) for *C. sakazakii* from milk, infant formula and other milk products.

The main source of *C. sakazakii* contamination appears to be the manufacturing environment.

Chromogenic technology enables faster detection and improved sensitivity compared with classical culture media, reducing the need for sub-culture or confirmatory tests. Already well known for their high quality chromogenic media for *Salmonella*, *Listeria* and *E.coli* detection, Lab M can reduce laboratory workloads even further by providing culture plates ready prepared with the HAL012 Harlequin™ *Cronobacter sakazakii* Isolation Medium.

Lab M broth lights up *E. coli* in milk

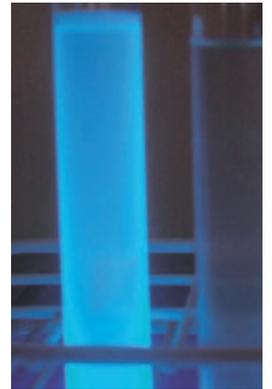
With routine testing of milk and milk products vital to ensuring consumer safety and product quality, detecting potential pathogens as quickly and easily as possible is of paramount importance. The ability to enumerate presumptive *E. coli* from presumptive coliforms, for instance, rapidly flags up any contamination problems.

Lab M's Modified Lauryl Sulphate Tryptose Broth with MUG & Tryptophan (ISO) is designed specifically for this purpose, enabling the presumptive enumeration of *Escherichia coli* from

milk and milk products using the Most Probable Number (MPN) technique according to ISO 11866-1:2005. ISO specifications indicate that this technique can be applied not only to milk, but also a wide range of liquid and dried milk products, cheese, butter, ice creams, custard, desserts and cream.

The key to *E. coli* enumeration is the addition of 4-methylumbelliferyl- β -D-glucuronide (MUG) to the standard Lauryl Tryptose Broth formulation, allowing positive discrimination of *E. coli* strains. The majority of *E. coli* produce β -glucuronidase enzyme, resulting in hydrolysis of MUG and the release of a fluorogenic compound.

Tubes which fluoresce under UV light are confirmed for *E. coli* by a positive indole reaction when Kovac's reagent is added to the tube. Modified Lauryl Sulphate Tryptose Broth with MUG & Tryptophan (ISO) is described in ISO 11866-1:2005 and is used in the HPA National Standard Method D5 for Enumeration of coliforms and presumptive *Escherichia coli* by the Most Probably Number (MPN) technique.



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Neogen Launches Campy Cefex Agar, a new culture medium for quicker easier detection of *Campylobacter*

Neogen Europe Ltd introduces a new culture medium for the isolation of *Campylobacter*. Campy Cefex Agar is a selective medium enabling quick and easy isolation and direct enumeration of the most clinically significant *Campylobacter* species such as *C. jejuni* and *C. coli*.

Campy Cefex Agar is quicker and easier to use than alternatives by providing "direct-to-plate" method. Traditional enrichment methods for *Campylobacter* require a 4-hour pre-enrichment step prior to inoculating media plates with sample cultures. Not only does Campy Cefex entirely eliminate the pre-enrichment step it has shown to both grow the *Campylobacter* in a culture and to effectively repress the growth of other microorganisms.

Campylobacter species are widely recognised as one of the leading identified causes of bacterial infectious disease throughout Europe.

According to European Food Safety Authority



figures (2005), campylobacteriosis has overtaken salmonellosis as the most reported animal infection transmitted to humans. The mishandling and/or consumption of raw or undercooked poultry is a major associated risk factor. Various surveys undertaken around the world have found the frequency of *Campylobacter* contamination of commercially available chicken to be between 50-80%.

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Invitrogen and Applied Biosystems Complete Merger

Invitrogen Corporation and Applied Biosystems Inc. have announced the successful completion of their merger transaction; the new company will be named Life Technologies Corporation.

Life Technologies is a global biotechnology tools company dedicated to improving the human condition. Our systems, consumables and services enable researchers to accelerate scientific exploration, driving to discoveries and developments that make life even better. Life Technologies customers do their work across the biological spectrum, working to advance personalised medicine, regenerative science, molecular diagnostics, agricultural and environmental research, and 21st century forensics. The company has historical sales of approximately \$3.5 billion, employs 9,500 people, has a presence in more than 100 countries, and possesses a rapidly growing intellectual property estate of over 3,600 patents and exclusive licenses.

"This is an exciting time in the history of Invitrogen and Applied Biosystems," said Greg Lucier, Chairman and Chief Executive Officer of Life Technologies. "By combining these two highly respected brands, we are not only creating a stronger company, but an industry thought leader, uniquely positioned to help our customers accelerate and drive new discoveries and commercial applications."

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New ProtoCOL UV is world's first automated UV/Visible colony counter and zone sizing system

The new multi-application ProtoCOL UV automated colony counting and zone measurement system from Synbiosis is now available.

The ProtoCOL UV has a high resolution camera inside a light tight darkroom with built in UV and white light, making it the only system currently on the market versatile enough to image fluorescing and visible colonies, as well as inhibition zones. The system is easy to connect to a PC allowing researchers to instantly capture, print and save accurate images in a BMP format. The images can then be used for archiving or analysis with new ProtoCOL V1.4 software, which is included with the system.

The ProtoCOL UV's universal darkroom is cleverly and safely designed for many different applications. For example, it can accommodate standard pour, spiral or surface inoculated plates, as well as large bioassay and Single Radial Immunodiffusion (SRD) plates. The darkroom has a sliding, auto-locking door to prevent accidental UV exposure and a filter drawer which means scientists can add filters for visualising naturally fluorescing bacteria, such as *Pseudomonas fluorescens*, or recombinant *E.coli* expressing green fluorescent protein.

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Thriving Exports for DWS

Don Whitley Scientific is riding out the UK 'credit crunch' with the help of its thriving export business.

To finish off the 2008 trading year, DWS received orders from the University of Leipzig for two Whitley MG1000 Anaerobic Workstations, a Whitley MG500 Workstation and a Whitley Jar Gassing System, and on the way to a customer in China are another three Whitley DG250 Workstations.

Paul Walton, Managing Director at DWS added: *"The weak pound and having an established export business has really helped the company to achieve our targets this year. However, despite the lack of funding in some UK sectors, research projects must continue and the equipment we manufacture and supply is designed to make laboratories more cost-effective in the long term."*

One new product is the improved Smasher blender, which is around 20 times quieter than other blenders. It now has quick release paddles (no tools required) for easier cleaning. Also, launched in January, is the Dilumat S gravimetric diluter with rotating central structure to allow the dispensing arm to rotate away from the sample preparation area providing more room to work and facilitate the addition of the sample.

further information

Visit: www.dwscientific.co.uk
Tel: +44 (0)1274 595728
Email: info@dwscientific.co.uk

Swabs for all Seasons and Reasons

Although best known for **Transwabs®**, the original and leading range of microbiology transport swabs, Medical Wire also manufactures a diverse range of dry swabs for every conceivable application, and not just for humans! Whether for

information

Are you a corporate member of the Society? If so, this section of *Microbiologist* is for you. Here you can publish short press releases, acquisition notices, news of new staff appointments, technical developments and much more.

Each corporate member of the society may publish **up to 200** words on a topic related to their field of activity in each issue of *Microbiologist*. For further information please contact Lucy Harper by email at: lucy@sfam.org.uk

Both corporate members and ordinary members of the Society will find a wealth of useful information and resources in this section.

frogs in the Amazon jungle being tested for chytridiomycosis, mares in the breeding season tested for *Taylorella*, ponies for flu, chickens or swans for *salmonella* or avian flu, salmon for aeromonas, or sheep and cattle for respiratory diseases, there will be a swab to fit. There are also swabs for environmental sampling, and for forensic investigations.

Swab buds can be rayon, polyester, calcium alginate, cellulose or polyurethane. Cotton is not recommended for microbiological specimens, but still has other applications. Rayon is preferred for specimens which are to be cultured, while polyester is often specified for molecular tests. Shafts can be short, standard (typically 15cm), long, very long (45cm) or very, very long (1.8m). Some environmental swabs have no shaft!

Medical Wire's new **Σ-Swab™ Sigma Swab™** features a polyurethane foam bud, with a highly absorbent open cell structure that not only collects well, but maintains viability for most microorganisms.

further information

Visit: www.mwe.co.uk
Tel: +44 (0)1225 810361
Email: sales@mwe.co.uk

SGL release new price list and product guide

Southern Group Laboratory (SGL) have just released their new 2009 UK Price List and Product Guide providing comprehensive details of the extensive range of ready prepared microbiological culture media, stains and reagents manufactured by SGL for a wide range of industry sectors such as:

- Healthcare Services
- Hospitals/ Pharmacies
- Food Companies
- Water Companies
- Contract Laboratories
- Pharmaceutical Companies
- Cosmetics Companies
- Research & Education Establishments

As an independent media producer we have the ability to manufacture in small batches and as such we can supply any commercial or custom-specified formulations to order. So, whether you require a completely new medium or simply a different pack or fill size, please contact our Customer Services Department.

further information

Visit: www.sglab.co.uk
Tel: +44 (0)1536 403815
Email: info@sglab.co.uk



Oxoid Rewards Young Microbiologist for Excellent Research Project

The Oxoid Prize for the Best Project in Microbiology (2008) at the University of Brighton School of Pharmacy and Biomolecular Sciences has been awarded to **Niamh Kilbride** for her investigations into the potential use of bacteriophage coatings for the prevention of microbial colonisation on medical devices.

Niamh's research investigated different methods for the immobilisation of phage K, with a view to developing a coating that would be effective against *Staphylococcus aureus* NCTC 10788.

She studied the retention of the bacteriophage when dried onto the surface of untreated and silanised glass. She then incorporated the bacteriophage into a hydrogel coating and investigated whether the coating was effective at preventing or inhibiting the growth of bacteria on its surface.

She concluded that it is possible to create a bacteriophage coating that can prevent microbial colonisation at sufficiently high concentrations of bacteriophage.

She also concluded that the development of a suitable bacteriophage coating could potentially reduce and prevent the growth of bacteria *in vivo*.

Alison Smith, pharmaceutical microbiology manager, Oxoid, presented Niamh with a certificate and cheque for £150.

further information

Visit: www.oxoid.com
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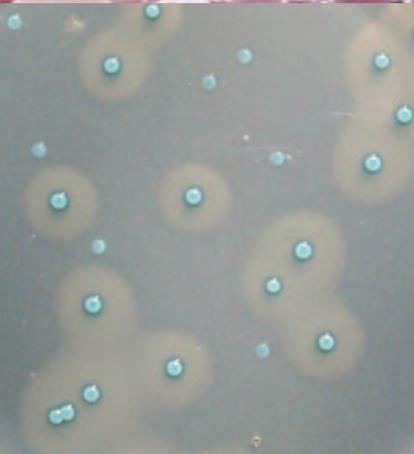
Oxoid Precis culture methods allow isolation, detection and confirmed identification of foodborne pathogens in just 2 days without the need for specialist equipment or training. Just follow these 3 simple steps:

1. Enrichment – a single broth combining resuscitation and growth in overnight incubation
2. Plating – a single medium incorporating enzyme specific inhibition and differentiation. Coloured colonies clearly indicate presumptive positive results
3. Confirmation – confirmed test result available in less than 10 minutes, direct from plate*



Combining the latest developments in culture media technology and rapid identification tests, the Oxoid Precis methods deliver a time to confirmed result normally only achieved with PCR and other automated methods.

Precis methods for *Salmonella* and *Listeria monocytogenes*, validated by AFNOR to ISO 16140, are now available. Contact us to find out more.



*ISO standard tests for confirmation have also been validated and approved by AFNOR, offering complete flexibility.



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