# Microbiologist

The magazine of the Society for Applied Microbiology ■ June 2010 ■ Vol 11 No 2 ISSN 1479-2699

# Parasites

## *Toxoplasma gondii* and schizophrenia

INSIDE

■ *Cryptosporidium* in humans ■ *Trichinella* ■ Historical perspectives — electron microscopy
■ Spring Meeting report ■ Summer Conference 2010 ■ Microbe 2010 ■ SfAM events in 2011
■ Careers: lawyers, microbiologists and food safety ■ PECS: events at the Summer Conference
■ Biofocus: science funding ■ MISAC competition 2010 ■ Mediawatch: science in the media — a debate

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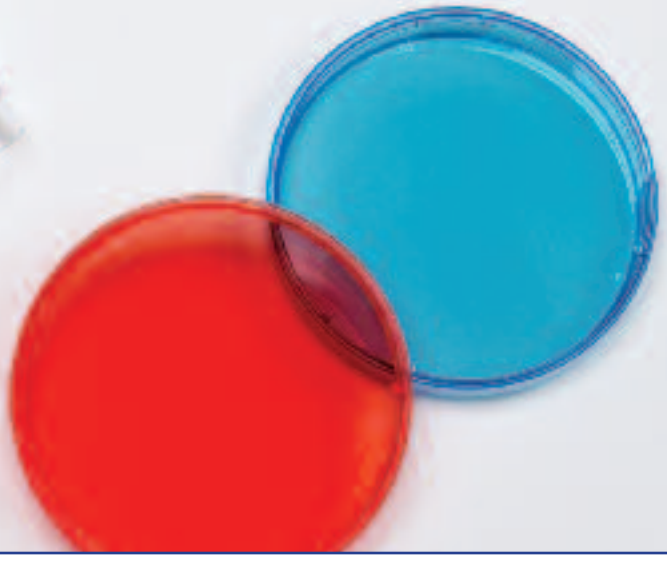

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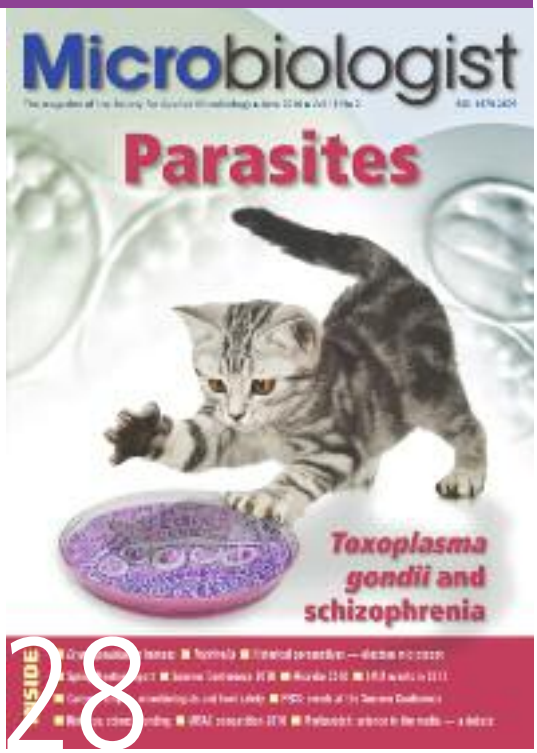
# contents





## members

04 **Editorial:** is science increasing in profile?

07 **Microbreak:** could you write a story in just six words?

09 **President's and CEO's columns**

11 **Membership matters**

44 **Careers:** lawyers, microbiologists and safe food

46 **In the loop:** PECS events at the Summer Conference

48 **Students into Work grant reports**

50 **President's Fund articles**

## publications

13 **JournalWatch**

## news

14 **Biofocus:** science funding—the need for common purpose

15 **MiSAC competition 2010:** food safety and barbecues

16 **MediaWatch:** science in the media—a debate

## features

28 ***Toxoplasma gondii* and schizophrenia**

31 ***Cryptosporidium* in humans**

35 **The future for detection and control of *Trichinella* in Europe**

39 **Historical Perspectives:** electron microscopy

42 **Statnote 21:** non-linear regression—fitting a logistic growth

## meetings

20 **Royal Society of Medicine meeting report:** zoonoses

22 **Spring Meeting 2010 report**

24 **Summer Conference 2010:** full programme and booking form

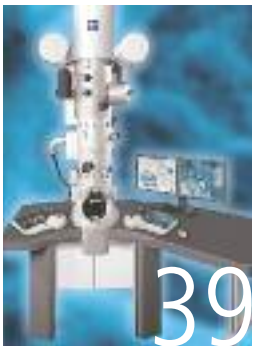
27 **Save the dates!** Forthcoming S*f*AM meetings in 2011

## commercial

52 **Advertisements and news from our Corporate members**



**Summer Conference 2010—BOOK NOW!**




**Historical Perspectives: electron microscopy**

## information

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# editorial

**Lucy Harper** talks about the increasing awareness of the importance of Science in the minds of non-scientists

Is it just me, or is science increasing in profile? Is "geek chic" a genuine phenomenon or am I biased? To me, it seems that science or scientific subjects seem to be appearing more and more on TV and radio—possibly, at least in part, due to 2010 being the BBC's 'year of science'. Take the brilliant documentary "*Wonders of the Solar System*" (I am aware this example is being taken from physics rather than microbiology, but do bear with me). This programme is encouraging an interest in science from many of my non-scientist friends who think the presenter, Brian Cox, is fast becoming the epitome of 'cool' and is taking science with him (whether this is despite or because of his previous life as keyboardist of '*D:Ream*' is yet to be confirmed). Hopefully, programmes like this and the recently aired series "*The story of science*" (BBC2) will continue past this year's promotion of science and will inspire a new generation's interest enough to pursue careers in scientific research.

Another example of science raising its profile comes in the form of the monthly science magazine '*Eureka*' produced by the *Times*. This time I am proud to say I can mention microbiology, as the very first edition of the magazine held an excellent piece by *Times* Science Editor, Mark Henderson, on the role of gut bacteria in human health. I must confess to only purchasing a newspaper on the first Thursday of each month to get hold of my copy.

I could list numerous examples of ways in which science is referred to in a positive way during entertainment programmes, from comedy shows such as "*Nerdstock*" (BBC Four) to Ricky Gervais' current tour: "*Science*". All these things raise awareness of the importance of science in the minds of non-scientists in the same way as do many public engagement activities.

Public engagement is recognised as an important way in which scientists can give something back and this is acknowledged here at S*f*AM through the provision of our innovative project/public engagement grant. If you think you could be microbiology's Brian Cox or you have ideas for ways in which you could engage the public in applied microbiology, do contact me or visit the S*f*AM website (www.sfam.org.uk/grants.php) for more information about our innovative project/public engagement grant.

One recipient of this grant this year held a novel event for World Malaria Day only two days ago*. The event was so recent that I'm afraid you'll have to be patient and wait until the next issue of Microbiologist before you can read the event report. That said, Malaria day does have some relevance to the theme of this issue of Microbiologist: parasites. We begin with a foray into the feline world of toxoplasmosis and a possible association with schizophrenia (page 28)—an article created through a chance encounter with Radio 4's health programme "Case Notes". Following this we have articles on Cryptosporidium (page 31) and the nematode parasite, Trichinella (page 35).

What do *you* think about the profile of science? Do you agree that in the minds of non-scientists science seems to be increasing in importance or relevance? Or do you think it's always been a subject of which people are aware? It would be great to hear your views, so contact me at the Society office and let me know what you think.

*accurate at time of going to press

## 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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**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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# 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 digitized 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 organizations 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

**www.sfam.org.uk**

# **micro**break

## could you write a story in just **six** words?

It is said that in the 1920s, Ernest Hemingway bet that he could write a complete story in just six words. He wrote: 'For Sale: baby shoes, never worn.'

**He won the bet.**

Now we would like to challenge you to do the same, but with a twist. This issue's microbreak competition is to write a story in six words with a microbiological link or theme.

The ten best entries will be published in *Microbiologist* and the winning entry will receive an Amazon voucher.

So all you budding writers out there get thinking and send your entries to the Editor (lucy@sfam.org.uk) **before 2 July 2010**.

The answers to the microbiology anagrams competition in the March issue of *Microbiologist* are shown below.

Congratulations to **Vydeki Shanmuganathan** whose correct answers were picked from the Editor's in-tray. A £30 Amazon voucher is on its way to you.

ACHE IS LICORICHE
*Escherichia coli*

ANY PROJECTILE CUB JAM
*Campylobacter jejuni*

ONE SUBLIMINAL PEAK EEL
*Klebsiella pneumoniae*

ILLUSIONARY MAT HELP MUM
*Salmonella typhimurium*

ACCUSATORY HUE CUP LOSS
*Staphylococcus aureus*

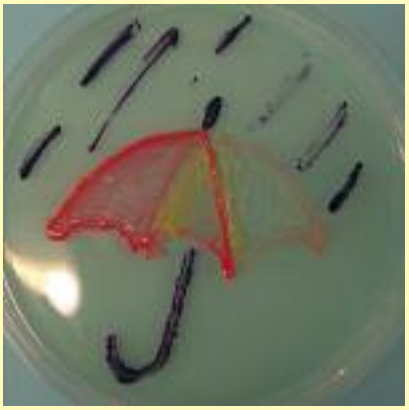
SARS — NICER CREAM TEAS
*Serratia marcescens*

MUSICIAN SEES OAR
*Neisseria mucosa*

ROUND APPLE MEAL TIM?
*Treponema pallidum*

# S*f*AM **Photo Competition**

Have you taken an outstanding photograph of your beloved bugs? Do you know someone who has and you'd like to see their work in print? Perhaps you've taken a photograph while attending a S*f*AM conference which you think is worthy of reproduction?

Due to popular demand, S*f*AM are running the photography competition again this year. We are looking for twelve eye-catching images to use for our 2011 calendar which we will be giving to all our members as a Christmas gift.

To enter this competition, please send your photographs to the Editor in the form of JPEG files which must be a minimum size of 7 x 7cm at 300dpi (800 x 800 pixels). Alternatively, you can send the original photographs in hard copy to the Society Office and we will return them to you once they have been scanned.

**Photographs will appear in one of two categories:**

1. Scientific — e.g., a colourful image using bacteria
2. Non-scientific but with a S*f*AM theme e.g., taken at a S*f*AM event

**The closing date for entries for this competition is Friday 1 October 2010**

## Erratum

The Microbreak competition in the March issue of *Microbiologist* (Vol. 11, No. 1), contained an incorrect anagram. The answer to the first anagram was *Escherichia Coli*, however, the anagram itself was missing a letter 'h'. The Editor would like to apologise for this error and thank the entrants to the competition who kindly pointed this out

# president's column

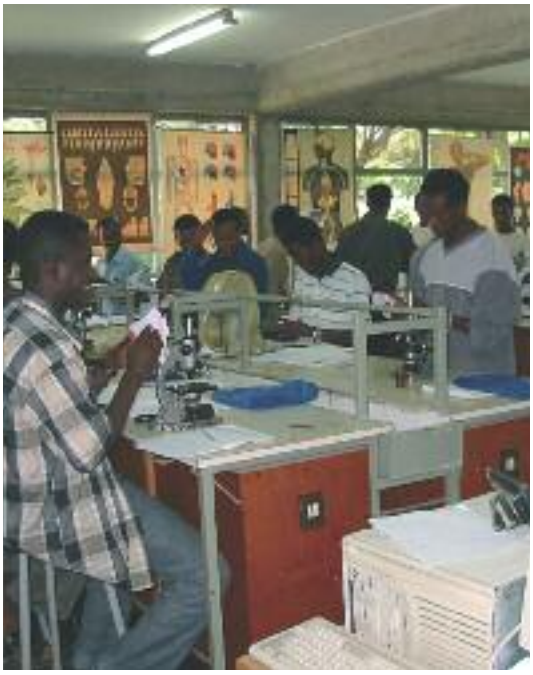
**Geoff Hanlon** updates members on the Society's process of reviewing its approach to International Capacity Building (ICB)

In the September 2009 issue of *Microbiologist* I outlined some of my preliminary thoughts on how we as a learned society might become more involved in International Capacity Building (ICB). This article generated some interest and I was contacted by several members who are keen to be involved in discussions aimed at moving this forward. The general response I've had has been positive which is very encouraging. I was also interested to learn that a number of members are already engaged in various aspects of capacity building and hopefully we can learn from their experiences.

The UK Collaborative on Development Sciences was set up in 2007 by the Secretary of State for International Development and is a collaboration of 13 UK funders and stakeholders with an interest in international development research. It was this organization that arranged the "*Sharing Our Experience*" Discussion Meeting on ICB activities by UK learned societies held in June last year which Phil Wheat and I attended. Following this meeting, the UK National Commission for UNESCO conducted a small survey of UK learned societies' capacity building activities and I thought you might be interested to see what other learned societies are doing in this area. The table on the right is a summary of their findings and more details are available on their website (**http://www.ukcds.org.uk/page-Learned__Societies-144.html**).

It is of interest that the majority of the initiatives outlined above are focused on Africa, leaving large areas of the globe unsupported which is clearly an aspect requiring careful consideration. The Society for Applied Microbiology was not asked to contribute to this survey but had we done so we could have specifically included both our Overseas Development Award (£5,000) and the Endangered Culture Collection Fund (£5000+ travel costs) thus demonstrating that we should not be too ashamed of our current position.

However, the purpose of this column today is to keep you up to date with where we have got to in the process of reviewing our approach to ICB. The first meeting of interested parties was held on 18th February 2010, where a number of pre-existing initiatives (mostly medically oriented) were discussed to see if there was scope for the Society to participate in these. However, any venture undertaken must comply with the objective of the Society: "*to advance for the benefit of the public the science of microbiology, in its application to the environment, human and animal health, agriculture and industry*". The main outcome of this first meeting was that resources could be made available for microbiology education and training in developing countries, particularly if these were centred on "training the trainers". In the short term this could be facilitated by an extension of the Overseas Development Award, but there was general consensus that the Society's grant structure requires revision and that this would be carried out with the aims of ICB in mind.



Attention was also paid to the idea of offering e-affiliate membership of S*f*AM to those microbiologists in the poorest countries of the world. This would be free of charge and they would have free access to society journals (already available via Wiley-Blackwell), a link to a pdf copy of *Microbiologist*, and access to specific grants allowing attendance at S*f*AM meetings. Since that meeting, Wiley-Blackwell has agreed to support the scheme and this will enable us to advertise the e-affiliate membership to nearly 50,000 individuals.

This is a very exciting initiative and I hope that a large number of members will feel they would like to engage with the process as the detailed structure emerges. I will keep you informed as more information becomes available and, as usual, please feel free to email me with any comments.

**Professor Geoff Hanlon**
President of the Society

## UK Learned Societies' International Capacity Building (ICB) activities

| Learned Society | Activities |
|---|---|
| **Biochemical Society** | E-mentoring between UK researchers and students in developing countries. |
| **British Academy** | International Partnership awards of up to £10,000 to support links between researchers. |
| **British Ecological Society** | Overseas Bursary for African researchers - grants up to £7,000 for ecologists in developing countries to carry out innovative ecological research.<br>Overseas fellowship scheme—continuation funding up to £15,000 for top candidates from the Overseas Bursary stage. |
| **Institute of Physics** | Physics for Development programme—a range of projects, events and resources for physicists and physics teachers in developing countries. |
| **Institution of Civil Engineers** | The Africa-UK Engineering for Development Partnership—a network to strengthen capacity and promote links between Africa and the UK. |
| **London Mathematical Society** | Mentoring African Research in Mathematics - developing professional links between mathematicians in the UK and Africa.<br>International Short Visits—grants of up to £2,100 to support a visit for collaborative research either to or from Africa. |
| **Physiological Society** | International Research and Teaching Grants<br>Meeting Grants of up to £2,000 to attend Society meetings in the UK. |
| **Royal Astronomical Society** | Astronomy for the Developing World—a new global strategy that aims to improve astronomy education and capacity in developing countries. |
| **Royal Academy of Engineering** | The Africa-UK Engineering for Development Partnership—a network to strengthen capacity and promote mutually beneficial links between the engineering community in Africa and the UK. |
| **Royal Society** | Royal Society Pfizer prize—annual award for early career scientists in Africa<br>Leverhulme Royal Society Africa Awards—grants for collaborative research projects between Ghanaian/Tanzanian research institutions and the UK.<br>Royal Society Pfizer African Academies Programme - three year programme of mentoring, training and support for national academies of science. |
| **Royal Society of Chemistry** | Pan Africa Chemistry Network—a range of programmes for universities, schools, scientists, teachers and students in Africa. |
| **Society for General Microbiology** | International Development Fund—Awards of up to £7,000 for travel and project support for microbiologists in developing countries. |

# ceo's column

**Philip Wheat** reports on the latest developments within the Society

As I have mentioned many times in this column, Society membership offers terrific value for money.

One benefit of membership is online access to our scientific journals which the Society publishes in partnership with Wiley-Blackwell. Members can access five journals: *Journal of Applied Microbiology*, *Letters in Applied Microbiology*, *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*. Online access to these journals is included in the basic cost of membership, a benefit which is not offered by all Learned Societies. When members first join they are sent two unique passwords by Wiley-Blackwell so that the new member can have personal access. Figures from Wiley-Blackwell, show that only approximately 15% of members make use of this personal online access. Whilst I do appreciate that a number of members will have access via institutional subscription, I do find it surprising that the uptake figure is so low. Should you be having difficulties accessing the journals in any way, or require further information, please contact me (pfwheat@sfam.org.uk).

A further major benefit of membership is the availability of grants to members. One of the grants available is the **Overseas Development Award** (ODA), the main purposes of which are:

- To support members to visit laboratories overseas and give lectures and training in appropriate areas of applied microbiology
- To support overseas members to visit UK laboratories to receive training in appropriate areas of microbiology

During 2010, the Society would like to enhance its activities and resources dedicated to International Capacity Building for scientists in developing countries. In particular we would like to further promote the ODA as an excellent way of achieving this aim. We are keen to support members who wish to visit developing countries to educate and train personnel from these countries, so that these people can then become trainers themselves. This will mean that the process has a better chance of long term success. Full details of the ODA can be found with details of all grants we offer on our website (www.sfam.org.uk/grants).


Members reading this in the UK will by now know the outcome of the UK General Election. I was fortunate enough to be invited to an event at the House of Commons in March 2010. The event was a political debate organized by the Royal Society of Chemistry concerning Science and the General Election. The protagonists for the debate were Lord Drayson (Minister of State for Science and Innovation), Dr Adam Afriyie (Shadow Minister for Innovation, Universities & Skills) and Dr Evan Harris (Liberal Democratic Party Spokesperson for Science & Technology). Each had an opportunity to address a large audience regarding their parties position on science and their aims for after the forthcoming General Election.

As you would expect from such a political debate, there were differences in the approach to science from the three parties. However, there was also overall agreement that science was essential if the UK was to have a prosperous future. Any economy that is to expand can only do so with investment in science for education, training and research. It was a fascinating evening and unique in that it was the very first time that a webcast had been transmitted live from the House of Commons.

The webcast is available online at: http://www.rsc.org/ScienceAndTechnology/Parliament/Events/2010Election.asp

**Philip Wheat**
Chief Executive Officer

# SfAM AGM AGENDA 2010

**The 79th Annual General Meeting of the Society for Applied Microbiology will be held on Wednesday 7 July at 4.30 pm at the Grand Hotel, Brighton**

**1. Apologies for absence**

**2. Approval of minutes**

Approval of minutes published December 2009 *Microbiologist* of the 78th Annual Meeting held in Manchester, 2009

**3. Matters arising from the previous minutes**

**4. Report of the Trustees of the Society 2009**

(i) Report of the Honorary President

(ii) Report of the Honorary General Secretary

(iii) Report of the Honorary Meetings Secretary

(iv) Report of the Honorary Treasurer

**5. Adoption of the 2009 Annual Report**

**6. Result of ballot and election of new Committee Members**

**7. Election of Vice President**

**8. Election of new members**

(including honorary members), deaths and resignations.

**9. Any other business**

## *Environmental Microbiology* Lecture 2010

The 2010 *Environmental Microbiology* lecture will be presented by **Professor Willy Verstraete** of Ghent University, Belgium. The title of his presentation will be "***Microbial resource management***". Professor Verstraete is the head of the Laboratory of Microbial Ecology and Technology (LabMet) at Ghent University. The lecture will take place on the 11 October 2010 at the Royal Society of Medicine, London and all members of the Society will receive an invitation in this issue. Further details about Professor Verstraete can be found at http://www.labmet.ugent. be/en/?staff:head. For members unable to attend, the lecture will be available online immediately after the event.

# membership matters

## Erratum

The Editor has had communication from the author of the article in the March issue of *Microbiologist* (**Vol. 11**, No. 1) entitled *Biofilms: an introduction to their significance and recalcitrance*. The author suggested that the *Irish Journal of Medical Science* was no longer in print. This resulted from an error in searching the older literature on bacterial persistence where the journal was listed as "*The Irish Journal of Medical Science* (1926-1967)". This is in fact one of four series and the journal continues to be active. The author would like to apologise for any confusion which may have arisen as a result.

# 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.**

### Armenia

N. Grigoryan

### Austria

B. Stessl

### Brazil

I. Dayo Owoyemi

### Chile

P. Junier

### Croatia

S. Mekic

### Finland

M. Pitkaranta

### France

R. Duval

### India

A. Gupta

### Ireland

R. Bonilla-Santiago; N. Clifford; S. Doll

### Nigeria

A. Obadina; J. Olaitan; O. Olaleye; O. Oyawoye; F. Tahir

### South Korea

S.W. Hong

### Spain

J. Maldonado; Z. Puyen

### Switzerland

H. Niculita Hirzel

### UK

R. Ahmed; P. Aimua; S. Al Kutby; N. Albaridi; N. Al-Kurdi; R. Al-Maaini; D. Anti; M. Arebiyi; R. Ayling; L. Birch; S. Bishop-Simon; I. Bohovych; K. Brathwaite; J. Bunce; T. Chinzowu; M. Clokie; J. Connolly; A. Cope; A. Cross; A. Dassuj; A. De Menezes; D. Diston; G. Downing; J. Drever-Heaps; Z. Duck; K. Emami; I. Ene; M. Fadaee-Shonada; N. Gicheva; N. Govindji; D. Handcock; H. Hardiman; M. Hichens; R. Hogg; N. Holling; P. Humphryes; N. Ikeda; S. Kolida; J. Lebeter; K. Lewis; V. Maisuria; R. Maluping; S. Marshall; A. Maule; W. McCully; J. Nzakizwanayo; I. Obidike; S. Onwuje; R. Quilliam; Z. Rees; S. Roberts; S. Salifu; D. Silva; P. Siringan; K. Skinner; S. Speight; A. Stapleton; D. Thomas; K. Tyagi; O. Uwasomba; M. Veses Garcia; C. Wabara; A. Waller; S. Whelan; E. White; U. Wijewardene; P. Williams; D. Wischer; J. Woolford; L. Yaliwal; A. Yates

### USA

M. Edwards; M. Maltz; D. Rao

### Corporate member

Germany: **Antibodies-online GmbH**
United Kingdom: **20/30 Labs Ltd**

### Losses

**We were saddened to learn of the deaths of the following members of the Society:**

Thomas Toomey and L. P. Hall

# journalWatch

News about the Society's journals


***Journal of Applied Microbiology***
***The following articles published in 2010 were the most downloaded articles from Journal of Applied Microbiology between January – March 2010:***

Filamentous fungal characterizations by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. C. Santos, R.R.M. Paterson, A. Venâncio and N. Lima, **Vol. 108**, No. 2, February 2010

Advances in enteropathogen control in poultry production. J.M. Cox and A. Pavic, **Vol. 108**, No. 3, March 2010

A synthetic furanone potentiates the effect of disinfectants on *Salmonella* in biofilm. L.K. Vestby, J. Lönn-Stensrud, T. Møretrø , S. Langsrud , A. Aamdal-Scheie, T. Benneche and L.L. Nesse, **Vol. 108**, No. 3, March 2010

The potential of flow cytometry in the study of *Bacillus cereus*. U.P. Cronin and M.G. Wilkinson, **Vol. 108**, No. 1, January 2010

Antimicrobial and antistaphylococcal biofilm activity from the sea urchin *Paracentrotus lividus*. D. Schillaci, V. Arizza, N. Parrinello, V. Di Stefano, S. Fanara, V. Muccilli, V. Cunsolo, J.J.A. Haagensen and S. Molin, **Vol. 108**, No. 1, January 2010


***Letters in Applied Microbiology***
***The following articles published in 2010 were the most downloaded articles from Letters in Applied Microbiology between January – March 2010:***

Screening and characterization of butanol-tolerant microorganisms. J. Li , J.B. Zhao, M. Zhao, Y.L. Yang, W.H. Jiang and S. Yang, **Vol. 50**, No. 4 April 2010

Preservation of probiotic strains isolated from kefir by spray drying. M.A. Golowczyc, J. Silva, A.G. Abraham, G.L. De Antoni and P. Teixeira, **Vol. 50**, No. 1, January 2010

The *in vitro* antibiofilm activity of selected culinary herbs and medicinal plants against *Listeria monocytogenes*. M. Sandasi, C.M. Leonard and A.M. Viljoen, **Vol. 50**, No. 1, January 2010

Cloning, expression and characterization of attachment-invasion locus protein (Ail) of *Yersinia enterocolitica* and its utilization in rapid detection by immunoassays. K. Balakrishna, H.S. Murali and H.V. Batra, **Vol. 50**, No. 1, January 2010

Development of a minimal growth medium for *Lactobacillus plantarum*. A. Wegkamp , B. Teusink, W.M. de Vos and E.J. Smid, **Vol. 50**, No. 1, January 2010


***Environmental Microbiology***
***The following articles published in 2010 were the most downloaded articles from Environmental Microbiology between January – March 2010:***

The Gram-positive side of plant–microbe interactions. I. Francis, M. Holsters and D. Vereecke, **Vol. 12**, No. 1, January 2010

Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. V. Kunin, A. Engelbrektson, H. Ochman and P. Hugenholtz, **Vol. 12**, No. 1, January 2010

Biofilms: the environmental playground of *Legionella pneumophila*. P. Declerck, **Vol. 12**, No. 3 March 2010

Metagenome and mRNA expression analyses of anaerobic methanotrophic archaea of the ANME-1 group. A. Meyerdierks, M. Kube, I. Kostadinov, H. Teeling, F. O. Glöckner, R. Reinhardt and R. Amann, **Vol. 12**, No. 2, February 2010

Diversity and population structure of sewage-derived microorganisms in wastewater treatment plant influent. S. L. McLellan, S. M. Huse, S. R. Mueller-Spitz, E. N. Andreishcheva and M. L. Sogin, **Vol. 12**, No. 2, February 2010


***Environmental Microbiology Reports***
***The following articles published in 2010 were the most downloaded articles from Environmental Microbiology Reports between January – March 2010:***

Web alert: Microbial metabolism prediction. L. P. Wackett, **Vol. 2**, No. 1, February 2010

Environmental reservoirs of *Vibrio cholerae* and their role in cholera. L. Vezzulli, C. Pruzzo, A. Huq and R. R. Colwell, **Vol. 2**, No. 1, February 2010

Environmental occurrence and clinical impact of *Vibrio vulnificus* and *Vibrio parahaemolyticus*: a European perspective. C. Baker-Austin, L. Stockley, R. Rangdale and J. Martinez-Urtaza, **Vol. 2**, No. 1, February 2010

Detection of toxigenic *Vibrio cholerae* O1 in freshwater lakes of the former Soviet Republic of Georgia. C. J. Grim, E. Jaiani, C. A. Whitehouse, N. Janelidze, T. Kokashvili, M. Tediashvili, R. R. Colwell and A. Huq, **Vol. 2**, No. 1 February 2010

Quorum sensing negatively regulates chitinase in *Vibrio harveyi*. T. Defoirdt, H.A. Darshanee Ruwandeepika, I. Karunasagar, N. Boon and P. Bossier, **Vol. 2**, No. 1, February 2010


***Microbial Biotechnology***
***The following articles published in 2010 were the most downloaded articles from Microbial Biotechnology between January – March 2010:***

Probiotics genomics. R. J. Siezen and G. Wilson, **Vol. 3**, No. 1, January 2010

LOVely enzymes—towards engineering light-controllable biocatalysts. U. Krauss, J. Lee, S. J. Benkovic and K. E Jaeger, **Vol. 3**, No. 1, January 2010

Metabolic engineering, new antibiotics and biofilm viscoelasticity. Craig Daniels, Manuel Espinosa-Urgel, J. Luis N. Arroyo, C. Michán and J. L. Ramos, **Vol. 3**, No. 1, January 2010

Metabolic engineering of cobalamin (vitamin $B_{12}$) production in *Bacillus megaterium*. R. Biedendieck, M. Malten, H. Barg, B. Bunk, J. H Martens, E. Deery, H. Leech, M. J. Warren and D. Jahn, **Vol. 3**, No. 1, January 2010

Advances in the field of high-molecular-weight polycyclic aromatic hydrocarbon biodegradation by bacteria. R. A. Kanaly and S. Harayama, **Vol. 3**, No. 2, March 2010

**Lucy Collister**
Wiley-Blackwell

# bio **focus**

**Mark Downs** talks about Science Funding—the need for common purpose

SOCIETY OF Biology

**The Society of Biology is a single unified voice for biology:**

- advising Government and influencing policy
- advancing education and professional development
- supporting our members
- engaging and encouraging public interest in the life sciences

**For further information visit:**

**www.societyofbiology.org**

Whatever flavour of new Government emerges from the general election, one thing is certain: they will not be over-endowed with scientific expertise. Partly as a fall out from the expenses scandal, the number of parliamentarians standing down at this election is significant. The result, however the electorate votes, will mean at least 300 new MPs. However, only a handful of these parliamentarians have any background in science. The scientific community will need to support these members and nurture an empathy with others to ensure the importance of science to our economy, health, the environment and social infrastructure is not lost.

They will need to be engaged in the issues rather than lectured to and above all, we need to avoid the trap of special pleading. The new Government and backbenchers alike need to hear a simple and consistent message about the value of science. The science budget must continue to be ring-fenced and the amount within it at least maintained. If we can win the wider argument, biology has a strong heritage to call upon to ensure the life sciences are not undervalued.

The Society of Biology is taking every opportunity to lobby. We have written to each parliamentary candidate to raise the profile of biology and the role of the Society but with a clear focus on the bigger picture. We are highlighting three key messages:

- **Recognition of the central role of science in the economy**, by maintaining or increasing funding for basic and applied research in real terms. We are calling on the new Government to develop a new 10-year funding framework for science, to underpin the UK's position as a world-leading scientific nation.

- **The importance of practical as well as theoretical skills in the training of scientists**. This will require financial support for a significant hands-on practical skills element, including laboratory and field skills in courses at school and university. School teachers should be enabled and supported to provide high quality laboratory and outdoor practical science teaching at all levels. We argue that particular attention is paid to this in forthcoming curriculum reviews.

- **The need to intensify efforts to ensure that scientific evidence is well used and communicated across government**. This is necessary to improve policy development and delivery where issues cross traditional departmental responsibilities. We recommend inclusion of principles guaranteeing academic freedom of scientific advisers in the ministerial code.

There are further, more detailed arguments to be made about equality of funding between the sciences, pointing out the absolute necessity that UK biology is funded in a way that enables us to retain our world-leading position. But it would be a mistake to lead the debate with special pleading for the biosciences. We need new parliamentarians to more fully understand the wide value of public financial support for UK science as a whole.

The recent Question Time style debate organized by the Royal Society of Chemistry in partnership with the Society and others, between the science spokesmen for the three main parties—Adam Afriyie (Cons), Lord Drayson (Lab) and Evan Harris (Lib Dem), did highlight some policy differences. It is right to provide the parties with more of the detail, focussing on biology. But it is evident that they at least have a good overview of the issues. It will be the lack of science understanding across key ministerial posts and parliamentary committees that is likely to lead to misunderstanding or a lack of urgency.

The Society will be working hard to build relationships with MPs and Peers post-election, pushing them on their science policy and seeking to represent biology on behalf of our membership. We will develop a range of case studies on the impact of biology to support the detailed arguments and we welcome your input. But the more MPs hear the same message the more likely it is that we will succeed. We encourage you to write to your local MP with these messages and stand ready to offer support wherever possible.

**Dr Mark Downs, Ph.D, FSB**
Chief Executive, Society of Biology

# MiSAC competition 2010: food safety and barbecues

The 22nd Annual Microbiology in Schools Advisory Committee (MiSAC) competition was to design a storyboard for a television advert to promote food safety at barbecues. S*f*AM provided this year's sponsorship for the competition, including £1,000 in prize money for the winning entrants and their schools

The topic and format of the entries were chosen to encourage the participants to develop an appreciation and understanding of the simple steps that can be taken to prevent food poisoning. Entries were invited in two age groups:

**11-14 years (Key Stage 3)**

**14-16 years (Key Stage 4)**

Almost 300 entries were received involving more than 400 students from nearly 50 schools and colleges in England, Wales, Scotland and Northern Ireland; an entry from a Ministry of Defence school in Cyprus maintained the international appeal of the competition.

KS3 1st prize winning entry from Henrietta Roseboom of Ipswich High School


KS4 1st prize winning entry from Katherine Stahl of Pocklington School, York

The concept of this year's competition was challenging, yet the high quality and creative approach of the entries in both age groups was very impressive and imaginative. Many entrants clearly relished the opportunity to express their artistic talents and as well as the prize winners for each age group, a number were awarded commendations for concept, content and design. For example, the judges were particularly impressed by the clarity and quality of the cartoons by Katherine Stahl of Pocklington School, York (KS4, 1st prize winner) and the artistic skills demonstrated by Ancella Ho of St Anselm's School, Canterbury (KS4, Commended for design). Judges also commented on the entertaining nature of the storyboard created by KS3 1st prize winner Henrietta Roseboom of Ipswich High School as well as the professional appearance of the entry by Joseph Brown of Portsmouth Grammar School (KS3 3rd prize winner).

There was an excellent level of adherence to the overall entry rule of submitting an A3-size storyboard containing six panels, and a good grasp of what makes an effective storyboard, aided by the guidance provided for entrants. The mention of foodborne microbes such as *Salmonella* and *Campylobacter* was a requirement and reference to correctly storing food, cooking it properly and preventing cross-contamination from uncooked to cooked foods was considered favourably. The judges looked particularly for originality, eye-catching design, a title that immediately indicated the purpose of the storyboard, a clear sequence to the panels that maintained viewers' attention, sound factual and scientific content, an appropriate approach to communicating science to a television audience, and use of the entrant's own words.

The judging panel consisted of S*f*AM representatives Professor Martin Adams of Surrey University, Dr Lucy Harper, the Society's Communications Manager, and Dr Anthony Hilton of Aston University and winner of the S*f*AM Communications Award 2009, together with the Chairman and other members of MiSAC. The facilities were provided by the Society for General Microbiology whose staff also bore the task of receiving and processing the entries at its headquarters in Reading.

In addition to the winners in 1st, 2nd and 3rd places in both age groups receiving money prizes, they and those awarded commendations were given a memory stick loaded with Micropod podcasts from S*f*AM. All students who participated were given a certificate of entry, a much appreciated feature of the competition, and their school received some microbiology teaching resources and a summary report on the competition.

Next year's competition will be on '**famous fungi**' and will be sponsored by the British Mycological Society.

**Dr John Grainger,** Chairman, MiSAC
**Dr Lucy Harper,** Communications Manager, S*f*AM


# media**watch**

microbiology and the media

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**

# Science in the Media — a debate

In the current economic climate, journalism is a profession (or trade, skill or crafts as Andrew Jack of the Financial Times describes it) which is not immune from deleterious effects. In the USA many publications have had to make cut-backs, with newspapers losing staff or closing their doors entirely. Science journalism is experiencing the same problems as all forms of journalism: with the explosion of web 2.0 and so-called 'citizen journalism' in the form of blogs and other direct-to-public forms of communication, the sustainability of the business model of journalism seems to have come into question.

Fiona Fox, Director of the Science Media Centre, with the help of a high profile working group and much consultation with journalists, press officers, scientists and others, wrote a report for Lord Drayson as part of the government's Science in Society Strategy entitled "Science and the media: securing the future" (http://interactive.bis.gov.uk/scienceandsociety/site/media/2010/01/21/comment-on-the-final-report/).

On the 31 March 2010 at City University, London, a panel of experts debated this report at a public event, and asked whether science in the media was in rude or ailing health (http://www.city.ac.uk/whatson/2010/3-mar/31032010-science-media.html).

The panel comprised Ed Yong, a scientist and blogger of 'Not Exactly Rocket Science' (http://blogs.discovermagazine.com/notrocketscience/), Natasha Loder, a trained scientist who is now a science correspondent for *the Economist*, Andrew Jack, Pharmaceuticals Correspondent for the *Financial Times* and Fiona Fox. Each panellist provided a response to the report, beginning with Fiona who began by saying that the working group had found science in the media to be in pretty good health. There are more science journalists than ever and more science in the headlines. She gave examples of excellent and accurate media coverage which gained as much exposure as the parliamentary expenses scandal:

- **Nutt-gate**—the sacking of Professor David Nutt from the governments advisory council on the misuse of drugs (ACMD)
- **Climate-gate**—leaked emails between climate scientists at the University of East Anglia
- **Swine flu**

Fiona looked back at the situation some years ago, citing examples such as the MMR scandal and the misreporting of GM, as cases where any deference to experts was missing from science reporting and subsequently journalists reported inaccuracies with real consequences. Nowadays, there are many examples of Editors going to their Science and Environment Correspondents for advice as to how to run a story, or asking them to take the lead—a really positive development.

Fiona went on to describe the crisis in journalism which is having a knock-on effect on science reporting, saying that even Rupert Murdoch, the person who originally wanted his content free online, doesn't know how to solve the problem with the business model in journalism and has introduced paywalls to his online content which are about to come into effect (see note[1] at end).

Time (or lack of it) is another problem and has resulted in so-called 'churnalism' (reporters not having the time to do their job properly and relying too heavily on the content of press releases as their only information sources). Fiona then went on to describe a couple of the recommendations contained in the report. The first point was training of journalists: "*time and time again we see science, health and environment correspondents doing it well, but then coming up against Editors and Sub-editors who don't understand science, the way it works, the difference between peer-reviewed and non-peer-reviewed study or communicating risk and all these things which we know are critical to accurate reporting*". As a result of their investigation, one of the recommendations is to offer training to Editors, Sub-editors and people who run newsrooms, who all expressed a desire to learn about the basic principles of science reporting. Fiona was delighted to announce that only that week, the government had agreed to fund this recommendation, leading to the imminent recruitment of a National Training Co-ordinator for science journalism.

Another of the recommendations was regarding the kinds of opportunities that are being opened up by web 2.0, blogging and so-called citizen journalism. She gave the example of Cancer Research UK (CRUK) who pay professional journalists to contribute to their online content and to prevent cancer scare stories being published or broadcast through mainstream media. Fiona said that instead of '*letting a thousand flowers bloom*', they recommend setting up a working group to identify the most effective of these kinds of initiatives, investing in these projects to enable them to grow.

Andrew Jack provided examples of good (Large Hadron Collider) and bad (MMR) science reporting. He then moved on to the good and bad of the internet: the good being the explosion in the number of sources and a form of democratization of information. This is good news for journalists and members of the general public who want to be '*granular*' and dig down to original sources. However, the idea that all this information is available free of charge enables the '*viral*' spread of misinformation which then

## SfAM 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.

attains credibility to which more authoritative, more respectable analysis potentially fails to catch up.

It would appear that there is more space online, but less in traditional media and even less time to fill any of that space. So rather than talk about a crisis in journalism, we should talk about a crisis in the structure of media generally and acknowledge that the next decade will be a difficult time when we anticipate seeing a real transformation.

Andrew put forward a few criticisms of the report, stating that it seemed very top-down in its approach, when the consumers of media are what we rely on to raise the level of interest and responsibility in reporting. He suggested that to stimulate an interest in the world around us there needs to be an inherent excitement about science in the school curriculum.

Andrew commented on the growth of PR as a beneficial development, as long as it's not influenced by financial, commercial or any other agenda. He then went on to reinforce the importance of positively encouraging scientists to communicate with the media. More needs to be done to encourage scientists to think about the long term consequences of their research and its impact on society.

Andrew finished by suggesting the availability of litigation support in science and science journalism. He cited the case of Simon Singh (see note[2] at end), saying "*if we can't modify the laws around libel we should at least provide support to ensure freedom of speech, so that open scientific debate and valid criticism isn't stifled by commercial or other vested interests*."

Natasha Loder began by expressing her gratitude that the research that went into the report had taken place, as she'd heard repeatedly that there was a crisis in journalism but that this hadn't seemed 'real' in the UK. In traditional media, journalists are working harder, under greater time constraints—there's a crisis in the business model of journalism. As Chair of the Association of British Science Writers (ABSW), Natasha spoke of the report lacking emphasis on continuing professional development (CPD): the explosion of information online has meant there are many changes taking place very quickly in the industry, but not everyone involved is able to keep up.

However, Natasha's viewpoint was that overall science journalism is in rude health because of the initiatives that Ed Yong and CRUK and other bloggers have undertaken. She described the futility of having a debate about whether blogging can be called journalism due to the public function of truth-telling that traditional journalists and bloggers all serve.

She highlighted that there are 82 full time journalists in the UK, so if this report is aimed at the traditional media, it has a very small audience, however, there are many more freelance journalists and bloggers who are essentially truth-telling as a public function—the thousand flowers which Fiona described are blooming and the public are receiving a lot more information.

Natasha mentioned that the recommendations in the report were looking at building initiatives around what's already there in science journalism —e.g. preparing scientists to talk to the traditional media—"*pimping scientists*". What's really needed is the acknowledgement that more broadly we are trying to get commercial organizations (e.g. *the Economist*) to serve a public function through the utilization of online developments.

Natasha went on to talk about science journalism and science communication courses saying it would be useful to know the outcome for graduates of such courses. The number of people we're training (around 65 per year graduate from City University and the same from Imperial College) seems to be disproportionate to the number of jobs available (82 full time journalists and 3-400 freelance science writers in the UK). Natasha ended by saying: "*Science journalism is out there and we need to recognize that and try to boost the quality of all of it*."

Ed Yong found a lot to like about this report, as he said: "*It's easy to read, it supports libel reform and has produced data from the research at Cardiff University and that certainly appeals to the scientist in me*". He thought some interpretation of the data was a little optimistic and voiced concerns in terms of the quality of science reporting—where the increased number of journalists is an indicator of quantity, but not of quality. He also raised the point that the report was derived from the opinions of science reporters which he likened to asking the pharmaceutical industry how drug development is doing. It is important to consult consumers of news because science journalism is about two way communication and as it stands there is confirmation bias in the report. It is premature to say that science in the media is in rude health as there are good and bad examples and everyone is in agreement that there could be improvements.

Ed's main issues with the report were regarding what he perceived it to be lacking, including the fact it is narrow in scope. The report says: "*We initially made a decision to rule out the explosion of direct-to-the-public science communication by way of websites, blogging, tweeting etc and concentrate on science communicated through journalism in mainstream media settings*." To Ed, that is the most interesting part—the fact that the internet is changing the face of science journalism and what it means to be a journalist—all this is acknowledged, but the ramifications of these

developments are not explored. He suggested that the drawing of a line between what is and isn't journalism has resulted in the report describing the present instead of, as its title suggests, "Securing the future". The title came under more scrutiny from Ed who took issue with "Science and the media". Ed explained that the report concentrates on (limits itself to) traditional mainstream media and talks a lot about journalism. The media is a channel through which journalism is practiced; it's not journalism itself which is about shared values of accuracy, scrutiny and objectivity in truth telling. Web 2.0 has enabled anyone with the skill and desire to talk about science to do so (this how Ed became a science writer/blogger/journalist). Tomorrow's journalists may well enter this way through what Ed termed the "*Cambrian explosion of science journalism—new species will come out of the blue, diversifying into new niches in order to explore new strategies*".

Ed went on to talk about training—he quoted Andrew Revkin, a well respected blogger and journalist formerly of the *New York Times*:

"*I want to help to make scientists better, more creative communicators in a world of shrinking specialized journalism, direct outreach will be more vital than ever*"—a sentiment with which Ed fully agrees. Rather than drawing lines in the sand it's better and more interesting to point out areas where those lines have been blurred because the future is all about blurred boundaries.

He provided some examples of situations where just such boundaries have been blurred, such as Mark Henderson, science editor of *the Times*, a trained journalist and excellent science editor who is now blogging.

Likening the blogger-versus-journalist argument to the film Titanic, he said: "*everyone's drawn to it, it goes on forever and never gets anywhere interesting*".

Ed explained there are so many exciting things going on that he's disappointed with the recommendation to create a new working group to keep a watching eye on these new initiatives. This sort of top-down approach is unlikely to produce much that's going to shape the future of science journalism. Ed then finished, saying: "*I think the really interesting stuff is being done by people from the bottom up who are trying out new things, diversifying into new niches and getting their hands dirty with new technology. I think that's the way one secures the future*."

A debate then ensued including sometimes heated discussion including whether a blogger who subscribes to all the tenets of journalistic scrutiny, fact-checking and thorough research can call themselves a journalist. Should we defend a trade/craft called journalism? Some thought the increased 'noise' of information means that traditional journalism is needed to help filter out the bad from the good. If traditional journalism isn't supported it may be lost. Others didn't think making a firm division between the two worked, with Andrew stating that one of the problems for journalists is that they are increasingly expected to do both, create objective pieces for traditional media, and write often opinionated pieces online.

When asked if blogging can be objective, Ed gave an example where journalistic skills and objectivity were at the forefront of his mind whilst blogging a story which will interest our members: "*there was a story in PNAS that came out a couple of weeks ago about bacterial forensics which suggested that by looking at the bacteria that you leave on the things you touch, you can work out who touched what object, because the bacteria on peoples skin vary considerably from individual to individual. This was all over the papers and only two places actually managed to bother to interview an actual forensic scientist to work out if this could ever be used in a criminal investigation and that was me and [the publication] WIRED. Both of our reports were the only ones which actually said no, it would never be used in a criminal investigation—it will never achieve that level of basic accuracy and everywhere else there were headlines about 'Cops use skin bacteria to catch crooks' and so on*".

Verifying sources online is easier than in traditional media through links, allowing the reader to do their own fact-checking of online information which isn't possible in print media. This "*encourages this culture of scrutiny and critical analysis which I think we're missing at the moment*", said Ed Yong.

Finally, the issue of training scientists to communicate more effectively was discussed, with some comments from science-trained Natasha who said: "*I think some scientists will be suited to it and some won't. You have to train them out of some of the things they've been trained to do as scientists. I felt that a lot of the things I'd learnt through my training on a PhD were contrary to a lot of the skills I needed to learn as a journalist...you make sure they've got what it takes in terms of character to be a journalist: sharp elbows, a bit of nous and the ability to get the job done*."

Scientists communicating with the media will be the topic of a session prior to the official start of this year's S*f*AM Summer Conference. If this article has stimulated some thought, you're attending the Conference and you'd like to join the debate, contact Lucy Harper for details.

**Lucy Harper**
Communications Manager

## note

- [1]Accurate at time of going to press
- [2]Simon Singh is a researcher who criticized certain aspects of chiropractic and alternative medicine. The British Chiropractic Association responded by drawing case against him which has subsequently recently been dropped.

# Royal Society of Medicine meeting
# Zoonoses: from wildlife to warfare

On the second of February S*f*AM exhibited at a fascinating meeting of the Pathology Section of the Royal Society of Medicine (RSM) entitled: **Zoonoses: from wildlife to warfare**

The day began bright and early with registration, coffee and plenty of opportunity for delegates to visit the S*f*AM stand. We were warmly welcomed by all RSM staff and the friendly host David Spratt. Then we piled into the lecture theatre for the first session of the day. The one-day meeting, chaired by Dr James Kirkwood began with Professor Richard Titball discussing *Zoonotic pathogens and biological weapons*. Richard referred to the US Health and Human Services list of infectious micro-organisms, noting that most are zoonotic pathogens. He described the properties of different organisms, highlighting how they could be useful as bioweapons. The method of dispersion of bioweapons was discussed and his talk illustrated this with a map showing the locations of documented attacks since the 1930s, such as the Aum Shinrikyo anthrax attack in Japan, 1993 (Keim *et al.*, 2001).

Richard then went on to describe a vaccination programme against *Yersinia pestis* which his group has brought to Phase 2 of clinical trials.

Following this, Dr Dilys Morgan of the Health Protection Agency (HPA) spoke on: *Assessing the threat to the UK population from emerging zoonoses*. Dilys described the work of the National Expert Panel for New and Emerging Infections (NEPNEI) in the identification of newly emerging infectious disease. She stated that 75% of emerging pathogens are zoonotic and went on to explain the four stages of risk analysis:

- Hazard detection—including the use of the ProMed database
- Assessment
- Risk management
- Risk communication—including managing perceptions of risk

Next, Dr Andrew Cunningham of the Zoological Society of London spoke about *Zoonotic disease threats from wildlife*. He began by telling us that 72% of zoonotic emerging infectious diseases (EIDs) originate in wildlife. Andrew continued with a recent series of high case-fatality EIDs with wildlife origins including Lyssavirus with bats as the reservoir host, and Hendravirus which uses horses as its amplifier host in Australia.

Following a coffee break, Maggie Tomlinson from the Department of Health kicked the session off with *Sick swans, crazy canines and risky rodents: integrating UK policy on zoonoses*. Maggie gave an interesting and informative introduction to UK policy. She discussed the different organizations involved in UK policy, how they are linked and their individual and group responsibilities. Professor Stephen Palmer of Cardiff University gave the next talk with the equally interesting title *Rats, lice, mad cows and history*. Stephen discussed his own personal career within zoonotic epidemiology and his time working for the HPA. Stephen took us back in time discussing the origins of milk pasteurization following the identification of bovine tuberculosis in milk and explained how we can relate this incident to modern zoonotic infections. He went on to discuss the role of veterinary practice in human health protection and the 'one health approach'. These talks were followed by a fascinating discussion session, including questions on petting zoos and who should undertake the responsibility of explaining risk.

Lunch provided another opportunity for delegates to chat to the S*f*AM representatives including Hon. Gen. Sec. Mark Fielder and Main Committee member Alison Kelly. After lunch Vincent Emery, Professor of Virology at the Royal Free Hospital. spoke on: *Viral zoonoses a continuing threat to man*. He discussed zoonotic viruses and how their continual evolution makes them so fascinating to study. His overview covered an enormous array of zoonotic viruses, yet they were just the tip of the iceberg. From HIV to Ebola, zoonotic viruses are extraordinary in their ability to survive and produce such catastrophic effects of infection.

Next, Marina Morgan of Royal Devon and Exeter Foundation Trust, took us on a whistle-stop tour of bacterial zoonoses. Hardly stopping for breath, Marina described many different bacterial zoonoses and showed us (often graphic) images of their clinical presentation. Her enthusiasm for the subject was clear as was her message: we should not blame the animals.

The meeting finished with a presentation from the distinguished Professor Francis Cox of London School of Hygiene and Tropical Medicine whose presentation was entitled: *Protozoan infections and emerging zoonoses*. Francis looked at protozoa and illustrated how only a small number of these which cause serious disease are zoonotic. He described the global distribution of parasitic diseases and lifecycles of a number of protozoa including African trypanosomes, malaria parasites and *Leishmania* spp.

The S*f*AM stand generated much interest from delegates, many of whom took away S*f*AM material for their colleagues. This was a great outcome and a suitable end to a fascinating day.

## reference

■ Keim, O., Smith, K.L., Keys, C., Takahashi, H., Kurata, T. and Kaufmann, A. (2001) Molecular investigation of the *Arum Shinrikyo* anthrax release in Kameido, Japan. *Journal of Clinical Microbiology*. **Vol. 29**, No. 12, pp4566-4567

**Lucy Harper and Clare Doggett**

# Biofilms 4 International Conference

1–3 September 2010, Winchester, UK

## "Communities Bridging Disciplines"

### Themes

Microbial communities in disease

**Community ecology and evolution**

Global scale biofilm systems

**Surface engineering and biofilm tribology**

Novel biotechnology and bioengineering

**Structural dynamics and emergent properties of biofilms**

Modulation of biofilm communities

**Signalling and communication in biofilms**

Biofilm development: a multidisciplinary approach

**KEYNOTE SPEAKERS:**

Bill Costerton, *Allegheny-Singer Research Institute, USA,* Staffan Kjelleberg, *University of New South Wales, Australia,* Søren Molin, *Danish Technical University*

A limited number of SfAM studentship grants are available to student members of the Society wishing to attend the conference. Download forms from the SfAM website.

Visit **www.biofilms4.com** for more information on the conference and to register.
Registration deadline: 16 July 2010.

**Contact Kinetix Events Ltd, 01234 761750, biofilms4@kinetixevents.co.uk**

# MICROBE 2010

*Sf*AM members will be delighted to know that following the success of the MICROBE 2008 conference, the organising committee has agreed that **MICROBE 2010** will be held at the same venue (Hilton Hotel, Sheffield) and in a similar format. Conference dates are **24-26 September, 2010**.

The venue is ideal for visitors, being located close to the main A57 Parkway into Sheffield, and a short distance from travel interchanges. Sheffield is well served by motorways and there are direct trains from major towns and cities. Many hotel rooms overlook the canal basin which is frequently full of colourful canal boats. All lectures, trade exhibitions and social arrangements are being held in the Hilton Hotel, avoiding the need for travel throughout the day. The hotel has a leisure club, which will be available to delegates.

There will be an extensive trade exhibition comprised of approximately 35 companies, which supply medical microbiology products. The companies will be promoting current and new technologies, and providing product information and corporate gifts. Delegates who attended the previous MICROBE conference will have appreciated the vibrant and enjoyable atmosphere in the trade exhibition areas.

The lecture programme is still being finalized but there will be a range of interesting speakers and topical themes from early Friday afternoon until late on Sunday morning. The provisional programme is diverse, including significant sessions on viral infections; novel antimicrobial treatments; new technologies; zoonotic infections; and clinical conditions & their microbiological complications. The conference is CPD accredited (14 credits).

Social activities, including visits to the trade exhibition are arranged for the evenings, providing the ideal opportunity to catch up with friends and acquaintances.

Further details can be obtained on the web site. This will include a programme of events, the final lectures and speakers, application information, road directions and any other information related to the conference. The single delegate fee is only £199, which hasn't changed since MICROBE 2008. A shared accommodation offer is also available at £150 for each delegate. Both packages include all accommodation and meals, plus access to the trade exhibition, lecture programme, social events and entry to the hotel health club.

All applications should be submitted as early as possible so that suitable accommodation can be booked.

**Alan Pease**
Secretary Microbe2010 committee

**Further details from: www.microbe.org.uk**

*Professor Peter Borriello receiving the Procter and Gamble Applied Healthcare Microbiology award from Alex Blanchard*

# Spring Meeting 2010 Report

# 4th broadening microbiology horizons in biomedical science meeting

## Latest developments in gastrointestinal infections

Stratford Q Hotel, Stratford-upon-Avon, UK, Friday 16 April 2010

Despite the Icelandic volcano spewing ash over northern Europe, delegates managed to make it to sunny Stratford-upon-Avon for the SfAM Spring Meeting. The venue was a delightful setting for a day of talks on a variety of gastrointestinal infections.

After a coffee and a chat, the morning sessions began with the Procter and Gamble Applied Healthcare Microbiology award lecture entitled "*There's brass in muck*". This was delivered by Professor Peter Borriello of the Veterinary Laboratories Agency (VLA), Surrey. Peter said that "*there are more gastrointestinal bacteria in our gut than people on the planet*". He then talked about, not only the hinderance of *Clostridium* causing antibiotic-associated diarrhoea (CDAD), but also the organism's effect on other diseases.

Peter talked about the history of *C. difficile* and how pseudomembranous colitis was determined to be caused by this bacterium, through detection of the toxin. He also re-emphasised that the risk of infection follows antibiotic treatment, which lowers the gut natural flora, and thus allowing exposure to toxigenic *C. difficile*.

Peter then discussed lignans which are oestrogen-like compounds cytotoxic to lymphoid cells. Lignans, which are derived by gut bacteria from foods such as soya, have been shown to lower oestrogen and have demonstrated some anti-tumour activity. Equol, also found in horse urine, is a weak oestrogen with anti-oestrogenic activity and has been known to cause reversible infertility in sheep. Diets rich in lignans could explain why some individuals find it difficult to conceive. Finally, Peter discussed the role of gut bacteria, in particular *Yersinia enterocolitica* in Grave's disease, through binding of thyroid-stimulating hormone (TSH). He concluded that "*there is some gold to be found among the muck*".

The next speaker was John Threlfall from the Health Protection Agency (HPA), Colindale, London who discussed recent developments in *Salmonella.* He stated that there are 2,500 antigenic serotypes with *Salmonella* Enteritidis the most common serovar. He reminded us that the main reservoir is in poultry and that cases rose during the early 1990s. However thanks to the *S.* Enteritidis vaccination between 1996 and 1999 cases of the disease fell by 40% between 2001 and 2009. John also discussed how the *Salmonella* genomic island 1 (SGI1) variants have been found in different serovars and is rapidly spreading worldwide. He also mentioned a tetracycline resistant strain that has recently emerged and is associated with frozen mice used to feed pet snakes. He finished by discussing how monophasic salmonellae are a worldwide emerging problem and good infection control measures need to be used at all times, due to the fæcal-oral route of transmission.

Geraldine Smith, from the HPA, updated us all on the identification and typing methods used to identify the Vero cytotoxin-producing *E. Coli* O157 (VTEC). She reinforced that this bacteria can cause uncomplicated diarrhoea and asymptomatic infection as well as Haemolytic Uraemic Syndrome (HUS). Published research would indicate an infectious dose of <100 CFU to cause disease, and the organism can cause long term health effects.

Geraldine explained that once samples reach the reference laboratory they are phage and serotyped to determine epidemiological information. The verotoxin typing is then carried out using PCR and subtyping. A new technique with good discrimination known as 'multi locus variable number tandem repeat (VNTR) typing' has been used recently. Geraldine finished by discussing a number of cases of

## 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 meetings on pages 24 to 27 of this issue of *Microbiologist*

infection, saying that these tend to be new outbreaks, but in the same setting.

Just before lunch Guy Robinson, a last minute replacement for Rachel Chalmers from the *Cryptosporidium* Reference Unit (CRU, Swansea) reminded us of this important protozoan. Guy gave us an overview which included the history of the disease, cryptosporidiosis, and focussed on the two infectious species, *C. parvum* and *C. hominis.* He also discussed the latest detection methods. *Cryptosporidium* has an extremely low infectious dose of one oocyst capable of causing an infection and has an incredibly robust outer shell—it is resistant to chlorine and infection can occur through contaminated water. Cryptosporidiosis is normally self-limiting; however, there is no official treatment. Cryptosporidiosis is a greater problem in developing countries and can have long term effects, such as reactive arthritis and Irritable Bowel Syndrome (IBS). Guy then described in graphic detail one patient's experience, making us all feel a bit queasy just in time for lunch.

**Samantha Price** ■ PECS Events Officer

The lunch break gave delegates the opportunity to chat to colleagues and trade representatives. The afternoon session then continued the day's theme of gastrointestinal infections. Nicola Elviss of the Health Protection Agency, Leeds gave us a food microbiologist's perspective on *Campylobacter* infections. She explained that the 50,000 cases reported annually from England and Wales probably represent only 10% of the total number of cases. Due to the difficulties in isolating *Campylobacter* spp. from food, it is not known how many of these infections are foodborne. She also proposed that apparently sporadic cases may be part of outbreaks.

We were shown data from a study of the outer packaging of chicken portions. In the study, 5% were contaminated with *Campylobacter* spp. Nicola also dispelled the myth that these organisms are only surface contaminants of food. Although they don't multiply within food, their bipolar flagella enable them to migrate into chicken muscle. Nicola concluded by suggesting reasons why infection is so prevalent, including the lack of legislation to motivate changing kitchen practice.

Alistair Brown of Thermo Fisher Scientific described how enteric culture media have evolved since the 1880s and used the isolation of *Salmonella* spp. to demonstrate their evolution. He focussed particularly on the principles of chromogenic media, then explained how the basis of this technology had been modified to develop inhibigen media.

In chromogenic media, coloured chromophores are bound to a sugar component which renders them colourless. If an organism produces a specific enzyme which hydrolyzes this complex, then the chromophore component is released, accumulates within the cells and results in the colonies taking on the colour of the chromophore. Knowing which organisms produce which enzymes enables differential agars to be produced. In a similar fashion, inhibitor molecules can be attached to sugars or amino acids. Hydrolysis by specific enzymes then releases the inhibitory component which kills the organisms. Thus, selective agars can be developed which inhibit targeted organisms. This is a more selective approach than incorporating broad-spectrum antibiotics. Alistair suggested that this technology is likely to become more widely used in the future.

Jim Gray of Birmingham Children's Hospital questioned whether we could be better at diagnosing *Clostridium difficile* infection (CDI). Although hospitals have introduced successful infection control measures to lower the incidence of CDI, further efforts are required. He explained the limitations of currently available tests and showed why it is necessary to use them in conjunction with each other. Jim described how his laboratory had moved from a two-step algorithm using glutamate dehydrogenase (GDH) and an immunotoxin test to a three-step algorithm incorporating a PCR test. This approach provoked some debate from the audience, demonstrating the fact that diagnosis of this infection is not straightforward.

The final speaker of the day was Martin Woodward of the Veterinary Laboratories Agency, Surrey. He described how an integrated approach using genomics, proteomics and phenomics was deepening our understanding of the gut health of animals and foodborne zoonoses. He focussed on *Salmonella* outbreaks stressing that surveillance is key to the successful reduction of the burden of disease.

Despite some successful interventions such as poultry vaccination, the time interval between the emergence of a new zoonotic problem and the implementation of control measures is undesirably long. Martin gave examples of how comparisons of epidemic and non-epidemic strains have been used in order to identify possible virulence factors.

Many delegates expressed their appreciation for the interesting talks that were presented and felt it had been a beneficial day.

**Louise Hill-King**

## Monday 5 - Thursday 8 July 2010

# Summer Conference

Applied microbiology with sessions on:

- **Biofilms:** buzzwords in biofilms
- ***Listeria*:** new perspectives on an old pathogen
- **Bacteriophages:** applied bacteriophage technology

■ Including the Lewis B Perry Memorial Lecture

**CPD ACCREDITATION**
**15 CPD POINTS**

## The Grand Hotel, Brighton, UK

## SCIENCE IN THE MEDIA

■ Delegates to this year's Summer Conference will be invited to attend a pre-conference workshop exploring **science in the media**.

■ Would you like to know how to publicise your research?

■ Do you want to find out how you can help journalists, press officers and journal publishers to ensure your research is reported accurately?

■ This workshop will explain the benefits of publicising your research through the media, the pressures under which journalists find themselves and the ways in which you can get involved to spread the word about your science.

■ The workshop will take place on **Monday 5 July 2010** at the Grand Hotel, Brighton.

■ For more details visit: **www.sfam.org.uk/summer_conference.php** or contact Communications Manager, Lucy Harper (**lucy@sfam.org.uk**) or Communications Officer Clare Doggett (**clare@sfam.org.uk**).

## REDUCED RATES!

| **Summer Conference delegate fees REDUCED for 2010!** | Full member | | Student, Associate Honorary and Retired member | |
|---|---|---|---|---|
| | Early bird before 7 June | From 8 June | Early bird before 7 June | From 8 June |
| Full conference with accommodation | £250 | £300 | £200 | £250 |
| Full conference no accommodation | £100 | £150 | £50 | £100 |
| Day rate | £50 | £100 | £25 | £75 |

■ We are also delighted to announce that the Summer Conference 2010 fee includes dinner in the hotel restaurant every evening.

■ Please note that there is a small supplement of **£20** payable to attend the drinks reception and conference dinner on the evening of Wednesday 7th July 2010.

**To register online for the Summer Conference please visit www.sfam.org.uk/summer_conference.php or contact Sally Cryer ■ Email: sally@sfam.org.uk ■ Telephone +44 (0)1234 761752**

# Programme

## Monday 5 July

**14.00 onwards** — **Arrive and register**

**Workshop: Science and the media**

**18.00-18.50** — **Lewis B Perry Memorial Lecture:**

**Contagious bovine pleuropneumonia: in search of the origins and virulence of lung sickness**
Robin Nicholas, Veterinary Laboratories Agency, UK

**19.00-20.00** — **Drinks reception**

**20.00** — **Evening at leisure**

**21.30** — **Quiz night**

## Tuesday 6 July

### Session 1: Applied bacteriophage technology

**Chair:** — **Andy Sails**

**09.00-09.35** — **Phage therapy in human infections**
Alexander Sulakvelidze, Intralytix, USA

**09.35-10.10** — **Uses of phage in the animal food production chain**
Ian Connerton, University of Nottingham, UK

**10.10-10.45** — **Bacteriophages for the treatment of *Pseudomonas aeruginosa* infections**
David Harper, Biocontrol, UK

**10.45-11.15** — **Coffee and Tea**

**11.15-11.50** — **Using bacteriophages to develop DNA vaccines**
John March, Big DNA, Edinburgh, UK

**11.50-12.25** — **Bacteriophages for the prevention of *Listeria monocytogenes* in food**
Steven Hagens, EBI Food Safety, The Netherlands

**12.25-13.25** — **Lunch**

**13.25-14.00** — **Optimized by evolution: phage and its enzmyes for detection and control of pathogenic bacteria**
Martin J. Loessner, Institute of Food Science and Nutrition, ETH, Switzerland

**14.00-14.35** — **The challenge of using phage in food and veterinary diagnostics**
George Botsaris, Cyprus

### Session 2: *Listeria*: new perspectives on an old pathogen

**Chair:** — **Christine Dodd**

**14.35-15.10** — ***Listeria* and listeriosis: emergence and re-emergence**
Jim McLauchlin, Health Protection Agency, UK

**15.10-15.45** — **The challenge of quantifying *Listeria* from foods and environmental samples**
Martin Wagner, Austria

**15.45-16.00** — **Coffee and tea**

**16.00-16.35** — **Molecular sleuthing and listeriosis**
Kathie Grant, Health Protection Agency, Centre for Infections, UK

**16.35-17.10** — **Ecology of *Listeria* in the environment and in animals**
Kendra Nightingale, Colorado State University, USA

**17.10-18.10** — **Student session**

**17.15-19.30** — **Trade show**

## Wednesday 7 July

### Session 2: *Listeria*: New perspectives on an old pathogen (continued)

**09.00-09.35** — **What does not destroy *Listeria*, makes it strong—adapting to stress *in vitro* and *in vivo***
Colin Hill, University College Cork, Ireland

**09.35-10.10** — **Renaissance Bacteria — *Listeria* persistence in the food environment**
Cath Rees, University of Nottingham, UK

**10.10-10.40** — **Coffee and Tea**

**10.40-12.00** — **Attended poster viewing**

**12.00-13.00** — **Lunch**

### Session 3: Buzzwords in Biofilms

**Chair:** — **Jo Verran**

**13.00-13.35** — **Gene transfer in oral biofilms**
Adam Roberts, Eastman Dental Institute, UK

**13.35-14.10** — **Biofilm development and dispersion**
Karen Sauer, Binghamton University, USA

**14.15-14.45** — **Coffee and tea**

**14.45-15.45** — **Student presentations**

**Chair:** — **Geoff Hanlon**

**15.45-16.15** — **W.H. Pierce Prize Lecture**

**16.15-16.45** — **AGM**

**19.00** — **Drinks reception and dinner**

## Thursday 8 July

### Session 3: Buzzwords in Biofilms (continued)

**Chair:** — **Geoff Hanlon**

**09.00-09.05** — **Introduction to the New Lecturer Research Grant**

**09.05-09.40** — **Inter-species communication between biofilm bacteria**
Alex Rickard, (recipient of a *Sf*AM New Lecturers Grant), Binghamton University, USA

**09.40-10.15** — **Evolution**
Daniel Rozen, Manchester University, UK

**10.15-10.45** — **Coffee and tea**

**10.45-11.20** — **Subpopulation interactions during *Pseudomonas aeruginosa* biofilm formation**
Tim Tolker-Nielsen, University of Copenhagen, Denmark

**11.20-11.55** — **Biofilm development as a neoplastic-like mode of microbial growth**
Jeremy Webb, Southampton University, UK

**12.00-13.00** — **Lunch and 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

## SfAM SUMMER CONFERENCE 5 — 8 July 2010

**CLOSING DATE FOR REGISTRATIONS: Monday 21 June 2010. EARLY BIRD DISCOUNT of £50.00** is applied to all bookings made before **7 June.**

**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.

| FEES BEFORE 7 JUNE 2010 | Full Member | Student, Honorary, Associate & Retired Member | Student Non -Member | Non - Member |
|---|---|---|---|---|
| Full Conference Rate inc. accommodation | £250.00 ☐ | £200.00 ☐ | £400.00 ☐ | £600.00 ☐ |
| Conference Rate exc. accommodation | £100.00 ☐ | £50.00 ☐ | £100.00 ☐ | £200.00 ☐ |
| Conference Day Rate: | £50.00 ☐ | £25.00 ☐ | £50.00 ☐ | £100.00 ☐ |
| **FEES BETWEEN 8 JUNE and 21 JUNE 2010** | **Full Member** | **Student, Honorary, Associate & Retired Member** | **Student Non -Member** | **Non - Member** |
| Full Conference Rate: (inc accommodation) | £300.00 ☐ | £250.00 ☐ | £450.00 ☐ | £650.00 ☐ |
| Conference Rate: (no accommodation) | £150.00 ☐ | £100.00 ☐ | £150.00 ☐ | £250.00 ☐ |
| Conference Day Rate: | £100.00 ☐ | £75.00 ☐ | £100.00 ☐ | £150.00 ☐ |

Conference Day Rate delegates please tick the day you wish to attend: Mon 5th ☐ Tue 6th ☐ Wed 7th ☐ Thur 8th ☐

**CONFERENCE DINNER:** full conference fees include dinner in the hotel every night; there is a small supplement of £20 payable to attend the conference dinner on Wednesday evening. If you wish to attend this please tick the box: £20.00 ☐

**SCIENCE MEDIA WORKSHOP:** please tick this box if you would like to attend the Science Media Workshop taking place on Monday 5th July 11am - 5pm ☐

***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!

## *ADD MEMBERSHIP TO YOUR BOOKING

**Add Student membership (£25.00):** ☐ **Add Full membership (£50.00):** ☐

## YOUR DETAILS

**Title:**_____ **First Name:**_____ **Family Name:**_____

**Address:**_____

**Postcode:**_____ **Tel No:**_____ **Email:**_____

**Special dietary or other requirements:**_____

## YOUR NAME BADGE

Please enter the information below in **BLOCK CAPITALS** as you would like it to appear on your name badge

**First Name:**_____ **Family name:**_____

**Organisation/Affiliation:**_____

## YOUR PAYMENT

● **For all participants: The Society DOES NOT INVOICE for conference fees. Please treat your completed booking form as an invoice. Cheques must be in £ STERLING ONLY and made payable to 'The Society for Applied Microbiology'. Foreign cheques/ drafts MUST be negotiable for the full amount due.** We accept payment **ONLY** by the following credit and debit cards: VISA, Mastercard, Eurocard, Delta, Electron, JCB, Maestro and Solo.

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

**TOTAL Amount enclosed/ to be charged: £**_____

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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**

# SfAM events in 2011 — save the dates!

## 12 January 2011

### Winter Meeting

- **Probiotics**
- **Anaerobic microbiology**

■ Including the Denver Russell Memorial Lecture

**The Royal Society, London, UK**

## April 2011 (exact date to be confirmed)

### Spring Meeting

**5th broadening microbiology horizons in biomedical science meeting**

■ Including the Procter and Gamble Applied Healthcare Microbiology Award Lecture

**The Stratford Q Hotel, Stratford upon Avon, UK**

## 4 - 7 July 2011

### Summer Conference

**Food microbiology**

■ Including the Lewis B Perry Memorial Lecture, followed by drinks, buffet and tour of the Guinness Storehouse

■ Conference dinner with Irish entertainment and tutored whisky tasting session at the Jameson Distillery

**Clontarf Castle, Dublin, Ireland**

**For further information on these events please visit sfam.org.uk or contact Sally Cryer**
**■ Email: sally@sfam.org.uk ■ Telephone +44 (0)1234 761752**

> ...recent genome-wide linkage analysis studies have reported a stronger association between schizophrenia and detection of *T. gondii* antibodies than for any human gene.

# *Toxoplasma gondii* and schizophrenia

Recognition of potential infectious causation across a broad range of not only acute, but also chronic diseases is taking place. One such proposed association, revealed through epidemiological and neuropathological studies, is that some cases of schizophrenia may be associated with exposure to the protozoan *Toxoplasma gondii*. Reasons for this include *T. gondii's* ability to establish persistent infection within the central nervous system, its ability to manipulate intermediate host behaviour, the occurrence of neurological and psychiatric symptoms in some infected individuals and an association between infection with increased incidence of schizophrenia. Several of the medications used to treat schizophrenia and other psychiatric diseases have also been demonstrated, both *in vitro* and *in vivo*, to possess anti-parasitic, and in particular anti-*T. gondii* properties. Furthermore, new findings, including those revealed from within the parasite's genome, may help to elucidate the potential mechanisms involved in enabling such behavioural modifications. New developments should prompt a fresh wave of research of both fundamental and applied importance.

## Parasites changing host behaviour

As specified by the 'manipulation hypothesis', certain parasites can alter host behaviour for their own selective benefit. Classic examples involve transmission through the food chain, where a parasite is immature in an intermediate host, which must be eaten by a predatory definitive host before the parasite can reach maturity and complete its life cycle. The parasite thus manipulates the behaviour of its intermediate host so as to enhance its transmission to the definitive host. Perhaps the most convincing, and yet the most rare, are cases of neurotropic

parasites, and hence parasites that manipulate host behaviour by acting upon the central nervous system (CNS). For instance, it appears that the rabies virus appears able, at least in some instances, to alter the function of pathways related to aggression in mammals, the consequence of which is increased biting and thereby facilitation of rabies virion transmission shed in the saliva. Another potential example is hypersexuality in some cases of neurosyphilis, which may, in theory, enhance further transmission. Clinically apparent CNS involvement can span the entire course of syphilis infection (where another manifestation of neurosyphilis is psychosis, which can be indistinguishable from psychotic features of schizophrenia with acute or insidious onset). Perhaps one of the most convincing examples of a manipulatory parasite with a predilection for the CNS of its host, is the protozoan *T. gondii*. Approximately 30% of the human population across the world are *T. gondii* seropositive, with ranges of, for example, between 22% in the UK to 84% in France. A consideration of the life-cycle of this parasite may explain why it may be predicted to alter host behaviour. Like many indirectly-transmitted parasites, *T. gondii* requires transmission from an intermediate host reservoir to predatory definitive host in order to complete its life-cycle. Members of the cat family (*Felidae*) are the only definitive hosts within which the parasite undergoes full gametogenesis and mating within the intestinal epithelium, culminating in the generation of oocysts that are shed in the cat's faeces. If oocysts are ingested by an intermediate host, such as a wild rodent, or another secondary host, such as a human or domestic animal, the parasite undergoes asexual reproduction, characterized by rapidly dividing tachyzoites and the more slowly dividing bradyzoites, the latter of which can encyst in the brain, heart, and other tissues. The bradyzoites can remain, potentially for the host's lifetime. Transmission back to the feline definitive host occurs when an immunologically naïve cat ingests bradyzoite-infected tissue through predation and/or consumption of contaminated meat. Since sexual reproduction of *T. gondii* can be accomplished only in felines, there are likely to be strong selective pressures on the parasite to evolve mechanisms to enhance transmission from the intermediate host to the definitive feline host and thereby complete its life cycle. The predilection of *T. gondii* for the brain of its intermediate host places it in a privileged position to enable such manipulation.

A convincing body of evidence now exists to indicate that *T. gondii* can achieve such manipulation. Studies on rats and mice have demonstrated, for example, that *T. gondii* causes an increase in activity and a decrease in neophobic (the innate fear of novelty) and predator vigilance behavioural traits, each of which may be proposed to facilitate transmission of the parasite from the infected intermediate host to the feline definitive host. Moreover, whilst uninfected rats show a strong innate aversion to feline predator odour, *T. gondii* appears to subtly and specifically alter rats' cognitive perception of cat predation risk, turning their innate aversion into a 'suicidal' fatal feline attraction.

Humans can also be secondary hosts for *T. gondii* and it is clearly established that congenital infections, especially early in pregnancy, can produce severe morbidity or mortality. Some cases of acute adult-acquired toxoplasmosis can also result in morbidity, including headache, myalgia, lymphadenopathy and occasionally seizures. Latent toxoplasmosis in immunocompetent humans (and animals) has been, in contrast, generally considered to be asymptomatic. However, subtle alterations in behavioural traits amongst latently infected humans have also been observed, similar to those characterized in *T. gondii*-infected rodents—such as increased activity, decreased reaction times and altered personality profiles. Furthermore, in a small number of cases, latent *T. gondii* infections in humans may have substantial implications for human health. For example, individuals with latent toxoplasmosis have been reported to be at increased risk of being involved in car accidents, and also of attempting suicide, relative to that of their matched uninfected counterparts.

## An association between Toxoplasma and schizophrenia

Whilst any association between *T. gondii* infection and the development of schizophrenia is likely to occur only in a very small proportion of infected humans, and is applicable only to some cases of schizophrenia, there is a gathering body of convincing evidence that link the two. For instance, both schizophrenia and toxoplasmosis have been demonstrated to have strong familial associations, affecting multiple members of the same family, and recent genome-wide linkage analysis studies have reported a stronger association between schizophrenia and detection of *T. gondii* antibodies than for any human gene. To provide a few epidemiological examples from across the world, for instance, analyses of serum samples obtained from mothers shortly before or after giving birth revealed a significantly raised proportion of antibodies to *T. gondii* in those whose children subsequently develop schizophrenia in later life, and individuals suffering from first-episode schizophrenia have been shown to have significantly elevated *T. gondii* antibodies, within both serum and cerebral spinal fluid (CSF), compared to uninfected control subjects. Likewise, a recent study of military personnel revealed significantly increased levels of *T. gondii* antibodies prior to the first onset of their schizophrenia. Studies have also demonstrated that *T. gondii* antibodies in patients with schizophrenia treated with antipsychotic drugs are intermediate between those of patients never treated and those of control groups, with a significant reduction in those patients undergoing current drug treatment, thereby suggestive that antipsychotic treatment may affect *T. gondii* infection levels. Indeed, antipsychotic drugs used in the treatment of schizophrenia have been observed to inhibit the replication of *T. gondii* tachyzoites in cell culture. Moreover, studies have demonstrated that *T. gondii*-exposed rats treated with antipsychotic or mood stabiliser drugs do not develop the suicidal feline attraction and altered behavioural profile displayed by their untreated but infected counterparts, nor was there the same level of parasite establishment within the brains of these drug-treated infected rats relative to their untreated infected counterparts. Such results, therefore, raise the hypothesis that the antipsychotic and mood stabilizing activity of some medications may be at least augmented through their inhibition of *T. gondii* replication, invasion and/or

subsequent modulatory impact in infected individuals.

## Potential mechanisms of behavioural manipulation

How *T. gondii* actually causes such manipulation of its host remains, however, a black box. Indeed, for so many manipulatory parasites, the current pressing need is to indeed elucidate the precise mechanisms involved. Nevertheless, studies performed to date do provide us with a few potential clues as *T. gondii'* mechanism of action. For instance, we do know that tissue cysts containing bradyzoite stage parasites predominate in neural cells. Gross pathology alone, however, is unlikely to account for the observed behavioural changes in the majority of cases, since other important behavioural characteristics, in infected rodents at least, such as social status and mating success, remain unaltered in *T. gondii*-infected individuals. One could, therefore, perhaps suspect that some form of differential localization of *T. gondii* within specific brain areas may account for the behavioural alterations observed. Indeed, whilst studies have generally found *T. gondii* to be distributed widely across the brains of infected individuals, there are several reports of predominant cyst locations in certain brain regions—including regions such as the amygdala and nucleus accumbens, which are areas thought to play an important role in traits such as reward, pleasure and/or fear. Furthermore, both these brain regions have also been proposed to be involved in the control of psychomotor behaviour and/or implicated in the aetiology of schizophrenia.

The subsequent impact of *T. gondii* presence on the neuromodulatory pathways may also be suspected to play a role in altering host behaviour. For instance, there is evidence that different forms of cerebral 'insult', as may perhaps be suspected to occur in areas of high cyst presence, to regions such as the nucleus accumbens can produce a hyperdopaminergic state. Altered dopamine levels in rodents have been associated with changes in activity and exploratory behaviour similar to those observed following *T. gondii* infection. Likewise altered dopamine levels have been reported as a consequence of both *T. gondii* infection and schizophrenia, and the aforementioned anti-psychotic drugs for use in schizophrenia which were shown to prevent parasite-altered behaviour in rodents, primarily haloperidol, are dopamine D2 antagonists.

Yet, how the parasite alters dopamine is still in question. One could perhaps propose that, directly or indirectly, *T. gondii* presence in the brain may alter the production or degradation of host dopamine levels. Alternatively, one could perhaps even speculate that *T. gondii* could produce its own dopamine levels and thereby modulate host behaviour. A recent paper by Gaskell *et al.*, (2009) revealed that, through screening of the parasite's genome, *T. gondii* was found to encode a copy of the mammalian enzyme tyrosinephenylalanine hydroxylase. This enzyme, never previously described in protozoa, represents the rate-limiting step in dopamine synthesis, through synthesis of the L-dopa precursor to dopamine. Moreover, *T. gondii* was found to possess two nearly identical genes with similarity to metazoan tyrosine hydroxylase, which exhibit very similar kinetic properties yet differ in their developmental regulation of expression. One copy of the gene is constitutively expressed across the parasite lifecycle and one copy is upregulated during the brain and muscle cyst forming stage and the enzyme is secreted into the host cells. The differential expression and two catalytic activities may allow this enzyme to fulfil multiple biological functions through the parasite life cycle. This appears to be the first description of an aromatic amino acid hydroxylase in an apicomplexan parasite. Furthermore, this gene was not found in any other apicomplexan except the closely related *Neospora caninum* in bioinformatics searches of available genome sequences.

## What next?

Such associative studies raise several important theoretical and applied implications. For example, they provide further support for the theory of *T. gondii* as a causative agent in some cases of schizophrenia. They could also predict that certain drugs may be expected to be particularly effective in individuals with schizophrenia who are also infected with *T. gondii* and/or may provide prophylactic treatments for certain groups at serious risk of developing psychiatric disorders in later life as a result of *T. gondii* infection. However, we still need to fully elucidate the parasite's potential mechanism of action. Moreover, we also need to understand why and how any potential effects of *T. gondii* on host behaviour, in particular in terms of the clinical outcome of human behaviour, may differ between individuals. The consequences of such interdisciplinary research may well also provide further understanding of the neurobiology associated with behaviour in general.

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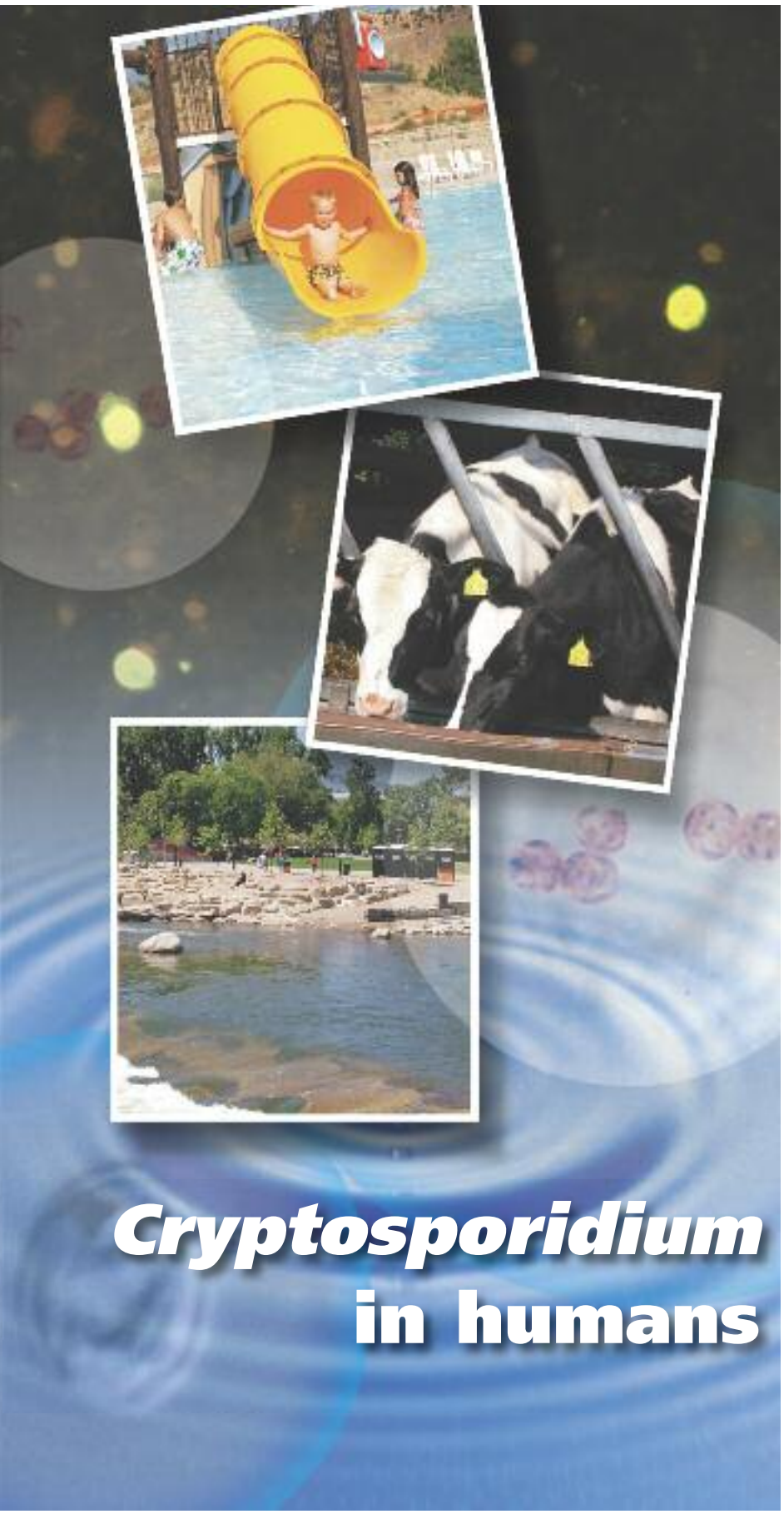


# Cryptosporidium in humans

The protozoan parasite *Cryptosporidium* has recently celebrated its centenary and is today regarded as a pathogen of veterinary and public health importance as well as causing potentially serious infections in immunocompromised patients. Its clinical and economic importance is recognized internationally, and in 2004 it was included in the World Health Organization Neglected Diseases Initiative (Savioli *et al.*, 2006). Such diseases "*exhibit a considerable and increasing global burden, and impair the ability of those infected to achieve their full potential, both developmentally and socio-economically*".

## Historical perspective

Back in 1907 the American parasitologist Ernest Edward Tyzzer observed and published detailed descriptions of asexual and sexual stages in the gastric glands of common mice and noted the presence of 'spores' in their faeces (Tyzzer, 1907). Although uncertain of the taxonomic status, he named the organism *Cryptosporidium muris*. Further observations in mice, rabbits and chickens and the naming of a second species, *Cryptosporidium parvum*, found in the small intestine of mice, followed. Tyzzer's remarkable reports contain exquisite descriptions of the parasite and remain inspiring reading today. Some ultra-structural details have been amended or added since, thanks to the fine detail afforded by transmission electron microscopy, but Tyzzer captured the elements of the *Cryptosporidium* life cycle. Despite having many features of the coccidia, some elements such as epi-cellular development, auto-infection, endogenous sporulation and lack of sporocysts set *Cryptosporidium* apart. In his 1907 paper, Tyzzer reported that in "*possession of an organ of attachment and of iodophilic granules, it resembles the gregarines"* and in *"morphology, lack of motion in the adult and in sexual dimorphism it resembles the coccidia*". Uncertainty regarding the taxonomic position of the genus within the Apicomplexa remains to this day as it possesses some structural features of both the gregarines and coccidia, and yet is typical of neither. However, with the current advantage of phylogenetic analyses, a distantly related lineage has been suggested (Barta & Thompson, 2006).

Today, 19 named species with supporting genetic data and over 40 genotypes have been identified (Fayer, 2010). The term genotype is widely used for isolates with significant genomic differences, but has no taxonomic status and is a temporary descriptor. Although often named for the host in which originally found, host specificity cannot always be inferred. This is illustrated by the finding of the skunk genotype in humans in the UK, one of which was an

asymptomatic child (Robinson *et al.*, 2008; Davies *et al.*, 2009), and the occurrence of a waterborne outbreak among residents of Northamptonshire, England caused by the rabbit genotype (Chalmers *et al.*, 2009a).

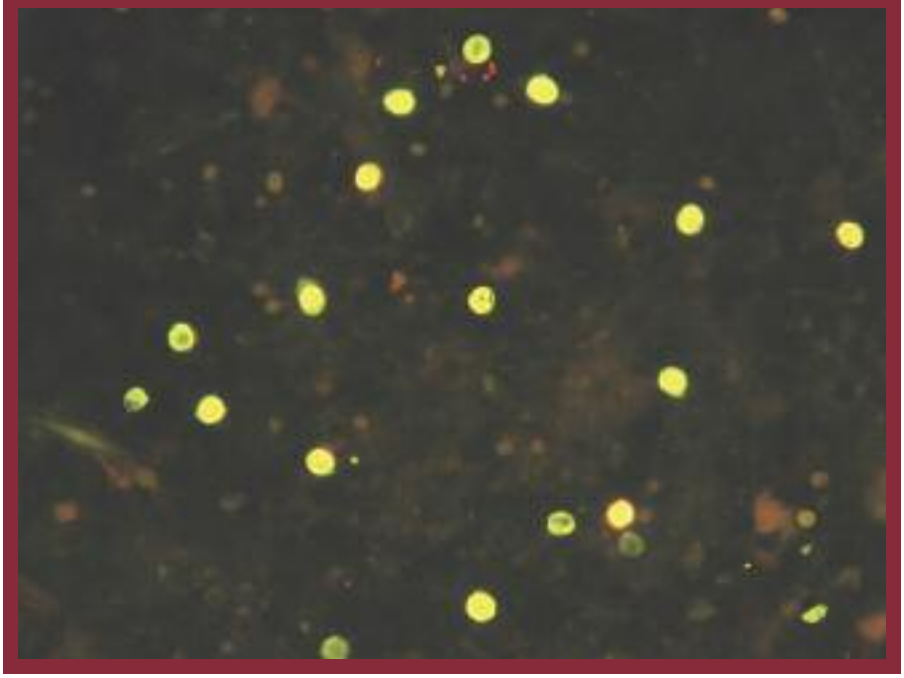
Largely because there had been no apparent linkage to pathology, there were few further studies of *Cryptosporidium* until the second half of the twentieth century. Even then, despite reports of pathological effects and death in turkeys caused by *Cryptosporidium meleagridis* and association of *C. parvum* with diarrhoea in calves, it wasn't until the 1970s that interest in the parasite increased. In 1976, reports of two human cases of illness were published. Although each of the two cases presented with severe watery diarrhoea, lived on cattle farms and had a dog, they were very different in their clinical histories. One was a three year old boy with no reported immunological defects (Nime *et al.*, 1976), the other an immunosuppressed adult (Meisel *et al.*, 1976). Two further cases diagnosed in the 1970s were also immuno-compromised. However, patient testing was not widespread and diagnosis involved lengthy histological examination of intestinal biopsy material. Two reports in the Centers for Disease Control and Prevention's Morbidity and Mortality Weekly Report in 1982 highlighted key issues, changing the perception of cryptosporidiosis. The first was of an outbreak among calf handlers at a university farm, which confirmed zoonotic transmission to apparently healthy adult individuals, for whom disease was apparently self-limiting (Anon, 1982a). At the time, cryptosporidiosis was an AIDS defining illness. The second report described the difficulties in managing such patients for whom underlying immune deficiency was not correctable, and the severe problems for patient management in the absence of effective anti-parasitic drug treatment (Anon, 1982b). Thus, although the AIDS epidemic demonstrated the potentially severe problems with cryptosporidiosis, understanding that *Cryptosporidium* was not solely an opportunistic infection was vital in establishing more widespread testing of the general population. A waterborne outbreak in Milwaukee in 1993 highlighted the need for a multi-agency and multi-disciplinary approach to addressing the problems presented by *Cryptosporidium*. These are largely due to its ability to evade conventional water treatment and chlorine disinfection and the lack of effective drug therapy.

## Current perspective

Today, widespread testing is common in diagnostic laboratories across the UK with 73% including a test for *Cryptosporidium* in routine diagnosis of gastrointestinal illness from community cases of diarrhoeal illness (Cryptosporidium Reference Unit (CRU), unpublished data). This is encouraging as even a test request for "ova, cysts and parasites" (OCP) would not include a *Cryptosporidium* assay; the oocysts are too small to be observed in OCP examination. Most laboratories use the Health Protection Agency's standard methods of auramine-phenol staining (76%) or modified Ziehl-Neelsen staining (22%) of stool smears for microscopical examination (Figures 1 and 2) (http://www.hpa-standard methods.org.uk/documents/bsopTP/pdf/bsoptp39.pdf), although there is an emerging trend towards adoption of commercially-available Enzyme Immuno Assays (EIAs) which facilitate high throughput testing. The sensitivity and specificity of the current generation of EIAs is comparable to standard diagnostic methods (CRU, unpublished data). EIAs are simple to perform, can be automated and are readily incorporated into laboratory workflows. The higher consumables costs can be offset by re-directing the work of skilled laboratory staff from time consuming microscopy. Furthermore, combination tests with other parasites such as *Giardia* are available. Good ascertainment of cryptosporidiosis from laboratory testing to surveillance and reporting is essential in understanding the risk factors for disease, identifying outbreaks and implementation of control measures.

All ages can be affected by cryptosporidiosis but in the UK most disease is in children under five years old and is most common in two year olds. Symptoms in immunocompetent cases are self-limiting, lasting one to two weeks, although sometimes longer, and relapse can occur during this period. In those seeking medical assistance, the main symptom is diarrhoea, often with abdominal pain, nausea and/or vomiting (particularly in children), mild fever and loss of appetite. Hospitalization rates are about 10% to 15%. In developing countries, children often acquire cryptosporidiosis in the first 24 months of life. There is substantial morbidity, including poor growth, especially where children are malnourished, even following apparently asymptomatic infection (Checkley *et al.*, 1997). Long-

***Figure 1.*** *C. parvum-sized auramine phenol staining with x50 objective*



term health effects of childhood infections are of concern and further study of these is required. Sero-negative reactive arthritis has been reported in adults and children, it has been suggested that infection may cause relapse of inflammatory bowel disease, and there are anecdotal reports of a link with irritable bowel syndrome (Davies & Chalmers, 2009).

Typing isolates to the species level is not undertaken in routine diagnostic laboratories but is carried out using molecular techniques on reference samples. At the UK CRU, using polymerase chain reaction–restriction fragment length polymorphism analysis (PCR-RFLP) of the *Cryptosporidium* oocyst wall protein and small subunit ribosomal RNA genes, we have shown that the most common infections, accounting for 96% cases, are with *Cryptosporidium parvum* and *Cryptosporidium hominis* (Chalmers *et al.*, 2009b). This knowledge has enabled us to develop a real-time Taqman PCR assay to type isolates for epidemiological purposes, incorporating pan-genus, *C. hominis* and *C. parvum* primer-probe sets. This assay replaced the conventional PCR-RFLP for clinical samples in April 2010. Infection with *C. hominis* may lead to more severe acute illness than *C. parvum*, and *C. hominis* has also been associated with joint pain, eye pain, headache and fatigue in the two months following infection (Hunter *et al.*, 2004a).

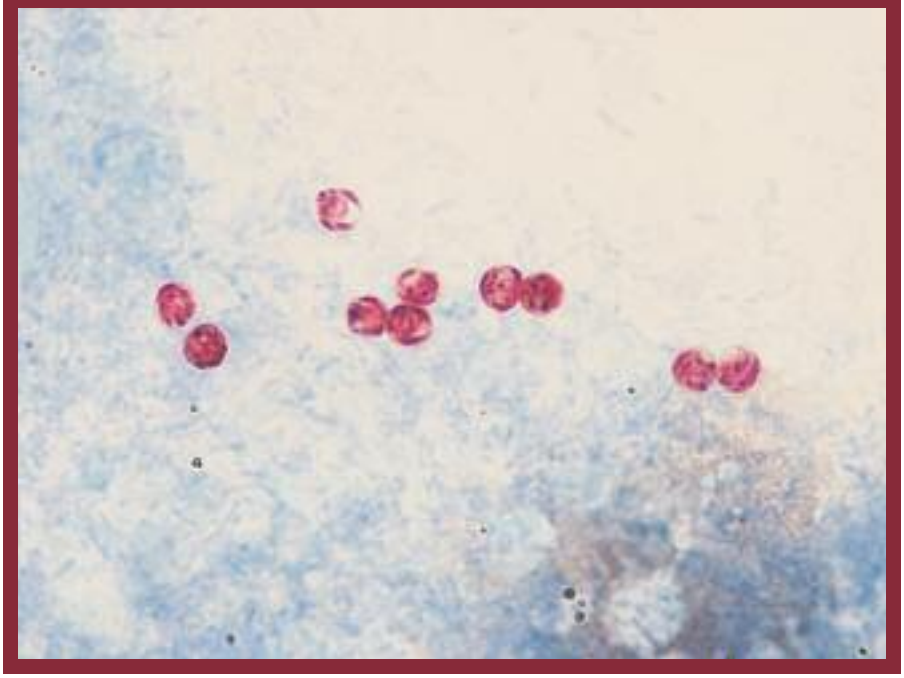
In the UK, *C. parvum* generally peaks in the spring and *C. hominis* in the late summer and autumn (Chalmers *et al.*, 2009b). Discrimination between these species can help reduce the noise in the surveillance data and aid epidemiological investigations. There has been a change in the epidemiology of cryptosporidiosis in the past ten years. The spring peak is much reduced, following improvements in catchment protection and drinking water treatment, especially filtration (Sopwith *et al.*, 2005). UV disinfection has also extended the options available for *Cryptosporidium* control.

Risk factors for acquisition of *C. parvum* and *C. hominis* differ; infections in patients reporting recent foreign travel, infants under one year, and females aged 15 to 44 years are more likely to be *C. hominis*, which, in a case control study was significantly associated with travel abroad, changing children's nappies, and contact with someone with diarrhoea (Chalmers *et al.*, 2009b; Hunter *et al.*, 2004b). *C. parvum* infections were associated with farm animal contact (Hunter *et al.*, 2004b) or visiting a farm (Goh *et al.*, 2004). Socio-economic factors were explored by Lake *et al.*, (2007) who found that *C. hominis* was associated with living in more densely populated areas. Environmental factors investigated in Scotland found not only the same geographical propensity for *C. hominis*, but also that *C. parvum* was more common in areas with lower human population densities, with a higher ratio of the number of farms to human inhabitants and with a higher ratio of the number of private water supplies to human inhabitants (Pollock *et al.*, 2009). Thus the epidemiological evidence is for anthroponotic acquisition of *C. hominis* and zoonotic acquisition of *C. parvum*. Despite these findings we are still unclear about the precise drivers for the increase in cases in the second part of the year, although foreign travel and swimming pool use are probably involved. Certainly, outbreaks related to swimming pools are more common at this time of year and currently present a major public health challenge in the UK. The lack of residual disinfection against *Cryptosporidium* in swimming pool water, and sometimes unsatisfactory pool water circulation and filtration contribute to this.

Typing is also useful in environmental investigations to assist in understanding sources of contamination and risks to public health, and has been useful in outbreak investigations (Chalmers *et al.*, 2009a; Chalmers *et al.*, 2010). Segregation of isolates into probable human, wildlife and farmed animal sources is largely possible, although improved understanding of host range is required. There is currently no recognised subtyping scheme for *C. parvum* or *C. hominis*. Multi-locus typing, investigating distribution of short tandem repeats, has identified human-restricted transmission of some *C. parvum* isolates and differences in the global phylogeny of *C. hominis*. In the UK, *C. hominis* appears to be much more homogenous than *C. parvum* but this is not reflected globally. Mining the published genomes for *C. parvum*, *C. hominis* and the non-pathogenic *C. muris* may provide identification of further markers for investigation but any markers, or combinations of markers, need to be validated prior to use for epidemiological purposes. Next generation sequencing capabilities may facilitate this.

Other species are also found in human infections, but in the UK these are very rare. Interestingly, in a study of asymptomatic carriage of *Cryptosporidium* in young children in

**Figure 2.** *C . parvum-sized mZN staining with x100 objective*



day care nurseries in the UK, we found that of the three out of 230 (1.3%, upper 95% CI 3.8%) children carrying the parasite only one was *C. hominis* and the others the cervine and skunk genotypes (Davies *et al.*, 2009). This proportion of unusual infections in asymptomatic carriers was far higher than seen in patients with gastroenteritis and indicated that unusual infections may be more common than previously thought and may have lower pathogenicity.

To return to the global importance of *Cryptosporidium* and one of the reasons why it was included in the WHO Neglected Diseases Initiative, treatment options are limited. In the US, nitazoxanide is licensed for treatment in the immunocompetent and there is some evidence for efficacy in immunocompromised patients, although not in the subgroup with most advanced HIV disease. Other modalities for immunocompromised patients are of unproven benefit and immune reconstitution remains the best hope for clearing the parasite. Those immunocompromised patients most at risk from chronic or intractable disease are those with T-cell immunodeficiency, specifically children with haematological malignancy, HIV infection and CD4 counts <200 and especially <50, and primary T-cell deficiencies such as CD40 ligand deficiency (hyper IgM syndrome) or severe combined immunodeficiency (Hunter & Nichols, 2002). Any part of the gastrointestinal tract can become infected, and complications can include pancreato-biliary infection leading to pancreatitis or sclerosing cholangitis and, rarely, biliary cirrhosis. Risks to bone marrow transplant patients seem to depend on the underlying diagnosis for which the transplant was performed.

Although the threat once posed by *Cryptosporidium* to HIV AIDS patients has diminished in developed countries where highly active antiretroviral treatment (HAART) is routinely prescribed, elsewhere the tragedy is that even if HAART is available, counterfeit medication and inconsistencies in administration mean vast numbers of people do not receive the benefits, and cryptosporidiosis is still a problem in this setting. Prevention and risk reduction remain the most important interventions in cryptosporidiosis, but even in areas where drinking water quality has been improved there may remain a background risk and patients who have compromised T-cell function may be advised to boil their drinking water. However, for those at most risk, including young children in much of the world, avoidance interventions are difficult and expensive to implement. The WHO initiative for neglected diseases that occur "*in developing countries where climate, poverty and lack of access to services influence outcomes*" readily applies to *Cryptosporidium*. The hope is that improvement in source water protection, drinking water treatment and drug therapies might be transferred to those who need it most wherever they happen to live.

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# The future for detection and control of *Trichinella* in Europe

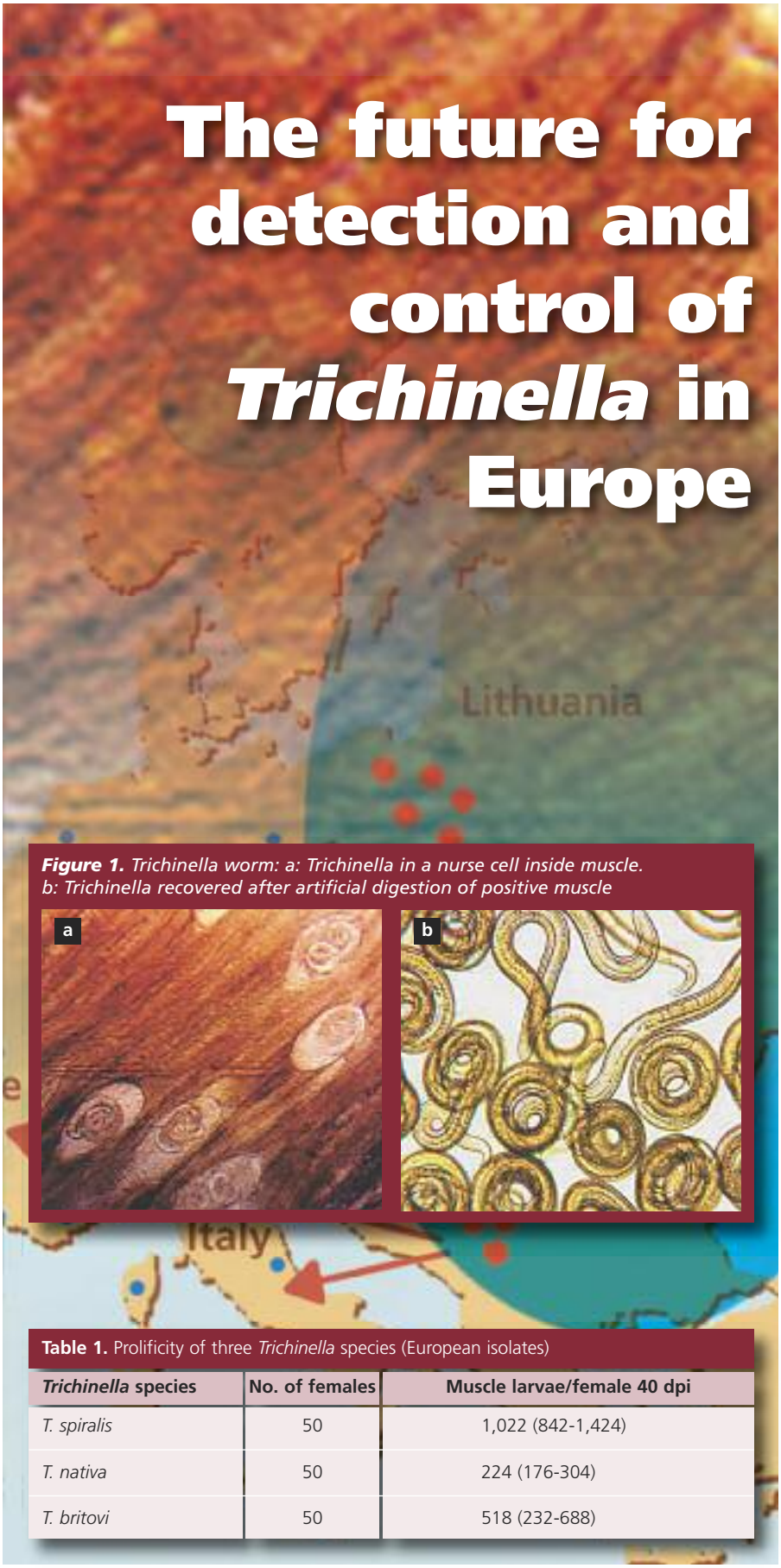



***Figure 1.*** *Trichinella worm: a: Trichinella in a nurse cell inside muscle. b: Trichinella recovered after artificial digestion of positive muscle*




**Table 1.** Prolificity of three *Trichinella* species (European isolates)

| *Trichinella* species | No. of females | Muscle larvae/female 40 dpi |
|---|---|---|
| *T. spiralis* | 50 | 1,022 (842-1,424) |
| *T. nativa* | 50 | 224 (176-304) |
| *T. britovi* | 50 | 518 (232-688) |

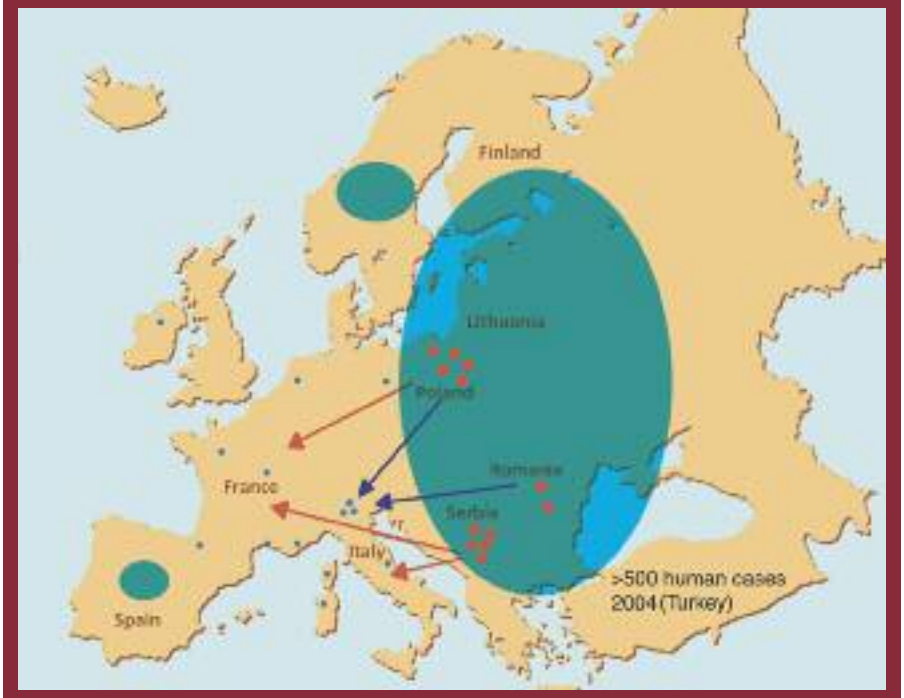
One of the most widespread parasites infecting both animals and humans in all climates is *Trichinella*. Human trichinellosis in the EU is characterized by a low frequency of infection, even though Romania has the highest prevalence of the disease in the world (Blaga *et al.*, 2009). With an incidence of 51.0 cases of trichinellosis per 106 persons per year over a period of 25 years, *Trichinella* and trichinellosis are a major concern in Romania (Blaga *et al.*, 2007). Four species (*T. spiralis*, *T. nativa*, *T. britovi* and *T. pseudospiralis*) have been involved in the domestic and/or sylvatic cycle in Europe (Pozio *et al.*, 2009). In Western Europe, horse meat, wild boar meat and pork were identified as the major source of infection for humans; whereas in Eastern European countries, pork was identified as the main source of infection (Pozio *et al.*, 2006, Djordjevic *et al.*, 2003). More than 2000 trichinellosis cases traced back to outdoor pigs, were notified from EU Member States, namely Spain, France, Italy and Germany, in the last three decades.

In spite of the low number of domestic animals bred in EU countries identified with *Trichinella* infection, direct and indirect costs to detect this infection at the slaughterhouse make trichinellosis one of the most costly of all parasitic zoonoses in the EU (several hundred million Euro/year). Furthermore, the endemic nature of infection in Eastern European countries, both in production animals and wildlife, represents a high risk for humans. The official methods (mainly artificial digestion of meat, according to the World Organization for Animal Health [OIE] methodology), based on the visual detection of the parasite, has prompted the improvement of the control method by the evaluation of quality control strategies (Vallée *et al.*, 2007).

## Biology and strain virulence of *Trichinella*

The life cycle of *Trichinella* enables the parasite to develop through all stages in the same host. Muscle larvae (ML1) (Figure 1a/b) are the infective stage, which are ingested by the host. ML1 are released from the muscle tissue—and their capsule—in the stomach by digestion. The larvae then reach the duodenum and jejunum where they penetrate into the intestinal

***Figure 2.*** *Trichinella spreading in Europe*



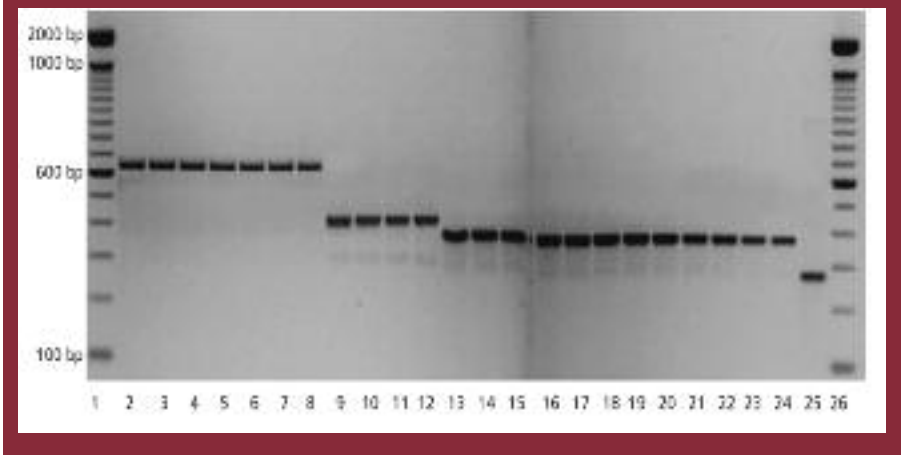

*The highest endemic area (blue spheres) in Europe can be defined by:*

- *presence in the wild carnivorous and omnivorous animals >1% (Romania, Serbia, Slovakia, Lithuania)*
- *emergence in outdoor pigs (Finland, France (Corsica), Italy (Sardinia)*
- *occurrence of positive horses as an unusual sentinel mammal (Serbia, Poland, Romania). Arrows indicate the origin of a positive horse that may have induced human cases in France/Italy or that was blocked before consumption between 1998 and 2008.*

*Spots represent specific isolates*

***Figure 3.*** *Trichinella typing:*

*Electrophoresis of the mitochondrial multiplex PCR products of 24 Trichinella isolates. Line 1 and 26 100 bp Ladder (Invitrogen); line 2-8 T. spiralis isolates (ISS 378, 004, 51, 104, 534, 596, 889, 1100); line 9-12 T. nativa isolates (ISS 10, 42, 70, 1611); line 13 -24 T. britovi (ISS 100, 137, 235, 1497, 1572, 1573, 1574, 1209, 1325, 1327, 1330, 1617); line 25 T. pseudospiralis (ISS 13) Palearctic population (Blaga et al., 2009)*




epithelium. Within 48 hours, they undergo four moults (L2, L3 and L4 stages) and finally develop into the adult. Males and females copulate within the intestinal epithelium, and six to seven days post-infection (dpi), females begin to release newborn larvae (NBL1). These NBL1 will be produced within one to two weeks depending upon the host species and the efficiency of its immune response. NBL1 move from the intestinal mucosa to muscle tissue through blood and lymphatic vessels. They reach only the striated muscle and penetrate actively into the muscle cell that will differentiate to become nurse cells where NBL1 grow and develop as ML1. Depending on the *Trichinella* species, a thick collagen capsule may or may not be produced.

Currently, twelve species/genotypes of *Trichinella* are recognized and most of them cause disease in humans. They are differentiated by genotyping (see Figure 3). Five species are described as encapsulated due to the production of a collagen capsule: *T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli* and *T. nelsoni*. Three species of *Trichinella* do not form a collagen capsule in muscle, namely *T. pseudospiralis*, *T. papuae* and *T. zimbabwensis*. Only *T. pseudospiralis* is a zoonotic agent amongst the non encapsulated group of *Trichinella* (Zarlenga *et al.*, 2006).

The results of immunohistochemical localization of the immunodominant antigens on the three different parasite stages of four European species of *Trichinella* have shown a high heterogeneity between parasite stages and species (Boireau *et al.*, 1997, Liu *et al.*, 2007). This observation suggests that the parasite has developed a highly variable antigenic structure during its life span to escape the host immune response. The evaluation of the virulence and pathogenicity of *Trichinella* species and of their strains is very complex and involves many variables, most of which are related to the host species, its immunological and biological status. During *Trichinella* infection most of the symptoms observed in humans are attributable to the parenteral phase, corresponding to the production of new born larvae, their migration through the host body, their penetration into the muscle cell and the modification of the host cell structure. The established score for the number of muscle larvae produced by the female of

three investigated species (Table 1), is a good parameter, which can represent a starting point to define the virulence of *Trichinella* species.

## Animal *Trichinella* infection and trichinellosis in Europe

During the last 10 years more than 1,000 isolates of *Trichinella* were collected by the Reference Community Laboratory for Parasites (ISS, Rome, E Pozio) from domestic (pigs and cats), synanthropic (cats, dogs and rats) and sylvatic ætiological (red foxes, wild boars, racoon dogs, wolves, martens, ferrets, brown bears and lynxes) animals from European countries. *T. spiralis* represents the main etiological agent (81%) in domestic pigs; however, *T. britovi* (18%) and *T. pseudospiralis* (<1%) can also be transmitted to domestic pigs (Pozio *et al.*, 2009). Differences in species distribution have been observed. For example *T. britovi* has been detected more frequently in wild boars, than in domestic pigs (47% versus 17%). *T. pseudospiralis* has been detected in wild boars, domestic pigs, and a lynx, but never in red foxes. *T. britovi* is the prevalent species in carnivores (92% in foxes, 100% in wolves etc.) (Pozio *et al.*, 2009). This suggests that this species of *Trichinella* is more easily transmitted in the sylvatic environment than *T. spiralis* (in both swine and carnivores) and is more adapted to the Carnivora order than to the order Artiodactyla. The detection of *T. spiralis*-positive foxes in Ireland and the identification of a wild boar infected with *T spiralis* in the South of France, are examples of how the lack of apparent infection among domestic animals and humans—even when for more than three decades—is not enough to assume an area is *Trichinella*-free (Figure 2). These results also confirm that the sylvatic cycle can last for decades, independently of the existence of a domestic cycle. *T. pseudospiralis* is more widespread among wildlife within Europe than was previously believed, consequently there is a real risk of transmission of this *Trichinella* species from animals to humans.

## The pathology of *Trichinella* in animals and human (trichinellosis)

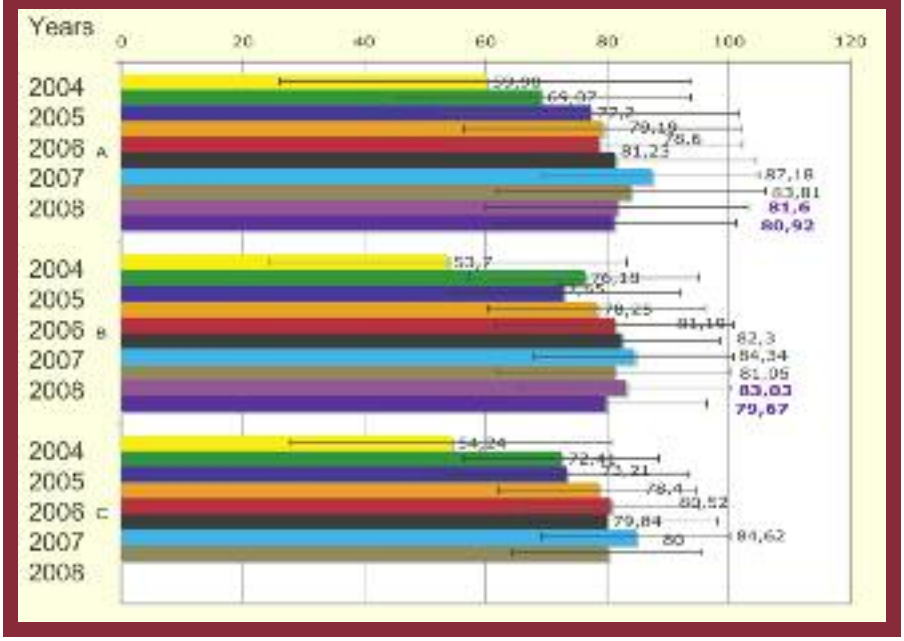
In animals the disease is considered asymptomatic whereas in humans, trichinellosis is a serious disease that can cause much suffering and occasionally may result in death. Human trichinellosis is directly linked to cultural food practices such as the consumption of raw or undercooked meat which has originated from carnivorous or omnivorous mammals, birds and in some cases reptiles. The clinical diagnosis of trichinellosis is difficult because there are no pathognomonic signs. Serological hyper-eosinophilia and increased creatine phosphokinase (CPK) activity are the most frequently observed laboratory features. The detection of circulating antibodies directed against *Trichinella* antigens using immunodiagnostic testing is used to confirm the presence of trichinellosis. Treatment is based on anthelminthics and corticosteroids. The earlier the treatment regime is started, the more effective will be the outcome. These drugs are also used to prevent complications due to eosinophil activation.

## Prevention of *Trichinella* infection.

Prevention of human infection is accomplished through thorough meat inspection, meat processing and prevention of exposure of food animals to infected meat (Gajadhar *et al.*, 2009). Game meats should always be considered as a potential source of infection, and therefore game meats should be tested and cooked thoroughly.

Domestic and wild animals intended for human consumption are subjected to veterinary controls to ensure the meat is *Trichinella*-free. The method for *Trichinella* detection is based on direct identification of the parasite after artificial digestion (Figure 1b) of muscle sample harvested from animal carcasses. The reference method is the magnetic stirrer method for pooled sample digestion (EU Commission regulation EC 2075/2005; International Commission on Trichinellosis (ICT) Recommendations). This method is described as the most reliable with best sensitivity for *Trichinella* detection. Sampling of carcasses (size and predilection of muscle to be tested) depends on the animal species and is described in both EU regulation and the ICT recommendations. Continued quality assurance is an underlying theme of effective diagnostic and control programs for all pathogens. In these

***Figure 4.*** *Sensitivity of Trichinella detection in routine laboratories (national interlaboratory assay of proficiency samples). Since 2004 the National Reference Laboratory has organized five national ring trials with two sessions per year per laboratory. Three types of proficiency samples are sent to each routine laboratory (sample A: 3 to 5 capsules; sample B: 5 to 10 capsules; sample C: more than 12 capsules (this last sample has not been used since 2008)).*
*Up to now this is the longest and widest ring trial in Europe for Trichinella control. The global sensitivity of routine laboratories increased from 52-54% (2004) up to 80% (2008) (I. Vallée, 2007, I.Vallée et al., 2009)*



conditions the quality assurance is based on a gold standard, the specific training for practitioners, accreditation (to follow ISO 17025) and ring trials to harmonize the sensitivity and specificity of the tests used. *Trichinella* control is based on a visual detection method with several readers within the same laboratory confirming the observed result without any control (positive or negative). Therefore it is important to undertake ring trials of routine and reference laboratories at least twice a year. For this purpose, proficiency samples were developed (Figure 4) to improve the consistency of routine diagnosis in France during a period of seven years (Vallée *et al.*, 2007). Meat control of domestic swine is no longer necessary when pig holdings have been officially recognized as *Trichinella*-free by the competent authorities (guideline described in EU Commission regulation EC 2075/2005) or are located in a *Trichinella*-free area (compartmentalization). Denmark was the first country in the EU where the risk of *Trichinella* in domestic pigs was recognized as negligible.

The prevention of livestock contamination is essential for ensuring *Trichinella*-free farms. Feed must be purchased from an approved company which produces feed following good production practices. Feed and feed storage must be maintained in closed silos where rodents cannot enter. Importantly, all contact between wild animal and pigs must be restricted to decrease the risk of contamination by wildlife within the sylvatic population.

Although digestion methods used for routine meat inspection in pigs are considered to be suitable for minimising the risk of clinical trichinellosis in humans, the indirect ELISA methods which are presently only used for surveillance, have been suggested to have a better sensitivity under specific conditions. Meat juice (tissue serum) offers an interesting alternative matrix because it is simpler to collect than drawing blood and processing the blood sample for serum (Nockler *et al.*, 2005). In Denmark, monitoring programmes based on meat juice ELISA have been successfully implemented for *Salmonella* diagnosis in pigs. New *Trichinella* ELISA tests based on purified recombinant proteins of infective stages (Boireau P & Liu M *et al*, 2006) are under validation to improve various aspects of control including early diagnosis, reliability and specificity, freeze resistant species diagnosis and also to delineate with security *Trichinella*-free production units/areas.

## Meat processing and trichinellosis.

Meat of domestic swine that has undergone a freezing treatment according to EU regulation or ICT recommendations and under the supervision of competent authorities, can be exempt from *Trichinella* examination. For example, pork of a thickness up to 15cm needs to be frozen at -15°C for at least 20 days to be considered *Trichinella*-free. However, *Trichinella* found in game meats (mainly *T. nativa*) may be resistant to freezing and therefore frozen meat may still pose a public health risk. If the quality of meat destined for human consumption cannot be controlled by direct examination, ICT recommends curing by cooking meat to an internal temperature of 71°C. *Trichinella*-free meat cannot be guaranteed by the use of microwaves, drying or smoking processes.

## Conclusions

The harmonization of veterinary control methods is very important in the light of new regulations on zoonoses, and is now a major focus of Community and National Reference laboratories in the EU. Ensuring standards with Quality Control ring trials is essential to eliminate *Trichinella* in meat for human consumption in the absence of certified *Trichinella*-free farms.

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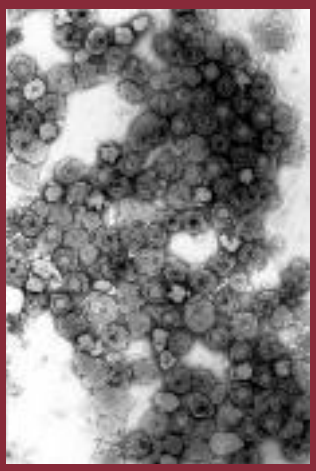
***Figure 1.*** *Comparison of poxvirus (left) and herpesvirus (right) morphology. Orthopox viruses (such as smallpox) are large brick-shaped viruses whereas herpesviruses are spherical (icosahedral) with tubular surface capsomers and often surrounded by an external membrane. Such morphological differences are easily and quickly seen under the electron microscope*



# Electron microscopy—an evolving technique for microbiology

Since the experiments of Antony van Leeuwenhoek in the seventeenth century, the light microscope has arguably been one of the most significant inventions within science, but by the middle of the nineteenth century it was realized that the limit of resolution could not easily be improved. The smallest feature that could be visualized measured about 200 to 250nm, the size of the smallpox virus, one of the largest viruses. The limitation was due to the nature of visible light which only covers wavelengths from 400 to 700nm. To see finer detail shorter wavelength radiation was required. Ultraviolet light and X-radiation were obvious candidates, but there were practical and theoretical difficulties in developing instruments using these radiation sources.

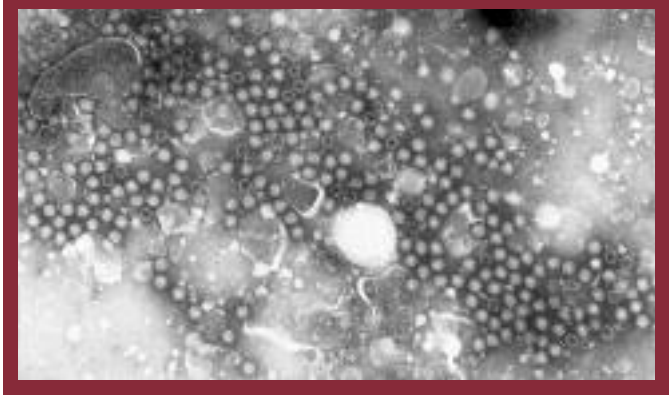
The breakthrough came in the 1920s with the realization that electrons also had wave properties. In an electron microscope electrons are accelerated in a high voltage field giving a beam of radiation. At 80,000 volts, which has been traditionally used in a biological setting, electrons have a wavelength of about 0.005nm giving a resolution up to one thousand times greater (about 0.2nm) than that of a light microscope. At higher accelerating voltages and with modern microscope design far greater resolution can be achieved down to atomic levels. Because of their negative charge, electrons can be deflected in magnetic fields. A series of electromagnets can be used to focus the electron beam in the same way that glass lenses focus a light beam in a light microscope. The human eye is not sensitive to electrons, but the electron image can be converted to a visible image by interaction with a phosphorescent screen, digital imaging sensor or conventional photographic film. This system forms the basis of the transmission electron microscope (TEM) as we know it today.

Experiments into electron optics began in Germany and elsewhere in the 1920s. Major developments came from the work of Ernst Ruska (1906 -1988), the 'father' of the modern electron microscope. Ernst Ruska was awarded the Nobel Prize in 1986 for his work in electron optics and design of the first electron microscope. Ernst's brother, Helmut was a doctor who saw the potential of using the new 'ultra-microscopy' technique to visualize viruses. Initially there were difficulties and new preparation methods had to be developed. Most biological material is low in contrast and this had to be increased to reveal specimen details. Metal shadowing was an early method used for adding contrast to viruses and bacteria. Under vacuum, metals, such as platinum, were evaporated and deposited at an angle onto the specimen. The technique is technically demanding and fine details of surface structure are not always visible. In the late 1950s, Brenner and Horne at the John Innes Institute at Norwich, UK developed the much simpler and rapid technique of negative staining. Unlike light microscopy where stain penetrates into the cells of a sample, in this technique the background of the sample is stained with heavy metal salts (e.g. phosphotungstic acid or uranyl acetate) producing a halo of stain around the microorganism. This method reveals far more detail of the surface structure of viruses and revolutionized the way in which viruses could be seen.

## Electron microscopy (EM) and viruses

Visualization of viruses in the TEM led to the realization that viruses could be grouped by their morphology; e.g. all adenoviruses have an identical icosahedral shape, but the different members of the group can cause a range of different diseases, including respiratory illness, eye infections, cystitis,

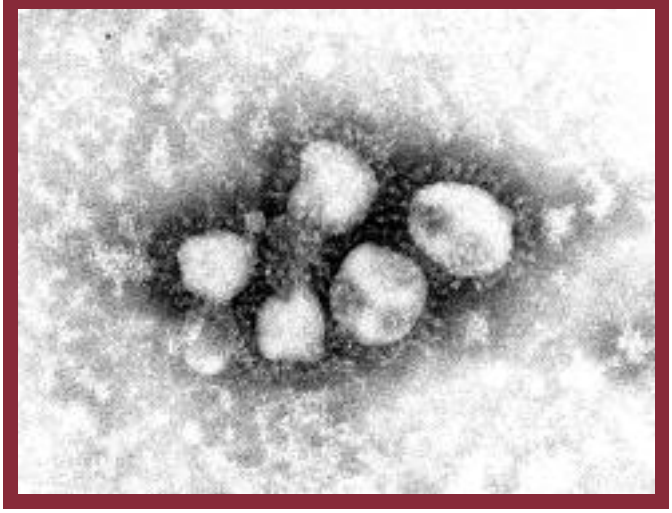
***Figure 2.*** *A large group of rotaviruses found in a diarrhoeal sample of a child with gastroenteritis. Rotaviruses are double-shelled viruses with 'spokes' between the inner and outer capsids layers – hence the name 'rota', meaning wheel*



gastroenteritis, and even tumours in some animal species. This morphological grouping was an important component of viral classification prior to development of molecular biology and it also came to play an important role in diagnostic virology, since viruses could be identified by their characteristic morphology.

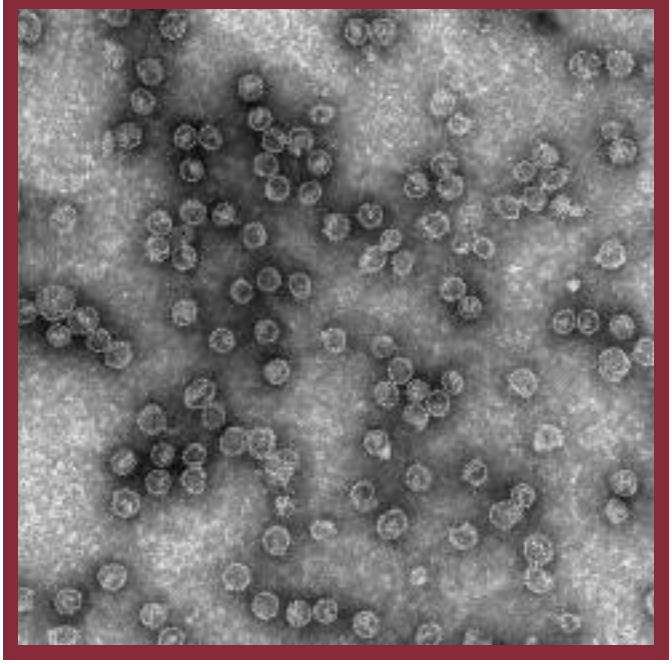
EM was the first "rapid" laboratory technique for diagnostic virology, since clinical specimens could be examined directly. Its value was first recognized in the 1950s and 1960s for the diagnosis of smallpox. Smallpox and chicken pox can easily be confused, particularly in atypical presentations. The skin lesions caused by these two diseases are very rich in virus particles. By mixing a drop of vesicle fluid with a negative stain, virus may be readily seen under the electron microscope. Smallpox is caused by an orthopox virus, but chicken pox is caused by a member of the morphologically distinct herpes virus group (Figure 1). Although seeing a poxvirus would not identify whether it was in fact smallpox, or the relatively harmless cowpox virus, seeing a herpes virus, however, removed the potential panic and the public health measures that would have had to be activated in the case of a possible smallpox infection. All this could be achieved in about 30 minutes of the sample arriving in the laboratory, rather than the three days required by older traditional tests. The advantages of this rapid technique led to the first electron microscope being purchased for the then Public Health Laboratory Service (now part of the Health Protection Agency) in the late 1960s and it is a technique that is still in use.

***Figure 3.*** *SARS virus—EM was important in determining that this new virus belonged to the coronavirus family, with its characteristic surface club-shaped projections (peplomers) giving the appearance of a corona*



Diagnosis of known virus infections by EM then led to a search for new viruses that were suspected of causing disease but could not be detected by traditional methods of growth in animals, eggs or cell cultures. Most notable was the discovery in the 1970s of several viruses that cause gastroenteritis. Rotaviruses (Figure 2), fæcal adenoviruses, astroviruses, caliciviruses (sapoviruses and the Norwalk group of viruses—noroviruses) were all discovered by electron microscopy. Electron microscopes were installed in many virology laboratories during this time for the diagnosis of viral gastroenteritis and continued to be used for this purpose until fairly recently when more sensitive methods, such as PCR, were introduced.

***Figure 4.*** *Recombinant papillomavirus capsids (type 16 used in cervical cancer vaccine). The capsid proteins are produced in an insect baculovirus and assemble to form virus-like particles (VLP): these are non infectious as they contain no nucleic acid*



EM has been instrumental in the discovery of other viruses, such as the viruses causing hepatitis A and B, and in 2003 it was EM that indicated that a coronavirus was the cause of SARS (Figure 3). Identification of the virus group responsible for SARS resulted in faster development of other diagnostic tests for this infection. EM has the advantage that the microscopist does not need a pre-conceived idea of what they are looking for. It is a catch-all method revealing what is there, unlike PCR and ELISA where the scientist has to decide in advance which antigen or nucleic acid to target.

EM is now used infrequently for routine diagnostic virology but it has a vital role in more specialized laboratories to support reference work and research and development (R&D). It is also being used to aid the development of new tests.

***Figure 5a.*** *Negatively-stained image of Helicobacter pylori showing the terminal location of the sheathed flagellar filaments*

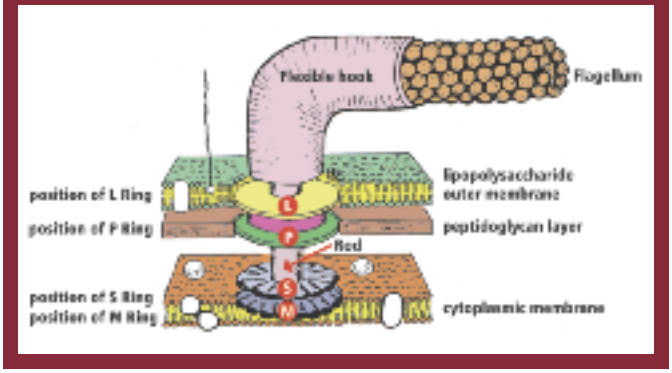
Recombinant virus capsid proteins, expressed in baculovirus systems, are being used increasingly for making monoclonal antibodies and developing new diagnostic tests and vaccines. The viral proteins are far more antigenic and effective if they can be made to assemble into virus-like particles (VLPs). EM is useful for monitoring VLP assembly, saving both time and expense (Figure 4).

## Bacteria and the bacterial flagellum

EM has been used extensively to visualize microorganisms and particularly viruses. In the field of bacteriology, EM is of little use diagnostically, but it has been used in elucidating certain mechanisms of bacterial biology, perhaps best illustrated by the investigations into bacterial motility.

Bacterial species show various arrangements of flagella on the bacterial cell surface, but the fundamental mechanism of flagellar movement of all bacteria is similar. At first sight the bacterial flagellum appears structurally simple, consisting of an external helical filament about 2-3$\mu$m in length. In some species, for example *Helicobacter pylori*, the flagellar filaments are sheathed (Figure 5a). The mechanism embedded in the bacterial cell wall, however, is quite complex, comprising tiny 22.5nm diameter rings or discs that either rotate or act as bearings to rotate the external flagellar filament. Interestingly Gram-positive bacteria and Gram-negative bacteria utilize this 'rotating motor' mechanism, but the number of rings is variable. Gram-negative bacteria have four such discs/rings corresponding to the various envelope layers (L, P, S and M rings). Gram-positive bacteria possess two rings or discs which correspond to the M and S rings of Gram-negative bacteria (Figure 5b). Spirochætes may appear to move differently from those bacteria with external flagellar filaments, but they possess similar flagellar filaments and motor complexes, which are located within a flexible external membrane (the internal axial filaments of spirochætes).

***Figure 5b.*** *Mechanism of bacterial flagellar movement: drawing showing the basal complex of discs and the hook, with the base of the filament (modified from DePamphilis & Adler, 1971*




## Conclusion

Over the last eighty years, EM has had a fundamental impact on our understanding of cells and microorganisms and continues to do so. Although widespread availability of diagnostic EM in microbiology is a thing of the past, EM is still advancing and there are currently many exciting developments taking place. Techniques that were once the preserve of specialized high-end research laboratories are now being introduced into more modest settings. Electron tomography is being used to establish the 3D structure of single virus particles and to find pathways of virus assembly and release from cells. In cryo-electron microscopy very few artefacts are introduced. Samples are rapidly frozen by plunging into liquid ethane at around -170$^{o}$C. Distortion is minimal as there is no drying of the sample, and no staining is required as sufficient contrast is produced in the vitreous ice suspending the virus. Use of these newer methods, however, is only available in laboratories and research centres willing to invest in this expensive technology. EM is alive and well, with committed and enthusiastic users, and will continue to produce significant advances to our understanding of microorganisms into the future.

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**Hazel Appleton[1] and Alan Curry[2]**

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

## *Non-linear regression: fitting a logistic growth*

# Statnote 21

There are a number of non-linear relationships that may arise in microbiological research that cannot easily be reduced to straight lines by data transformation (see Statnote 19) or are not well fitted by a general polynomial-type curve (see Statnote 20). Examples of such curves include the 'asymptotic' regression and 'logistic' growth curves described in Statnote 19. Asymptotic or logistic regressions are best fitted using statistical software employing 'non-linear estimation methods'. Non-linear estimation is a general curve fitting procedure that can usually provide a fit to any kind of relationship between two variables *X* and *Y*. As an example of the method, we fitted a logistic regression equation to a bacterial growth curve obtained in liquid culture.

### Scenario

Bacteria can move freely through a liquid medium either by diffusion or active locomotion. Hence, as the cells grow and divide they are commonly dispersed throughout the medium which often becomes increasingly cloudy as the population grows. Hence, a few bacteria were introduced into a liquid nutrient medium and placed under optimum growth conditions. At regular intervals, a small volume of medium was removed and a count made of the cells. Plotting numbers against time enables a growth curve to be constructed for the bacterium under test. In such an experiment, cell division may not commence immediately, i.e., there is a 'lag phase' as the bacterium adapts to the new environment. Cells then begin to divide and grow at a rate maximal for the species under test and this phase is known as the 'exponential phase'. As they grow, however, cells use up the nutrients and produce waste products and eventually growth slows down or may even cease. The resultant curve is known as the 'logistic' growth curve. The data obtained comprise estimates of the numbers of bacteria in a culture (*Y*) measured at different time intervals (*X*) and are presented in Table 1.

### How is the analysis carried out?

The statistical model for a general non-linear regression can be written:

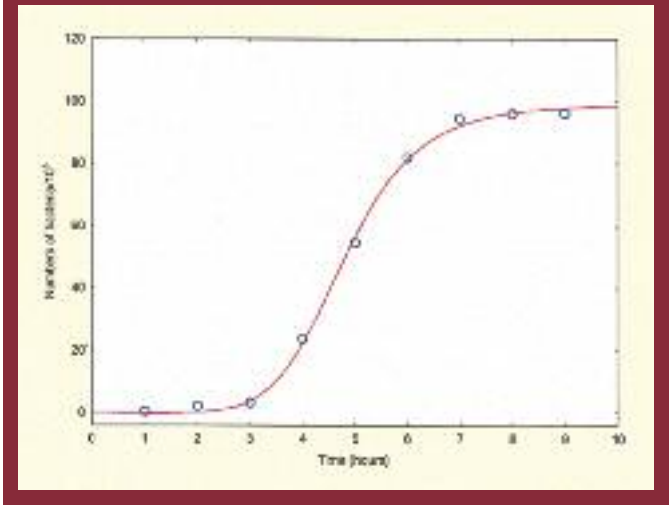
$$Y_i = f(\alpha, \beta, \gamma, X_i) + \varepsilon_i \ (\ i = 1,2 \ldots n) \ldots\ldots\ldots (1)$$

Where '*f*' is a regression function containing $X_i$ and the parameters $\alpha$, $\beta$, $\gamma$, while the errors $\varepsilon_i$ have zero means and constant variance. The least squares method can be used to estimate the parameters $\alpha$, $\beta$, $\gamma$, by minimizing the relationship:

$$\Sigma\ (I = 1\text{-}n)\ [Y_i - f\ (\alpha, \beta, \gamma, X_i)]^2 \ldots\ldots\ldots (2)$$

The problem in fitting this type of regression is the non-

***Figure 1.*** *A logistic regression curve fitted to the data in Table 1*




linearity in one or more of the parameters $\alpha$, $\beta$, $\gamma$. Note that the second-order polynomial regression $\alpha + \beta x + \gamma X^2$ is linear in the parameters $\alpha$, $\beta$, $\gamma$. Hence, initial estimates $a_i$, $b_i$, $c_i$ are made by the computer software of $\alpha$, $\beta$, $\gamma$ and then Taylor's theorem is used to refine the estimates (Snedecor & Cochran, 1980). The user will normally have to specify initial estimates of the parameters defining the model. The program may also have a 'stopping rule', i.e., the analysis will terminate when the residual SS changes by less than a specified amount on each of a number of successive iterations.

To carry out the analysis, the equation for the logistic curve is entered into the software and the 'least squares method' selected as the non-linear estimation method. There are a number of methods available for non-linear estimation; the least squares method being the most widely used. There are several different formulations of the logistic growth curve, one of the simplest being given by the equation:

$$Y = b_0/\{1 + b_1 exp(-b_2 X)\} \ldots\ldots\ldots (3)$$

In equation 3, '$b_0$' represents the upper limit of population growth, '$b_1$' the lower limit, and '$b_2$' the growth rate of the population. Reasonable estimates of the 'starting values' for the parameters $b_0$, $b_1$, $b_2$ and are also entered. Most statistical packages incorporating a general linear modelling option will be able to carry out this type of analysis. We used STATISTICA software (Statsoft Inc., Tulsa, OK, USA) to analyze the present data.

## Interpretation

Estimates of the parameters $b_0$, $b_1$, $b_2$ together with their standard errors and statistical significance are shown in Table 2. The resulting fitted logistic curve is shown in Figure 1. The statistical significance of the parameters ($P < 0.001$) suggest the curve is a very good fit to the data. This method can be used to fit any model whose mathematical equation is known and will provide estimates of the defining constants and a test of the goodness of fit of the curve to the points.

## Conclusion

The techniques associated with regression, whether linear or non-linear, are some of the most useful statistical procedures that can be applied in microbiology. In some cases, there may be no scientific model of the relationship between $X$ and $Y$ that can be specified in advance and the objective may be to provide a 'curve of best fit' for predictive purposes. In such cases, the fitting of successive polynomials is the best approach. The investigator may have a specific model in mind that relates $Y$ to $X$ and the data may provide a test of this hypothesis. Some of these curves may be converted to straight lines by transformation, e.g., the exponential growth and decay curves. In other cases, e.g., the asymptotic or logistic curve, the regression will need to be fitted by a more complex process involving non-linear estimation.

**Table 1.** The number of bacterial colonies ($Y$) derived by the dilution plate method measured at different times ($X$) in liquid culture

| Time in hours ($X$) | *Bacterial colonies (divided by $10^3$) ($Y$)* |
|---|---|
| 1 | 0.5 |
| 2 | 2.3 |
| 3 | 3.4 |
| 4 | 24 |
| 5 | 54.7 |
| 6 | 82.1 |
| 7 | 94.8 |
| 8 | 96.2 |
| 9 | 96.4 |

**Table 2.** Estimation of the parameters of the logistic curve by non-linear estimation methods

| *Parameter* | *Estimate* | *Standard error* | *'t'* | *P* |
|---|---|---|---|---|
| $b_0$ | 99.94 | 1.57 | 63.51 | < 0.001 |
| $b_1$ | 6.76 | 0.42 | 16.02 | < 0.001 |
| $b_2$ | 4.80 | 0.05 | 95.67 | < 0.001 |

'$b_0$' = Upper limit of population growth, '$b_1$' = Lower limit, '$b_2$' = The growth rate of the population, '$t$' = t test, $P$ = probability

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**Dr Anthony Hilton[1] and Dr Richard Armstrong[2]**

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careers

# Lawyers, microbiologists and safe food

**William Marler** describes how his career in law has developed to encompass microbiological food safety. He then goes on to explain the ways in which microbiology features in his day-to-day life as a plantiff's attorney

There must be a good joke about a lawyer who sidles up to a bar and strikes up a conversation with the microbiologist on the next stool. I don't know the punch line, but rest assured it will have something to do with the microbiologist peering through a microscope, and the lawyer chasing ambulances. The punch line will insult both, but mostly the lawyer. As with any joke, it will have some basis in reality. Microbiologists spend some time with microscopes, and while I've never literally chased an ambulance, I make my living representing people who have spent time inside ambulances.

## Education and training

My parents were teachers, so I took my education very seriously and aspired to become a lawyer. I studied my share of science, but I gravitated toward law and politics, and it would be some years before science became a crucial component in my work. I studied political science, English and economics at Washington State University (WSU) and while still in school, I was elected to the Pullman City Council—the youngest person and first WSU student to be elected to that office. I went on to law school at Seattle University, receiving my law degree in 1987 and from there I joined a Seattle law firm.

Six years later, my career took off when I represented many of the families injured by the infamous outbreak of *E. coli* O157:H7 linked to undercooked hamburgers at a chain of restaurants in the USA. Eventually the company settled for millions of dollars, which helped those families deal with the medical consequences to their loved ones, and enabled me to set up my own firm, and a new direction.

My firm specializes in cases involving foodborne illness. As the founding partner, I oversee a staff of about 20, including seven lawyers. Over the years we have represented thousands of people and families injured by *E. coli*, *Salmonella* and other potentially harmful microbes, and we are involved in virtually every outbreak across the USA. In many respects, my job is similar to other lawyers—dealing with clients and opposing lawyers, gathering information, negotiating settlements and sometimes trying cases in court. The main difference is that I am also deeply involved in consumer education and in the political effort to reduce the risk of foodborne illnesses.

My days usually begin before 5am, when I spend 90 minutes or so responding to emails, monitoring developments in my firm, and posting new items on my personal blog—Marlerblog.com, and several other informational sites that have become an important part of my work. By the nature of my specialty, I rely heavily on advances in science. My job would be far more difficult, if not impossible, without the advances made in food safety and microbiology.

The most obvious way we interact with scientists is through culture and isolation of bacteria, the very foundation of foodborne illness detection and surveillance. Molecular microbiology methods, such as pulse field gel electrophoresis (PFGE), allow us to build solid claims on behalf of our clients. To represent our clients, we must be able to trace a foodborne illness to its source, whether that is a pizza parlor in Florida or a meat processing plant in Nebraska, and that source must be established with enough certainty that a jury is left with little doubt about the source of an outbreak illness.

Until just a few years ago, this was difficult to establish. Outbreaks of *Salmonella* or *E. coli* O157:H7 or other serious illnesses were generally investigated by interviewing victims and searching for a common denominator—a local restaurant or scouts club or church event. In a complex society, it was difficult to detect widespread outbreaks, let alone trace those outbreaks to a specific source. PFGE changed everything. This method establishes a DNA "fingerprint" that distinguishes specific strains, confirming that these people were sickened by this batch of hamburger or that batch of peanut butter. PulseNet is a nationwide network of state and local public health agencies, coordinated by the Centers for Disease Control, which enables epidemiologists and public officials to detect national outbreaks that might normally be missed. So both the science and bureaucracy surrounding PFGE help us to represent our clients far more effectively.

There are other less obvious, but equally important ways in which we use microbiology. The popular misconception of my work goes something like this: somebody feels they have been injured or damaged, so they hire a lawyer, who argues the case before a judge and jury who awards them a great deal of money. Case closed. In fact our work is much more complicated. Every day, we hear from people who believe they have been sickened by foodborne illness. They have a stomach ache, or diarrhoea, or their grilled cheese sandwiches "*tasted funny*" or "*didn't look right*." They read in the newspaper about an outbreak of foodborne illness linked to grilled cheese sandwiches, and they say: "*Ah ha! Obviously, that's what made me sick*." So they call us. Some of those people have legitimate claims, but most are not supportable. The illnesses may be psychosomatic, or coincidental, or downright fraudulent, and the first task of a good lawyer is to sift through the claims and determine which cases are sound. As I mentioned, most claims are not supportable, and we reject the great majority. When lawyers fail to use good judgment in assessing these claims, there are repercussions throughout the system. No lawyer can make a living by arguing false or fraudulent claims; it merely wastes their own time and money, that of his clients and the courts, and that of the companies forced to defend them. Pursuing illegitimate claims undermines the system, so that the food industry is more likely to deny legitimate claims by people who have actually been sickened by their products. This, in turn, makes it more difficult to push through important measures that would improve food safety. In the long run, pursuing false claims only increases the risk that more people will get sick. Our first task is to weed out the fraudulent complaints and thereby increase the chances of

achieving success with the legitimate ones. For foodborne illness claims, we have developed a series of legal and scientific screens, derived from years of experience. Here are some of the factors we consider:

**Incubation period**: When claimants say the hamburger they ate this morning sickened them, they are generally out to lunch. Incubation periods—the time between eating and the onset of symptoms—are only ranges, and wide ranges at that. But they are still important. So, the claimant who says she got *E. coli* O157:H7 from today's hamburger simply does not have a winnable case, because of the incubation period for *E. coli* O157:H7 (one to ten days, typically two to five days). In 2004, a claimant who had stopped for a cheese sandwich contacted us saying: "*within two hours of eating that sandwich I became very ill*," he wrote. "*My fever went up from 98.6 to 100.2; I got diarrhoea, stomach cramps, headache and chills. I am still very sick…can you please help me?*" The answer was, no. Based on incubation periods, this person's lunch from this restaurant is most likely not the source of his illness. The major culprits—*Salmonella*, *Shigella*, *Campylobacter*, or *E. coli* O157:H7—are all subject to incubation periods longer than two hours, which rules out the cheese sandwich.

**Smell and taste**: other potential claimants complain that something they ate tasted funny, or didn't smell right. We try to be sympathetic, but most bacteria are odourless and tasteless, and customers who suspect a meal because it tasted funny are usually wrong. Others file what we call "*gross-out*" claims. In one case, a consumer complained that she had opened a box of "*buffalo wings*" and "*an unusually shaped piece caught my eye…when I saw that the piece had a beak, I got sick to my stomach. My lunch and Diet Coke came up and I managed to christen my carpet, bedding and clothing. I want them to at least pay for cleaning my carpet etc. What do you think*?" We thought she chose wisely not to eat the wings, but this is probably not a legitimate personal injury claim.

**Health department investigations**: while statutes and regulations vary from state to state, most health departments monitor outbreaks of foodborne illness. In most cases, a positive lab result from a human sample triggers a report to the local health authority and some type of follow-up investigation. The length, breadth, and documentation vary depending on the pathogen involved, the type of food, the number of persons who may be sick, the local jurisdiction, and other factors. Usually, the results of the investigation are either made public by the health authorities or can be obtained through public records. It is difficult for food-processing companies to dispute such investigations. Rarely has a defendant avoided liability where the local health department concluded that the defendant's food was the source of an outbreak. In general, public health officials are extremely cautious not to prematurely assign blame for an outbreak. They operate with a much higher burden of proof than the civil justice system. Most epidemiologists require 95% confidence in a particular conclusion, while a jury requires only 51 percent confidence. Take, for example, the *E. coli* outbreak at a school in eastern Washington State in 1998. Local and state health officials concluded that the source of the outbreak was a ground-beef taco meal prepared and served at the school. We represented the families of 11 children who were identified as victims of the outbreak. All but one of them attended the school. Four of the children developed haemolytic uraemic syndrome (HUS), which resulted in varying degrees of permanent kidney damage. However, the child with the most severe injuries did not attend the school and did not eat the implicated meal. Health officials concluded that she had been infected through exposure to her sister or another student - a secondary infection. The school district disagreed, but in doing so, the district had to challenge the health department's conclusions. The case went to trial, and testimony by health department officials proved to be crucial in the jury's decision in favour of the plaintiffs.

Health officials will not report a confirmed outbreak, or pinpoint a restaurant or supplier as its source, without being virtually certain of that conclusion. Without 95% confidence, based largely on PFGE and other biological analyses, they are likely to identify outbreaks as possible but not certain. That standard of evidence works both ways; if health officials conclude that an outbreak did not come from a particular source, plaintiffs face an uphill battle to prove their case.

**Prior health inspections**: most state and local health departments enforce health regulations by inspecting restaurants and other food services, and imposing fines or other sanctions for violations. These inspections provide an important tool for establishing the source of an outbreak. Documents may include reports of prior incidents or accusations of food contamination, and those documents can be acquired through the discovery process or through public record requests. Health department documents may provide evidence of improper food handling, suggesting how food may have become contaminated. They may help document a history of improper techniques and code violations that can serve as a tool for limiting a defendant's trial options. Such documentation can lead to an early and favourable settlement, and a history of repeated violations can build a case for punitive damages.

When consumers claim to have been sickened by restaurant meals, health officials or lawyers may find contaminated leftovers, but that is unusual. Far more frequently, lawyers will build their cases on a documented pattern of health code violations. For example in 2001, a young girl suffered a particularly severe *E. coli* O157:H7 infection. She had eaten a hamburger at a California fast-food chain. But, by the time health officials investigated, the original case of frozen hamburgers was long gone, and officials did not find any food on site that tested positive for *E. coli*. However, a thorough review of the restaurant's current and prior inspections revealed a crucial flaw in the firm's cooking method. In six reports spanning three years, health officials had warned the restaurant of the dangers of cross contamination. The matter settled shortly after the presentation of this information.

Clearly, advances in microbiology have helped the world understand which pathogens cause illnesses, foods that are vehicles for transmission of pathogens, and how those illnesses can be avoided. Those advances make it easier for public health and the legal system to trace an outbreak of illness to its source, and to impose sanctions that encourage food processors to minimize risk. This is one of the ways that science and the law conspire to make the world a safer and better place to live.

**William Marler**

# PECS Events at the Summer Conference 2010

## The Grand Hotel, Brighton, UK 5-8 July 2010

In the loop pecs

News from the S*f*AM Postgraduate and Early Career Scientist Committee

This year the Summer Conference will be held at the Grand Hotel in Brighton—a great location and venue and we're sure to have a good time with the sun, sea and pebbles (for those who don't know, Brighton is distinctly lacking in sand).

The student ice breaker session on the Monday is going to be a fun opportunity for everyone to get to know each other (with a microbiology theme). We don't want to ruin the surprise and tell you now, so we're keeping the ice breaker session a secret until the conference! All you need to know is it's called "Find your microbe!"

On the Tuesday, following the afternoon session on *Listeria*, we will be holding a student session. We decided that it would be a great idea to give this session the topic: "*What to do with your PhD?*" We have gathered a number of people to talk about what they have done and career options available including; Industry, Enterprise, Communications, NHS, Lecturing and Post-doctoral research.

Our first speaker, Steve Davies, will be talking about options within the NHS and his role as Laboratory Manager. After this, Sarah Odoi will then discuss her decisions after completing her PhD and her role in Enterprise. There will be a talk about options within Industry (TBA). S*f*AM's Lucy Harper will also be discussing her role in Communications. Finally we will have talks from Simon Gould and Alison Kelly about two roles within academia: post-doctoral research and lecturing.

We feel this session will be beneficial for most students who are not aware of the range of paths to take after completing their PhD. Academia is the most well-known route, as, naturally, most PhD supervisors work in academia. However, there are many options for PhD graduates who naturally develop many transferable skills including writing, communication skills, analytical and research skills.

Graduates can go on to work within the National Health Service (NHS) as senior biomedical/clinical scientists and if they choose, go into further research and development within the health service. Enterprise, based at Kingston University London, aims to bridge the gap between business and academia, providing amongst other things, funding and business opportunities. The function of most University Enterprise departments is to identify commercially viable research and through consultancy, courses and funding incentives they aim to assist entrepreneurs to launch businesses or business ideas. PhD students develop skills such as project design, problem solving and communication skills throughout their project. So if you're entrepreneurially minded enterprise projects and funding are worth exploring.

PhD graduates have training in communication and develop their communication skills through the writing of articles, production of posters and oral communication through presentations—so if you excel in this area, communications may be an ideal role.

Post-doctoral research is a common route for graduates and can lead to. Lecturer positions. If you enjoy the teaching aspect of academic life then this may be a route you wish to choose.

After the student session and trade show we are planning a tour of the local bars. So if you're up for a bit of a chat, a drink and a giggle then come to the student session and we'll let you know the plan of action.


**Emmanuel Adukwu**

PECS Events Team
University of Northampton

**Samantha Price**

PECS Events Team
De Montfort University

# PECS Events at the Summer Conference

## Monday 5 July

**(Time TBA)** **Ice Breaker Session "Find your Microbe!"**
Let's get everyone talking with this fun ice breaker!

**(Time TBA)** **Fish and chips on the pier**

**21.30** **Quiz Night**

## Tuesday 6 July

**17.10-18.10** **Student Session "What to do with your PhD?"**
**A variety of Speakers talk about their career paths.**

**Steve Davies** (NHS, Northern General Hospital)—Laboratory Manager

**Sarah Odoi**—Enterprise

**(Name and Company TBA)**—Industry

**Lucy Harper** (S*f*AM)—Communications

**Simon Gould** (Kingston University)—Post-doc research

**Alison Kelly** (Kingston University)—Lecturer

**20.30-21.00** **Tour of the local bars**

## Congratulations!

Congratulations go to **Vicki McCune** who has been awarded her PhD.

If you know a S*f*AM student or early career scientist who has been awarded a PhD/Prize/award then get in touch, email Clare Doggett clare@sfam.org.uk

## Get involved!

PECS committee members would like your ideas for PECS social events during the Summer Conference.

If any post-graduate or early career scientists have suggestions of things to do in Brighton then please let us know.

There is a discussion board on the S*f*AM facebook fans page set up for your ideas http://www.facebook.com/pages/SfAM/94913188313?v=app_2373072738&ref=ts#!/topic.php?uid=94913188313&topic=13719 or alternatively you can contact: pecs@sfam.org.uk

# Students into Work Grant reports

## am I eligible — can I apply?

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*.

For further information visit: **www.sfam.org.uk/grants.php**

## The effectiveness of various disinfectants on bacteria in the presence and absence of organic load

**I am a Biomedical Sciences student** at the University of the West of England (UWE) in Bristol and, having just completed the second year of my studies, I was eager to use my summer holidays gaining practical scientific experience rather than the alternative option of spending them behind a desk in an office job. I was, therefore, delighted to be given the opportunity to spend 10 weeks in the Microbiology Research Lab at UWE conducting a comparative investigation into the effectiveness of various disinfectants on bacteria in the presence and absence of organic load. The topic of disinfection is of great interest to me because, as well as being a much covered subject in the media, the increasing incidence of nosocomial infections and the burden it is placing upon the NHS is particularly relevant to me owing to the fact that I am spending my placement year in an NHS Pathology Laboratory.

The initial phase of my studentship involved training in fundamental microbiological techniques such as learning how to work aseptically, how to make up nutrient agar and broth, pour plates and sterilize equipment—things which as an undergraduate student I hadn't previously considered in much detail as these procedures were always carried out for us in advance of our teaching practicals.

My introductory training was followed by a period of getting to know the target bacteria that I was going to be working with, namely *Escherichia coli* pGLITE (an *E. coli* which had been genetically modified to bioluminesce). To achieve this, the growth of the bacteria was analyzed by various means such as recording its optical density, performing viable counts and recording its bioluminescence. Growth curves were then constructed, comparing these methods and also comparing the growth with that of the wild type *E. coli* in order to fully understand its behaviour. Once this detailed investigation into the growth of the target bacteria was complete, the remainder of my time was spent performing the planned experimental work.

Bioluminescence occurs throughout nature in many different organisms including fungi, fish, insects, algae, squid and in the most abundant and widespread bioluminescent organisms, bacteria (Meighen, 1993). Bioluminescent bacteria are found in marine, freshwater and terrestrial environments as free-living species in the oceans, saprophytes growing on dead fish or meat, gut symbionts in fish, parasites in insects and as light organ symbionts in a number of aquatic species. All naturally occurring bioluminescent bacteria that have been characterized are motile, Gram-negative rods and all are capable of facultative anaerobic growth (Meighen, 1991).

Bioluminescence provides a bioreporter system that produces a physical rather than chemical signal, and avoids accumulation that may lead to toxicity or instability. Light emission can be accurately measured, with a high level of sensitivity, in a non-destructive fashion and in real-time (Billard & DuBow, 1998) without the need for interference with samples as would be required by traditional counting methods. Insertion of the lux operon from the terrestrial bioluminescent species *Photorhabdus luminescens* into *E. coli* using the plasmid pGLITE (Parveen *et al.*, 2001) produces a strain of *E. coli* that is constitutively bioluminescent. Since the operon encodes not only the luciferase but the enzyme complex responsible for the synthesis of the substrate, all that is needed to produce light in a recombinant aerobic bacterium is the expression of the *lux* operon (Hill *et al.*, 1993).

The experiment involved growing the *E. coli* pGLITE each morning from an overnight culture to a specific optical density, treating the bacteria with a disinfectant at three different concentrations and following the death of the bacteria at each concentration level by recording its bioluminescence continuously over a two minute period using a single-tube luminometer. This was also done in the presence and absence of foetal bovine serum (which represented organic loading) at two concentrations—5% and 10%. Six replicates of each were performed and each experiment was carried out on two independent

occasions. The disinfectants investigated were: (1) Electrochemically Activated Solutions (ECAS), produced on-site at concentrations of 80%, 10% and 1%; (2) ethanol at concentrations of 80%, 10% and 1%; (3) bleach at concentrations of 80%, 10% and 1% and (4) commercially available disinfectant at concentrations of 10%, 1% and 0.1%. ECAS are generated from low halide salt solutions using specially designed electrolytic cells. Electrochemical generation of active killing solutions has been exploited widely outside of Europe, particularly in Russia where electrochemically activated water is the subject of over 300 patents and in excess of 20,000 ECAS generators are in use within hospitals (Marais & Williams, 2001).

The luminometer proved to be extremely reliable and standardizing the bacterial inoculum provided very consistent results. Analysis of the results in brief suggested that, in the absence of serum, ethanol is only effective at high concentration (80%); bleach has an effect at all concentrations but does not completely kill the bacteria, whereas ECAS and commercially available disinfectant are very effective biocides especially at mid to high concentrations, killing bacteria within 10 seconds at their highest concentration. With the introduction of 5% serum, all disinfectant kill rates were slowed although commercially available disinfectant, ECAS and ethanol retained their complete killing of the bacteria at mid and high concentrations. At a serum level of 10% all disinfectants needed to be at their highest concentrations to achieve a complete kill of the bacteria. Ethanol at 80% and ECAS at 80% proved to be the most rapid disinfectants in the presence of 10% organic load.

I thoroughly enjoyed the 10 weeks of my summer studentship, and feel that it gave me excellent practical experience of working in a laboratory which will stand me in good stead, not only during my placement year but also when I return to university to carry out my final year project. I learned to appreciate the planning involved in setting up an experiment. This is particularly relevant in microbiology where careful planning is essential in order to ensure that all equipment needed is sterilized in advance and that target bacteria are maintained in an optimal environment to allow for their growth. Experiencing first-hand what a research laboratory is like will also allow me to make a much more informed decision about whether to undertake postgraduate study once I finish my degree.

I would like to thank S*f*AM for providing the funding which enabled me to have this really worthwhile experience and thanks must also go to Dr Darren Reynolds, Dr Gareth Robinson and their PhD student Su Lee for their help.

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**Kathy Tonks**
UWE

## The effectiveness of essential oils in wound dressings to prevent infection

**I would like to say a special thank** you to S*f*AM and Dr Alan Edmondson for giving me an opportunity to carry out a seven week summer research project. The research focused on exploring alternative therapy as a method of prevention of wound infections which are a serious problem in hospitals and in the community. In my final year honours research project my findings showed that several essential oils (clove bud, thyme and cinnamon) worked well in combination with dicloxacillin against *Staphylococcus*

*aureus*. However, their concentrations were still too high for use intravenously in combination therapy with an antibiotic. These findings led me to explore the use of oils in wound dressings or washes to prevent infection. This could alleviate the extra suffering of already ill individuals as well as minimizing hospital stay, reducing the large economic costs imposed by such infections.

I chose the three essential oils from my honours project for further study in this project. It is also worth noting that these oils have been shown to be effective, to an extent, in other areas of microbiology. For example, they have shown some effects against mycotoxigenic moulds, as discussed in a President's Fund report in the March 2008 issue of *Microbiologis*t. To better evaluate the extent of their antibacterial action I also compared them to the effects of silver dressing, an existing product for prevention of wound infections. To investigate the matter further I explored the combined effects of silver and oil. I tested these against three pathogens commonly associated with wound infections: *S. aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

In my preliminary work I carried out zone of inhibition tests for each oil against each pathogen. Thyme essential oil showed the highest level of inhibition against *S. aureus* and *E. coli*. Thyme oil still produced a zone of inhibition at a concentration of 5% (v/v) against both pathogens, whereas the other two oils were inactive at this concentration. Thyme oil concentration of 5% achieved the same size zone of inhibition as the dressing containing silver. *P. aeruginosa* was resistant to all the oils even when they were used undiluted. This drastic

difference could be at least partially attributed to the difference in bacterial cell wall structures of Gram-positive and Gram-negative microorganisms.

For my main experiment I measured the reduction in bacterial growth seen after application of thyme oil. I chose this oil alone for further investigation as it was the only oil to show good antimicrobial effect in the preliminary investigation. I added an inoculum containing known quantities of bacteria to the dressing containing oil. After 5, 10, 15 and 20 minutes I measured the quantity of bacteria surviving on the dressing. I also carried out this procedure with silver dressing for comparison and with silver dressing containing oil for further insight. The silver was shown to be more effective than thyme oil with a greater reduction in bacterial growth. The silver dressing produced a reduction of $10^2$, while the oil showed a reduction of $10^1$. Interestingly, the combination of the two inhibited bacterial growth completely, suggesting a good synergistic effect. Strangely, reduction in bacterial growth did not increase with time.

Thyme oil contains high amounts of the chemical compounds thymol and carvacrol, which are thought to bind to proteins on the bacterial cell wall leading to increased permeability and loss of essential ions and/or breakdown of the cell wall. This mode of action could explain why there was no correlation with time. The oil compounds would quickly bind to the susceptible bacterial cells, executing their effects on the cell wall soon afterwards. Antimicrobial properties of silver seem to arise from varied mechanisms of action, due to the charged silver ions interacting at many points within the cell. This could explain why silver showed a better antibacterial effect than oil, as oil exhibits only one mechanism of action, making it easier for the bacterial cell to counteract. The silver seems to have several mechanisms and therefore a more challenging environment for the cell to deal with. The synergistic effect of silver and thyme oil could be due to the oil making the cell more permeable and allowing more silver ions to enter the cell with greater ease. These ions can then disrupt the cell from within leading to a bactericidal effect.

These results led to the use of a wound wash containing thyme oil before the application of a wound dressing containing silver. As there was no correlation between the time of exposure and the amount of microorganisms eliminated, it may be sufficient to wash the wound out thoroughly with oil solution before applying the silver dressing and this would certainly be an interesting area to explore further.

This project has helped me to develop the many skills involved in research, from planning an investigation and effectively dealing with problems as they come along, to collecting, analyzing and evaluating the data. It has been a valuable and insightful experience to manage a whole project from start to finish.

**Elina Williams**
Leeds Metropolitan University

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## The role of viral aggregation in disinfection

**Disinfection and preservation** have been used for centuries, even though people were unaware of the existence of microorganisms. Ancient records show that the Egyptians, Chinese and Persians practised preservation, drinking water sanitation and antisepsis for wounds. Chemicals such as pitch, wine, copper and silver were the earliest disinfectants, as well as mercury which was used by Arab physicians as an antiseptic, and a wood preservative in the fourth century. In the nineteenth century, the knowledge about microorganisms and their transmission became clearer. Louis Pasteur was credited with dispelling the belief in spontaneous generation of illness and in 1857 he developed the process known as pasteurization, showing that souring of milk was due to microorganisms.

The twentieth century was characterized by the discovery of several new chemical biocides, such as cationic compounds (biguanides and quaternary ammonium compounds), aldehydes, phenolics and peroxygens (Maillard, 2005). The use of biocides during disinfection, preservation and to some extent sterilization is important in controlling and preventing the transmission of infectious diseases in public places, healthcare centres and hospitals. However, the effect of biocides against viruses is usually poorly documented.

Mechanisms of action and viricidal properties of biocides can vary greatly depending on several factors; environment-related and virus-type-related. Viruses present multiple target sites to biocides, which can be identified into four groups: (1) the envelope; (2) the capsid; (3) the glycoprotein receptors and (4) the viral genome.

The envelope, being highly lypophilic and negatively charged, is susceptible to a wide range of membrane-active agents.

Hence, the enveloped viruses appear to be more sensitive to biocides than the non-enveloped ones. The capsid is the most important part and protects the viral genome from the outer environment. The efficacy of biocides also depends on the size of the capsid (a larger capsid provides more target sites than smaller ones) and of its amino acid composition; for instance $–NH_2$ and –SH groups are targets respectively of glutaraldehyde and of iodine and hydrogen peroxide disinfectants. The alteration of glycoproteins can cause a loss in viral infectivity due to their role in virus specificity and in releasing the viral genome into the host cytoplasm. Finally, the genome, which is the infectious component, is well protected and can vary in size, appearance, content and degree of bonding to the capsid. Compounds such as chlorine-based, peracetic acid (Maillard *et al.* 1996), and glutaraldehyde have been found to alter the viral nucleic acid.

The development of viral forms of resistance which alter the kinetics of the time/biocide efficacy reaction has been described, although not extensively. Viruses show a variety of intrinsic and acquired mechanisms of resistance. The acquired mechanisms are distinct owing to single or multiple genetic mutations. The intrinsic ones are due to the structure of the viral particles, for example, small non-enveloped viruses are considered to be more resistant than enveloped viruses, due to the greater interaction between the biocide and the viral particles.

Clumping could be quite an effective response against the action of biocides. Viruses generate clumps whilst in the cell, in the form of crystals and, after being released from the host, they can remain clumped in water or can stay associated with cellular debris. It has been proposed that clumps of 16 identically sized virions are required to form a protective coat around one virus of the same size; enough to protect it from the contact with biocides. Particulate matter (soiling) can form a layer around viruses protecting them from the environment (Thurman & Gerba *et al*, 1988) and enhance the aggregation phenomenon. Viral aggregation depends on (1) the virus species and serotype, particularly the viral surface charge, (2) viral concentration and (3) chemical properties of the surrounding medium, such as pH, ionic strength, and the nature of the ions present (Gassilloud & Gantzer, 2005). Studies showed that aggregation is correlated to the isoelectric point (pI) of viruses. In particular, virions tend to be dispersed at their pI, even though it is not possible to find a general pattern because each virus responds differently to different buffers and pH (Floyd & Sharp, 1979).

During treatment with biocides, aggregation can greatly influence their efficacy and can cause the underestimation of viral particles, particularly during biocide testing. Formation of clumps, in fact, protects viruses which are trapped inside, avoiding the contact with the biocide. As a consequence, some resistant subpopulations remain present in the medium or on the surface. For example, this was demonstrated with reovirus during bromine disinfection in water (Sharp *et al*, 1975). One of the reasons of aggregating after the treatment with biocides, particularly with charged compounds (e.g. cationic compounds) is the change of hydrophobicity of viruses in the medium. It is likely that biocides might attach to the polar components of the capsid or envelope, and therefore decreased virus surface polarity encourages virions to group and form micelle-like particles.

There are still few studies on viral aggregation as a form of resistance to disinfection, but understanding the mechanisms of interaction helps to increase the efficacy of biocides and hence the quality of disinfection.

We are currently investigating the interaction of a common biocide, polyhexamethylene biguanide hydrochloride (PHMB) with viral particles. The viricidal activity of this cationic biocide against a non-enveloped bacterial virus (MS2) which is used as a surrogate for picornaviruses, was shown to be limited to a 2 $\log_{10}$ reduction in viable number, although we demonstrated that the biocide interacted strongly with viral proteins and damaged the viral capsid. In order to further understand the reason for a limitation in viricidal activity, one line of investigation concentrated on the formation of viral clumps during disinfection. Kinetics of inactivation showed a tailing effect which might indicate the presence of a resistant sub-population of viruses. Further investigations, including changes in hydrophobicity during biocide exposure, electron microscopy and light scattering analysis, confirmed the presence of clumps after the disinfection treatment. We believe that the formation of these aggregates is responsible partly or completely for the limited viricidal activity of PHMB against MS2. To confirm this hypothesis, we are currently investigating ways to decrease or eliminate clumps during treatment.

I would like to thank SfAM for the award of the President's Fund, which gave me the opportunity to attend the 108th General Meeting of the American Society of Microbiology in Boston.

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**Federica Pinto**
Cardiff University

## What lies within the rinse water: efficacy of high-level oxidising disinfectants against persistent bacteria isolated from hospital washer-disinfectors.

**Flexible endoscopes are a valuable** diagnostic tool in the healthcare setting. These are sophisticated and expensive medical devices that have to be cleaned and disinfected to the highest standards

possible in order to ensure patient safety. Among all medical devices used, endoscopes are thought to be the ones which are most likely to be linked to hospital-acquired infections (Rutala & Weber 2004). These devices are reusable and therefore the way in which they are cleaned and disinfected is important due to the extent of microbial contamination (bioburden) present on the endoscope after a procedure. Endoscopes are heat sensitive and decontamination must be achieved through the use of high-level disinfectants. These devices are exceedingly difficult to clean due to their complex design. If there is a breach in the rigorous disinfection procedure then there is potential for patient-to-patient transmission of infection. For example, this happens when insufficient cleaning and disinfection of the endoscope occur, when the wrong concentration of disinfectant is used, when sterile water used to rinse the endoscope is contaminated or when endoscope channels are not dried appropriately.

The approach to endoscope decontamination is under regular review in order to optimise the process, to avoid manual error and to reduce the risk of cross-infection to patients. Endoscopes can be disinfected in washer-disinfectors or manually; washer-disinfectors increase the efficacy of disinfection and can reduce the chances of endoscopy staff coming into contact with the high-level disinfectants.

There have been noted instances where endoscope disinfection failure is due to the washer-disinfector. Recently Gamble and colleagues (2007) conducted a review into decontamination incidents in England. Seven instances involving washer-disinfectors were observed and causes ranged from mechanical faults to incorrect connectors being fitted. It was also established that microbiological testing was not done following national guidelines. An outbreak of multidrug-resistant *Pseudomonas aeruginosa* was linked to a contaminated bronchoscope and the source of the contamination was potentially linked to the washer-disinfector used to disinfect the endoscope (Schelenz & French 2000). The washer-disinfector was found to be encrusted with limescale on its inner workings and *P. aeruginosa* isolates were found to be identical to the ones isolated from the bronchoscopes.

Disinfection procedures can also fail when staff are unfamiliar with new protocols, when disinfectants are incorrectly prepared before use or when machinery malfunction occurs and goes unnoticed (Honeybourne & Neumann, 1997; Gamble *et al.*, 2007). In 2004 in Northern Ireland there were reported instances of failure to decontaminate an endoscope effectively. An auxiliary channel within an endoscope was not identified and as a result it has not been cleaned or disinfected since purchase (Gamble *et al.*, 2007). Subsequently an investigation was launched as there was the potential transmission of bloodborne viruses and 1300 patients were contacted.

The aim of our study was to establish if microbial contaminants could survive high-level disinfection within washer-disinfectors and, if so, which resistance mechanisms are involved in these microorganisms.

Bacterial strains were isolated from washer-disinfectors used for the reprocessing of flexible endoscopes. The effects of various oxidising agents on their survival were tested using a standard suspension test method. Three high-level disinfectants were used: 7.5% hydrogen peroxide formulation, 2.5% peracetic acid formulation and 0.03% chlorine dioxide formulation. A higher concentration of chlorine dioxide was investigated as isolates were found to be insensitive to the recommended in-use concentrations. Disinfectant efficacy at 0.5, 1, 5, 30 and 60 minutes were tested. When a five $\log_{10}$ reduction was achieved no other contact times were investigated for that disinfectant and bacterial isolate (Martin *et al.*, 2008).

Any bacterial isolates that showed unexpected resistance to typical disinfectant contact times with the oxidising agents were then studied to understand the mechanisms of survival and resistance. These investigations included scanning electron microscopy (EM).

Sampling retrieved 14 bacterial isolates in total over three visits to an endoscopy unit. Two of these isolates (*Bacillus subtilis* and *Micrococcus luteus*, both found in the rinse water of the washer-disinfector) were shown to have a greater resistance towards chlorine dioxide, the oxidizing agent used in the washer-disinfectors sampled. Vegetative *B.subtilis* isolates showed resistance to chlorine dioxide disinfectant at higher than recommended in-use concentrations and cross-resistance with the other oxidizing agents tested. The standard strain which was used to compare the efficacy of the oxidising agents was found to be more susceptible to the biocides than the *B. subtilis* isolate. EM investigations highlighted the difference in the presence of exopolysaccharides (EPS) between the resistant clinical isolate and the standard counterpart strain (Martin *et al.*, 2008). Current investigations are focusing on the role of EPS and aggregation as a means of survival in the presence of oxidizing agents, together with inactivation mechanisms of these types of biocides. The *B. subtilis* isolate was prone to aggregation and produced a large quantity of EPS when compared to the standard counterpart strain.

This study demonstrated that bacteria can survive high-level disinfection within washer-disinfectors and highlighted the diversity of mechanisms of resistance to oxidizing agents. So who knows what might be lurking in the rinse water in your endoscope washer-disinfector or for how long it will survive.

I would like to thank S*f*AM for awarding me the President's Fund grant to attend the 108th General Meeting of the American Society for Microbiology in Boston, Massachusetts where this work was presented.

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**Deborah Martin**
Cardiff University

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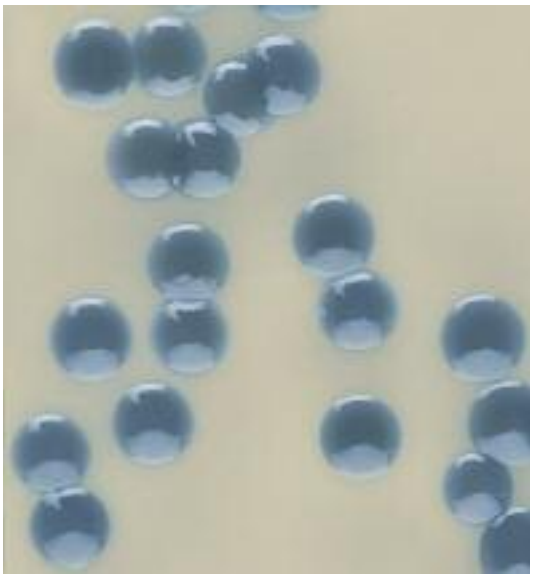

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Each corporate member of the society may publish ***up to*** 200 words on a topic related to their field of activity in each issue of *Microbiologist*. For further information please contact Lucy Harper by email at: **lucy@sfam.org.uk**

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


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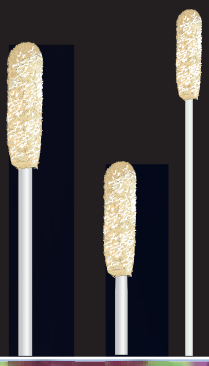

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