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Abstracts

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Contents

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Plenary session

Molecular pathogenesis of virus infections

The regulation and maturation of antiviral T cell responses

J. Lindsay Whitton

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CD8+ T cells play a key role in controlling primary virus infections, and protecting against subsequent exposures to the same virus. The CD8+ T cell response to acute virus infection is triphasic. During the expansion phase, virus-specific cells proliferate rapidly, peaking at around 7–9 days after infection. Thereafter, ~90% of them die (the contraction phase); and the residual ~10% survive long-term (the memory phase). To complement these quantitative analyses, we have undertaken qualitative studies, to evaluate the biological functions of CD8+ T cells over the course of infection. We have identified a number of ways in which the antiviral activities of these cells are improved: they become better able to respond to low levels of viral antigen; they elaborate different patterns of antiviral cytokines; and the rapidity with which they produce these effector molecules increases. Finally, we have found that one of these antiviral molecules, IFNγ, is responsible for regulating the abundance of CD8+ T cells, and we propose a model in which evolution has killed two birds with one stone, ensuring that the cells best-suited to clearing virus infection are also those that are most rapidly expanded during the primary response, and are selected to enter the memory pool.

The immune response to dengue virus infection

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Dengue virus infection is major threat to public health in a number of tropical and subtropical countries.

The prevalence of dengue infection is increasing and presents a huge health and financial burden to a number of developing countries. Around 2.5 billion people are at risk and there are estimated to be up to 50 million infections per year. Dengue is a flavivirus which circulates as 4 major serotypes and is passed on following a bite from an infected mosquito. Dengue haemorrhagic fever and dengue shock syndrome, the more severe manifestations of dengue infection, do not tend to occur during a primary dengue infection. Instead they occur when an individual is re-infected with a virus of different serotype to the primary infection.

Antibody dependent enhancement has been demonstrated both *in vitro* and *in vivo* to drive higher viral replication and is thus thought to be a major mechanism driving higher viral loads in secondary infection or in the neonatal period as maternally derived antibody titres fall. One further factor which may contribute to the severity is the T cell response to infection. T cells can secrete a large number of inflammatory cytokines and also be directly cytotoxic. It may therefore be that the coincidence of a large antigen load driven by antibody dependent enhancement with a high amplitude T cell response may lead to some of the downstream tissue damage/dysfuntion seen in dengue. We will present a detailed examination of T cell responses in dengue infection and combine this with an analysis of cytokine secretion and virus loads across a time course of infection.

The immune response to HIV

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Primary human immunodeficiency virus type 1 (HIV-1) infection is characterised by an acute burst of viral replication, followed by establishment of a 'set-point' persisting viral load that is predictive of the subsequent rate of disease progression. Recent studies have begun to give insight into the immune response induced during this critical phase of infection and why it fails to contain viraemia more completely. Innate responses are activated prior to the peak in acute viral replication, and may have a key influence on early events, but remain poorly characterised. HIV seroconversion occurs as acute viraemia is contained, although neutralising antibody production is typically delayed. HIV-specific CD8 T cell responses are induced during acute infection, and play an important role in control of early virus replication. Rapid selection for viral variants able to escape CD8 T cell recognition can occur in early HIV infection, and factors influencing the extent to which the primary CD8 T cell response is evaded by this mechanism may impact on the persisting viral load established. Sustained HIV-specific CD4 T cell responses are associated with good control of virus replication. Identification of immune correlates of efficient control of virus replication is of importance for HIV vaccine design and evaluation.

Persistent RNA virus infections

John Fazakerley

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How RNA viruses are able to persist in their host is in many cases poorly understood. Replication within specific sites and cell types, avoidance or active suppression of host cell suicide and of innate and adaptive immune responses are important factors in the persistence of many RNA virus infections. The nature of persistence and its detection are important issues which need careful consideration. The importance of tropism, apoptosis and immune responses in the persistence of RNA viruses will be illustrated by studies on Semliki Forest virus (SFV), an Alphavirus and Theiler's virus (TV), a Picornavirus. The outcome of SFV infection in the mouse brain changes from widespread, destructive panencephalitis to focal, non-destructive potentially persistent infection upon neuronal differentiation. Antibodies are required to eliminate SFV infection from the mature mouse CNS. In the case of TV, virus persistence is linked to MHC haplotype and depends upon the quality and quantity of adaptive immune responses.

Pathogenesis of Ebola and Marburg viruses

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Zaire and Sudan ebolaviruses and marburgvirus are associated with epidemics of rapidly progressing high fatality hemorrhagic disease in humans. Although vascular dysfunction is a important disease feature, initial symptoms are frequently non-specific (fever, generalized body pains etc.) which can lead to initial misdiagnosis and virus transmission to those in close contact with patients. Macrophages and dendritic cells are the primary targets of virus infection. In fatal cases, virus-specific immunity is often suppressed, suggesting that early infection of these antigen presenting cells may result in impaired induction of adaptive immunity. The VP35 protein of Zaire ebolavirus is essential for virus RNA replication and has also been shown to inhibit the host cell Type I interferon (IFN) response. More recently, VP35 has been shown to block phosphorylation of IRF-3, (a transcription factor that induces expression of many antiviral genes, including IFN-β and a C-terminal basic amino acid motif is required for inhibition of both ISG56-reporter gene expression and IFN-β production. This basic amino acid motif displays high sequence identity with the N-terminal RNA-binding domain of another interferon-antagonizing protein, NS1 of influenza A virus. Reverse genetic systems are being used to assess the contribution of VP35-mediated IFN-antagonism to ebolavirus replication and pathogenesis.

Although vascular dysfunction is a central feature of the disease, initial symptoms can be relatively non-specific (fever, generalized body pains etc.) which can lead to initial misdiagnosis and virus transmission to those in close contact with patients. Marked immunosuppresion is a hallmark of filovirus infections. Most strikingly, little or no virus-specific humoral antibody responses are detected in fatally infected patients, and although Ebola virus infection induces cytokine responses, distinct differences can be seen between fatally and non-fatally infected patients. Early detection of anti-inflammatory cytokines such as IL-10 in patient plasma and high levels of neopterin and IL-1 receptor A (IL-1RA) are common features of fatal infections. Conversely, early detection of IL-1β and elevated IL-6 in patient plasma is commonly found in survivors. Fatally infected patients display prominent signs of bleeding and coagulation defects, and signs of disseminated intravascular coagulation (DIC) and multi-organ failure become increasingly evident close to death. Important factors leading to poor and dysregulated immune response and high pathogenicity include the virus initial targeting of dendritic cells and macrophages, and synthesis of the virus encoded VP35 protein which acts as an interferon antagonist.

Coronaviruses and SARS

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Abstract not received

Transmissible spongiform encephalopathies – practical issues and basic mechanisms of disease

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The Transmissible Spongiform Encephalopathies have been a major focus for concern and for research activity since the emergence of BSE in the late 1980's. Nearly two decades later how has our understanding of these diseases progressed? While the BSE epidemic appears in decline in both UK and Europe, new challenges for TSEs have appeared. BSE has been reported in countries not previously affected. Moreover a number of issues surrounding vCJD, concerning the susceptibility of the population to vCJD, the incubation time of disease and the extent to which human to human transmission is likely to occur remain unanswered. A new epidemic of TSEs has appeared in the US in the form of CWD in deer and new strains of TSE agent are now being detected in Europe in both sheep and cattle due to increased surveillance.

Much progress has been made in the study of the PrP protein which accumulates in the CNS and other tissues during the course of a TSE infection. PrP is known to be central to the TSE diseases since animals without PrP do not develop disease. The disease associated form of PrP (PrP^{Sc}) has been hypothesised to be the infectious agent but the precise molecular structure of this agent and of the toxic species in these diseases remains to be defined. PrP is also thought to be involved in determining the TSE strain and in defining a host susceptibility of resistance to disease although the mechanism by which this is achieved remains to be defined. Our ability to diagnose, control, eradicate or treat these diseases is dependant on providing answers to these fundamental questions.

Influenza virus pathogens

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Pandemic influenza is a zoonotic disease caused by the transfer of influenza A viruses or virus gene segments from aquatic bird reservoirs to humans and domestic animals. In wild aquatic birds – the natural hosts of influenza viruses – these viruses are in evolutionary stasis. After transfer to other species influenza viruses evolve rapidly. The 1918 Spanish influenza killed upwards of 50 million persons worldwide and the currently emerging H5N1 influenza virus is killing 3/4 of infected young persons in Vietnam and Thailand. Other pandemic strains are much less pathogenic. The disease resulting from influenza viral infection is a complex event involving both the virus and the host and the immune status. The viral genes responsible for high pathogenicity are better resolved in avian H5 and H7 strains subtypes than in humans. The hemagglutinin (HA) is of critical importance but a combination of genes including the polymerase gene (PB2) and the non-structural gene (NS1) are involved. There is increasing evidence for the role of the NS1 gene and a cytokine imbalance. The failure of H5N1 viruses to transmit human to human and the genes involved remain largely unresolved.

Antiviral potential of RNA silencing

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RNA interference (RNAi) or RNA silencing is a double-stranded RNA (dsRNA) guided mechanism that mediates sequence specific degradation of RNA. The recent demonstration that RNA interference can be used to inhibit virus replication has initiated an exciting field of research. First, it provides a novel antiviral therapeutic approach and, second, it may constitute a hitherto unrecognized natural antiviral defense mechanism.

We wish to evaluate the antiviral potential of RNA interference and focus on three specific areas: 1) The therapeutic potential of RNAi; 2) RNAi as a natural antiviral defense mechanism in animals; and 3) Systemic spread of RNAi inhibitory effect. We have employed positive stranded RNA viruses as model organisms. We have demonstrated that dsRNA can effectively protect human cells against infection by this type of rapidly replicating and highly cytolytic RNA virus. To examine the ability of viruses to escape RNAi inhibition we recovered and

sequenced polioviruses that escaped inhibition by several siRNAs directed against viral sequences. Analysis of these viral escape mutants shows that RNAi recognition is sensitive to subtle point mutations within the central region and the 3' end of the target RNA. Even single transition mutations resulting in G:U mismatches are effective in overcoming viral inhibition from defined siRNAs. However, simultaneous targeting of multiple viral sequences overcame these resistance mechanisms to RNAi and prevent viral escape.

$CD8^+$ T cell regulation of HSV-1 latency

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Abstract not received

Evasion of innate immune responses by hepatitis C virus

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Toll-like receptors (TLRs) bind pathogen-specific ligands early in infection, initiating signaling pathways leading to expression of protective cellular genes. Many viruses have evolved strategies blocking protective mechanisms induced through these signaling pathways, but interference with proximal receptor interactions has not been identified. We show that the NS3/4A protease of hepatitis C virus (HCV) inhibits TLR3 signaling induced by the synthetic dsRNA analogue, poly I:C, in HeLa cells containing replicating subgenomic HCV replicons. Disruption of TLR3 signaling is associated with proteolytic cleavage of the TLR3 adaptor protein, TRIF, (but not TLR3, TBK1 or IKKε kinases) by NS3/4A, and is reversed by a specific peptidomimetic protease inhibitor. TRIF proteolysis suppresses dsRNA activation of interferon regulatory factor 3 (IRF-3) and NF-κB dependent promoters controlling expression of many antiviral defense genes and may thus contribute to HCV persistence. However, poly-I:C does not activate IRF-3 or NF-κB in Huh7 hepatoma cells, which are uniquely permissive for HCV RNA replication, due to insufficient expression of TLR3. Virus activation of IRF-3 and NF-κB in these cells occurs via a distinct, TRIF-independent, signaling pathway involving the cellular DExH/D helicase RIG-I, that is also disrupted by the viral protease, suggesting that it targets a second, yet unidentified, signaling molecule.

Papillomaviruses and human neoplasia

C. Simon Herrington

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The human papillomavirus genome encodes a relatively small number of proteins but these proteins exert numerous effects on cellular pathways that control cell cycle progression and apoptosis. The aim of these interactions is to subvert host cell functions and allow replication of the viral genome with subsequent production of virions. However, the high-risk HPV types induce changes that are associated with the accumulation of cells with chromosome abnormalities and that inhibit the elimination of these cells by apoptosis. Under these circumstances, progression to neoplasia may occur. The study of these events has provided considerable insight into the mechanisms of neoplastic progression and will guide the development of therapeutic intervention in HPV-associated disease.

Intracellular antiviral defence mechanisms and viral escape

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After infection of their vertebrate hosts, viruses encounter a formidable task. They have to multiply in the face of a vigorous host immune response. Among the first obstacles are antiviral proteins which operate in a predetermined fashion at the level of the single target cell. Thus, upon entry into cells, viruses may encounter preexisting antiviral restriction factorsthat block virus growth, such as TRIM protein family members. Virus-infected cells then synthesize and secrete type I interferons (IFN-α/β) which warn the body of the dangerous intruders. Secreted IFNs circulate in the body and cause susceptible cells to express potent antiviral mechanisms which limit further viral growth and spread. The dynamin-like MxA GTPase is a good example of such a cell-autonomous IFN-induced effector protein. Human MxA is partly associated with membranes of the endoplasmic reticulum and inhibits the multiplication of several single- or double-stranded RNA viruses. Interestingly, it blocks the infecting virus soon after cell entry by recognizing the viral nucleocapsid protein in a mechanistic process similar to that proposed for the intrinsic antiretroviral protein TRIM5a. MxA is antiviral against a number of arthropod-borne viruses which need extensive replication in the vertebrate host to guarantee their transmission to new arthopods by a blood meal. To succeed, arboviruses have evolved specific viral proteins that counteract the IFN system. As invertebrates do not have equivalent IFN genes, these viral IFN antagonists are most likely an adaptation to vertebrate host, allowing trans-species transmission. It is becoming increasingly clear that most, if not all, viruses have ways to subvert the IFN response by various means. They either inhibit IFN synthesis, bind and inactivate secreted IFN molecules, block IFN-activated signaling, or disturb the action of IFN-induced antiviral proteins. Evidently, viruses and their host's innate immune responses have coevolved, leading to a subtle balance between virus-promoting and virus-inhibiting factors. A better understanding of virus-host interactions is now emerging with great implications for vaccine development and drug design.

Poxvirus immune evasion: tumour necrosis factor and chemokine binding proteins

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Poxviruses encode secreted proteins that bind cytokines and chemokines to modulate the host immune response. Poxvirus soluble tumour necrosis factor receptors (vTNFRs) are thought to act as decoy TNFRs during infection, blocking the antiviral effect of this cytokine. These proteins are differentially distributed amongst poxvirus species, a fact that is thought to be related to the host range and/or evolutionary history of each virus. Ectromelia virus (EV) and variola virus (VaV) both encode a single vTNFR predicted to be active, named CrmD and CrmB, respectively. The N-terminal region of both proteins contains the four cysteine-rich domains (CRDs) characteristic of the cellular TNFRs and we show that this region is sufficient for binding of TNF and inhibition of its cytotoxic activity *in vitro*. In addition, CrmB and CrmD have a C-terminal domain with no similarity to host proteins. We have identified novel chemokine binding activity in the vTNFRs and in three other related proteins encoded by poxviruses. These findings uncover a novel virus-encoded protein domain that binds chemokines.

Evasion of host defence systems by African swine fever virus

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African swine fever virus (ASFV) causes an acute haemorrhagic fever with high mortality rates in domestic pigs and asymptomatic, persistent infections in its natural hosts, warthogs, bushpigs and soft ticks of the species *Ornithodoros*. The disease causes major economic losses in many African countries.

The virus replicates primarily in macrophages. Widespread cell death caused by apoptosis occurs in both T and B lymphocytes in lymphoid tissues and endothelial cells in arterioles and capillaries. Disseminated intravascular coagulation (DIC) develops during acute infections and this may lead to the characteristic haemorrhagic syndrome.

ASFV is a large, cytoplasmic DNA virus and is classified as the only member of the *Asfarviridae*. The virus genome encodes about 150 proteins including enzymes and factors required for replication and transcription of the virus genome. Amongst the other proteins encoded are many which interfere with host defences to facilitate virus persistence. Several of these interfere with activation of host transcription factors and can thus prevent activation of host immunomodulatoy gene transcription. The functions of virus immune evasion genes will be discussed as well as recent data on effects of virus infection on macrophage transcriptional responses.

Molecular pathogenesis of gammaherpesviruses

James P. Stewart

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Gammaherpesviruses such as Epstein–Barr virus and the Kaposi's sarcoma-associated herpesvirus are important pathogens of man. The study of murid herpesvirus-4 (MuHV-4 or MHV-68) infection of laboratory mice has been developed as an amenable model system for studying gammaherpesvirus pathogenesis. In particular, the use of genetically modified mice and targeted gene knockouts in the virus are producing important insights into the mechanisms underlying pathogenesis. Even so, the mouse model does not recapitulate all aspects of gammaherpesviruses pathogenesis and gene knockouts have not been universally successful in assigning functions to viral gene products. We have therefore developed an experimental model system for gammaherpesvirus pathogenesis using the natural host for MuHV-4, that is the wood mouse (*Apodemus sylvaticus*). There are significant differences in the pathogenesis of MuHV-4 in the wood mouse as compared with the laboratory mouse. Analysis of viral mutants in the wood mouse system has now allowed the assignment of functions to specific gene products. Since wood mice are the natural host for MuHV-4, this is an attractive alternative system with which to study gammaherpesvirus pathogenesis.

Special symposium

Emerging diseases of wildlife and farmed animals

Wildlife infections and emerging diseases

M. Bennett

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Abstract not received

Application of genomics to the understanding and control of bovine tuberculosis

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In Great Britain there has been a dramatic increase in bovine tuberculosis over the past fifteen years. A greater understanding of the way *M. bovis* is spread and the development of new tools such as improved diagnostic tests and vaccines are urgently needed to combat the spread of this consummate pathogen.

It has long been thought that human tuberculosis had its origin as a zoonosis, with *M. bovis* jumping the species barrier and host adapting to humans to become *M. tuberculosis* at the time of cattle domestication 10,000–15,000 years ago. However, the completion of the genome sequences of *M. tuberculosis* and *M. bovis* has shown that *M. bovis* evolved from a progenitor of the *M. tuberculosis* complex as a host-adapted clone. Analysis of the genome suggests that the host-adaptation process did not rely on the presence of specific virulence genes per se, but rather on alterations in gene expression, metabolism and exposed components of the cell envelope. The genome sequence will therefore have a major impact on our understanding of the evolution, host adaptation and pathobiology of tuberculosis and, in the longer term, on the generation of vaccine candidates and diagnostic reagents to combat disease. Current approaches towards these goals will be discussed.

Development of vaccines against influenza H5N1 virus

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Cases of highly pathogenic avian influenza virus infection of man have, within the past nine years, raised international concern that we may be on the brink of a global influenza pandemic. If the H5N1 viruses acquire the ability to transmit efficiently from person to person, the effects will be devastating. In response to this threat, early in 2004 the WHO called upon their influenza laboratory network to develop suitable H5N1 vaccine strains which could be made available to influenza vaccine manufacturers. The immediate problem for vaccine development was the fact that the H5N1 viruses were far too dangerous to use for vaccine production. Recent technological developments (reverse genetics) have allowed us to manipulate the influenza virus genome, so that we are able to clone virus gene segments, manipulate the gene segments and reassemble the virus so that it is fully infectious. We can also insert the modified H5 HA and N1 neuraminidase gene segments into a genetic background supplied by the attenuated human influenza virus strain A/PR/8/34. In other words we now have the tools to produce safe vaccine strains from dangerous H5N1 viruses. However the transition of reverse genetics technology from the research laboratory to the manufacturing environment has posed new challenges for WHO laboratories and vaccine manufacturers as well as for veterinary and public health authorities.

In recent years there have been some clinical trials conducted with vaccines derived from novel avian viruses to suggest that different immunisation strategies are needed in order to stimulate protection in man. Such strategies will be discussed.

Progress in fish vaccinology

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Abstract not received

Emerging clones of *Salmonella*

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Salmonella is an important food borne pathogen and a considerable cause of production losses in farm animals. Over time we see a dynamic change in relative importance of clones of *Salmonella*. For example *S. Enteritidis* PT4 and *S. typhimurium* DT104 have caused wide spread outbreaks, but are now not so commonly isolated from farm animals. While we have a considerable insight into molecular aspects of host bacteria interaction in general, we lack information on the particular characteristics that contribute to the success of new clones of *Salmonella*. This presentation will illustrate the dynamics of clones of *Salmonella* using historical data from surveillance in Denmark, introduce the molecular pathogenesis of *Salmonella*, and present results of investigations performed in order to understand which factors contribute to differences between serotypes. Characterization of clones of *S. typhimurium*, *S. enteritidis*, *S.* 4,12:b:-, *S.* Liverpool and a few selected clones of other serotypes was performed in terms of virulence, colonization ability and stress adaptation. Comparison was performed on both phenotypic and gene expression level.

Are we eating our way to antibiotic resistance?

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The principle that resistant bacteria can spread between animals and humans is illustrated by results with nurseothricin, an antibiotic used in livestock in East Germany, and without any analogue used in human medicine. Here plasmid-mediated resistance was selected in animal *E. coli*, which then spread to farm staff; moreover their resistance transferred to *Shigella* spp., which lack an animal reservoir. Similarly, identical resistant *Salmonella* strains circulate in animals, where they were first selected, and in the human population. Counterwise, most clinically-important resistances – e.g. methicillin-resistance in *S. aureus,* penicillin resistance in pneumococci, and pan-resistance in *Acinetobacter* and *Pseudomonas* spp. – involve bacteria without major animal reservoirs. The most contentious cases are enterococci and *E. coli.* The emergence of vancomycin-resistant enterococci (VRE) in humans is partly attributed to the use of avoparcin as a growth promoter in animals, even though the human VRE problem is greatest in the USA, where avoparcin was not used.

Following an EU ban on avoparcin use, the prevalence of VRE in animals has declined, but there is no decline yet in human VRE infections. Resistance to fluoroquinolones and cephalosporins is increasing in human and animal *E. coli*, with particular concern about the proliferation of CTX-M extended-spectrum β-lactamases (ESBLs). Nevertheless we lack proof that the resistant *E. coli* causing e.g. urinary infections in humans originated in animals; moreover the particular ESBLs found in human and animal *E. coli* in the UK are mostly distinct. In summary, antibiotic use in animal husbandry does contribute to the total burden of clinical resistance, but seems a much smaller factor than clinical antibiotic usage itself.

Emerging disease threats to amphibians

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Over the past decade, there has been a growing recognition of the role played by infectious disease on the population dynamics of wild amphibians. Emerging infectious diseases threats to amphibians are predominantly due to ranavirus infections and cutaneous chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis*.

Ranaviruses have been known since the 1960s, but it is only recently that they have emerged as threats to amphibians. In Britain, ranavirus disease has caused recurrent annual epidemics since the late 1980s, characterised by the mass mortality of common frogs (*Rana temporaria*) with death due to systemic haemorrhaging or cutaneous ulceration. While mortality incidents can involve several hundred animals over a short time period, it is unclear how much of a threat ranavirus poses to amphibian populations.

Cutaneous chytridiomycosis is considered to be the most important emerging disease threat to amphibians world-wide. It has low host specificity, infecting at least 14 families and 93 species on 5 continents and causing severe population declines in many of these, including a small number of global extinctions. The origin of *B. dendrobatidis* is unknown. It was first discovered in 1996 and remains the only chytrid fungus known to infect a vertebrate host.

Morbillivirus infections in aquatic mammals – origins and predisposing factors

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Abstract not received

Systematics & Evolution Group / Clinical Microbiology Group joint session

Molecular typing and epidemiology

Genomic analysis and its use in the study of epidemiology

S. Andersson

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Abstract not received

The evolution of virulence and host specificity in the *Pasteurellaceae*

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The *Pasteurellaceae* includes important pathogens of man and domesticated animals. Bacterial species within this family colonise the upper respiratory tract and characteristically have a very narrow host range; in many cases bacteria are adapted to a single host species or to a group of closely related host species. The close relationship between pathogen and host suggests long periods of co-evolution and adaptation. Evidence for this is provided by the fact that certain virulence factors (e.g. leukotoxin, transferrin receptors) are highly specific for cells or molecules (e.g. leukocytes, transferrin) of the affected host species. *Mannheimia* (*Pasteurella*) *haemolytica* is an important respiratory tract pathogen of cattle and sheep. However, disease-causing strains of *M. haemolytica* from cattle and sheep represent distinct sub-populations that are specifically adapted to one or other of these host species. Therefore, *M. haemolytica* provides an extremely useful model organism in which to study the molecular evolutionary genetics of host adaptation and strain evolution. Comparative sequence data from various housekeeping and virulence genes will be presented to show that host-switching of strains from cattle to sheep, and *vice versa*, together with horizontal DNA transfer and recombination, have played important roles in the evolution of *M. haemolytica* and in the emergence of new host-adapted strains.

Rapid sequence-based identification of gonococcal transmission clusters

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Gonorrhoea has increased considerably in the last five years and is most prevalent among high-risk groups predominantly in inner city areas. Tracing of contacts, to identify outbreaks and transmission chains, has been traditionally performed by partner notification and is often problematic. Molecular typing has the potential to identify linked patients that are not evident by epidemiological information alone, and exploits the genetic diversity of gonococci to resolve a large number of genotypes. Isolates from patients who are linked contacts or part of a transmission chain will then appear indistinguishable and, provided the typing method is highly discriminatory, clusters of identical isolates can be used to identify linked patients within communities. If this approach is to be useful to inform interventions it is essential to use a method that has high throughput and is robust. We have used nucleotide sequencing of two hypervariable genes, *por* and *tbp*B, to provide a highly discriminatory method, NG-MAST, that produces unambiguous data that is analysed through www.ng-mast.net. NG-MAST was validated using retrospective analysis of gonococci from known sexual contacts and for its ability to identify clusters. The feasibility of using NG-MAST to identify clusters of linked cases of gonorrhoea in London will be discussed.

Candida albicans: the role recombination plays in diversification

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Recently, components of a mating system were described for *Candida albicans*. This mating system exhibits truly unique features, some of which suggest that mating is intimately integrated into host–pathogen interactions. Mating has been demonstrated *in vivo* and *in vitro*, and occurs readily between unrelated strains, even strains from different clades. However, a number of population genetics studies have indicated that propagation is primarily clonal, although there are indications of low levels of recombination. These low levels of recombination, however, do not necessarily have to be the result of mating. The number of genes involved in *C. albicans* mating is quite large, and the unique developmental programs that have been integrated into the mating system complex. One must therefore wonder why maintain this complex system if recombination is so rare? Two answers are considered. First, although rare, recombination events generated by mating may be necessary for the success of the species. Second, mating may be involved in a process other than recombination that is equally essential for success, namely pathogenesis.

Analysis of the gene clusters for capsular polysaccharide biosynthesis from all 90 serotypes of *Streptococcus pneumoniae*

S. Bentley

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Strains of *Streptococcus pneumoniae*, a major cause of morbidity and mortality, worldwide, are divided into 90 individual serotypes based on immunochemical differences in their capsular polysaccharide (CPS). These serological differences are crucial to diagnosis and epidemiology and multi-valent conjugate vaccines based on the CPS from the serotypes most commonly associated with invasive disease have proved to be effective. Genes for the biosynthesis of CPS are, in general, located between *dexB* and *aliA*. We have cloned and sequenced the DNA from this region for all 90 serotypes. Combining annotation and analysis of this data with the available polysaccharide structures, and the patterns of serological reactions with typing sera, have allowed us to explore the genetics of capsular diversity in this major pathogen. We are able to account for differences between CPS within serogroups and\demonstrate close relationships between previously unlinked serotypes. Clustering of protein sequences has increased our understanding of the function and specificity of biosynthetic enzymes and the nature of genetic differences between close relatives give clues to the evolution of the gene clusters. The availability of the sequences of all 90 *cps* loci will allow molecular serotyping of pneumococci, either by targeting individual gene sequences diagnostic for each serotype, or using microarrays that include all *cps* genes and recognise each serotype by its characteristic gene profile. However, the close similarity among some serotypes may necessitate additional tests to distinguish them.

Molecular epidemiological approaches to the study of foodborne diseases

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Foodborne diseases are estimated to cause 76 million illnesses in the United States each year, leading to 325,000 hospitalizations and 5,000 deaths. These illnesses occur as sporadic cases or outbreaks. While the causes of sporadic illnesses are rarely identified, foodborne disease outbreaks present unique opportunities to identify the etiologic agent, its food vehicle of transmission, and the circumstances that led to the contamination of the food.

PulseNet USA was established in the United States in 1998 as an early warning system for foodborne disease outbreaks. Four bacterial pathogens (*E. coli* O157:H7, *Salmonella*, *Shigella* and *Listeria monocytogenes*) are routinely monitored by PulseNet by their DNA 'fingerprints' generated using highly standardized pulsed-field gel electrophoresis protocols. As soon as a cluster of clinical isolates of a pathogen with indistinguishable DNA fingerprints is identified, the patients in the cluster are rapidly interviewed. If preliminary findings indicate potential epidemiologic links between patients, that cluster is designated as an outbreak and a detailed investigation is initiated. This strategy has enabled the U.S. public health system to identify foodborne disease outbreaks that would not have been previously identified and has allowed outbreak identification and recall of a food product with as few as four cases in a cluster.

Emergence and evolution of MRSA

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most serious hospital-acquired pathogen in many countries and in the UK isolation rates have risen dramatically, from ~1.5% (of *S. aureus* bloodstream infections) in 1989 to 46.1% in 2001. An increasing proportion of these isolates are multiply antibiotic resistant and there is concern that MRSA isolates may soon emerge which are virtually untreatable with currently licensed antibiotics.

We have developed a multilocus sequence typing (MLST) scheme for *S. aureus* and this has been invaluable in many studies of MRSA epidemiology by scientists in many different countries. Pandemic MRSA clones, which cause the vast majority of nosocomial infections worldwide, have emerged in only five lineages of *S. aureus* and detailed phylogenetic analysis using MLST (housekeeping genes) loci and more variable targets (genes coding putative surface-exposed proteins) have allowed us to construct detailed evolutionary models for each of these. The results of these analyses show fundamental differences in background genotype between isolates causing community- and hospital-acquired infections. Worryingly however there is evidence that genetic exchange within the species is blurring the distinction between hospital and community isolates. This has resulted in the spread of 'hospital' clones into the community and it may allow more virulent and transmissible community isolates to enter hospitals – increasing the host range of MRSA.

VNTR analysis of *Yersinia pestis* delivering new perspectives in its phylogeny

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Yersinia pestis, the agent of plague, is a young and highly monomorphic species. Little is known about the origin and phylogenetic evolution of its three present biovars. Variable number of tandem repeats (VNTR) provide valuable polymorphic markers for genotyping and performing phylogenetic analyses. A collection of 200 *Y. pestis* isolates investigated by multiple locus VNTR analysis (MLVA) falls into four main groups. In particular, the Antiqua biovar is split into strains from Africa and strains from Asia. We also investigated the organisation of three remarkable repetitive elements called CRISPRs, consisting of repeats interspaced with non-repetitive elements or 'spacers'. In the different strains, the order of these repeats is always the same but some are deleted from an ancestral locus and others represent new acquisitions. In *Y. pestis* of the Orientalis biovar investigated in detail, addition of new spacers is observed and is polarized. The CRISPR structure provides a new and robust identification tool which may be of particularly high value to investigate ancient DNA. The data generated by the two approaches point to a *Y. pestis* evolution scenario in which all three biovars originate from Asia.

Helicobacter diversity and what it reveals about human migration

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Abstract not received

Clinical Microbiology Group / British Society for Antimicrobial Chemotherapy joint session

Antibiotic resistance

Efflux pumps: mechanistic aspects and regulation of expression

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Several families of multidrug efflux systems have been described in bacteria, with the Resistance-Nodulation-Division (RND) family being the most widely distributed and clinically significant in Gram-negative bacteria. These tripartite pumps comprise an inner membrane drug-proton antiporter (the RND component) an outer membrane (OM) channel (the OM factor; OMF) and a periplasmic link protein that joins the membrane components (the membrane fusion protein; MFP). The opportunistic human pathogen *Pseudomonas aeruginosa* elucidates several tripartite RND family efflux systems of which four, MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM have been implicated in intrinsic and/or acquired multidrug resistance in lab and clinical isolates. Hyperexpression of the various RND pumps in acquired multidrug-resistant strains is achieved via mutation in a number of different regulatory and structural genes, highlighting the complexity of RND-type multidrug efflux gene regulation in *P. aeruginosa*. Moreover, transposon insertion mutagenesis and gene cloning studies have identified a number of additional genes required for/involved in efflux gene expression and/or efflux-mediated multi-drug resistance. These studies provide evidence for novel functions for these broadly-specific efflux systems whose roles in processes other than antimicrobial export have been suggested for some time.

Antimicrobial efflux pumps: clinical and veterinary significance?

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Genomic analyses have revealed that multi-drug efflux pumps are ubiquitous throughout nature. Genetic and biochemical experiments have revealed that some of these export antimicrobial agents from the bacterium. Typically, if the organism is Gram negative the pump protein is from the RND class, and if Gram positive the pump is from the MFF. Some agents are also exported by ABC transporters. Efflux has been shown to confer inherent resistance to some agents in some bacteria, e.g. Pseudomonas aeruginosa, but in other species increased MIC values are only observed when there is over-expression of the efflux pump. Enhanced efflux usually gives rise to 2–8 fold increases in the MIC. Whether this increase is sufficient to cross the recommended breakpoint concentration 'barrier' is species and drug dependent. Over-expression of efflux pumps coupled with other resistance mechanisms (e.g. mutation(s) in gyrA) give rise to highly resistant bacteria. *P. aeruginosa*, Acinetobacter spp, *S. pneumoniae*, *S. aureus, E. coli*, *S. Typhimurium* and *C. jejuni* with these multiple mutations have been isolated from clinical and /or veterinary specimens. Data suggests that increased efflux in these isolates is due to mutation in genes controlling the expression of efflux pump genes or the presence of IS elements up-stream of these genes.

Serine beta-lactamases: X-ray structures, mechanism, inhibition, and mutation

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Atomic-level structures of class A β-lactamases (effective penicillinases), class C β-lactamases (cephalosporinases) and class D β-lactamases (oxacillinases) will be compared. Differences in their specificities and catalytic efficiencies for β-lactam substrates will be related to differences in binding site architectures. Crystallographic structures of complexes with β-lactam reaction intermediates and with tazobactam-type inhibitors will be examined, with a brief outline of possible hydrolytic and inhibition mechanisms. The consequences of natural mutations providing extended-spectrum activity or inhibitor resistance will be rationalized on the basis of structural changes seen by X-ray crystallography or proposed from molecular modeling. A goal of the talk is to help microbiologists and microbial geneticists understand how changes in antibiotic or inhibitor reactivity are exquisitely tied to mutational changes in three-dimensional enzyme structure.

Beta-lactamase induction mechanisms

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The primary mechanism of resistance to β-lactam antibiotics in bacteria is the production of β-lactamase enzymes. Many bacteria carry β-lactamase genes on their chromosomes, and have evolved sophisticated mechanisms to regulate the expression of these genes in response to β-lactam challenge. This talk will give a detailed overview of β-lactamase induction mechanisms, primarily in Gram-negative bacteria. It will describe the link between β-lactamase induction and peptidoglycan recycling in the AmpR-mediated regulatory system present in many clinically important bacteria, and will show how knowledge of this mechanism can be used to develop β-lactamase expression inhibitors. The talk will also discuss recent experiments concerning the β-lactamase regulator in *Aeromonas* spp., which centres on a two-component system, BlrAB, and responds to β-lactam-mediated inhibition of DD-carboxypeptidase activity. This mechanism has a number of important properties; for example: it regulates the expression of multiple genes, including three β-lactamase genes. Recent work concerning the β-lactamase regulatory system in the human pathogen, *Stenotrophomonas maltophilia* will also be outlined. This bacterium carries two β-lactamase genes, and appears to possess two separate induction mechanisms; one is mediated by AmpR, and the other by an unknown regulator.

Extended-spectrum beta-lactamases (ESBLs): clinical implications

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Extended-spectrum beta-lactam (ESB) antibiotics (e.g. ceftazidime, etc.) were introduced into clinical practice in the mid-1980s and are used extensively for the empirical treatment of serious infections caused by Gram-negative bacilli and surgical prophylaxis. Very shortly after their introduction, mutations occurred in the TEM and SHV beta-lactamase genes which altered the active site of the enzymes enabling hydrolysis of ESBs. These enzymes are termed extended-spectrum beta-lactamases because they are associated with transposons/IS elements and have become widely distributed amongst Gram-negative *Enterobacteriaceae,* particularly *Klebsiella* and *E. coli.* The range of mutations seen in both TEM and SHV is now extensive and, in different countries and districts

within those countries, different sub-types of ESBLs have become prominent. In addition, recently CTX-M ESBLs became widely dispersed in both South America and the Far East. Both the proportion of bacteria producing ESBLs and the genetic diversity of the genes encoding them are expanding rapidly and they now pose a major threat to the clinical use of the major group of antibiotics active against Gram-negative bacteria.

Molecular mechanisms of AmpC gene expression and the clinical implications associated with Gram-negative pathogens

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The therapeutic usefulness of β-lactam antibiotics is severely limited when bacterial pathogens overproduce an AmpC β-lactamase. The overproduction of AmpC renders most Gram-negative organisms resistant to all β-lactam antibiotics except cefepime, cefpirome, and carbapenems. The genes encoding AmpC can be found on the chromosome and/or plasmids of Gram-negative organisms. Studies have focused on examining the regulation of *amp*C expression from chromosomal sources including *Escherichia coli*, *Enterobacter cloacae*, *Citrobacter freundii*, *Serratia marcescens*, and *Morganella morganii*. In all of these organisms except *E. coli*, the chromosomal *amp*C gene is inducible. Since the late 1980s, chromosomal genes of inducible gene origin have been detected on plasmids but in most cases without the ability to be induced. These plasmids have been detected in organisms, such as *Klebsiella* spp., which do not express an AmpC. Recently, studies have examined the mechanism of plasmid-mediated gene expression and how expression levels influence the susceptibility of the organism. Plasmid-mediated AmpC β-lactamases complicate the job of clinical microbiologists due to the difficulties of accurately detecting AmpC-producing organisms. Because overproduction of AmpC is critical for the resistant phenotype it is imperative that we understand the mechanisms of overexpression and how those mechanisms influence the susceptibility and therefore detection of this resistance mechanism.

Metallo β-lactamases: structure, function and clinical significance

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The arsenal available to bacteria to avoid the action of antimicrobials can take on many guises but the prolific of these are β-lactamases, enzymes that bind and hydrolyse β-lactam antibiotics, rendering them functionally impotent. β-Lactamases can be arbitrarily categorised into two groups: serine- and metallo-. Serine β-lactamases include enzyme types such as TEM (molecular class A), SHV (class A), AmpC (class C), OXA (class D) and hydrolyse β-lactams by binding to and forming a covalent intermediate with the substrate. In contrast, metallo-β-lactamases (class B) do not physically engage the substrate but co-ordinate zinc molecules that instigate the hydrolysis of the β-lactam via a polarised water molecule. The majority of these enzymes have two zinc molecules per molecule of enzyme although their binding affinity can vary considerably.

A number of metallo-β-lactamases are intrinsically encoded but these are often produced by environmental bacteria, the majority of which are not clinically relevant. Those metallo-β-lactamases encoded by mobile genes are usually found in *Pseudomonas aeruginosa* and *Acinetobacter* spp., although a number have recently been found in Enterobacteriaceae. At present, there are four types of metallo-β-lactamase whose genes are mobile: IMP, VIM, SPM and GIM. There are approx. 18 different types of IMP, 11 types of VIM and, to date, just the single variant of both SPM and GIM. The genes that encode these enzymes vary in the way in which they are mobilised. The bla_{VIM}, bla_{IMP} and bla_{GIM} genes form gene cassettes imbedded into integrons (usually class 1) and can be associated with transposons, often flanking the integrons. In contrast, the South American metallo-β-lactamase, bla_{SPM}, is not associated with an integron or transposon but a genetic element known as a 'common region(s)'; their role in the mobility of these genes has yet to be elucidated. Metallo-β-lactamases are capable of hydrolysing all classes of lactams and, at present, no clinical inhibitor exists – nor is one likely to within the next 6–8 years.

The clinical laboratory is faced with a selection of choices both to screen and detect metallo-β-lactamases. These range from screening plates, double disk diffusion and more specialised methods like the Etest. The clinical importance and recent dissemination of metallo-β-lactamase necessitates the implementation of effective screening and surveillance systems, the data from which will predict their potential problem.

Methicillin and vancomycin resistance in *Staphylococcus aureus*, mechanisms and evolution

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The historically first isolates of MRSA already carried resistant factors to several antibiotics (penicillin, streptomycin and tetracycline and often to erythromycin as well) that have been used in clinical practice prior to the introduction of oxazolin-penicillins, such as methicillin. The methicillin resistance determinant *mecA* encodes a low affinity penicillin binding protein – PBP2A – which is assumed to take over the cell wall synthetic transpeptidase (TPase) functions of the four native PBPs of *S. aureus* when these become inactivated by the antibiotic in the medium. How this surrogate TPase can generate high-level resistance for the bacterium is much less clear. Virtually all early isolates of MRSA exhibit the peculiar heterogeneous phenotype in which the great majority of the cells in a culture exhibit only modest levels of resistance. In some exceptional cases such as MRSA strain COL extremely high-level resistance is shared by all cells of the culture. However Tn*551* mutagenesis has identified over 25 genes in the background of strain COL each one of which is essential for the expression of high-level resistance. The profound influence of these so called auxiliary genes has lead to the conceptually novel proposal that the methicillin resistant phenotype is the product of a stress response pathway in which several background genes have to cooperate with *mecA*. Recent probing of the nature of this cooperation has already identified functional cooperation between the transglycosylase (TGase) domain of the native staphylococcal PBP2 and the TPase domain of PBP2A. An evolutionary 'attempt' to integrate PBP2A into the regulatory circuitry of cell wall synthetic apparatus of the host cell is also indicated by the recently observed parallels of the transcription of the staphylococcal murE, pbpB and the acquired mecA. The worldwide spread of multidrug resistant MRSA clones increasingly lead to the virtual universal use of vancomycin as the last resort antibiotic against such infections. Predictably this enormous selective pressure has lead to the recent emergence of two kinds of vancomycin resistance mechanisms: one involving the 'entrapment' of the antibiotic molecule in the cell wall and the second one which involves the acquisition of the enterococcal Tn1456 (vanA). Such highly vancomycin resistant strains produce an abnormal cell wall precursor unrecognizable by the antibiotic molecule. This development brings the prospect of uncontrollable *S. aureus* infections uncomfortably close.

Clinical Virology Group session

Emerging infections: dangers to human and animal health

Epidemiology and prediction methods for emerging viruses

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Novel pathogens continue to emerge in human, domestic animal, wildlife and plant populations, yet the population dynamics of emergence remain poorly understood. This paper considers the epidemiological and evolutionary processes underlying the initial introduction and subsequent spread of a pathogen in a new host population, with special reference to pathogens that originate by jumping from one host species to another. A review of the literature suggests that, although pathogen emergence is inherently unpredictable, emerging pathogens tend to share some common traits, and that directly transmitted RNA viruses may be most likely to jump between host species. Well known examples include the viruses causing Ebola, SARS and HIV in humans and phocine distemper in seals, but there are counter-examples, e.g. canine parvovirus in dogs. Other factors associated with emergence include a broad host range, use of phylogenetically conserved cell receptors, and occurrence in areas experiencing ecological, demographic or social change. Regarding the subsequent spread of an emerging pathogen in a new host population, recent work on SARS, avian influenza and foot-and-mouth disease virus has concentrated on providing timely estimates of the basic reproduction number, which provides a measure of the potential for a major epidemic.

Zoonoses and virus evolution

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Abstract not received

Avian influenza

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Influenza A viruses cause natural infections of humans, other mammals and birds. Few of the 15 haemagglutinin and 9 neuraminidase subtype combinations have been isolated from mammals, but all have been isolated from birds. There are enormous pools of influenza A viruses in wild birds, especially migratory waterfowl.

In the 20th Century there were 4 pandemics of influenza due to the emergence of antigenically different strains in humans: 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and 1977 (H1N1). Influenza A viruses contain 8 distinct RNA genes and reassortment of these can occur in mixed infections with different viruses. The 1957 and 1968 pandemic viruses differed from the preceding viruses in humans by the substitution of genes that came from avian viruses, suggesting they arose by genetic reassortment of viruses of human and avian origin.

Up to 1995 there had been only three reports of avian influenza viruses infecting humans, in 1959, 1977 and 1981 [all H7N7], two were the result of laboratory accidents. Since 1996 there have been regular reports of natural infections of humans with avian influenza viruses: in England in 1996 [H7N7], Hong Kong 1997 [H5N1], 1999 [H9N2], and 2003 [H5N1], in The Netherlands 2003 [H7N7], Canada 2004 [H7N3], Vietnam 2004 [H5N1] and Thailand 2004 [H5N1]. The H5N1 virus is alarming as 40% [37/90] of the people confirmed as infected have died. Although there has been very little human to human transmission, these infections are a concern since if people infected with an 'avian' virus were infected simultaneously with a 'human' virus reassortment could result in the emergence of a virus capable of spread in the human population, but with an HA for which the human population was immunologically naive.

Emerging paramyxoviruses

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During the last two decades paramyxoviruses have emerged as causes of spectacular disease outbreaks. While some viruses, such as morbilliviruses from marine mammals were classified in existing genera, novel properties of others such as Hendra (HeV) and Nipah (NiV) led to the establishment of a new genus, *Henipavirus* within the subfamily *Paramyxovirinae*. The natural hosts of HeV and NiV are fruit bats in the genus *Pteropus*. These so-called flying foxes also harbour two new members of the *Rubulavirus* genus, Menangle and Tioman viruses. Flying foxes are distributed from Madagascar, through the Indian subcontinent to Australia and are found in greatest diversity in Sulawesi and eastern New Guinea. They are the largest bats in the world, do not echolocate and navigate by eyesight and smell. The emergence on a number of occasions of both HeV in Australia and NiV in Malaysia and Bangladesh will be described and their novel molecular characteristics summarized. Attributes which make both Menangle and Tioman viruses unique among rubulaviruses will be identified. The recent genomic analyses of several rodent paramyxoviruses isolated over two decades ago indicate that paramyxoviruses continue to hold surprises for those interested in the evolution and epidemiology of this virus family.

International surveillance for transboundary animal diseases

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Abstract not received

Laboratory preparedness for dealing with epidemics of foot-and-mouth disease

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The foot-and-mouth disease (FMD) epidemic of 2001 graphically illustrated the difficulty in providing sufficient capacity and speed of testing to confirm multiple outbreaks and to demonstrate disease freedom at the end of the epidemic. The requirement to slaughter animals on infected premises within 24 hours of suspicion being notified meant that the results of laboratory tests were not available in time to inform such decisions. Serosurveillance of more than 3 million sheep, to prove freedom from infection, was urgently needed after the epidemic so that trade restrictions could be lifted and this placed heavy burdens on the National Laboratory, requiring additional laboratories to be commissioned. Since then, the options for FMD control have been widened to permit emergency vaccination followed by the

retention of vaccinated animals providing they can be confirmed as free of infection. Progress will be reported on the subsequent development of laboratory contingency plans with emphasis on the development and validation of new test methodologies to increase throughput and speed and in support of the vaccinate-to-live policy.

A novel approach to characterise pathogen candidate mutations involved in clinical outcome

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As infectious diseases are responsible for 48% of deaths worldwide, understanding the interaction between disease and host is crucial for early diagnosis and optimised treatment. With the extraordinary development of gene mapping, the focus has been on the detection of susceptibility and resistance alleles in humans. In contrast, very little has been done to investigate the role of pathogen genotypes, even though its influence is widely acknowledged. Here, we propose a new methodological framework to detect candidate mutations within the pathogen that could influence the outcome of an infection, whilst controlling for host factors. We use a phylogeny-based approach coupled with a generalised linear modelling that allow us modelling the role of viral genotype without loosing statistical power. We illustrate this new method by an application on a dataset of hepatitis B polymerase genes. The statistical model retains age at infection as well as six candidate mutations as predictors for clinical outcome (acute, chronic and fulminant). Our method proved user-friendly, and had low computational cost. This framework could easily be applied to other pathogens and might find even broader application outside host-pathogen interaction to clarify link between genotype and phenotype while controlling for environmental factors.

Potential for hantavirus transmission in the UK

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Hantavirus disease is an important worldwide zoonosis, the incidence of which appears to be increasing. In NW Europe, the serotypes associated with human infection are Puumala virus (PUUV), transmitted by *Clethrionomys glareolus* (Bank vole) and Dobrava virus (DOBV), transmitted by *Apodemus* flavicollis (Yellow-necked mouse). Both rodents are present in the UK, however there is currently insufficient evidence of transmission of the disease to humans from small mammals. This may be due to limited surveillance or ecological/social scenarios that prevent or limit virus transmission to humans. This work investigates the current ecological scenario for PUUV transmission in NW Europe and assesses the potential for hantavirus transmission in the UK.

An analytical review of human and rodent hantavirus outbreaks in NW Europe, together with knowledge of the distribution and population dynamics of UK mammal fauna in response to (a)biotic factors, has enabled an assessment of the potential for PUUV transmission to humans.

(A)biotic risk factors for PUUV have been identified and a semi-quantitative assessment developed of the spatio-temporal dynamics of potential transmission in the UK .

This work strengthens evidence that, in the UK an ecological scenario, involving climate, tree mast and *C. glareolus* dynamics exists, which is similar to scenarios in PUUV-endemic areas in NW Europe.

European bat lyssavirus type-2: risks of spillover from bats to other mammals

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European Bat Lyssavirus (EBLV) type-2 is a member of the genus *Lyssavirus*, family *Rhabdoviridae* and was responsible for a human case of rabies in Scotland (2002). We have investigated the ability of EBLV2 to cross the species barrier into domestic ferrets and sheep, which are species where natural cases of spillover have been reported. These animals showed 'sterilising immunity' probably mediated via the induction of neutralising antibodies. Our data implies that not all EBLV2 exposures are lethal with a significant proportion leading to an 'abortive infection' suggesting that EBLV2 pathogenicity may differ compared with strains of classical rabies virus (RABV). These data provide further evidence to support the observation for the widespread prevalence of EBLV2 specific antibodies in Daubenton's (*Myotis daubentonii*) bats in the absence of live virus. These observations may explain why rabies epizootics as a result of EBLV host switching has never been reported whereas epizootics due to host switching by RABV are reported regularly. However, new lyssavirus bat variants have recently been identified in Europe therefore surveillance is essential. The threat to public health, especially in high-risk groups is significant and the possibility of adaptation of a bat variant to a susceptible host should not be underestimated.

Single nucleotide polymorphisms (SNPs) associated with symptomatic infection and differential human gene expression in normal seropositive persons each implicate the cytoskeleton, integrin signalling and oncosuppression in the pathogenesis of human parvovirus B19 infection

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Background The host response to parvovirus B19 is important in development of symptoms during acute infection and consequences of viral persistence.

Methods A. Genomic DNA from 42 cases of symptomatic B19 infection was compared to 53 normal controls using the Affymetrix Hu-SNP chip. B. Total RNA from peripheral blood mononuclear cells from 57 B19 seropositive and 13 B19 seronegative blood donors was analysed using a Nimblegen gene array representing 9,522 human genes.

Results A. 57 SNPs were significantly associated with symptomatic infection. This association was confirmed by PCR for 5 SNPs. Twelve SNPs occurred within genes with a role in the cytoskeletal/integrin signalling and three occurred within genes that negatively regulate cell growth. There was no association between carriage of particular SNPs and development of particular symptoms. B. 92 genes were differentially expressed in normal seropositive persons; this was confirmed by PCR in a different group for 6/42 genes. Four of these 6 confirmed genes play a functional role in integrin signalling/ cytoskeleton and one is a tumour suppressor.

Conclusion Our findings suggest involvement of integrin signalling, the cytoskeleton and oncosuppression in the pathogenesis of B19 infection, both during the acute phase and after resolution.

The application of bioinformatics in determining the status of high grade HPV

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High risk human papillomaviruses (HPV) are associated with cervical intraepithelial neoplasia (CIN) that can progress to cervical cancer. The physical status of HPV DNA plays an important role in malignant transformation. The integration of HPV DNA into the host genome is indicative of advanced genital tumour. The virus persists in episomal form in the early stages (CIN I), however transformation to malignant state (CIN III), involves disruption of viral *E2* gene and integration of the *E6* and *E7* genes into human chromosomes. This results in the up-regulation of oncogenic proteins. The increase in viral load has been correlated with the increase in CIN. Primers were designed, according to phylogenic relationship using NCBI BLAST search, multiple sequence alignment and homology using ClustalW, for the *E2* gene for 10 'high-risk' HPVs to determine any malignant tendencies. A set of primers of 20 bases were designed, which incorporated the signature sequences at the 3' end of the oligonucleotide.

These primers were tested on 50 Liquid Based Cytology (LBC) samples by both conventional and real time PCR using SYBR Green as a fluorophore to detect the amplification products. The results obtained suggest that the *E2* gene of high risk HPV is not very well conserved and that it may require a cocktail of primers to differentiate each phylogenetically related group.

Detection and quantification of high risk HPVs from LBC using real time PCR

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Cervical cancer is one of the major malignancies in women causing 250,000 deaths worldwide each year. Persistent infection with high grade HPV is one of the major factors for the pathogenesis of cervical cancer. However, a large proportion of females infected with HPV are able to clear the infections and do not show any changes in cervical intraepithelial neoplasia.

Early detection and quantification of the viral load can be useful in monitoring the progression of HPV infection and therefore preventative action can be taken.

Previous work carried in our laboratory by Rughooputh and co-workers (2004) showed that Liquid based cytology (LBC) can be used for the detection of high grade HPV in conventional PCR followed by gel electrophoresis.

This study focuses on the use of SYBR Green in quantitative PCR for the detection of high grade HPV. DNA from 50 samples were tested for the presence of high grade HPV using GP5+, GP6+; a set of degenerate primers that target the *L1* gene of high grade HPV instead of specific primers which can present complexities in detection and quantification of the viral load. *Gamma actin* a house-keeping gene was used as an internal control to determine the quality of DNA extracted. Positive and negative controls were included. All the results were confirmed by running the amplicons on 1% agarose gel electrophoresis.

Investigation of human respiratory specimens for the presence of human metapneumovirus

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Human metapneumovirus (hMPV) produces seasonal respiratory illness in infants and children worldwide. Our aims have been to assess the hMPV disease burden in Newcastle-upon-Tyne, to identify the virus strains responsible for epidemics in successive years and to evaluate the replication of different virus genotypes in a human bronchiolar cell line, 16-HBE-40.

In 2002–3 between December and March, 383 respiratory specimens submitted to the Newcastle HPA and found negative for other respiratory viruses were tested by RT-PCR using primers in the fusion (F) and nucleoprotein (N) gene of hMPV. N gene amplicands were sequenced. Twenty nine (7%) specimens were positive of which 74% were classified as group A1 (15% as A2, 11% as B1 and 0% as B2). In the second season (2003–4) specimens were screened by both RT-PCR and immunofluorescence using a polyclonal rabbit antiserum to a B1 hMPV strain and all positive specimens were inoculated onto 16-HBE-40 cells. 6% (21/346) of specimens were RT/PCR positive with 81% falling into group B1 (0% B2, 0% A1 and 19% A2). Two specimens were only positive by RT-PCR and 9 only positive by immunofluorescence. All but one of the sequenced B1 strains and all of the sequenced A2 strains were isolated on 16-HBE-40 cells.

Genetic characterization of hepatitis A virus circulating in Seoul, Korea

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Hepatitis A viruses infection have been increasing for adults in South Korea in spite of improving quality of personal hygiene and environmental sanitation. To characterize the genetic variability of HAV circulating in South Korea, sequence analyses for 168 bp of VP1/2A junction of HAV obtained from stool specimens from 26 patients were performed and compared with those of 10 Korean and 99 foreign isolates derived from GenBank. Of those, 15 (57.7%) samples were positive for HAV RT-PCR amplification. Phylogenetic analysis revealed that 14 strains were an unique genotype 1A, which is the majority type all over the world. Interestingly, genotype IIIA with homology of 97.6% of Indian strain was detected from one patient who had a history of traveling in India before symptom manifested. This finding provided valuable new data on the genetic relatedness of HAV from South Korea and suggested the possibility of endemic transmission for existing of an unique cluster.

Genotypes of enteroviruses causing aseptic meningitis and febrile illness in Kuwait

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Enteroviruses are important causative agents of various diseases such as myocarditis, febrile illness, encephalitis, acute hemorrhagic conjunctivitis, hand foot and mouth disease and particularly aseptic meningitis. This study aimed at investigating the role of enteroviruses in causing aseptic meningitis and febrile illness in Kuwait and to determine the prevalent enterovirus types in these diseases.

'Semi-nested Reverse Transcription PCR (snRT-PCR) based-assay was used for the detection of enteroviruses directly in clinical specimens obtained from patients presenting with aseptic meningitis or pyrexia of unknown origin. This assay was developed to rapidly detect as few as 10 copies of enteroviral genome in clinical specimens. The presence of enteroviral RNA in the snRT-PCR positive clinical specimens was confirmed by Southern hybridization of the amplified product with an enterovirus specific probe. The typing of the enteroviruses in the positive specimens was performed by amplification of 575 bp of the 5'-UTR, followed by DNA sequencing.

Of the 437 CSF samples referred to our laboratory from patients suspected of aseptic meningitis (80% were from children less than 12 years), enteroviral RNA was detected in 134 of 437 (30%) of these cases. Further, enteroviral RNA was also detected in the blood of 25 of 88 (28%) patients presenting with pyrexia of unknown origin. Genotyping of the positive isolates of enteroviruses from cases of aseptic meningitis and febrile illness showed a predominance of echovirus type 9, followed by coxsackievirus A7.

The snRT-PCR assay proved to be useful in investigating the prevalence of enteroviruses in cases of aseptic meningitis and febrile illness. Identification of enterovirus types based on sequencing showed a predominance of echovirus type 9 and coxsackievirus A7 in the enteroviral cases that were investigated.

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Development of a quality assured, real-time PCR containing amplification controls for foot-and-mouth disease virus detection

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Foot-and-Mouth Disease (FMD) affects several livestock species of economic importance. The causative agent is the Foot-and-Mouth Disease Virus (FMDV), a *picornavirus* consisting of a single-stranded, 8 kb (+) RNA genome. Rapid and accurate identification of FMDV within these target animals is critical for controlling FMDV dispersion. We have developed a quality assured, one-step real-time PCR assay for FMDV to accompany our existing virus identification protocols. This assay was successfully utilized on three different real-time PCR platforms. PCR specificity was demonstrated as 23 FMDV isolates representing all seven serotypes amplified successfully without detection of other vesicular viruses.

To ensure FMDV positive reactions are not contaminants from preceding reactions, a synthetically-derived oligonucleotide positive amplification control containing the primer and probe sequences was constructed. The resulting 130 bp amplicon can be size-differentiated from the 88 bp FMDV product. To safeguard against false negative results, a parallel assay to monitor RNA integrity from the sample was developed. Primers and a probe were developed across several target species to detect endogenous beta actin RNA within the isolated sample. The FMDV and beta actin PCR assays were applied to detect FMDV in several diagnostic specimens from experimentally infected cows, pigs, and sheep.

Unusual CNS diseases in renal transplant patients

Judith Timms

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These 2 patients presented during 2004 with signs of CNS disease.

The first was an 18 year old, transplanted 12 years previously, who initially appeared to have benign intracranial hypertension. However, the patient subsequently deteriorated and died from fulminant meningitis and cerebral ischaemia.

The second patient received her renal transplant 26 years ago. At first she was generally unwell and with a fall in Hb and platelets, but then developed focal neurological signs.

The same virus was detected by PCR in the CSF of both patients. The clinical features, CSF and radiological findings of each case are discussed.

Education & Training Group session

Virology: is it practical?

Virology teaching: how safe is it?

J.D. Williamson

Abstract not received

Hands on/hands off practicals

R.A. Killington

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This presentation overviews the aims and objectives of laboratory practicals in the context of virology. Both formative and summative examples of practical exercises will be discussed. Experiments include those involving tissue culture, comparative virus infectivity assays, particle:infectivity ratios, virus structure and phage one-step growth curves. A range of diagnostic case studies will also be discussed.

Practical demonstrations of virus growth cycles and antigen evolution: many problems and some solutions

A.J. Easton

University of Warwick

Abstract not received

Simplifying and illustrating virology concepts: bacteriophage & virtual learning environments

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Laboratory practicals are an essential part of the university learning environment. They not only enable students to develop basic laboratory skills, which are essential for honours research projects, but they also provide an opportunity for students to analyse real data. Though this might be important to them for future job prospects, it also enables students to visualise techniques and equipment that are outside their personal experience, and so enhances their ability to comprehend lecture material. However, there have been reductions in practical teaching at many institutions due to budgetary restraints, greater student numbers and more stringent safety regulations. Two possible solutions to teaching practical virology involve unrelated methods: the use of bacteriophage with their respective bacterial hosts and the use of computer-based resources. Amongst other possibilities for bacteriophage-based practicals, replication of the one step growth curve experiment as performed by Ellis and Dulbruck in 1939, which examines viral replication using *E. coli* and bacteriophage T4, is a good teaching tool. This experiment routinely performs well and not only illustrates several important viral concepts but also requires that students work to a strict timescale and provides students with feedback on their experimental technique that is easily interpreted. However, no matter how simple and useful bacteriophages are to manipulate in the laboratory, there are always concepts in virology lectures that cannot be demonstrated in large practical classes or explained sufficiently in a one hour lecture slot. Virtual environments enable students to have a chance to analyse experimental data they may not have been able to obtain themselves due to reasons of time, safety or expense, or they can allow the lecturer to demonstrate concepts from a lecture in an applied manner. They can be designed to provide formative assessment while functioning at a pace tailored to the individual student, or they can simply be used as a revision tool. Platforms like Zope (www.zope.org) and Blackboard (www.blackboard.com) enable multiple users to prepare self-assessment questions, presentations slides, and various other file formats to create an easy to use virtual learning environment without the need for computer programming or advanced computer skills.

Computer simulations and analyses of hepatitis B virus in patients

B.A.B. Martin

University of Birmingham

In these days of increasing concern about safety, finding interesting and informative things to do with viruses is becoming increasingly difficult. This talk will focus on how virological principles can be learned and virus sequences manipulated without handling virus at all. We have developed a computer practical which teaches both the principles of bioinformatics and those of virus development of resistance of certain viruses to drug therapy. As part of a final year BSc module in Molecular Virology, students carry out various techniques on virus proteins and DNA. They then analyse the data obtained by use of bioinformatics software. We first introduce the package and help the students learn the necessary keyboard commands. In this part of the practical, students learn to manipulate, analyse and compare sequences. The practical builds up gradually over a week, with the students eventually being able to take raw sequence data from clinical specimens and make sense of how the virus is behaving in response to antiviral chemotherapy. I will describe how this practical enables students to discover the power of bioinformatics and to understand something of the dynamics of virus resistance to antiviral agents.

Environmental Microbiology Group session

Microbe–pollutant interactions: ecology, function and application

Microbial adaptation to poorly bioavailable environmental chemicals

H. Harms

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The poor bioavailablity of hydrophobic organic compounds (HOCs) such as petroleum hydrocarbons and polycyclic aromatic hydrocarbons is the most frequent explanation for the slow bioremediation progress of these priority environmental chemicals. Whereas microbiologists, environmental engineers and 'owners' of contaminated sites became aware of the physical limits of biodegradation in natural systems only two or three decades ago, micro-organisms face this problems since several hundreds of millions of years. It is thus likely and has been shown that micro-organisms developed powerful physiological adaptations to the permanently low flux of HOCs and to the necessity to actively improve the physical availability of these compounds in environmental systems. This paper will give an overview of recent advances in our understanding of physical limitations of biodegradation, how the limitations are overcome by adapted organisms and how physiological and physical adaptations of micro-organisms may help to improve engineered bioremediation.

Metals, minerals and metalloids: bacterial and fungal transformations

Geoffrey M. Gadd, Euan P. Burford, Marina Fomina, Christopher White & Simon Hockin

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Microorganisms are intimately involved in metal biogeochemistry. The balance between metal mobilization and immobilization varies depending on the organisms and physico-chemical conditions. In terrestrial environments, fungi contribute to the dissolution of mineral aggregates through excretion of H^+, organic acids and other ligands, or through redox transformations of mineral constituents. Main mechanisms of metal mobilization by free-living and symbiotic mycorrhizal fungi are acidification and ligand-promoted dissolution mediated by organic acid anions. However, if oxalic acid is produced the production of metal oxalate minerals may result. Sulfate-reducing bacteria (SRB) are key participants in the biological metal cycling. While much interest has focussed on insoluble metal sulfide precipitation, SRB can also reduce metalloid oxyanions. We have found that SRB can mediate formation of elemental sulfur in the presence of selenite. Co-precipitation of S and Se appears to be a generalised ability of SRB, and the sulfur-selenium deposits formed nanometer-scale aggregates within the biofilm. We have also quantitatively determined SRB biofilm interactions with metabolisable substrates and Cd. A mathematical model of bioprecipitation, in which CdS formation rate was determined by two steps – sulfide production and colloidal CdS flocculation, indicated that rates of sulfate reduction and flocculation were the key variables in optimising the biofilm system. This presentation will detail the above examples of metal-mineral transformations, and their ecological and applied relevance.

Influence of the rhizosphere on microbe-pollutant interactions: legume rhizodeposits and the enhanced mineralization of 2,4-D

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The rhizosphere is the unique environment at the plant-soil interface and a zone of intense soil-plant-microbe interaction. One recorded consequence of this interaction is often the enhanced microbial biodegradation of organic xenobiotics although few studies have attempted to unravel the mechanisms responsible.

We report that the mineralization of 2,4-D is enhanced in rhizosphere soil from *Trifolium pratense*. We monitored both numbers and diversity of 2,4-D degraders in pristine *Trifolium*-planted and non-planted soil. For planted and non-planted soil, most probable numbers of 2,4-D degrader were low (<100 MPN g^{-1}) and not related to plant treatment. Plant treatment also had no impact on the diversity of 2,4-D catabolic genes (*tfd*Aα), although an impact of 2,4-D application was recorded. Our results indicate that the enhanced mineralization was not due to selection for 2,4-D degraders in the rhizosphere but due to the action of a rhizodeposit as an inducer of the catabolic pathway.

To narrow down the identity of the stimulatory rhizodeposit, we investigated the influence of soil and plant properties on its production. Most significant was that production depended on the presence of micro-organisms and had legume specificity. We discuss the possibility of flavonoid promotion of 2,4-D mineralization. Further research is needed to investigate the role of flavonoids in the legume-enhanced mineralization. Such research could have implications for understanding not only the mechanisms behind rhizosphere-enhanced biodegradation, but also the 2,4-D degradative pathway in an evolutionary context.

Anaerobic dehalogenation of organohalide pollutants in estuarine and marine environments

Max M. Häggblom

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Estuarine and marine sediments as significant sinks for organohalide pollutants. Microbial degradation of organohalides in anoxic sediments is widely observed and generally proceeds via initial reductive dehalogenation. We have demonstrated that anaerobic microbial populations in estuarine and marine sediments can dehalogenate and mineralize chlorinated and brominated aromatic compounds, and that the dehalogenating populations are distinct under different redox conditions. The availability of suitable electron donors and acceptors affects the biodegradability of organohalides, and anaerobic dehalogenation are influenced by the microbial communities active in different redox zones. Although organohalide compounds are typically considered to be anthropogenic compounds of industrial origin, they have their counterpart in thousands of naturally occurring biogenic and geogenic organohalides. The marine environment is also a particularly rich source of biogenic organohalides, suggesting that these are natural substrates for dehalogenating micro-organisms. For example, marine sponges are natural sources of brominated organic compounds. We showed that these sponges were harbor a large

population of anaerobic dehalogenating bacteria and also demonstrated the presence of several putative reductive dehalogenase genes in the sponge-associated micro-organisms. These fundamental studies are providing an understanding of how dehalogenation processes are incorporated into a global 'halogen cycle' and are serving as a base for developing new methods for bioremediation of sediments contaminated with halogenated flame retardants, polychlorinated dibenzo-*p*-dioxins and halogenated pesticides. Enhancement of microbial dehalogenation is an attractive remediation alternative that could potentially detoxify sediments and avoid the problematic redistribution of contaminants that is associated with dredging.

Molecular approaches to quantifying catabolism of organic contaminants in the environment

M.J. Larkin

The Questor Centre, Queen's University of Belfast

The possibility that most organic compounds can serve as possible energy sources for a wide diversity of micro-organisms underpins our approach to employing micro-organisms for the bioremediation of many compounds. Accumulated evidence suggests that the diversity of catabolic pathways largely matches that of the organic compounds available. Whilst the potential for catabolic activity can be well defined under laboratory conditions, quantifying this under remediation conditions in a field process presents a formidable challenge. Dynamic changes in microbial populations, cellular physiology, gene expression and physicochemical conditions all contribute to the overall biodegradative activity. The assessment of microbial gene diversity has become an integral part of monitoring bioremediation processes under development. Improvements in the methods for the extraction of nucleic acids, the use of techniques such as gene microarrays and quantitative PCR to monitor changes in gene diversity and expression hold out the promise of faster quantification of catabolic ability. The use and limitations of molecular techniques will be critically reviewed with reference to some data acquired from the remediation of contaminated ground water at two former gasworks sites. These will be compared with the role of conventional culture techniques and chemical methods to assess *in situ* catabolic rates.

Biodegradation of the high explosive RDX by *Rhodococcus* sp.

James Edwards[1], Helena M.B. Seth-Smith[2], Deborah A. Rathbone[1] & Neil C. Bruce[1]

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Royal Demolition Explosive (RDX) is an important and widely manufactured high explosive but is now a recognised environmental pollutant owing to its toxicity and recalcitrance. Concern is growing regarding the large areas of land and ground water contaminated worldwide as a result of the continued manufacture, use and disposal of this compound. We have isolated 19 strains of bacteria belonging to the genus *Rhodococcus* that are capable of growth on RDX as a nitrogen source. We have cloned the two genes responsible for RDX degradation from *R. rhodochrous* strain 11Y, shown to be a highly unusual cytochrome P450, *xplA* and its partner oxidoreductase, *xplB*. Investigations of the remaining strains using a P450 inhibitor (metyrapone) greatly reduced RDX degradation suggesting P450 involvement in these strains. Furthermore, by using a combination of Southern Hybridisations and PCR, we have evidence to show that the *xplA* and *xplB* genes are present in both our RDX degrading strains, and in other strains isolated from different geographical sites from the UK and Australia. Interestingly, the homologues cloned thus far have a remarkable degree of similarity to one another (>99% amino acid identity).

Transformations of toxic metal minerals in the mycorrhizosphere

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The physico-chemical environment of the mycorrhizosphere is different from that of the bulk soil and this can affect toxic metal speciation. The aim of this research was to examine the transformation of toxic metal minerals by ericoid and ectomycorrhizal fungi and to determine how they affect metal speciation and mobility. Of the tested insoluble cadmium-, copper-, lead- and zinc-containing minerals, zinc phosphate was of least toxicity and the most easily solubilized by ericoid and ectomycorrhizal fungi. Solubilization of minerals was related to metal tolerance of the fungal species and toxic metal avoidance (e.g. decreased accumulation) appeared to be an important strategy in metal tolerance of the isolates. Zinc, copper and lead mobilized from minerals were oxygen-coordinated within fungal/ectomycorrhizal biomass. 'Heterotrophic leaching' of toxic metal(s) from insoluble minerals combined acidification and ligand-promoted dissolution mediated by different organic acid anions: if oxalic acid was produced, precipitation of metal oxalates resulted. Zinc phosphate solubilization by *Paxillus involutus/Pinus sylvestris* ectomycorrhizal associations and protection of host plants against toxic metals mobilized from the minerals depended on fungal tolerance and the phosphorus status in the growth matrix.

Mines, metals and micro-organisms – a case of going round in cycles

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Whilst metals have been used by mankind for a few thousand years, micro-organisms have exploited metals as sources and sinks of electrons on planet earth for eons. Many heavy metals, such as copper, lead and zinc, occur primarily as sulfide ores, and other (primarily iron) sulfides are often associated with ores of other metals (e.g. gold), and also with coals.

Mining of metals and coals produces large amounts of waste materials, increasingly so as the grade (metal content) of primary ores decreases. Abandoned mines, spoils and tailings constitute a serious environmental hazard, and major catastrophic pollution incidents at mining sites have occurred in recent times, for example at Wheal Jane (U.K.), Aznalcóllar (Spain) and Baia Mare (Romania). Two elements – iron and sulfur – are of primary importance, from a microbial perspective, in these situations. Both exist in variable oxidation states (3 in the case of iron, 9 in the case of sulfur) and can act as either electron donors or electron acceptors for specialized prokaryotes. Consequently, iron and sulfur are major energy 'currencies' in these situations, and cycling of these two elements (sometimes coupled) is a common and important phenomenon. The microbially-catalysed oxidative dissolution of sulfide minerals, usually (though not necessarily) coupled to the reduction of oxygen, can result in the production of highly acidic, metal-rich liquors. Whilst previously it had been thought that few micro-organisms were able to live in such extreme environments, the biodiversity and ecology of 'acidophiles' are now recognized to be extensive and complex. This area of environmental and applied microbiology will be described, together with new and established biotechnologies (biomining, bioremediation etc.) that make use of iron- and sulfur-metabolising acidophiles.

Role of the periplasmic space in bacterial heavy metal resistance

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To understand bacterial heavy metal resistance, the function of several groups of different metal efflux systems and their physiological interplay was analysed in *Escherichia coli* and the metal-resistant bacterium *Cupriavidus (Wautersia, Ralstonia, Alcaligenes) metallidurans*. RND (resistance, nodulation, cell division) systems form a first line of defense. Evidence from several RND systems indicates that RND systems *in vivo* protect the periplasm against superfluous heavy metal cations. CDF (cation diffusion facilitators) and P-type ATPases transport toxic metals from the cytoplasm across the cytoplasmic membrane, forwarding them to the RND systems. Contribution of at least one of the two the chromosomal Zn/Cd-exporting CPx-type ATPases of *C. metallidurans* is essential for full cadmium resistance, but not for full zinc resistance. Three groups of CDF proteins can be differentiated. Each group contains transporters with a broad substrate specificity, which is respectively centered around the central substrates Fe(II), Zn(II) or Co(II). The function of CDF proteins is essential to obtain cobalt resistance in *C. metallidurans*, which demonstrates that RND proteins are unable to detoxify substrates from the cytoplasm, but gather them in the periplasm instead. The contribution of the RpoE-sigmulon to metal homeostasis in *E. coli* demonstrates the importance of this cellular compartment for metal resistance.

Spatial variability of pesticide degradation in soil: mechanisms and implications

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Large amounts of pesticides are applied to agricultural land, with over 12,000 tonnes typically applied in Great Britain each year. The key processes controlling the fate of pesticides in the soil are sorption to soil organic matter and rates of biodegradation, which together control the extent to which pesticides persist in the soil, and the potential of compounds to leach from soil and contaminate ground- and surface-water. Biodegradation of pesticides can occur through 2 processes; Growth–linked metabolism, in which organisms proliferate during degradation, and cometabolism, in which there is no apparent proliferation of degraders. For compounds degraded by both growth-linked and cometabolic degradation, we have demonstrated substantial spatial variability in degradation rates across farms and within single agricultural fields. Soil pH has been found to be particularly critical in controlling this variability, acting by direct effects on the specific microbial communities responsible for degradation. For weakly sorbed compounds there are implications for patterns of leaching losses. However, for other compounds, spatial variability of degradation rate may affect the extent to which the compound exterts non-target effects on the soil microbiota. Overall the data suggests that there are 'hotspots' within the agricultural landscape in which rates of pesticide degradation processes are particularly slow, and in which there is the greatest potential for environmental impacts.

Response of microbial biosensors to Cu- and Zn-contaminated forest litter

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Application of biosolids (sewage sludge) to plantation forests avoids potential human food-chain issues. However, because the biosolids cannot be incorporated into the soil without causing extensive tree root damage, repeated biosolids application may result in the build up of very high concentrations of heavy metals in the surface litter layer. We applied metal-spiked (Cu, Ni, Zn) biosolids to plots under mature *Pinus radiata* trees and measured the impacts of the metals in the litter on sensitive bioluminescence-based fungal (*Armillaria mellea*) and bacterial (*Escherichia coli*) biosensors.

Water-soluble Zn explained 62% of the variance of luminescence of the bacterial biosensor and the EC_{50} concentration was 9.0 mg Zn l^{-1}. Similarly, water-soluble Cu explained 52% of the variance of luminescence of the fungal biosensor and the EC_{50} was 4.4 mg Cu l^{-1}.

Luminescent microbial biosensors are sensitive indicators of heavy metal toxicity in *P. radiata* litter. Data suggest that more than one indicator is required to accurately gauge toxicity, with differential sensitivity being observed between the two biosensors used.

Can bioavailability be measured chemically?

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Traditionally, analytical soil extraction techniques have been used in the determination of 'total' chemical concentrations using organic solvents. These measured concentrations have been and still are routinely used to assess contaminated land for risk and remediation. In light of the increasing body of knowledge relating to compound availability and ageing, such methods have little relevance to the concentration of contaminants, which is considered to be bioavailable. More recently, less-exhaustive techniques have been investigated with the aim of determining a method, which more closely relates to contaminant bioavailability; perhaps even mimicking microbial interactions with soil-associated compounds. Techniques include solid phase extraction using Tenax resins or XAD-4 and/or persulfate oxidation. Recent work at Lancaster University and the Universities of East Anglia and Aberdeen has pioneered the application of cyclodextrin extractions of organic contaminants in soils. Cyclodextrins are highly water-soluble; however, owing to their molecular structure they also contain a hydrophobic cavity, it is possible to form an inclusion complex between the cyclodextrin macrocycle and a hydrophobic organic molecule i.e. the cyclodextrin acts as a 'molecular bucket'. Unlike the portion of contaminants extracted by the use of organic solvents, cyclodextrins have been shown to correlate closely with key biological fractions, such as the portion of the contaminant that is mineralizable. This research has seen the development of a soil extraction technique that allows the determination of the biodegradable fraction of contaminants in soil. The extraction technique requires 'shaking' of contaminated soil using aqueous solutions of cyclodextrins. In this way, the mass transfer processes (soil surface to soil solution to microbial degrader) that dictate biodegradation may be mimicked. Data will be presented highlighting the applicability of this technique to chemically determine microbial degradation. To conclude, it is well known that contaminant bioavailability in soil is controlled by the physico-chemical properties of the contaminant(s), the physico-chemical properties of soil and the activity of soil biota. In light of this and other studies, bioavailability has been shown to differ for different biota. Further, the degree to which a contaminant is determined to be bioavailable may be dependant upon the desired temporal endpoint, i.e. the length of time an assay is carried out. When all of these factors are considered, it may well be impossible to develop a single method which measures contaminant bioavailability for soil biota.

Leading lights: exploiting biosensor technology to optimise remediation of BTEX contaminated groundwater

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Volatile organic pollutants, such as BTEX compounds, are of concern globally due to their toxic effects even at low concentrations. Bioremediation can offer a cost-effective and environmentally sound alternative for their clean-up. This approach can be limited by environmental constraints and the concentration/toxicity of the pollutants themselves. Biosensors, in this case genetically modified bacteria, can provide a reliable means of assessing the bioavailability and toxicity of contaminants, as well as integrating the associated physical parameters on site. A biosensor 'toolkit' was employed utilising toxicity based metabolic biosensors for overall assessment of remediation potential; and catabolic biosensors for addressing hydrocarbon bioavailability. Additionally, a novel approach for studying the *in situ* microbial community was employed, using inert carbon matrices to encourage the formation of biofilms from groundwater. These were then examined by molecular probing for functional genes, and analysis of 16S sequences by DGGE. This work has identified the influence of contaminant plumes on the structure of microbial communities *in situ*. This integrated approach provides an indication of the timescale for remediation, predicts an environmentally relevant end-point of a chosen remediation strategy; and identifies areas of a contaminated site, which support microbial activity.

Stimulating microbial remediation

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Given sufficient time there are few synthetic compounds that can resist microbial degradation, a fact exploited in environmental clean-up. Despite this the performance of micro-organisms in remedial technologies is often sub-optimal and rarely reliable. There are many reasons for the failure of indigenous microbial communities to reduce contaminant concentrations, including issues of bioavailability and the in ability of the contaminants to switch on catabolic genes. The application of molecular approaches, such as gene probing, has improved our ability to identify the diversity and activity of catabolic genes *in situ*. However, such insights represent only part of the problem. Even if the presence of the required catabolic genes is confirmed, there continues to be a significant need to develop procedures to stimulate their activity.

In this presentation, we describe several novel approaches whereby microbial degradation activity has been stimulated by engineered applications including electricity (electrokinetics) and the addition of macro-organisms, such as worms, that preferentially stimulate specific functional microbial groups. In addition, we have also demonstrated that the addition of some secondary plant metabolites, such as terpenoids and flavanoids, can be effective at stimulating rates of pesticide and pharmaceutical degradation in water. A major motivation for the identification of 'natural' inducers is their low toxicity and amenability to field applications, and their ability to favour the degradation of specific pollutant enantiomers.

Degradation of organic pollutants by plant-colonising bacteria

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Phytoremediation of organic soil pollutants is being developed as an environmentally friendly soil cleaning technology. Most researchers agree that its mechanism is based on an interaction between plants and degrader bacteria. However, it is still not clear, what is the environmental fate of some pollutants, which portion of the pollutants is degraded by the plants or by the bacteria, and precisely where on the plants the bacteria are active.

Laboratory investigations, combined with mathematical modelling, revealed that the contribution by plants *vs*. bacteria, as expected, greatly depends on the hydrophobicity and volatility of the pollutant. Recent studies suggest that the biomass of root-colonising degraders is smaller than expected. Most rhizosphere bacteria are in the stationary phase, but the degradation activity of their individual cells is still measurable. We also recognised that degrader bacteria not only colonise the root surface, but may inhabit the xylem vessels of plants. Surprisingly, such endophytic bacteria can contribute to the degradation of some toxic pollutants, with the indirect effect of enhancing the plants' pollutant tolerance.

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Microbial tools for sustainable remediation solutions

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Chemical analysis alone is inadequate at placing the toxicity/potential for remediation associated with a contaminated site in a suitable context. The term bioavailability can only be applied in a context specific to a target receptor or a proven chemical surrogate. Coupling biological and chemical data can often yield significant advances in hazard assessment and act as a suitable baseline for site assessment to be made. Here, using specific examples, the value of luminescence-based biosensors is discussed and their application in hazard assessment and bioremediation programmes described. The sites considered are an ionic works, a former manufactured gas plant and a textile factory. Risk reduction was achieved by linking biosensor technology to risk assessment models. In terms of remediation, three examples are considered- a manufactured gas plant, a pesticide holding area and an oil sludge depot. The sensors predicted a remediation end-point allowing commercial confidence and responsible environmental custody. As stakeholders, regulators and site owners require cost-effective and highly focussed information about individual sites, biosensors offer a unique and increasingly widely applied technology.

Food & Beverages Group session

Evolving bacteria and emerging foodborne disease

Major factors in the emergence of foodborne pathogenic bacteria

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The broad spectrum of foodborne infections has changed dramatically over time, as well-established pathogens have been controlled or eliminated, and new ones have emerged. The burden of foodborne disease remains substantial: we estimate that each year 1 in 4 Americans has a significant foodborne illness. The majority of these illnesses are not accounted for by known pathogens, so more must remain to be discovered. One may expect foodborne pathogens to emerge from animal reservoirs. In addition to the emergence or recognition of new pathogens, other trends include the emergence of highly resistant strains, and the increasing identification of large and dispersed outbreaks, and of global pandemics. New pathogens can emerge because of changes in ecology, technology or trade that connect a potential pathogen with the food chain. They also can emerge *de novo* by lateral transfer of mobile virulence factors, often through bacteriophage. Better understanding phage transmission should help us understand the appearance of new pathogens. The successes of the 20th century and the new challenges we face mean that public health vigilance, rapid investigation of new problems, and partnerships to bring about effective control measures from farm to table will be needed for the foreseeable future.

How do foodborne bacteria cause disease?

G. Dougan

The Wellcome Trust Sanger Institute, Cambridge

Abstract not received

Campylobacter invasion success model

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Studying the mechanisms of *Campylobacter* pathogenesis is complicated by the lack of simple animal models that mimic the disease seen in humans. *In vitro* cell culture methods provide a useful alternative to investigate the interactions between *Campylobacter* and the host epithelium that occur during infection. The mechanisms of pathogenesis are not yet well defined but are multifactorial and involve factors such as motility, chemotaxis, colonisation, adhesion, invasion, iron acquisition, and the formation of toxins (Ketley, 1997; Wassenaar & Blaser, 1999). In the genomics era there is an increasing use of *in vitro* cell culture techniques to interrogate the potential role of different genes in pathogenesis. The suitability and limitations of the various experimental approaches used to study *Campylobacter* invasion and the influence of cell-specific as well as bacterial factors on invasion will be discussed. The involvement and effects of phase variation on the results of invasion studies in cell culture emphasise the need to verify observed strain variations. We present the use of a mathematical Invasion Success Model to analyse *Campylobacter* invasion and show that it can be used to derive three strain dependent characteristics I_{max}, k, and I_0. Even by combining data from independent experiments the Invasion Success Model can be used to statistically compare *Campylobacter* strains for their invasion of epithelial cells. Recommendations are given for the adoption of standard assay parameters and analytical methods such as the Invasion Success Model in order to facilitate comparison of data generated in different laboratories.

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Determining the prevalence of antibiotic resistant bacteria from pigs exposed to different farming practices

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The use of antibiotics with food-producing animals has raised concerns about the levels of antibiotic resistant bacteria carried by farm animals. Antibiotics administered at sub-therapeutic levels exert a selective pressure on the flora of the animal allowing for resistant bacterial populations to increase. Food-producing animals carrying antibiotic resistant bacteria may pose a risk to human health. Therefore, farming is an area that requires constant monitoring to assess the levels of resistance. The bacterial flora found in pig faeces was assessed to determine resistance levels to various groups of antibiotics. Samples were taken from one week old piglets, and then every two weeks until weaning. The pigs involved in the study were exposed to different farming practices which included indoor and outdoor growth, weaning at the age of four and six weeks, and a post-weaning diet with and without sub-therapeutic levels of antibiotics. The faecal samples were diluted and inoculated onto media containing one type of antibiotic. Growth, which was carried out both aerobically and anaerobically, was compared with growth on antibiotic free media to determine the percentage of resistance. The aim of the study was to determine the influence of different farming practices on the prevalence of antibiotic resistance.

Mechanisms of genetic variability in bacteria

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The increasing availability of genome sequence information and the advent of microarray technology are providing new insights into the genetic basis of bacterial diversity and the mechanisms involved in gene transfer that lead to the acquisition of new genetic traits in different species and genus. The introduction of DNA into microbial genomes, referred to as horizontal DNA transfer, can occur in several ways i.e. by transformation, bacteriophages, or via conjugative transposons and plasmids and this has the potential to radically alter the life-style of a bacterium. Surveys of microbial genomes have revealed that up to 20% of the genome of some bacteria constitutes horizontally transferred DNA and the retention of this DNA over evolutionary time contributes to species diversification. Clusters of genes or islands that are important in virulence and have an average base composition different from the bulk of the genome are commonly referred to as pathogenicity islands (PI) and they have been found in a variety of Gram-positive and Gram-negative bacteria. In many cases PI are flanked by sequences associated with DNA transfer indicating that they have been spread among members of the bacterial kingdom by

horizontal transfer, especially via plasmids. There are also examples where particular genes have been lost from bacterial lineages either because they fail to provide any further benefit or because they interfere with adaptation to a new ecological niche. In this lecture the contribution of lateral gene transfer to the evolution of food-borne bacterial pathogens will be discussed including recent data on the use of DNA microarray hybridisations to investigate uncharacterised of *Campylobacter jejuni*, *Salmonella* and *Listeria*. The variety of possible DNA transfer and transposition mechanisms operating in these pathogens will also be highlighted.

Relating pathogen genome sequence to biology

J. Parkhill

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Abstract not received

Emergence of *Campylobacter jejuni*/*Campylobacter coli* in humans

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Campylobacter emerged as an important human pathogen nearly 30 years ago. Campylobacter infection has proved to be a major public health problem and its epidemiology is complex. There are extensive animal and environmental reservoirs and multiple risk factors for infection. Although epidemiological patterns, such as marked seasonality and a paucity of recognized outbreaks, are well described, their underlying explanations are still obscure despite much study.

Foodborne transmission is well recognized and poultry appears to be an important source of infection. Eating food, including poultry, on commercial catering premises has been identified as a risk in several case-control studies of sporadic infection. Important progress has been made in reducing the role of the food chain as a vehicle for campylobacter infection.

However, in addition to the contribution of poultry to human campylobacter infection, many studies also point to numerous other sources and vehicles. Contaminated drinking water, contact with animals (either domestic pets or farm animals), or reported problems with the home sewerage system, have also been implicated in infection. In seeking to reduce the human toll of campylobacter infection it is very important that these additional risk factors are not overlooked.

Pathogenesis of *Campylobacter jejuni*

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Campylobacter jejuni is one of the major causes of foodborne illness in the world. Despite twenty years of intensive research and genomic sequences, little is understood about molecular pathogenesis. The organism is generally considered to be invasive for intestinal epithelial cells, although the levels of invasion vary considerably among strains. Strain 81-176 is more invasive than most other strains of *C. jejuni* that have been examined, including strains isolated from documented cases of dysenteric-like disease. Motility is critical for intestinal colonization and invasion in vitro, perhaps because the flagellar apparatus may function to secrete virulence determinants. Some of these virulence determinants appear to be part of the flagella regulon. The surface of campylobacter is covered with a variety of novel carbohydrate structures, including LOS, capsule and the glycans decorating the surface of flagella, all of which contribute to virulence in distinct ways. In contrast to the variability observed in levels of invasion of intestinal epithelial cells, most strains *C. jejuni* are capable of survival and replication within human macrophages. The process results in release of high levels of proinflammatory cytokines and apoptosis, events that likely contribute to the disease symptoms.

Significance of *Campylobacter* in animals

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Campylobacter spp. have their reservoir in a broad range of animal species. Some of the species are host related though not restricted. *C. jejuni* seems to have the broadest range of hosts, occurring in all mammals and birds. As it is a very successful colonizer of the poultry gut, contaminated poultry meat is one of the most important sources for human campylobacteriosis. Besides colonizing birds, it also occurs in pigs, cattle, sheep and companion animals like dogs and cats. *C. coli* is mainly associated with pigs, and also with poultry and sheep. Other *Campylobacter* species, like *C. upsaliensis, C. helveticus, C. fetus* and *C. hyointestinalis* show a more restricted host relationship.

The common characteristic of all *Campylobacter* spp. is that, although they survive in the environment, their main location of multiplication is the animal gut. Except for *C. fetus*, *Campylobacter* mainly colonizes the gut without any clinical relevance for the animals. However, due to direct animal contact and contamination of meat as final product of the animal production chain, animals play a crucial role in the epidemiology of human *Campylobacter* infections.

Intervention strategies to prevent human campylobacteriosis will be discussed in the presentation.

Emergence of *Salmonella enterica* serovar *enteritidis* and of antibiotic-resistant *Salmonella* serotypes in humans

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Salmonella enterica serovar *Enteritidis* became a major health hazard in the mid-1980s following the apparent adaptation of the organism to survival in the oviduct of laying hens, followed by intra-ovarian transmission through laying hens. Several different phage types were involved in different countries but the most important phage type (PT) globally was PT4. PT4 remained the most common strain causing salmonellosis in humans in the UK until 2002. Subsequently PT 4 has been replaced in prevalence by several different phage types, many of which have been associated with outbreaks linked to raw shell eggs imported from Spain. Although these phage types are different to PT4 the molecular characteristics of the strains involved are very similar. The history of multiple-resistant (MR) *Salmonella* in the UK has been dominated by three clones, *S. Typhimurium* DT29, DT 204/193 and DT104. These strains are zoonotic in origin and have caused numerous infections in humans. In DTs 29 and 204/193 resistances have for the most part been plasmid-mediated; in DT104 the predominant resistances – ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines, (= R-type ACSSuT) have been chromosomally-encoded in a 13 kb resistance island – *Salmonella* Genomic Island 1 (SGI1). Antimicrobial usage in food production animals has been regarded as having a key role in the development of resistance, although for DT104 of R-type ACSSuT, SGI1 was probably present in the strain before its establishment in food production animals.

Pathogenicity and virulence of *Salmonella* serovars in humans and other animal species

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Control of *Salmonella* within food animals is a good strategy to eliminate these pathogens from human food. Vaccines are available for the control of *Salmonella*, however they vary in efficacy. Within *Salmonella enterica* there are over 2,300 different serovars that cause infections ranging in severity. Furthermore different serotypes vary in host-specificity. Understanding the molecular basis of how *Salmonella* colonise intestines and cause disease is essential for the development of effective vaccines. Bacterial Type Three Secretion Systems (TTSS) have been shown to be major virulence factors influencing the colonisation and pathogenicity of *Salmonella* in some but not all animal species. TTSSs act by delivering effector proteins into host cells. Such proteins can act to modify signalling events causing alterations to the cell cytoskeleton and vesicular trafficking, which can increase *Salmonella*-induced enteritis and persistence and thus transmission. Disruption of TTSSs and/or related secreted effector proteins can be adopted as a strategy for the attenuation of live vaccine strains. Furthermore secreted effector proteins have the potential to be incorporated into sub-unit vaccines. As many of such effector proteins are conserved between different serotypes, such vaccines offer the hope of cross-serotype protective immune responses. The role of other virulence factors influencing colonisation and pathogenesis will be reviewed.

Impact of *Salmonella enteritidis* and antibiotic-resistant salmonellas on the food chain

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In the last two decades there have been a number of international pandemics of human salmonellosis. Principal amongst these have been those caused by *Salmonella enteritidis*, which was mainly egg-associated, and *S. typhimurium* DT104, which was associated with infection of a range of food animals and which showed multiple antibiotic resistance. The success of these two *Salmonella* serovars as zoonotic pathogens is related, in part, to their invasive behaviour in food animals, which means that food interiors can be contaminated. Global trade in food and animals exacerbated international spread. As well as causing millions of cases of infection and thousands of deaths, these pandemics had and continue to have a major impact on the food chain. Thus egg-associated infection in the UK was only brought under control by the vaccination of laying hens under the Lion Code and the identification and slaughter of breeding flocks in both the egg and broiler sectors. The UK egg industry has spent ~ £20 million pounds vaccinating layers against *S.* enteritidis. In common with most *Salmonella* serovars, *S. enteritidis* and *S. typhimurium* are capable of prolonged persistence in the farm environment and continue to pose a threat to livestock because of this. The problems for the food chain that this may cause is also potentially made worse by Global trade. For example, the UK has seen a large number of *S.* Enteritidis outbreaks in the last four years, principally caused by imported eggs. This trade has also introduced more than 20 phage types (PTs) either never seen or only rarely seen before in the UK. These can now be isolated from the UK farm environment. The imported egg-associated outbreaks can be characterised by the very high contamination rates seen in eggs. This may well be due to new virulence factors expressed by these novel *S. enteritidis* PTs. Their altered LPS structures, which leads to a change in PT, may also compromise the protection given by the current vaccines, which are based on PT4, which was most prevalent in the UK until these new ones arrived. These and other matters will be discussed.

Food preservation stresses and the development of antibiotic resistance

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Modern food preservation processes involve the application of bacteriostatic (sublethal) stresses e.g. high/low temperature, extremes of pH and A_w to slow or prevent bacterial growth. However, such stress can induce resistance to subsequent challenge with the same stress (stress hardening) or a wide range of different environmental stresses (cross-protection). Resistance may involve short-term phenotypic changes e.g. expression of shock proteins, or more permanent genetic changes resulting from stress induced increased genetic plasticity. This study investigates the possibility that such stress induced genetic plasticity alters antibiotic resistance (ABR) in food related pathogens.

ABR (zone diameter/MIC) to a range of antibiotics in sublethally stressed and unstressed food related pathogens (*E. coli, S. typhimurium,* and *S. aureus*) was determined. Sublethal food preservation stresses include high/low temperature; osmotic and pH stress.

Some sublethal stresses significantly changed antibiotic resistance. Sublethal high temperature (45°C) stress decreased ABR. However, high salt (above 4.5%) or low pH (>5.0) stress increased ABR by up to 32X(MIC). Some increases were stable during repeated post-stress culture.

Increased use of bacteriostatic (multiple hurdle technology), rather than bacteriocidal food preservation systems, may be contributing to the development and dissemination of ABR among important food borne pathogens.

Outbreaks of vero cytotoxin-producing *Escherichia coli* O157 (VTEC O157) infection in England and Wales associated with food or water

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Infection with VTEC O157 is acquired through ingestion of contaminated food or beverages, by animal contact or by person-to-person spread. Between 1995 and 2004, 150 general outbreaks of VTEC O157 infection in England and Wales were analysed by transmission route, the properties of the causative strain and the genotypic comparison between human and non-human isolates. The percentage of total outbreaks that were food-borne fell in the period 2000–2004 compared with 1995–1999. A food vehicle was implicated in 37 of 50 food-borne outbreaks, most commonly milk and dairy products. There were 10 water-associated outbreaks, four of which were linked to drinking water. Strains of phage type (PT) 2,8 or 21/28 caused 44 of the 50 food-borne outbreaks with PT2 and PT8 outbreaks predominant before 2000. Since then, PT21/28 has caused 69% of the food-borne outbreaks, a reflection of its position as the most common PT in England and Wales. In 20 outbreaks, isolates from implicated food or water or from dairy cattle associated with the food source had PTs and pulsed field gel electrophoresis profiles that matched those of human strains. Food-borne general outbreaks appear to have declined but phenotypic and genotypic typing is required for specific investigation of infection sources.

Emergence of verocytotoxin-producing *Escherichia coli* in humans and in animals

P.M. Griffin

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Abstract not received

Pathogenicity and virulence of verocytotoxin-producing *Escherichia coli* in humans and animals

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Enteropathogenic (EPEC) and enterohaemorrhagic *E. coli* (EHEC) are extracellular human pathogens that exhibit unique virulence properties. Like many Gram-negative pathogen, they also use a type III secretion systems (TTSS) which mediate bacterial attachment to, and injection of effector proteins into, the eukaryotic host cells. TTSSs are sophisticated macromolecular structures, which play an imperative role in bacterial infections and human disease. The TTSS needle complex is conserved amongst bacterial pathogens and shows broad similarity to the flagellar basal body. However, TTSS of EPEC and EHEC is unique in that it has an ~12 nm diameter filamentous extension to the needle which is composed of the secreted translocator protein EspA. In this presentation I will report our investigation on EspA filament assembly and delivery of effector proteins across the bacterial cell wall. In particular I will concentrate on the mechanism through which EspA filaments are elongated and how their length can be modulated. In addition, new evidence will be presented that EspA filaments are hollow conduits through which effector proteins are delivered to the extremity of the bacterial cell and subsequently into the host cell.

Impact of verocytotoxin-producing *Escherichia coli* (VTEC) on the food chain

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The identification of *Escherichia coli* O157:H7 as a human pathogen in 1982 has led to the recognition of a new category of diarrheagenic *E. coli*, which have the common trait of verocytotoxin (Shiga toxin) production. Subsequently, cattle and other ruminants were identified as principal sources of VTEC associated with human illness and, in the United States, ground beef has been the leading vehicle of *E. coli* O157:H7 food-associated outbreaks. Considerable attention has been focused by the U. S. Department of Agriculture on reducing *E. coli* O157:H7 in ground beef with intriguing results in reducing human illnesses. In contrast, some countries have focused regulatory attention on the presence of VTEC in ground beef which encompasses a considerably larger number of *E. coli* of which many are of unknown human pathogenicity. There is a public health and economic need to develop criteria for differentiating significant human pathogenic VTEC from other VTEC commonly associated with animals. Furthermore, a farm to table risk assessment of human pathogenic VTEC is needed to risk rank the primary sources of VTEC infections. This will enable public health and regulatory agencies and the food industry to better focus their resources on points in the food chain where interventions and regulatory oversight are likely to have the greatest impact on reducing human VTEC infections.

Microbial Infection Group session

Alternative models of infection

Zebrafish embryos to model *Salmonella typhimurium* and *Mycobacterium marinum* infections

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The zebrafish, and especially the use of the zebrafish embryo, is rapidly gaining ground in infection models. The major advantages of the zebrafish model are (i) the unique possibility for real-time analysis of microbial infections at the single bacterium level in a vertebrate host, and (ii) the possibility for high-throughput screens, such as screens of bacterial- and also zebrafish-mutants, but also screens of chemical libraries for their *in vivo* effect on bacterial replication. The bacteria that have been studied in detail are the intracellular pathogens *Salmonella typhimurium* and *Mycobacterium marinum*. The latter bacterium is the causative agent of fish-tuberculosis and frequently used as a model system for tuberculosis. For *S. typhimurium* extracellular multiplication was shown to be an important aspect of its life cycle in the zebrafish, especially in the later stages of disease. To monitor the interaction of the pathogen with its host a number of zebrafish immune markers have been obtained. To evaluate the infection model we have knocked-down the expression of components of the TLR signal transduction pathway by the injection of modified antisense oligos. Subsequently, these embryos have been challenged with mutants of *S. typhimurium* and *M. marinum*.

Bronchial organ cultures for modeling bacterial infection

Andrew M. Middleton

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The interaction of bacteria with mucosal surfaces of the respiratory tract is thought to be critical in their pathogenesis. Bacteria must overcome local defences which include: physical barriers such as mucus, beating cilia and an intact epithelial surface; antibacterial substances such as defensins, secretory immunoglobulin A and lysozyme; and resident phagocytes. Organ cultures have a near normal ratio of differentiated cell types that maintain their three-dimensional relationships with the extracellular matrix and submucosal tissues, and by incorporating an air interface they closely simulate *in vitro* the physiological conditions of the respiratory mucosa found *in vivo*. Our organ culture model can remain structurally and functionally intact for up to three weeks, with maintenance medium replaced daily. Tissue can be removed from the model and examined by light microscopy, scanning electron microscopy, transmission electron microscopy and culture. Organ cultures can be used to study the interaction of bacteria with the mucosal surface. They are sufficiently sensitive to discriminate between the interactions of closely related bacterial strains and so provide a powerful tool by which to investigate the importance of a bacterial toxin or a surface structure involved in adherence during infection of the mucosa.

Symbiosis: infection without disease

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Bacterial symbiosis in insects is widespread, with approximately 15–20% of insect taxa living in association with symbiotic partners. Symbiotic bacteria in insects are generally regarded as non-pathogenic comprising of both mutualists (i.e. bacteria advantageous to the insect) and commensals (bacteria of no apparent significance to the insect). Many of these symbiotic bacteria are intracellular with tropisms to specific insect cells and some can persist over multiple insect generations by transovarial vertical transmission, i.e. the transfer of bacteria to the unfertilised egg in the maternal ovaries. In a number of cases the evolution of symbiotic relationships is thought to have been a key factor in the rapid diversification and huge reproductive success of insect species.

Genomic analysis of the tsetse fly symbiont *Sodalis glossinidius* has revealed the presence of a number of pathogenicity islands (PI) i.e. type III secretion system and siderophores for iron acquisition. These PI are utilised in symbiosis, but result in infection without disease. The importance of symbiont interactions with insect cell lines as an alternative model for studying bacterial infections will be explored.

Plant models for *Burkholderia cepacia* complex infections

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A simple plant model using alfalfa seedlings has been developed to aid in the analysis of virulence of strains of the *Burkholderia cepacia* complex (Bcc). After germination overnight, seedlings are planted on water agar plates, wounded, and inoculated with Bcc strains. Symptoms are observed 5–7 days post infection and include yellowing of the leaves, stunted roots and necrosis. This model has been used to assess the relative virulence of seven species of the Bcc that have been found to cause respiratory infections in people with cystic fibrosis. Comparative studies were performed using a chronic infection model in rats. Most of the strains tested that were virulent in the alfalfa model were also virulent in the rats. We subsequently developed a semi-high throughput assay using 24 well plates with three seedlings planted in each well. The observation time was shortened to five days. The alfalfa infection model has been used to screen transposon libraries and signature tagged mutagenesis libraries for mutants that are avirulent or have reduced virulence as indicated by a lack of symptoms in the alfalfa seedlings. This model in conjunction with animal studies can be used to identify multi-host virulence factors conserved through evolution.

The nematode *Panagrellus redivivus* is susceptible to killing by human pathogens at 37°C

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Increasingly *Caenorhabditis elegans* has been used as a host for the study of bacteria that cause disease in mammals. However, a significant limitation of the model is that *C. elegans* is not viable at 37°C. We show that exposing final larval stage *C. elegans* to temperatures greater than 25°C markedly decreases life expectancy. Similar experiments were performed using the nematode *Panagrellus redivivus*. The gonochoristic nematode *P. redivivus* was capable of surviving at 37°C and maintaining its life cycle at temperatures up to and including 31.5°C. *Pseudomonas aeruginosa* reduced *P. redivivus* lifespan at 25°C and 37°C to a similar extent. Four

strains of *Burkholderia multivorans* were investigated for virulence at the two temperatures. One strain reduced nematode lifespan at both temperatures, one strain was avirulent at both temperatures and two strains only reduced *P. redivivus* lifespan at 37°C. The mechanism by which one strain of *B. multivorans* kills *P. redivivus* at 37°C but not at 25°C was investigated in further detail. We found that killing requires live cells, does not involve bacterial invasion of tissues and is not associated with heightened numbers of live bacteria within the intestine of the worm.

Ciliated epithelia for modelling pneumococcal infection

C. O'Callaghan

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The ciliated respiratory epithelium has evolved to protect itself from the constant bombardment of pathogens and toxins. It is less well known that the ventricular surface of the brain, the cerebral aqueducts and the central canal of the spinal cord are also covered by a ciliated ependymal layer. This layer of cells is of interest as it separates the infected CSF in meningitis from the underlying neuronal tissue of the brain.

We have developed methods that allow us to culture cells from the brain and the respiratory tract and differentiate these back into ciliated respiratory epithelia. This has allowed us to assess ciliary function using high speed video photography and to determine the effect of the pneumococcus and its virulence factors.

Using brain ependymal cilia, we have shown that both pneumococcus and the pneumococcal toxin, pneumolysin, cause ciliary stasis at levels encountered during clinical meningitis. We have also shown that hydrogen peroxide produced by the pneumococcus is toxic to ependymal cilia. These effects were attenuated by the combination of anti-pneumolysin antibody and catalayse.

To determine if *ex vivo* findings were replicated *in vivo*, a rat model of meningitis has been developed to study the effect of pneumococci on the ependyma. Studies confirmed that the ependyma was disrupted and ciliary beat frequency affected following meningitis with wild-type pneumococci. However, pneumococci unable to produce pneumolysin had little affect on the ependyma and in a number of cases, these bacteria were cleared from the CSF.

Three-dimensional tissue assemblies: novel models for the study of infectious disease

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Our understanding of the mechanisms of infectious disease in humans will be significantly advanced by the continued development of physiologically relevant *in vitro* models of human cells and tissues suitable for studying the pathogenesis of micro-organisms in a controlled environment. Development of such models should recognize that organs and tissues function in a three-dimensional (3-D) environment, and that this spatial context is required for cells to display bonafide tissue and organ specificity as would be found in ideal models of pathogenesis. We have used innovative bioreactor technology, the rotating wall vessel (RWV), to develop 3-D cell cultures that mimic the differentiated form and function of tissues *in vivo* as models for microbial pathogenesis. We first reported the use of 3-D human tissue aggregates cultured in the RWV as a model for microbial infectivity by a bacterial pathogen. The human intestinal epithelial cell line Int-407 generated well-differentiated 3-D cultures that exhibited apical/basolateral polarity, well-formed tight-junctions and desmosomes, and mucous production. These *in vivo*-like phenotypes were not observed in Int-407 monolayers. When used as models to study *Salmonella*-induced enteric disease, we observed important differences in adherence, invasion, apoptosis, cytokine profiles, and tissue pathology between the 3-D cells and monolayers. Many of these differences were more reflective of an *in vivo* infection. We have since generated other biologically meaningful 3-D cell models including colon, lung, placenta, bladder, and periodontal ligament that we have successfully used to study a variety of microbial pathogens. Collectively, our results indicate that 3-D cell cultures have wide applications in the modeling of infectious disease.

Screening a *Streptococcus suis* STM library using a novel, alternative porcine respiratory infection model

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Background *Streptococcus suis* causes septicaemia in neonatal pigs and respiratory disease, meningitis and polyarthritis in older pigs. There is at present little detailed information on the molecular basis of its pathogenicity.

Methods We generated a library of 1,500 signature tagged transposon mutants of *S.suis* type 2 (strain P1/7) using the Tn*917* mutagenesis vector pTV408. Southern blotting and sequence analysis of a large number of mutants revealed that transposition had occurred singly and near-randomly into the chromosome. Using standard STM protocols the library was screened in pool sizes of 48 for colonisation-deficient mutants using a novel air interface porcine respiratory organ culture system. In this system, in contrast to traditional submerged cultures, there is differential regional colonisation of the respiratory tract: *S.suis* colonises the nasal turbinates but is rapidly cleared from trachea.

Results Screening of the library using air interface organ cultures identified approx. 2% of mutants that were consistently unable to colonise pig respiratory organ cultures. Growth characteristics of the colonisation-deficient mutants were the same as that of the wild type. Sequence analysis of the chromosomal transposon insertion sites is in progress.

Conclusion Air interface organ cultures provide a valid *in vitro* method of screening STM libraries.

Artificial gut models

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The human gut microbiota is the subject of much research. Gut microbes may provide colonisation resistance to pathogens, but their exact role is unclear. Intestinal flora studies have necessitated the development of *in vitro* gut models, from simple test-tube to complex continuous culture systems. A triple-stage continuous culture model was previously developed reflecting the spatial, temporal, nutritional and physicochemical characteristics of the proximal to distal colon and validated against gut contents of sudden-death victims. We studied this system as an *in vitro* model of antibiotic-associated *Clostridium difficile* infection (CDI) and investigated the predisposition to CDI by certain antimicrobials. Antibiotic-mediated impairment of colonisation resistance may be a major factor.

We investigated the effects of predisposing and non-predisposing antibiotics and no antibiotic (control) upon gut microflora and *C. difficile* growth and toxin production. *C. difficile* germination and toxin production were associated with cefotaxime and clindamycin, but not with piperacillin-tazobactam instillation or control, reflecting clinical observations. Antibiotic-mediated depletion of gut bacteria was not associated with *C. difficile* germination and toxin production.

The system is a successful *in vitro* model of CDI. Impairment of colonisation resistance may be only partially responsible for CDI.

Gut models are a useful means of studying pathogenesis.

Physiology, Biochemistry & Molecular Genetics / Cells & Cell Surfaces / Microbial Infection / Education & Training Groups joint session

Bacteriophage evolution, ecology and applications

Phage genomics and evolution

Evolution of dsDNA phages

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We are using phage genome sequencing together with comparative analysis of the sequences to make inferences about how phages evolve, about the genetic structure of the global population of ~10^{31} dsDNA tailed phages, and about novel biological functions revealed by the sequences.

The most dramatic evolutionary process we detect is rampant horizontal exchange of genes among phages by non-homologous recombination. This together with homologous recombination and mutational divergence of sequences can account for the differences we observe among genomes. As we sequence more genomes we see more phages that are intermediate in inferred phenotype between previously known phages. The global phage population is looking more like a genetic continuum of types than was previously apparent, and it is still unclear how well the traditional view that phages are clustered into distinct types will survive continued sequencing.

Among numerous biological insights that come from the genome comparisons, one that has led to progress in a non-genomic area is the translational frameshift found in the tail genes of long-tailed phages. This function is strongly conserved in this group of phages, and its investigation has led to a new mechanism of chaperone-mediated assembly that is a previously unsuspected central feature of tail assembly.

Comparative genomics of mycobacteriophages

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We have determined the genome sequences of 30 mycobacteriophages and analysed them using comparative genomic approaches. These represent a diverse group of phage genomes that differ greatly in their genome size, GC% content, numbers of genes, virion morphology, transcriptional organization, and putative gene functions. Most of these phages contain a cluster of genes involved in virion structure and assembly occupying approximately 25kbp of genome space; the remaining parts of genome space are typically replete with open reading frames of unknown function (and few or no database matches) in mosaic-like arrangement, with modules typically represented by single genes. A small number of relatively recent recombination events provide clues as to how this genomic mosaicism is generated.

A high proportion of mycobacteriophage genes have no database matches and are of unknown function. However, a high proportion of those that do have database matches are related to genes that have not previously been associated with bacteriophages (mostly bacterial genes). Many of these suggest novel ways in which bacteriophages may influence the physiology of their hosts.

The genome and proteome of phage ϕKMV

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Bacteriophage ϕKMV, isolated from a small Moscow suburban pond, infects 17% of 72 worldwide collected *Pseudomonas aeruginosa* isolates. It has a linear G+C-rich (62.3%) double stranded DNA genome of 42,519 bp with direct terminal repeats of 414 bp.

After *in silico* genome analysis the proteome was explored by a combination of liquid chromatography mass spectrometry (LC-MS/MS), recombinant expression and enzymatic assays. The 48 open reading frames of ϕKMV are arranged in a T7-type genome organization. Currently, 21 ϕKMV gene products show significant similarity to proteins from phages including T7, SP6, K1-5, PaP3, gh-1 and Xp10. Using LC-MS/MS, we identified twelve structure-related proteins in the ϕKMV particle, confirming *in silico* annotation (for 7 genes) and revealing 5 new gene products involved in phage morphology. Recombinant expression in *Escherichia coli* and functional assays revealed the ϕKMV DNA ligase (gp17), a thermostable, structure-related lysozyme (gp36) and the lysis cassette, with a possible holin (gp44) and signal-arrest-release (SAR) lysin (gp45).

The data suggests that ϕKMV is a T7-like *Podoviridae* member, notwithstanding protein domain reorganizations in the internal core and injection needle, as in SP6 and K1-5. Also, infection (non transcription-driven DNA entry) and progeny release (SAR) mechanisms may differ from T7.

Prophages in bacterial genomes

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Whole genome sequencing has brought phage biology back into the limelight and provided us with an unprecedented insight into the genomic architecture and evolution of bacterial pathogens. Bacterial genomes are 'peppered' with prophage or phage-like elements which not only contribute to the overall sequence diversity, but also have a significant influence on the pathogenic potential of the host bacterium. Perhaps the most important observation made from genome sequencing is not the mere presence of phage but the number and diversity of phage that have been discovered. Even though we are still only scratching the surface it is clear that phage biology is once again recognised as being fundamental to our understanding of long and short term bacterial evolution. An overview of the contribution of phage to pathogenomics will be presented.

Sequence analysis of the lactococcal bacteriophages 712 & jj50

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Bacteriophages of *Lactococcus lactis* disrupt dairy fermentation processes, which may result in considerable economic losses. Lactococcal (bacterio)phages isolated from the dairy environment are principally members of the c2, 936 or P335 species of the genus, *Siphoviridae*. Phages of the c2 and 936 species employ a lytic life cycle, while the P335 species is heterogeneous and involves virulent and temperate phages. The 936-type phages account for approximately half of all industrial isolates. Therefore, this species warrants the level of sequence and functional analyses that have been invested in these phages in recent years. Among the 936-type phages, the complete genome sequence of ϕsk1 and bIL170 have previously been determined. In order to advance our knowledge of the genetics and evolution of this phage species, sequencing of the small isometric-headed phages 712 and jj50 was undertaken. Comparative genomic analysis of the available 936-type phages reveals that insertions/deletions in the early expressed genes are the main source of divergence of these phages.

Phage ecology and phage–host response

Interactions between bacteriophage and their hosts in a terrestrial habitat: adapting to a life in soil

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Phage capable of infecting *Streptomycetes* can be readily isolated from most soils, reflecting their ubiquity in soil ecosystems. The actinophage ΦC31 is the most studied *Streptomyces* phage. The interactions between *S. lividans* TK24 and *S. coelicolor* J1929 and ΦC31 wt (N) and derivatives mutants were studied using soil microcosms. The dynamics of the interaction was monitored by assessing the spores, total propagules and free phage populations. Soil microcosms at low multiplicity of infection (0. 1 MOI) showed, that the host population was unaffected by the presence and activity of the phage population. A decline in the free phage numbers was observed to occur between day 3 and 4 from a 7 days experiment. Interaction dynamics of non-sporulating mutant *S. lividans* J1725 revealed the importance of mycelia age for the adsorption of phage. Overall results showed that germinating spores are the most susceptible form of the organism to infection by phage. Little is know about the infection process between actinophage and *Streptomyces* at microscopical level. In order to study the infection process, the green fluorescent protein (*gfp*) was used to mark the genome of ΦC31. The molecular strategy and its implication for time-lapse imaging of the infection process will be discussed.

Marine cyanobacterial phages

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The prokaryotic component of the marine picoplankton is dominated by unicellular cyanobacteria belonging to the genera *Synechococcus* and *Prochlorococcus* and makes a significant contribution to primary production, particularly in the oligotrophic regions of the world's oceans. Phages infecting these organisms were first characterized in 1993 are now thought to affect the genetic diversity and abundance of their hosts and to drive a significant fraction of fixed carbon into the microbial loop. Two of the key factors determining the nature of phage host interactions are host population density and host quality and a theoretical consideration of these factors can yield interesting predictions regarding the pressures that have shaped the evolutionary trajectory of these phages and consequently determine the current nature of their interactions with hosts. Evidence from a very different approach, genomics, also provides novel insights into phage-host interactions and extends current ideas and models based solely on the study of phages infecting heterotrophic bacteria. Data will be presented from a clear example of phage diversity driving cyanobacterial diversity. A genomic analysis of phages infecting *Synechococcus* and *Prochlorococcus* has shown that they appear to be capable of subverting and modifying the host's photosynthetic physiology in a variety of ways.

Effect of light on the interaction between cyanophage S-PM2 and *Synechococcus* sp. WH7803

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S-PM2 is a bacteriophage, with a double-stranded, circular genome, which infects the ecologically important marine cyanobacterium *Synechococcus* sp. WH7803. We are currently investigating fundamental features of phage-host interactions. An initial investigation of the role of light on phage attachment has indicated striking light-dependence. In the darkness, phages were not capable of adsorbing to cells, but adsorption resumed as soon as the light switched on. A collection of marine cyanophages is being screened in order to see if this is a common phenomenon. This will offer a significant insight into one of the factors affecting cyanophage and host dynamics under natural environmental conditions. Because of the time-consuming and labour-intensive nature of the plating method, we are developing an alternative technique that allows us to count phages stained with SYBR Green I by flow cytometry. This approach has been verified on CsCl-purified phage, and we are attempting to apply it to adsorption experiments.

The arms race 1: restriction and anti-restriction

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In this presentation I will survey the known types of DNA restriction and modification (R/M) systems found in bacteria and the various mechanisms they use to protect the host cell from phage infection. In addition to the well known DNA cleavage and DNA methylation function of these systems, many restriction enzymes are capable of additional functions such as binding to multiple DNA sequences simultaneously or coupling the hydrolysis of ATP with extensive translocation of DNA using molecular motors. R/M systems present a formidable barrier to phage and have forced the evolution of numerous anti-restriction capabilities by phage. Of particular interest is the synthesis of a protein by phage T7 which physically resembles the DNA duplex recognised by the type I class of R/M systems. This DNA mimic effectively inhibits the restriction enzyme allowing successful propagation of the phage.

An abortive phage infection (Abi) system in the enteric phytopathogen, *Erwinia carotovora* subsp. *atroseptica*

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We have identified a cryptic plasmid in a strain of *E. carotovora* subsp. *atroseptica* (Eca). The plasmid was isolated and a ~4.4 kbp *Eco*RI digestion fragment was cloned into pUC19 (pABI1) and used to transform Eca SCRI1043. The pABI1 transformants were resistant to ϕM1 (a generalised transducing phage). Sequencing of the fragment identified an ORF encoding a 172 aa protein showing sequence similarity with an Abi protein originally identified in *Lactococcus lactis*. Abi systems have been intensively studied in *L. lactis*, with over twenty systems described to date. In Abi$^+$ bacteria, phage adsorption

and DNA injection occur but the lytic cycle is perturbed and host cell death occurs. The mechanism by which this occurs is not understood. Eca SCRI1043 carrying pABI1 showed varying levels of 'resistance' to 20 of 27 *Erwinia* phages tested (e.o.p of 10^{-2} to 10^{-11}). A frameshift mutation in the ORF abolished resistance. This discovery of an Abi system in *Erwinia* suggests that abortive phage infection systems are more widespread than previously thought.

Phage–host interactions in *Streptomyces*

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Bacteria are under constant threat of infection by bacteriophages resulting in strong selection to acquire and maintain phage resistance determinants. Phages are under strong selection pressures to overcome these resistance mechanisms. The end result is a perpetual cycle of attack and counter-attack, sometimes referred to as an evolutionary 'arms race'. Very diverse mechanisms to overcome phage infection have evolved and include restriction-modification, abortive infection and phage exclusion. Bacteria in the genus *Streptomyces* are known for their ability to protect themselves from competitors/predators by the production of antibiotics. Here we examine what these bacteria do to protect themselves from phage infection. *Streptomyces coelicolor* A3(2) contains a completely novel type of phage resistance system called phage growth limitation (Pgl). The Pgl phenotype is characterised by the ability of Pgl$^+$ hosts to support a phage burst on initial infection but subsequent cycles are severely attenuated. Chinenova *et al* originally proposed that phage were modified during the first burst and the modified phage are restricted in the second and subsequent infectious cycles. Four genes, *pglW, pglX, pglY* and *pglZ* are required for Pgl, and we are currently studying the functions of their predicted protein products. Whilst the target of Pgl in the Pgl-sensitive phage, ϕC31, remains unknown at present, we have identified a putative anti-Pgl system within another *Streptomyces* phage, ϕHAU3. Recent results indicate that Pgl is a common phage resistance system occurring frequently in a wide variety of other bacteria.

Phages and virulence

Role of *Vibrio cholerae* phages in virulence

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Abstract not received

The prophages of *Staphylococcus aureus*: widespread distribution and extensive recombination as detected by microarray

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Prophages in *S. aureus* genomes are common, with some strains carrying up to three types. Many virulence genes are carried on prophages, including those encoding Panton-Valentine leukocidin, enterotoxin A, staphylokinase, chemotaxis inhibitory protein and exfoliative toxin A. We have built a seven-strain *S. aureus* microarray that includes every gene from 13 sequenced prophages. We can use the microarray to detect the presence or absence of each of these genes in any given strain. In population studies of over 200 community and hospital isolates of *S. aureus* and MRSA, we found evidence of widespread distribution of different phage genes in a range of different clonal backgrounds. Most surprising was the incredible amount of recombination seen in the form of conserved mosaic fragments, and evidence of many phage with large fragments of unknown/unsequenced genes. Our results suggest horizontal transfer and recombination of *S. aureus* prophage is extremely common.

Shiga toxin and phages

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Genes encoding Shiga toxin (*stx*) in STEC (Shiga toxin-producing *E. coli*) are located in partial or complete genomes of lambdoid prophages downstream of the phage late promoter P_R'. Expression and/or release of Stx in a number STEC strains requires transcription from P_R' rather than the *stx* promoter. Productive transcription from P_R' requires transcription from the early P_R phage promoter which is controlled by the phage repressor. Hence, Stx expression and/or release require prophage induction. We postulate that in these cases production and/or release of Stx derives from a subpopulation of STEC in which the prophage is spontaneously induced; i.e., in the absence of overt DNA damage the prophage repressor is inactivated resulting in phage gene expression.

Using SIVET, a reporter system for studying the fraction of a lysogen population in which the prophage is induced, we find that those members of the lambda family encoding Stx spontaneously induce more readily than family members not encoding Stx. The sequences of the operators and repressor of *stx*-encoding phage 933W have an unusually close similarity with those of the non-*stx* encoding phage HK022. Nevertheless, lysogens of 933W fail to repress growth of HK022. However, lysogens of HK022 repress growth of 933W. This observation has potential significance in understanding how repressor-operator variants evolved.

Mechanisms underlying the dissemination of Shiga toxin genes by bacteriophages

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The production of Shiga toxin (Stx) is the most important phenotype in distinguishing STEC from other pathogenic *E. coli* strains, and is key to the lethality of the STEC pathogenicity profile. This phenotype is conferred on *E. coli* following infection by Stx-phage, which are a very heterogeneous that are essentially genetic mosaics. Intracellular recombination events in multiply infected cells is a likely driver of this genetic diversity, but generally, bacteria lysogenised by lambdoid phages are immune to subsequent infections by related phages. We have obtained data that explains how these multiple infections may occur in the host environment, which involves unusual immunity mechanisms, multiple integrases with different insertion sites and host receptor ligands conserved across a broad spectrum of bacterial species. Considered together, these data help to explain the emergence of STEC-mediated disease as a serious health problem worldwide. Furthermore the data could provide an explanation for those reports of Shiga toxin-mediated disease by other enterobacterial species.

The contribution of phage to *Salmonella* pathogenicity

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Salmonella enterica harbors a number of prophages whose genetic mosaicism and variable distribution constitute major sources of diversity between strains. Most prophages are fully functional and the carrier strains continuously produce phage as a result of spontaneous induction. This creates a regimen that positively selects for the spread

of phage to new, susceptible strains. Prophages often include loci that participate in pathogenesis, creating conditions that favor maintenance of phage sequences in the lineage. Variability in the assortment of phage-associated loci is predicted to modulate strain- and/or serovar-specific features such as host adaptation, tissue tropism and infection pathways. Some of these genes encode effector proteins translocated inside eukaryotic cells by type III secretion system [SopE, GogB, SseI, (SrfH) SspH1]; others encode proteins that influence intracellular survival and proliferation (SodC1, GtgE). The properties of some of these proteins as well as the phages carrying them will be described.

Mechanisms involved in the infection of *Escherichia coli* by short tailed Shiga toxin phage

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University of Liverpool

The major virulence factor for enterohaemorrhagic *E. coli* (EHEC) is Shiga toxin (Stx) production encoded by genes on lambdoid bacteriophage. We induced such a short-tailed Stx phage ($\Phi 24_B$) from a clinical isolate of *E. coli* O157:H7 and following inactivation of the toxin gene by insertion of selectable markers, have proceeded to characterise the phage infection process. An *E. coli* mutant resistant to phage infection was identified, and this defect subsequently complemented by a gene now known to encode an outer membrane protein of unknown function (*vpr*). We purified the 810 amino acid protein and produced polyclonal rabbit antibody. A phage adsorption assay was used to demonstrate that occlusion of Vpr with the immune sera prevented phage infection. Vpr is very widely distributed amongst all Gram negative bacteria, but highly conserved within the *Enterobacteriaceae* and is encoded by an essential gene. We have also used multiple novel primer sets to examine tail fibre genes from a variety of short tailed Stx-phages. These phages have nearly identical tail fibres and should therefore all bind to Vpr to initiate phage infection. This suggests that Vpr serves as the primary target recognition molecule for a great many Stx-encoding bacteriophage and is a key element in the dissemination of Shiga toxin genes amongst *E. coli* populations.

Phage therapy

Phage therapy: history and prospects

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Felix d'Herelle discovered phages in 1917 while fighting dysentery in French soldiers, and soon successfully applied them in treating a range of diseases. Many entrepreneurs jumped into the field, but with inconsistent results due to inadequate testing inadequate testing and poor understanding of the heterogeneity of both phage and bacteria. The escalating incidence of antibiotic-resistant bacteria and growing concern about side effects of antibiotics have led to broad renewed interest in therapeutic phage applications, taking advantage of molecular tools, growing knowledge about the ecology and biology of phages, and the extensive clinical experience in Eastern Europe. Phage therapy there has been particularly successful against purulent infections like osteomyelitis and diabetic ulcers; the phage keep multiplying and moving deeper into pockets of infection, whereas antibiotics penetrate poorly, creating selection for antibiotic resistance. Long-lost relevant US experiments from the 1940s unequivocally showed that phage injected intraperitoneally or intramuscularly could reach and multiply in pockets of infection anywhere in the body – even in the brain – and block otherwise-fatal infections. (see www.evergreen.edu/phage) While such reports are tantalizing, finding funding for controlled clinical trials, in Tbilisi, Poland or the US, is challenging – different phages attack different bacteria, phage cocktails are needed, and intellectual property issues are complex. Though phages do not easily fit into our current pharmacological system, they hold important potential for dealing with MRSA, reducing amputations in diabetics, controlling food-safety problems.

Treatment of methicillin resistant *Staphylococcus aureus* (MRSA) infection by immobilised bacteriophage

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Cases of methicillin resistant *Staphylococcus aureus* (MRSA) infections are on the increase. This study has investigated the effects of chemically bonding (immobilising) a lytic bacteriophage derivative of 9563 (NCIMB), which is effective against strains of MRSA to polymers including nylon.

Results show that this derivative of 9563 (NCIMB) can be immobilised onto nylon where it can infect and lyse host bacteria and release new bacteriophage particles. The bacteriophage strain used is active against 5 internationally recognised clones of MRSA (PF015a, PF016a, PF099b, PF105a, PF111-29, supplied by Scottish MRSA Reference Laboratory) and *S. aureus* strain 8588 (NCIMB) and 7 of the 17 epidemic MRSA (EMRSA) strains (NCTC). The nylon can be in different forms including strips, sutures and beads.

There is no decline in the infectivity of the immobilised bacteriophage under differing temperature and humidity conditions where bacteriophage in suspension lose activity. Immobilising the bacteriophage acts as a stabiliser to the viability of the bacteriophage, which could contribute to their use as therapeutic agents in order to overcome some of the limitations of their use including the clearance by the immune system and inability to survive in arid environments.

Preliminary data from trials of immobilised bacteriophage on sutures show that such sutures can prevent wound infection in an infected rat model.

SAR domains and the regulation of phage lysis

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Phages effect and regulate lysis through 3 types of proteins: holins, endolysins (lysozymes) and, in some cases, antiholins. Holins are small membrane proteins that accumulate in the membrane until suddenly triggering at an allele-specific time. Holin triggering results in a lethal permeabilizing lesion, the 'hole', in the cytoplasmic membrane. Antiholins are proteins that bind to and inhibit holins. Many lysozymes accumulate in the cytoplasm and are released through the holes after holin triggering. Recently endolysins have been described which are exported by the *sec* system. Lyz, the lysozyme of coliphage P1, accumulates in an inactive form in the periplasm, tethered to the membrane by an N-terminal transmembrane domain (TMD). When holin-triggering depolarizes the membrane, the N-terminal TMD exits the bilayer, and the protein becomes activated; the domain is thus called a SAR domain ('signal anchor-release'). The unusual ability of SAR domains to exit the membrane is used in other aspects of the regulation of lysis. Recent results indicating that SAR domains regulate the functions of both holins and antiholins will be described. SAR domains appear to be uniquely suited for regulation of lysis because of the linkage of their topology to the energy state of the membrane.

The lytic genes from mycobacteriophage Ms6: role in cell lysis

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Ms6 is a temperate bacteriophage that infects *Mycobacterium smegmatis* cells. As it happens with all double stranded DNA phages, it produces lytic enzymes that quickly destroy the cell wall of the host bacterium to release progeny phage. The mechanisms of lysis have been studied either for Gram negative or Gram positive bacteria but no information was previously available on phage lysis in Mycobacteria. We have identified genes *lys*A(endolysin) and *hol*(holin) from phage Ms6, which are clustered in a transcription unit together genes *gp1, gp3* and *gp5*. Our goal is to understand the role of each ORF in lysis. In this study several recombinant strains were constructed in order to assess the contribution of different combinations of the five genes to cell lysis. Expression was carried out in *E.coli* and in *M. smegmatis* and the growth kinetics was measured. The results showed that plasmids harbouring the five genes are able to induce a rapid and lethal effect on *E.coli* cells, and that the presence of gp1 is necessary to this severe effect. A different phenotype was observed when gp1 is absent. This data suggests that *gp1* has an important role in *E. coli* lysis, and that *gp4* (holing) is not essential to achieve that phenotype.

Phages as vaccines

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Whole bacteriophage λ particles have been used as DNA vaccine delivery vehicles. In this system, the gene encoding the antigen, under the control of a suitable eukaryotic promoter, is cloned into the bacteriophage genome and the whole bacteriophage particle is used to inoculate the host. 'Phage' DNA vaccines have been successfully tested with both single gene constructs and genomic libraries, in mice, rabbits and sheep, and using a variety of delivery routes, including intramuscular, intradermal, and most interesting, orally. Phage-based DNA vaccines offer several significant advantages over standard 'naked' DNA vaccination, including much larger cloning capacity – up to 23kb in size can be inserted (or 50kb if a cosmid-containing bacteriophage is used). Unlike eukaryotic virus vectors, there should be no possibility of bacteriophage replication within host tissues, and no antibiotic resistance genes are carried. In theory, phage particles can be rapidly, easily and inexpensively prepared to high yields sufficient to deliver millions of doses. In addition, they are highly stable under ambient conditions, facilitating transport and storage. Bacteriophages have been used in eastern Europe since the 1930s as systemic bactericidal agents, and thus there is a long history regarding large scale manufacturing and use in humans.

Bacteriophage library screening for the rapid identification of DNA vaccine candidate genes

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It has been shown that whole bacteriophage particles can be used to efficiently deliver DNA vaccines. A vaccine gene in a eukaryotic promoter-driven expression cassette is cloned into a standard lambda (λ) cloning vector. Whole phage particles are used to vaccinate the host. In a development of the system, we have screened phage whole genome libraries with convalescent serum to identify putative vaccine candidates.

A genomic library of the bovine pathogen *Mycoplasma mycoides* small colony type was cloned into the λ-ZAP Express vector, which allows both prokaryotic and eukaryotic expression of the cloned insert. Immunoscreening of phage grown on *E. coli* with convalescent antisera was used to identify phage expressing immunodominant proteins. Positive plaques were picked, amplified and the inserts characterised by Western blotting and sequencing and mice vaccinated with two selected clones. In both cases, specific antibody responses against the vaccine genes were seen, as measured by ELISA and Western blot. In one case protection against challenge was also observed. Sequence and structural analysis of the protein expressed by the protective clone suggested it was a putative adhesin.

This screening method allows new phage DNA vaccines to be developed, with no prior knowledge of the protective antigens involved.

Virus Group session

Virus cell tropism and host range

Molecular determinants of adenovirus tropism

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The 51 human adenovirus types are divided into six species A to F with different tropisms. The distal knobs of the 12 fibers protruding from each vertex interacts with the primary receptor. Vertex capsomeres express five RGD containing loops interacting with $\alpha_v\beta_3$ $\alpha_v\beta_5$ integrins – the secondary receptors. Fiber knobs of all species but B contain CAR binding motives.

CAR is an integral part of tight junctions with limited in vivo accessibility and has a role during unzippering of epithelial cells during release of adenovirus.

Non CAR receptors: Species B adenovirus receptors are abundantly expressed on hematopoietic, neural, epithelial and endothelial cells. Ad11p, Ad35 & Ad3 (species B) showed high affinity to CD34(+) cells.

CAR binding amino acid residues are not conserved in fiber knobs. Consequently CAR cannot be used as a receptor, instead CD46; CD80, CD86 & EDTA sensitive primary receptor are used. Species C: Heparan sulphate affinity of the fiber shaft. Species D: Both CAR (Ad9) and non CAR mediated transduction (Ad30 and Ad37) has been noted.

The crystal structure of the fiber knob of EKC causing Ad37 and sialic acid is available.

Cell-type specific leukaemogenicity of avian leukosis viruses

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Avian leukosis viruses (ALV) are members of the alpharetrovirus genus of the family *Retroviridae*. *Structurally*, ALVs are simple viruses carrying just three genes *gag/pro-pol-env*, that encode the virion group-specific antigens and protease, the enzyme reverse transcriptase, and the envelope glycoproteins respectively. On the basis of the envelope properties, ALVs are classified into 10 subgroups designated A to J. Chickens are infected by the subgroups A, B, C, D, E and the most recently emerged J subgroup. Unlike ALV subgroups A to D, that mainly induces lymphoid leukosis (LL), ALV-J with a distinct *env* was unique in inducing myeloid leukosis (ML). The induction of LL occurs by the activation of the c-*myc* gene insertional mutagenesis. Our studies show that ALV-J-induced ML by also occurs by insertional mutagenesis of the c-*myc* gene, although there was a much higher frequency of transduction of *myc* sequences leading to the generation of v-*myc*-containing acutely transforming viruses in the tumours. In order to examine the basis for the unique myeloid tropism for ALV-J, we studied the oncogenicity of chimeric viruses that expresses A or the J *env* sequences. Our studies showed that the viral *env* is a major determinant of cell type-specific oncogenecity.

Aspects of molecular biology of HIV-1

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HIV-1 replication in human cells is a complicated process. Following fusion and uncoating, the virus encounters several blocks at the level of reverse transcription, where DNA-editing effects of APOBECs 3G and 3F are ovecome by Vif. After integration, its transcription must be initiated by NF-κB and maintained by Tat, both of which recruit P-TEFb to the preinitiation complex. P-TEFb is composed of CycT1 and CDK9, which phosporylates the CTD or RNAPII, Spt5 from DSIF and RD from NELF. These actions allow RNAPII to elongate, copy the viral genome and cotranscriptionally splice viral transcripts. P-TEFb itself is carefully regulated by HEXIM1 and 7SK RNA in cells. The balance between LMW and HMW P-TEFb complexes dictates the state of proviral latency in the host. This issue must be addressed to eliminate the reservoir of HIV-1 from the body. Should the virus be transcribed, then complicated splicing and export processes ensue that are dictated by alternative splicing factors and Rev. Finally, HIV-1 buds into MVBs via its Gag and Nef subunits. In this scenario, Nef also binds GagPol, cholesterol and is targeted into lipid rafts. This complicated assembly ensures that optimally infectious viral particles are released from cells.

The interferon response against hepatitis C virus

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Hepatitis C virus (HCV) is a positive strand RNA virus belonging to the *Flaviviridae* family of viruses. In contrast to other members of this family, HCV exhibits restricted tissue tropism for hepatocytes and replicates poorly or not at all in established cell lines. Even in infected liver tissue, HCV replication could so far only be detected after amplification of cDNAs with the polymerase chain reaction, suggesting that only a fraction of hepatocytes produce virus during an infection. Nevertheless, the sequelae of HCV infections are fibrosis, cirrhosis, and liver cancer. Disease progression can be reversed or prevented with alpha interferon (IFN-α) therapy that, in combination with ribavirin can cure 50–90% of infected patients. In this regard, the HCV system is unique because infections with related viruses, including West Nile virus (WNV) and Japanese encephalitis virus are resistant to IFN-α therapy. Investigations with a cell culture system for the replication of HCV variants revealed that HCV is very sensitive to the antiviral program induced by IFN-α. In contrast, WNV replication blocks the IFN signal transduction pathway, which explains the observed resistance to IFN therapy in infected animals and people. Why a fraction of HCV infected patients does not respond to IFN-α therapy remains a major unresolved question that could be explained with two models. The first predicts the existence of HCV variants that are resistant to the antiviral program induced by IFN-α. The second proposes that all, or a fraction of infected hepatocytes have lost the ability to respond to IFN-α and hence, fail to establish an antiviral state necessary to inhibit HCV replication.

Chemokine receptors and co-stimulatory molecules: unravelling FIV infection

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The feline immunodeficiency virus (FIV) induces a disease similar to AIDS in cats yet, in contrast to HIV, it does not utilise CD4 as a cell surface receptor. Here, we identify the primary receptor for FIV as CD134 (OX-40), a helper T cell activation antigen and co-stimulatory molecule. CD134 expression promotes viral binding and renders cells permissive for viral entry, productive infection and syncytium formation by FIV. Furthermore, CD134-dependent infection is species-specific with FIV displaying a strong preference for feline CD134 compared with human CD134. CD134-dependent infection required co-expression of CXCR4 and was inhibited completely by the CXCR4 antagonist AMD3100. Usage of CD134 as a receptor was widespread amongst FIV strains from diverse geographical origins and of distinct subtypes (clades). Using a panel of chimaeric CD134 molecules generated between feline and human CD134, the binding site for FIV was localised to the first cysteine-rich domain (CRD-1), a region outwith the predicted binding site for the natural ligand. Further, using a cross-species reactive anti-human CD134 antibody, we confirmed that feline CD134 was expressed on both activated T cells and monocyte-derived macrophages; the principal target cells for the virus. Despite the evolutionary divergence of the feline and human lentiviruses, both viruses utilise cell surface molecules that target the virus to a subset of cells that is pivotal to the acquired immune response.

Coordination of virus entry and innate immune activation by human cytomegalovirus

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Human cytomegalovirus (HCMV) enters cells via a series of interactions between viral envelope glycoproteins and cellular molecules that serve as entry receptors. We recently discovered that certain cellular integrins serve as entry receptors for HCMV and that the interaction is mediated via a highly conserved disintegrin-like domain in glycoprotein B. At some point in the entry pathway, however, immune sensors known as Toll-like receptors detect structures on envelope glycoproteins and activate signal transduction pathways that lead to expression of genes involved in innate immunity. An important questions is how are the two process coordinated or are they? We have found that molecules that inhibit entry also inhibit induction of innate immunity. Further virions appear to traffic to a pH neutral, intracellular compartment that may be the site of productive infection and activation of signal transduction. One possibility is that entry receptors (EGFR, integrins and signaling accessory molecules) and innate immunity machinery (TLR2, its membrane-associated partners, cytoplasmic adaptors and signaling machinery) coalesce into specialized membrane microdomains with integrins playing central ligating role. Concentration of all of these cell surface receptors into a defined platform likely facilitates cell signaling events, some of which are optimal for replication and others of which are clearly hostile to the virus. Evidence in support of this hypothesis will be presented.

Virus evasion of the interferon system

Steve Goodbourn

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The type I interferons (IFNs) comprise the host's primary (innate) defence against viruses. The IFN-induced antiviral state blocks or limits virus replication by up-regulating the synthesis of a number of cellular proteins that interfere with transcription and protein synthesis, render cells sensitive to apoptosis in response to infection, cause cell-cycle arrest, or up-regulate MHC molecules and antigen processing. To establish infections viruses must replicate in the face of this powerful defence mechanism. Over the last 5–6 years it has become clear that many viruses attempt to evade innate immunity by either blocking the induction of type I IFN, or by blocking signal transduction in response to IFN. We will describe results using paramyxoviruses as examples that show that the ability to limit the production of, and response to, IFN, can affect both pathogenicity and host range. Many types of paramyxovirus inhibit cellular signalling in response to IFN, but show surprising differences in the means by which they achieve this. Rubulaviruses inactivate IFN signalling by targeting STAT molecules for degradation by proteasomes in a mechanism in which the virus V protein recruits normally stable STAT molecules to an ubiquitin E3-ligase. Our results show that the V protein directly interacts with cellular STAT2 and that the species-specificity of this interaction may play a role in host range. The V proteins of paramyxoviruses also act to limit the production of IFN-β by direct interaction with a novel cellular RNA helicase called mda-5. This is a property of all paramyxovirus V proteins we have tested and we are currently investigating the contribution of this property to host range restrictions.

How high are species jumps for paramyxoviruses?

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Most paramyxoviruses are transmitted by the respiratory route, which facilitates cross species infection (CSI) and hence species jumps. CSI requires first that hosts of different species get in close proximity. The next barrier to CSI may be unavailability of entry (co)receptors. In the case of the morbilliviruses, this barrier is very low as all members of the genus appear to be able to use homologues in other species. Inside the cell, the viruses have to overcome the host's innate immune response and paramyxo-viruses use at least part of the surface of non-structural proteins to inhibit the interferon response. This inhibition can be abrogated by very small sequence changes. However, it is not clear how easy viruses can attain the ability to inhibit innate immune response in a new host. The introduction of viral RNAs into the host's cytoplasmic RNA complement may trigger interference signals and this may provide another barrier to CSI as is the presence or absence of specific cellular accessory proteins required for viral replication and transcription. Finally, the degree to which the viruses can bud from appropriate epithelial membranes or from other cells present in the lungs will determine whether they can be transmitted to other members of the new host species.

Picornaviruses

D.J. Evans

University of Glasgow

Abstract not received

Special lectures

Colworth Prize Lecture

Gas gangrene; an open and closed case

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Gas gangrene, especially caused by the bacterium *Clostridium perfringens*, is one of the diseases that contributed to significant mortality during past armed conflicts – it is often stated that more servicemen died indirectly from gas gangrene than directly from ballistic injuries during World war I. Gas gangrene is no longer a major cause for concern on the battlefield, but it is a disease of increasing incidence in the civilian population especially in individuals who have sustained traumatic injuries or undergone some forms of surgery. The elderly and diabetics are especially vulnerable to disease. A period of intense research in the 1940s resulted in the identification of the alpha-toxin as a key virulence factor in *C. perfringens* gas gangrene. However, it is not until very recently that the role of this toxin at a molecular level has been determined. This knowledge has allowed novel genetic toxoids to be devised which offer the potential to protect against disease.

Fleming Prize Lecture

Understanding the latent-lytic switch in gamma-2 herpesviruses

A. Whitehouse

University of Leeds

Gamma-2 herpesviruses are an important sub-family of herpesviruses with oncogenic potential, particularly as a result of the identification of the first human gamma-2 herpesvirus, Kaposi's sarcoma-associated herpesvirus (KSHV). Adrian's current research focuses on the molecular basis of gamma-2 herpesvirus latency and the virus-host cell interactions that regulate the transition to the lytic phase of virus replication. In addition, a second focus of his research involves the development of herpesviruses as episomally maintained gene delivery vectors.

Posters

Clinical Microbiology Group

CM 01 A comparison between a phenotypic and a molecular-based typing strategy for *Campylobacter jejuni* and *Campylobacter coli*

C. Swift[1], R.J. Owen[1], H.R. Smith[1], N.C. Elviss[2], L.K. Williams[2], F. Jorgensen[2], T.J. Humphrey[2], D.J. Griggs[3], M.M. Johnson[3] & L.J.V. Piddock[3]

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A typing strategy for *Campylobacter jejuni* and *Campylobacter coli* based on *fla*A short variable region (SVR) sequence typing, PFGE and speciation by real-time PCR detection of *ceu*E and *map*A genes, was applied to 52 *Campylobacter* isolates from five different broiler flocks. All isolates had previously been characterized phenotypically using direct agglutination of heat-stable (HS) antigens and phage-typing.

Twelve different *fla*A SVR types, from a database of 405 defined types, and two novel types were identified. Phenotypic typing gave eleven HS serotypes and six phage-types. Together, these gave fourteen distinct sero-phage type combinations. Six isolates were untypable by HS serotype and one was untypable by phage-type. The *fla*A SVR sequencing approach sub-divided four distinct groups of isolates of identical sero-phage type. Conversely, two groups of isolates of identical *fla*A type were sub-divided by sero-phage type combinations. Interestingly, those isolates of *fla*A type 105 all shared identical PFGE patterns.

Typing *C. jejuni* and *C. coli* using a genotypic approach circumvents some problems associated with expression of antigens and surface structures inherent in phenotypic typing methods. This study shows the important advantages of genotyping are that all strains are typable and that greater discrimination is possible between strains than by sero-phage type.

CM 02 A novel ELISA detecting anti-pertussis toxin IgG in oral fluids for diagnosis and surveillance of *Bordetella pertussis* infection in children and young adults

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Protection afforded by infant vaccination against *Bordetella pertussis* wanes after 5–10 years and older children and adults can become reinfected, causing a prolonged (non-whooping) cough. Diagnosis of infection in this population is important for treatment, surveillance and for preventing transmission to unvaccinated babies, for whom consequences are severe.

Serum IgG titres against pertussis toxin (PT) are routinely used as a marker of recent or persisting *B. pertussis* infection. In order to increase the convenience of sample collection, we have devised an IgG-capture ELISA capable of detecting anti-PT IgG in oral fluid. The assay was evaluated by comparison to our serum ELISA, using paired serum and oral fluid samples from 80 children (aged 5–16 years) with a history of prolonged coughing, whose serum anti-PT titre had already been determined. Results showed that the oral fluid assay detected seropositive subjects with a sensitivity of 79.1% (95%CI 64.0–90.0%), a specificity of 94.6% (95%CI 81.8–99.3%) and a positive predictive value of 86.3% (assuming 30% prevalence in this population). When used to distinguish between the seropositive coughing children and 220 normal control children, its specificity rose to 96.4% (95%CI 93.0–98.4%). This oral fluid ELISA will greatly assist in the convenience of *B. pertussis* disease diagnosis.

CM 03 Multiplex PCR-based identification of *Salmonella enterica*

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Introduction The current basis for identification of *Salmonella enterica* is the Kauffmann-White scheme, which recognises over 2500 serotypes by exploiting the expression of distinct surface antigens. *Salmonella* express flagellar (H1 and H2), somatic (O) and virulence antigens that contribute to the pathogenicity of the organism and vary between strains. This variation is detected via agglutination with known antisera, and it may also be detected at amino acid and nucleotide sequence levels. DNA amplification techniques circumvent the need for antisera production using animals.

Method A panel of 500 *S. enterica* isolates was tested using three different multiplex-PCR assays in a blind trial and the results were compared with traditional serotyping methods. The antigens identified included H:b, H:d, H:e,h, H:G, H:i, H:l,v, H:r, H:z10, H:1,2, H:1,5, H:1,6, H:1,7, H:1,w, H:e,n,x H:e,n,z15 (flagellar) and B, C1, C2, D and E (somatic groups).

Results / conclusions This work assesses the feasibility of a DNA sequence-based approach to identification. The results show that sequence variation at loci of flagellar and somatic antigens can be exploited to complement existing phenotypic methods of identification. The multiplex PCR approach is rapid, specific and reproducible. It is also independent of the phase expressed by *S. enterica* at the time of testing.

CM 04 Improved diagnosis of listeriosis by real-time 5' nuclease PCR assay

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The foodborne pathogen *Listeria monocytogenes* is capable of causing severe and fatal infections in certain high-risk patient groups including pregnant women and neonates. Clinical features may include abortion and perinatal infections.A definitive diagnosis of listeriosis depends upon culturing the organism from blood or cerebral spinal fluid (CSF). However, low numbers of the bacterium may be present in both samples and problems with culturing this bacterium after commencement of antimicrobial therapy are well recognised.

Real-time 5'nuclease PCR (TaqMan) assay (both nested and un-nested) to specifically amplify a fragment of the *L.monocytogenes hly* gene was established.

The assay proved useful for the rapid confirmation of *L. monocytogenes* colonies growing *in vitro* and was specific for this bacterium. The assay was assessed for detecting *L. monocytogenes*

in serum and CSF samples from patients with clinical symptoms of listeriosis. The sensitivity of the assay was further improved by performing a 25 cycle PCR assay prior to the real-time assay, using primers external to those used for the assay.

These results show that 5'nuclease PCR (TaqMan) assays are useful for rapid confirmation *L. monocytogenes* of cultures growing *in vitro*. The nested assay provides an additional tool for establishing a non-cultural diagnosis of listeriosis, especially in patients with suspected neurological infection.

CM 05 Human gene expression in peripheral blood mononuclear cells (PBMC) from chronic fatigue syndrome (CFS) patients

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Background Chronic Fatigue Syndrome (CFS) is a multisystem disease, the pathogenesis of which remains undetermined.

Methods To gain further insight into the pathogenesis of CFS, we analysed gene expression in peripheral blood mononuclear cells (PBMC) from 25 CFS cases diagnosed according to the CDC criteria and 25 normal blood donors matched for age, sex and geographical location, using a single-colour microarray representing 9,522 human genes. After normalisation, average difference values for each gene were compared between test and control groups using a cut-off fold-difference of expression ≥1.5 and a P value of 0.001. Genes showing differential expression were further analysed using Taqman real-time PCR in fresh samples.

Results Analysis of microarray data revealed differential expression of 35 genes. Real-time PCR confirmed differential expression in the same direction as array results for 16 of these genes, 15 of which were upregulated (ABCD4, PRKCL1, MRPL23, CD2BP2, GSN, NTE, POLR2G, PEX16, EIF2B4, EIF4G1, ANAPC11, PDCD2, KHSRP, BRMS1, GABARAPL1) and one was downregulated (IL-10RA). This profile suggests T-cell activation, and perturbation of neuronal and mitochondrial function. Upregulation of NTE and EIF4G1 may suggest links with organophosphate exposure, and virus infection, respectively.

Conclusion Our study suggests that CFS patients have reproducible alterations in gene regulation.

CM 06 An international comparison of ciprofloxacin resistant *Neisseria gonorrhoeae* by sequence based genotyping

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Tracking important strains of bacterial pathogens is increasingly being performed by sequence-based methods, allowing strains to be compared via a central database. We have used the highly discriminatory sequence based NG-MAST procedure, which indexes sequence variation at two hypervariable genes, *por* and *tbpB*, to compare 475 ciprofloxacin resistant *N. gonorrhoeae* (QRNG) isolates from five countries. The por and tbpB genes were sequenced and alleles and sequence types (ST) were assigned from www.ng-mast.net. The QRNG were from London (2000–2003 surveillance isolates), Scotland (all QRNG in 2002), France (2002–2003 surveillance isolates), Italy (2003 surveillance isolates) and Durban, South Africa (November 2003). STs were compared.

There were 164 different STs. 125 isolates (26%) had a unique ST and there were 34 STs which included multiple isolates (2 to 93), 23 of which were unique to one country and 11 were from multiple countries.

NG-MAST has illustrated the different phases of the spread of QRNG within a country: predominantly unique STs and small clusters from importation due to travel, such as South Africa, to the emergence of large clusters as strains become endemically transmitted, such as London and Scotland. NG-MAST is a useful method for tracking changes in the epidemiology of gonorrhoea over time and across countries.

CM 07 Identification of *Acinetobacter baumannii* by PCR

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Acinetobacter species, particularly *A. baumannii*, have become increasingly important nosocomial pathogens in recent years. *A. baumannii* is difficult to identify using methods currently in use in clinical laboratories and a rapid, specific method of identification would be of great benefit to infection control teams.

Method PCR assays based on the 16S rRNA gene were developed to enable identification of the *Acinetobacter* genus and, following that, of the *A. baumannii* species. The assays developed were tested against a range of clinically significant Gram-negative isolates, which included all known *Acinetobacter* species. These assays have been applied to a large number of acinetobacter isolates from hospitals and results compared to those obtained using fatty acid analysis by gas chromatography.

Results Preliminary tests showed that the genus PCR was highly sensitive for *Acinetobacter* species, but cross reactivity was observed with strains of *Moraxella osloensis*. However, optimisation of the annealing temperature rendered the assay specific for the *Acinetobacter* genus. This protocol was able to detect all *Acinetobacter* species, except the newly described species *A. ursingii*. The species PCR was found to be specific for *A. baumannii*.

Conclusions The combination of the two PCR assays allows the confirmation of isolates as *Acinetobacter spp*. and unequivocal identification of *A. baumannii*.

CM 08 The effect of antibiotics on mutation frequency in *Streptococcus pneumoniae*

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The use of antimicrobial agents in medicine provides a selective pressure that favours the development and spread of resistant organisms. In some situations antimicrobial exposure may be at concentrations below the MIC for the micro-organisms exposed. This study has examined the effect of sub-inhibitory concentrations of antimicrobials on mutation- and transformation-frequency in *Streptococcus pneumoniae*.

Three clinical isolates were grown with and without ampicillin, ciprofloxacin, erythromycin, streptomycin and trimethoprim at the maximum concentrations that allowed a normal growth rate for each isolate. Two isolates were further examined for differences in transformation frequency in the presence and absence of the same antimicrobial agents.

In all antibiotic treatments the median frequency of mutants was increased. This increase was significant for results pooled fom the 3 isolates when grown in ciprofloxacin ($p<0.01$), streptomycin ($p<0.001$) and trimethoprim ($p=0.05$), although significance was not found for each strain when the results were examined separately. Antibiotic treatment had little effect on transformation frequency.

Sub-inhibitory concentrations of ciprofloxacin, streptomycin and trimethoprim leads to an elevated frequency of mutation in at least some isolates of *Streptococcus pneumoniae*. This suggests that antibiotic treatment may result in increased mutability in pneumococci, as well as selecting for resistance *per se*.

CM 09 Can pulsed-field gel electrophoresis be used to monitor nosocomial patient-to-patient transmission of methicillin resistant *Staphylococcus aureus*?

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Methicillin resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital acquired infection. At the Royal Free Hospital, cross-transmission is said to have occurred if a patient has contact with an MRSA-positive patient then becomes positive themself. However, this does not take into account that the two patients maybe colonised with different strains. The aim of this study was to determine if phage-typing and pulsed-field gel electrophoresis (PFGE) could be used to more accurately identify cross-transmission between patients with MRSA.

Patients on two surgical wards were screened for MRSA and the daily bed occupancies were recorded. Isolates were typed using phage-typing and PFGE. Fifty isolates from 32 patients were phage typed and six different phage types (PT) were identified. PFGE typing of the most common phages types, PT16 and PT15, showed six different PFGE profiles of PT 16 and nine different profiles of PT 15. Phage typing data suggests 42 instances of cross-transmission on Ward C, although only 17 involved patients colonized with isolates with the same PFGE type. On Ward J, phage-typing data showed ten possible instances of cross-transmission. However, PFGE data illustrates that isolates had the same PFGE type in only one of these cases. This study shows the potential of PFGE to monitor cross-transmission of MRSA on hospital wards.

CM 10 Investigation of rifampicin resistance in strains of *Mycobacterium tuberculosis* isolated during an outbreak in North London

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Rifampicin resistance can occur as a result of mutations on the *rpo*B gene that encodes the β-subunit of the RNA polymerase. Over 95% of these mutations occur on an 81 bp fragment of the gene between bases 1276 and 1356. This region is known as the Rifampicin Resistance Determining Region (RRDR) or 'hotspot', and is used as a target for direct sequencing and commercial line probe assays. *Mycobacterium tuberculosis* isolates cultured from six patients associated with an isoniazid-resistant *M. tuberculosis* outbreak were demonstrated to have developed rifampicin resistance using phenotypic methods. The Line Probe resistance determining hybridization assay, Inno-LiPA (Innogenetics, Belgium) failed to identify rifampicin resistance in these strains. The aim of this study was to determine the basis for the rifampicin resistance in these six isolates by sequencing the entire *rpo*B gene.

The *rpo*B gene sequences revealed that resistance was associated with three different, rare mutation in each of the six isolates. Three isolates showed rare mutations within the RRDR. Two strains had the same mutation and the third was different. The remaining three isolates had the same mutation outside the RRDR, illustrating the need to use techniques that can detect mutations both inside and outside the rifampicin resistance-determining region.

CM 11 Identification of a putative adhesion gene in *Mycoplasma amphoriforme*, a novel mycoplasma

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In 1999 *Mycoplasma amphoriforme* was identified from a primary antibody deficient patient with unexplained bronchitis. Subsequently this organism has been detected in both immunocompromised and immunocompetent patients with respiratory tract symptoms, but not in healthy controls. Phylogenetically *M. amphoriforme* belongs to the *M. pneumoniae* group. Members of this group are known to adhere to cells via adhesin proteins, which are located in terminal organelles. To investigate if *M. amphoriforme* adheres to cells using a similar mechanism, PCR primers based on homologous regions of known mycoplasma adhesin genes were designed. Using these primers amplimers were obtained for 5 different *M. amphoriforme* isolates. The sequences of these amplimers showed 99.6–100% homology with each other and 50%, 53% and 45% homology with the P1-like adhesin gene, the MgPa adhesin gene and the P1 adhesin gene respectively. For this region of sequence the P1-like gene shares 49% and 45% homology with MgPa and P1 respectively. Using a GenomeWalker kit the entire gene is now being sequenced. This study has identified a putative adhesin gene in *M. amphoriforme*, which suggests that it may adhere to human cells by a similar mechanism to *M. pneumoniae*.

CM 12 CSP of *Streptococcus pneumoniae*: moonlighting as a novel virulence protein?

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The *com* locus (competence locus) of *S. pneumoniae* has a high degree of sequence homology to *Sil* (Streptococcal invasion locus) of *S. pyogenes*. A gene within *Sil, silC,* is found to have moonlighting properties with roles as a virulence protein and as a competence regulator. *SilC* shares significant sequence homology to *comC* which codes for CSP (competence stimulating factor), a protein that is excreted by *S. pneumoniae* and acts in a pheromone manner to regulate competence. We sequenced *comC* from carriage, pneumonia, bacteraemia and meningitis case isolates. In all sequences the first 22 amino acids are homologous with a double glycine at positions 23 and 24 – the putative cleavage site. This is followed by a variable sequence of 18 amino acids for all disease isolates and some carriage strains. In the majority of carriage strains, however, the homologous region is followed by 23 amino acids. This truncated sequence in the disease isolate group is due to a stop codon at position 42. In the majority of carriage strains the stop codon is at position 47. The variation in *comC* sequences that we have identified may indicate a role in invasiveness for CSP and further studies are underway to investigate this hypothesis.

CM 13 The potential for veterinary antibiotic use to cause glycopeptide resistance in human *Staphylococcus aureus*

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Staphylococcus aureus is a common pathogen of nocosomial and community acquired infections. The increasing prevalence of multi-resistant *S. aureus*, particularly that of methicillin-resistant *S. aureus* (MRSA), is a growing public health concern. Infections caused by MRSA are currently treated with vancomycin, the only remaining reliable antibiotic available. However, the development of vancomycin-resistant enterococci (VRE) has since contributed to the emergence of high-level vancomycin-resistant *S. aureus* (VRSA), with the first of three VRSA cases being documented in 2002. This study attempted to address the risk posed by transferable glycopeptide resistance in animal husbandry to human *S. aureus*, by looking at the mobilisation of resistance genes through conjugal elements. Preliminary results demonstrated 95% transferability of the tetracycline resistance gene, *tetM*, via conjugative transposition, from a laboratory strain of *E. faecalis*, JH2-2, to twenty animal *S. aureus* strains. The efficiency of transfer ranged from $2.5x10^{-9}$ to $1.04x10^{-4}$ conjugants per donor. This establishment of antibiotic resistance markers in animal *S. aureus* suggests the possibility of horizontal transmission of these genes to human *S. aureus*. To this end, the ability of animal *S. aureus* to act as intermediaries in horizontal gene transfer from animal enterococci to human *S. aureus* is currently being investigated.

CM 14 Genotypic comparison of *Yersinia enterocolitica* from humans and livestock by AFLP and comparative microarray analysis

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Yersinia enterocolitica is a well-recognised cause of human acute enteritis, epidemiologically-linked with consumption of contaminated pork. Recent national abattoir studies have detected *Y. enterocolitica* in sheep and cattle as well as pigs. To assess the relationship between veterinary and disease strains, 88 strains isolated from colonised livestock or associated with disease in humans over the same time period, were compared using Amplified Fragment Length Polymorphism and comparative genomic DNA microarrays. A pan-*Yersinia* array was used which consists of sequences representative of all known ORF's identified in *Yersinia spp*. The AFLP analysis groups isolates into 2 distinct groups consisting of biotype 1a isolates (classically designated non-pathogenic) and biotype 2-5 isolates (considered main pathogenic isolates in the UK). Within these groups sub-clusters formed according to serotypes. Microarrays are a powerful technology which can be used to study all genetic elements present within the genome simultaneously. Data produced through comparative genomics can be subjected to phylogenetic analysis and can reveal specific linkages and relationships between isolates. The results of the microarray analysis and their comparison to AFLP and biotyping data will be discussed here.

CM 15 Expression of *marA*, *soxS* and *acrB* in *Salmonella enterica*: role in cyclohexane tolerance

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Multiple antibiotic resistance (MAR) and cyclohexane tolerance in *Escherichia coli* has been attributed to the over-expression of *marA* and/ or *soxS* and consequent over-expression of the AcrAB-TolC efflux system. In *Salmonella enterica*, cyclohexane tolerance has also been associated with MAR and it has been hypothesized that this association is mediated as in *E. coli*. To determine the role of *marA*, *soxS* and *acrB* in cyclohexane tolerance, the expression of these genes were measured using comparative RT-PCR and DHPLC WAVE analysis from 46 isolates of *S. enterica* including cyclohexane tolerant and sensitive strains. Twenty-one isolates were tolerant to cyclohexane of which eight were resistant to ciprofloxacin. Twenty-five isolates were cyclohexane sensitive of which 16 were ciprofloxacin resistant. Nine isolates were found to over-express *soxS*, 10 isolates over-expressed *marA* and two isolates over-expressed *acrB*. No association was found between over-expression of *marA*, *soxS* and/or *acrB* and tolerance to cyclohexane. These data suggest that AcrB is not the effector for organic solvent tolerance in *S. enterica*, despite the great similarities between the genomes of *E. coli* and *S. enterica*.

CM 16 Effect of growth media and growth phase upon expression of genes conferring multiple antibiotic resistance

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The effect of ciprofloxacin, growth medium and growth phase upon the expression of genes involved in efflux-mediated antibiotic resistance was investigated.

RNA was isolated from growing cultures in either rich or minimal medium after 30mins incubation with antibiotic. RT-PCR was performed and data analysed using DHPLC.

Expression of *16S* rRNA remained constant at all conditions tested other than an expected increase associated with increased viable count during late logarithmic phase. Expression of *marA* decreased in minimal media at both growth phases, and decreased further in the presence of ciprofloxacin. Expression of *soxS* decreased in minimal media during early logarithmic phase only, and no change was seen in the presence of ciprofloxacin. However, expression of *soxS* in minimal media during late logarithmic phase was decreased in the presence of ciprofloxacin. Expression of *acrB* increased in minimal media during both early and late logarithmic growth phase. In the presence of ciprofloxacin, expression decreased dose-dependently.

Clear, statistically significant differences in gene expression between the different media were observed. In conclusion, expression of *marA* and *acrB*, genes which confer antibiotic resistance, had decreased expression in the minimal media, and more so in the presence of ciprofloxacin.

CM 17 Sulphonamide resistance in agricultural soils

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A study was carried out to assess the environmental impact of veterinary medicines released into the environment through the spreading of slurry. Plate counts and bacterial isolations were carried out to assess the numbers of resistant bacteria present in the soil before and after application of tylosin fed pigs' slurry, amended with sulphachloropyridazine and oxytetracycline.

Isolated bacteria were screened for sulphonamide resistance genes and the *intI* gene. Data has shown a pool of resistant bacteria has formed both in the presence and absence of a selective pressure.

2.5% of isolates contained a previously unpublished genotype of *sulI*, *sulII* and *sul3* genes. Exogenous isolations have been carried out to confirm whether they are carried on mobile elements.

One isolate, an *Arthrobacter* sp., carries a transferable plasmid, conferring sulphonamide resistance. This 23kb incQ plasmid contains a class 1 integron with a *sulI* gene, having 99.8% homology to pTET3 from *Corynebacterium glutamicum* and a second sulphonamide resistance gene, *sulII*.

CM 18 Detection and identification of bacterial presence in idiopathic disease using broad-range 16S rDNA PCR

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There are several forms of juvenile idiopathic arthritis (JIA). An infectious aetiology has been implicated, and there is indirect evidence that antigens, including those from Gram negative enteric bacteria may drive intra-articular inflammation in some forms of JIA. To address this question, we have used an optimised nested broad-range bacterial 16S ribosomal DNA PCR to detect bacterial DNA in synovial fluid (SF) and peripheral blood (PB) from patients with JIA. Potential environmental contamination of specimens was monitored. Bacterial DNA was detected in 25/76 JIA patients (33%, 16 SF positive), 2/4 children with other inflammatory disease, and 4 of 20 adult controls (0/11 non-arthritic, 0/5 rheumatoid arthritis, 4/4 reactive arthritis). A wide range of bacteria were identified in SF from patients with JIA, including species of *Bacillus*, *Micrococcus*, *Nocardia* and *Staphylococcus* (associated with arthritis), *Clavibacter* and *Herbaspirillum* (not known to be human pathogens), and several unrecognised bacterial sequences. Statistical correspondence analyses indicated associations between 2 clinical subgroups of JIA (systemic and oligoarthritis) and bacterial genera, but no associations with any subgroup were demonstrated at the species level. Analysis of TLR4 polymorphisms in some patients did not reveal any associations with potential genetic predisposition to infection. This study supports the concept that bacteria may be involved in the pathogenesis of chronic inflammation.

CM 19 The Oxford invasive pneumococcal infections surveillance programme

Angela B. Brueggemann, David T. Griffiths, Andrew J. Pollard, Richard Moxon, Kyle Knox, Derrick W.M. Crook & the Oxford Pneumococcal Surveillance Group

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Nine years of data and ~2600 pneumococcal isolates have been collected since this ongoing programme was established. Pneumococcal isolates causing invasive disease in Oxfordshire are collected from ten regional laboratories, with an emphasis on serotypic characterisation of all isolates to monitor the prevalence of individual capsular types causing invasive disease, and susceptibility testing to monitor the emergence and spread of antimicrobial resistance among invasive pneumococci. The ten most prevalent serotypes (in decreasing order) are as follows: ≥5 yrs, 14, 1, 9V, 4, 23F, 3, 8, 6B, 12F and 19F; children <5 yrs of age, 14, 18C, 6B, 19F, 23F, 9V, 19A, 6A, 1 and 7F; and children <1 yr, 14, 6B, 19F, 23F, 18C, 9V, 19A, 6A, 8 and 7F. Heptavalent vaccine coverage among children <5 yrs and <1 year is 77% and 69%, respectively. The percentage of all isolates that are antibiotic resistant is low: penicillin (4.7%), cefotaxime (0.4%), tetracycline (2.1%) and chloramphenicol (0.4%); although 12.9% of isolates are erythromycin resistant. This programme is particularly useful for the detection of any significant changes in the prevalence of vaccine and non-vaccine serotypes in the population following implementation of the pneumococcal vaccine, or any significant increases in antibiotic resistance in Oxfordshire.

CM 20 Bacterial (BACT) and viral (VIR) causes of conjunctivitis (CONJ) in children presenting to a primary care physician

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We explored the aetiology of CONJ as part of a clinical trial. 326 children with CONJ (CA) were recruited; the worst affected eye was swabbed once for BACT culture and once for molecular testing. Swabbing was repeated 7 days later. Likewise, 90 healthy children (CN) were recruited and swabs were taken from one eye. Blood and chocolate agar plates were inoculated with the BACT swab and incubated at 37°C+5% CO_2 for 48 h. Every colony type was counted and identified using standard methodology. 28 different BACT species were identified among CA, 15 of which were also found among CN. Each BACT species was defined as a pathogen if it was significantly ($p \leq 0.05$) more prevalent among recruitment swabs of CA v. CN. Only *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* were significant. 9% (CA) v. 26% (CN) of recruitment swabs were culture negative ($p < 0.001$). 6, 7, 0 and 0% (recruitment swab) and 4, 9, 0 and 0% (2^{nd} swab) of CA swabs were positive for adenovirus, picornavirus (P), herpes simplex virus and C. trachomatis, respectively, by PCR assay. One CN was positive for (P). These results have implications for the appropriate clinical management of CONJ.

CM 21 Molecular detection of community-acquired respiratory viruses, including human metapneumovirus (hMPV), in children with cough and fever

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Respiratory disease is common in children during the winter months. We explored which viruses are predominant in the community at this time using PCR-based detection methods. 443 children 0.5–12 yrs of age with cough and a fever in the past 72 h were recruited during the winters of 2000–04. A nasopharyngeal aspirate (NPA) was obtained and transported to the laboratory. 425 NPAs were collected and stratification by season was: 57 (2000–01), 100 (2001–02), 158 (2002–03) and 98 (2003–04). The overall viral detection rate was 77%, stratified by organism (n,% of 425 NPAs, range per season): influenza A (99, 23%, 14–37%); influenza B (36, 9%, 0–35%); respiratory syncytial virus (RSV) A (36, 9%, 4–15%); RSV B (23, 5%, 0–7%); hMPV (34, 8%, 7–9%); parainfluenza 1 (18, 4%, 1–7%); parainfluenza 2 (10, 2%, 0–6%), parainfluenza 3 (22, 5%, 0–16%); parainfluenza 4 (8, 2%, 1–4%); adenovirus (10, 2%, 2–4%); and picornavirus (79, 19%, 18–23%). A virus was detected in a high proportion of children using molecular assays. One-third of cases were positive for influenza A or B, an infection that is vaccine-preventable. hMPV was detected in 8% of cases.

CM 22 Impact of antibiotic therapy on distribution of antibiotic resistance ICEs in *Haemophilus* spp.

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Background & objectives Integrative and conjugative elements (ICEs) encode resistance to antibiotics among nasopharyngeal *Haemophilus* spp. There is no knowledge about the effect antibiotics have on the distribution of ICEs. The aim of the study was to determine the impact of ampicillin treatment on ICE distribution.

Methods 150 Children (aged 0.5–12 years) with upper respiratory infection were entered into an observational cohort study. Antibiotic treatment, if given, was administered for up to a week. Throat swabs were taken at recruitment, 2 weeks and 12 weeks (visit 1, 2 & 3) and inoculated onto enriched Columbia + bacitracin agar. Up to 5 morphologically different haemophilus colonies were picked, purified and tested for antibiotic susceptibility and the presence of ICEs detected using PCR.

Results 99/150 children received antibiotics. At visits 1, 2 & 3, ICEs were detected in 32.2%, 66.7%, and 36.4% of children receiving amoxicillin respectively compared with 38.5%, 35.9%, and 37.5% in controls respectively. The proportion of haemophilus isolates harboring ICEs increased from 16% to 35% in treated children and no increase amongst isolates from control children was seen (Anova, p = 0.002).

Conclusion Antibiotic exposure is strongly associated with increased detection of ICEs among nasopharyngeal commensal haemophili.

CM 23 Enhanced virulence of neonatal group B *Streptococcus*

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A study of the human pathogen, group B *Streptococcus* (GBS), was carried out over a 3-year period in a defined geographical area (Oxford, UK). Three hundred and sixty nine isolates of GBS were studied using two methods, capsular serotyping and multilocus sequence typing (MLST). 190 isolates were from asymptomatic carriage in pregnant women, 109 from diseased neonates and the remainder from adults >60 years.

Twenty percent of pregnant women were carrying GBS. Invasive GBS disease was seen in 0.9/1000 live births and in 11/ 100,000 population > 60 years per annum. Four sequence types (STs) (ST-17, ST-19, ST-23 and ST-1) identified using MLST accounted for over fifty percent of carried and invasive strains.

A single ST (ST-17), was significantly associated with hypervirulence in neonates (p=0.00002), particularly those with late-onset disease. Virulence associated with ST-17 was independent of capsular serotype III. No STs were identified as being hypervirulent in adults > 60 years, where STs commonly identified in carriage were also prevalent in disease.

The identification of a hypervirulent clone (ST-17) causing neonatal infections, presents an opportunity for the further study of pathogenesis of neonatal infection and potentially for the design of effective preventative strategies.

CM 24 Carriage of group B *Streptococcus* in pregnant women from Oxford, UK

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Asymptomatic vagino-rectal carriage of group B *Streptococcus* (GBS) in pregnant women in Oxfordshire, UK, is investigated. Epidemiological data for a subset (n=167) of the pregnant women participating were examined and the distribution of capsular serotypes of the GBS identified was determined. Two microbiological methods for isolation of GBS from vagino-rectal swabs were compared.

21.3% (159/748) women were colonised with group B *Streptococcus* (GBS). Recognised risk factors for neonatal GBS disease (maternal fever, prolonged rupture of membranes and preterm delivery) were present in 34 of 167 women (20.4%). The presence of these factors correlated poorly with GBS carriage. Capsular serotypes III (26.4%), IA (25.8%), V (18.9%) and IB (15.7%) were identified. Selective broth culture of vagino-rectal swabs was superior to selective plate culture, but the combination of both methods was associated with increased detection of GBS (7.5%).

In conclusion, GBS carriage is high in Oxfordshire, UK. The poor correlation between risk factors and GBS carriage requires further investigation on larger groups, given that the identification of these surrogate markers is recommended to guide administration of intrapartum prophylaxis by the Royal College of Obstetricians (UK). Selective broth culture detects more GBS carriers than a selective plate culture.

CM 25 Molecular analysis of methicillin resistant *Staphylococcus aureus* from an Intensive Therapy Unit

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Methicillin-resistant *Staphylococcus aureus* (MRSA) are nosocomial pathogens and are a common cause of post-operative infections, costing over £1 billion per year in healthcare. Methicillin resistance in *S. aureus* is mediated by the production of a penicillin-binding protein, PBP 2a, encoded by the *mecA* gene. In the hospital environment it is essential to rapidly identify MRSA. Traditional methods involve culture, which is time-consuming but now molecular techniques can be applied which circumvent these problems. The aim of this research was to characterise *S. aureus* isolates sampled from an Intensive Therapy Unit. DNA was directly extracted from environmental swabs and PCR amplified using primers that targeted the 16S-23S intergenic spacer region (ITS). Polyacrylamide gel analysis of the PCR products classified the *S. aureus* isolates into different sub-types. In conjunction with the culture-based approach, PCR amplification of the *mecA* gene was also performed and approximately 10% of samples were MRSA positive. To determine the efficacy of the hospital decontamination procedures, we selected several MRSA and methicillin-sensitive isolates and monitored the survival and recolonisation of MRSA following treatment with different biocides. This will allow the development of more effective strategies to control hospital MRSA infection.

CM 26 Changing patterns in *Candida* infection

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Introduction Although *Candida albicans* remains the most common candidal fungaemia, treatment resistant, non-albicans species are becoming increasingly prevalent. There is little data available on the epidemiology of these non-albicans species

Aims To examine the incidence of candida colonisation in ITU patients with emphasis on prevalence and infection patterns of non-albicans species.

Method A retrospective analysis of patients admitted to the ITU in Dumfries and Galloway Royal Infirmary, over a 6 month period.

Results A positive sputum culture was isolated from 54 patients. All patients had routine sputum samples sent, tracheal aspirates were sent from ventilated patients. 52% of patients were colonised with *C. albicans*. 48% of patients were colonised with non-albicans species, 74% of which were male patients. 85% of the non-albicans species were *C. glabrata*. *C. tropicalis* was isolated in 19% of patients (5 patients, 4 out of 5 of which was in combination with a second candida species). 24% of patients were colonised with both *C. albicans* and C. *glabrata*. The average age of patients with C. *glabrata* was 75 years, much higher than for patients with *C. albicans* (62 years). The mean APACHE II scores for patients with *C. glabrata* was 28 (range 79 to15), significantly higher than for patients with *C. albicans* 18, (range 32 to 8). All patients with *C. glabrata* had been treated with broad spectrum antibiotics, 78% with more than one type. Of the patients with non-albicans species, 77% had been ventilated, 82% had intravascular or urinary catheters in-situ. 26% had undergone abdominal surgery and 26% had severe underlying respiratory problems. Only 5 patients with initial *C. albicans,* who were treated with triazoles, subsequently became colonised with *C. glabrata*. Over the six month period, results suggest clustering of *C. glabrata* in months 1, 5 and 6.

Discussion Studies suggest an increasing trend of *C. glabrata* in the acute clinical setting. Isolates are possible in the absence of *C. albicans*. Frail, immunocompromised patients, in particular those who have had interventional therapy, broad spectrum antimicrobial or antifungal therapy seem to be at a highest risk. Clustering may implicate contamination from health-care workers in its transmission. This may be an understated risk factor in the acquisition of *C. glabrata* infection and emphasizes the need for strict infection control measures.

CM 27 Toxic shock syndrome – a diagnostic difficulty

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Introduction Toxic shock syndrome (TSS), is an acute, non-contagious systemic illness. It is caused by the toxin producing strains of *S. aureus* and the β-haemolytic *streptococci* and can occur in any non-immune person exposed to a TSS toxin. TSS is commonly associated with menstruation and tampon use, however can also be related to skin or soft tissue infections, particularly post surgical, skeletal infections or respiratory tract infection. TSS is often non-immunising and recurrent menstrual-associated TSS is well-described. Literature suggests that TSS is extremely rare, but diagnostic difficulties can lead to misdiagnosis and TSS can be fatal if left undiagnosed. We report a series of three cases of TSS, presenting within a short period of time.

Case reports

Case 1 17 year old female, presented with sudden onset collapse, diarrhoea, vomiting and abdominal pains. She gave no history of menstruation and an initial diagnosis of severe gastroenteritis was made. She failed to respond to conservative management and required ITU support. She was discharged with no firm diagnosis and re-presented one month later with similar symptoms, when a diagnosis of *staphylococcal* TSS was confirmed.

Case 2 15 year old female, presented during menses with sudden onset rash, rigors, severe diarhhoea, vomiting and abdominal pains. She was diagnosed with *staphylococcal* TSS on admission.

Case 3 28 year old female. Presented with sudden onset severe diarrhoea, vomiting, pyrexia and rash. She gave no history of menstruation. She responded poorly to treatment, required ITU support, high dose steroids and was eventually diagnosed with *streptococcal* TSS.

Discussion Diagnostic criteria for TSS include high fever, hypotension, erythematous rash and a complicated multisystem disfunction. Patients often require aggressive management. The Public Health Laboratory Service reports an average of 18 cases of diagnosed TSS in the UK per year. However, because of the uncommon and difficult nature of the diagnosis, many cases are misdiagnosed and therefore go unreported. It is essential to maintain a high level of suspicion for patients who are epidemiologically at high risk, but importantly, also the less ill patient with suggestive symptoms who fails to meet all diagnostic criteria but are in an at-risk group. High morbidity and mortality has been reported for undiagnosed and untreated cases.

CM 28 *Erysipelothrix rhusipathiae*: septic arthritis of the knee – a case report

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Introduction *E. rhisiopathiae* is a rare pathogen found worldwide. It is a commensal of a wide variety of vertebrate and invertebrate species but its major reservoir is domestic swine. The greatest commercial impact of infection is due to swine erysipelas. Serious infection in humans in extremely rare and associated with cutaneous infection in those who have had occupational exposure. Clinical manifestations include brain abscesses, osteomyelitis and chronic arthritis. Systemic infection can often be complicated by infective endocarditis, in which the mortality rate appears to be higher than for other organisms. We report a rare case of *E. rhusiopathiae* joint infection.

Case report A 74 year old male patient with known rheumatoid arthritis presented to a routine rheumatology clinic with an isolated knee sepsis, associated with a raised CRP and WCC. The knee was aspirated and *E. rhusiopathiae* isolated from culture broth after failing to grow on initial culture media. There were no other systemic manifestations. No direct occupational exposure was reported. A repeat knee aspirate failed to grow *E. rhusiopathiae*, but a third aspirate taken at arthroscopy once more isolated the organism. It was assumed that a soft tissue infection with *E. rhusiopathiae* had led to a bacteraemia which seeded into the rheumatoid knee joint. He underwent prolonged treatment, with a six week course of Clindamycin. Unfortunately, a low grade chronic infection persisted leading to complete disorganisation of the joint. A joint replacement is being considered.

Discussion This report is of interest because of the exceptionally unusual nature of the pathogen and because of the lack of systemic symptoms or history of any social or occupational exposure to explain the presence of the organism. Diagnosis of *E. rhusiopathiae* was problematical but is essential to ensure effective treatment as most strains are resistant to vancomycin, which is often used empirically to treat bacteraemia due to gram positive organisms.

CM 29 MRSA contamination in hands of healthcare workers – results of a 12 month audit from Wycombe General Hospital, Buckinghamshire

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Introduction MRSA infection within hospitals has increased dramatically in the last two decades and constitutes a serious burden to NHS resources. MRSA associated hospitalizations result in increased patient morbidity and mortality and substantially higher medical costs. An important mode of transmission of MRSA is via contaminated healthcare workers hands. Knowledge of the scope of the problem is essential for hospital administrators and medical personnel, who make policy decisions on control measures to prevent the spread of MRSA.

Aims To detect MSSA and MRSA carriage rate on the hands of healthcare staff.

Methods Handprints from hospital healthcare workers were sampled during working periods and cultured on MRSA specific isolation media. MRSA, MSSA and normal skin flora were identified using standard lab methods. The audit was carried out in 2 phases. In the first phase, handprints of 150 personnel were sampled. The audit was then repeated after 6 months with 160 samples to determine the effectiveness of infection control measures and hand hygiene policy introduced in the intervening period.

Results In the initial audit phase MRSA was isolated from the hands of 6% of staff, (doctors 5%, nurses 6%, porters 17%) and MSSA from the hands of 21%, (doctors 22%, nurses 17%, physiotherapists/OT's 28% and porters 33%). In the repeat cycle, MRSA was isolated from the hands of 9%, an increase of 3%, (doctors 13%, nurses 9%, physiotherapists/OT's 5%, porters 40%) and MSSA from the hands of 24%, an increase of 3%, (doctors 22%, nurses 21%, physiotherapists/OT's 30%, porters 40%).

Discussion Health care associated infections cost the NHS >£1 billion per year in England alone. 5000 deaths annually are a direct result of HAI's and a further 15,000 deaths annually have HAI's as a contributory factor. Our results suggest that although standard hospital procedures are being followed with respect to hand hygiene, they are failing to combat the MRSA problem. Alcohol gels in wards should be more in number, more easily accessible, i.e. at every bedside and available in high visibility areas throughout the hospital. A formal induction programme for new medical staff and revalidation study day for existing doctors and nurses may be helpful.

Conclusion Hand hygiene is the single most important measure to reduce transmission of healthcare associated infections. ALL HCW's need greater awareness of the potential to spread organisms via their hands. A national campaign is needed to improve hand hygiene across all areas of healthcare with senior clinical and management support.

CM 30 International proficiency programmes for the molecular detection of pathogens

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Objectives Quality Control for Molecular Diagnostics (QCMD) is an independent 'not for profit' EQA organisation whose primary aim is to establish and develop proficiency programmes for the evaluation of nucleic acid amplification techniques (NATs) in the diagnostic laboratory.

Methods & results In 2004, proficiency programme panels were distributed to over ~1400 participating laboratories in ~40 countries worldwide. Seventeen different panels were distributed for the molecular diagnosis of respiratory, blood-borne, STD and CNS pathogens as well as molecular genotyping. Of these, pilot proficiency programmes for *Legionella pneumophilus*, *Toxoplasma gondii* and respiratory viruses were offered for the first time.

Conclusion At present, over 1600 individuals from around 70 countries worldwide have registered an interest in QCMD programmes. The programme portfolio continues to grow as demand for EQA in molecular diagnostics increases. In 2005, in addition to the existing programmes, five new pilot proficiency programmes will be offered for JC & BK viruses, Noroviruses, West Nile Virus, Human Papillomavirus and *Bordetella pertussis*.

CM 31 Genetic characterisation of serogroup A meningococcal disease within Scotland

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Neisseria meningitidis is an important cause of meningitis and bacteraemia worldwide. Serogroup A meningococcal disease is a major problem in the 'Meningitis Belt' of sub-Saharan Africa and has been responsible for many epidemics. However, Meningococci of serogroup A is not common in present day Europe and has not been prevalent in Scotland since the 1970s. In this study, Multi-locus sequence typing (MLST) and *porA* typing methods were used for the retrospective characterisation of invasive serogroup A meningococci. Nucleotide sequences for seven housekeeping genes (*abcZ*, *adk*, *aroE*, *fumC*, *gdh*, *pdhC*, *pgm*) and the class I OMP (*porA*) were determined. Alleles and sequence types were assigned using the MLST database (http://neisseria.org/nm/typing/mlst/) and ST's were assigned to lineages using the BURST program. Three variable regions were examined within *porA* VR1, VR2 and VR3 and variant names were assigned using the *porA* variable region databases (http://neisseria.org/nm/typing/pora/ and http://www.show.scot.nhs.uk/smprl/).

There were 120 cases of Serogroup A meningocci present within Scotland from 1973 to 2004, with over 90% of those case being from 1973–83. Serogroup A was shown to be highly clonal with only 8 different STs present, the most common being ST-1 which has been responsible for epidemics and sporadic cases in Africa since the 1960s.

CM 32 Genomic library of *Streptomyces rimosus* M4018 using pSuperCos 1

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Streptomyces are Gram-positive, filamentous actinomycetes that exhibit a complex life cycle. They produce around 70% of naturally-occurring antibiotics. The industrial strain, *Streptomyces rimosus* M4018, produces the clinically-useful antibiotic oxytetracycline (OTC). The genes for OTC biosynthesis are in a 30kb cluster, around 600kb from the end of the single linear chromosome.

A number of post-genomic tools are now available for *Streptomyces*, including tagged transposon mutagenesis and conjugal transfer from *E. coli*. To capitalize on these resources, a new cosmid library of *S. rimosus* 4018 has been constructed in pSuperCos 1 – which has been chosen principally for its proven stability for *Streptomyces* DNA.

The library has been screened using Southern hybridization. Two resistance genes that flank the cluster have been used as probes to identify clones that contain the entire cluster. The library is also being used to clone genes of central metabolism from *S. rimosus*, using the genome sequence of *S. coelicolor* as a guide.

CM 33 Bacterial biofilm formation on gentamicin loaded polymethylmethacrylate bone cement

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Bacterial infection primarily with *Staphylococcus* spp. and the anaerobic bacterium, *Propionibacterium acnes*, remains a significant complication following total hip replacement. In an attempt to reduce the risk of recurrent infection, gentamicin loaded bone cement is routinely used for prosthesis fixation at revision surgery.

The aim of this study was to compare the effects of gentamicin-loaded and unloaded bone cement on *in vitro* biofilm formation by clinical *Staphylococcus* spp. strains. Biofilms of 10 aerobically grown *Staphylococcus* spp. clinical strains were formed in triplicate over various time intervals (6, 24, 48 & 72h) on sections (1 cm^2) of unloaded Palacos R® and Palacos R® with gentamicin bone cements. At each time point, the bone cement sections were removed, washed and biofilm formation quantified by total viable count. After 6 hours incubation, gentamicin-loaded bone cement showed either prevention of (3 strains) or a reduction in (7 strains) biofilm formation relative to the unloaded cement. However, by 72 hours biofilms were formed in similar numbers on gentamicin-loaded and unloaded cement by all strains tested.

This study has shown that although gentamicin loading of bone cement reduces initial biofilm formation, *Staphylococcus* spp. clinical strains are able to grow and form biofilms on gentamicin-loaded bone cement.

CM 34 Susceptibility of methicillin resistant *Staphylococcus aureus* in biofilm to tea tree oil

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Methicillin resistant *Staphylococcus aureus* (MRSA) is acknowledged as a major nosocomial pathogen causing significant morbidity & frequent mortality, with the UK having one of the highest rates of MRSA in Europe. Tea tree oil (TTO) is known to possess antibacterial activity and has previously been used for MRSA decolonisation when the use of standard antiseptics is unsuitable. The aim of this study was to determine the activity of TTO against MRSA isolates growing in a biofilm on a glass surface. Twelve MRSA strains were used to form biofilms over 24hr in Mueller Hinton Broth on glass discs. Biofilm discs were then treated with either 5% TTO or water (control) for a period of 1 hr and, following washing, biofilm formation on the discs was quantified by total viable count. The biofilms formed on the glass discs were not affected by treatment with water with total viable counts ranging from $1.8x10^2$ to $4.85x10^5$ cfu/cm^2. In contrast, treatment with 5% TTO eradicated biofilm growth on the discs for all of the strains tested. The results of this study suggest that 5% TTO possesses rapid and significant antibacterial activity against MRSA in biofilm and could be an effective treatment for MRSA decolonisaton.

CM 35 Interspecies transfer of antibiotic resistance genes in bacteria

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The increased prevalence of antibiotic resistance among bacteria isolated from clinical samples is often attributed to horizontal gene transfer via conjugation. This spread of antibiotic resistance has lead to a decrease in efficacy of many therapeutic antibacterial drugs.

It has been demonstrated in the laboratory that naturally occurring plasmids such as pIP501 have a wide host range among Gram-positive bacteria. pIP501 is a self transmissible plasmid carrying resistant markers for chloramphenicol and erythromycin. It also has multiple origins of replication, *tra* genes, and *mob* genes. Using filter mating the transfer of the chloramphenicol resistant marker was demonstrated to a variety of bacteria including *Enterococcus faecalis*, *Lactococcus lactis*, *Lactobacillus helveticus*, *Bacillus subtillis* and *Staphylococcus aureus* and *Escherichia coli*. The level of transfer of the chloramphenicol resistant marker from *E. faecalis* to *E. coli* was $4.07x10^{-7}$ per recipient. However, no evidence of the intact plasmid could be detected in *E. coli* using, plasmid extraction, PCR, Southern hybridisation or exconjugation from putative transconjugants. Thus it is not possible to confirm the transfer of pIP501. Therefore, although there is genetic evidence for transfer of the CmpR marker there appears to be no physical evidence that this is associated with intact plasmid pIP501.

CM 36 Increased density of *Staphylococcus epidermidis* biofilms in the presence of vancomycin

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S. epidermidis is a potential cause of nosocomial infections, by, for example, colonising intravascular catheters, and vancomycin is often used to treat such infections. The change in biofilm densities in the presence of vancomycin for selection of clinical strains known to form biofilms was studied, together with the reference strain RP62A. The biofilm density of *S. epidermidis* strains grown in TSB was assessed using a microtitre plate technique. Two approaches were used: 1, incubating the culture for 20 hours with various concentrations of antibiotic to assess changes in initial establishment and 2, incubating the culture without antibiotic for 20 hours then with antibiotic for a further 24 to assess the affect on established biofilms. Two of three established biofilms showed increased densities at high vancomycin levels. In addition, over half the strains tested showed increased initial biofilm densities at subinhibitory vancomycin levels. In conclusion, exposure to vancomycin may increase the biofilm density of some *S. epidermidis* strains.

CM 37 Methicillin-resistant *Staphylococcus aureus* outbreak identification using spa repeat determination

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Staphylococcus aureus is an important nosocomial and community-acquired pathogen. Its genetic plasticity has facilitated the evolution of many virulent and drug-resistant strains, presenting a major and constantly changing clinical challenge.

Among these strains are Methicillin-Resistant *Staphylococcus aureus* (MRSA), which are widely spread in hospital environments.

The eradication of MRSA from the health facilities needs a reproducible typing method that does not have the excessive cost and labour limitations. Consequently, there is a strong need for a rapid, highly discriminatory and comparable typing method.

In this study, the *spa* gene of *Staphylococcus aureus* that encodes protein A was the target. Sequence typing of the *spa* gene X region repeats was used to study the epidemiology of 41 MRSA isolates from Central Manchester and Manchester Children's University NHS Trust.

The study confirmed the utility of *spa* typing with regards to typeability, discriminatory power and inter-laboratory comparability. The characterisation of isolates demonstrated a predominance of two related sequence types with sporadic occurrence of other strains that may correlate to outbreak situations. The method shows promise as a rapid tool for outbreak detection and investigation. Analysis of the succession of repeat units suggests that local evolution may be occurring in the *spaA* X region.

CM 38 Development of a preliminary DNA microarray for diagnosis of *Staphylococcus aureus* and characterisation of virulence factors

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Rapid diagnosis of staphylococcal infections is an important objective for medical microbiologists. Microarray technology represents an exciting opportunity for economically combining rapid diagnosis while obtaining detailed information relating to virulence factors and resistance mechanisms possessed by individual isolates. A low-density amplicon-based DNA microarray has been developed that contains 21 gene probes including toxins, antibiotic resistance genes, adhesins and identification genes from *S. aureus* along with appropriate positive and negative controls. Suitable primer pairs for the genes included on the array were designed based on aligned sequences from published genome sequences of different *S. aureus* isolates. PCR amplification was performed and PCR products were purified and sequenced before being spotted onto microarray slides. Cy3-labelled genomic DNA from well characterised *S. aureus* isolates was hybridised to preliminary microarray slides and the results obtained were compared to those of PCR. The three *S. aureus* isolates examined to date produced the expected pattern of results on the microarray using a fluorescence threshold of three times the highest negative control value for identifying positive spots. These results provide a proof of principle for the use of DNA microarrays for diagnosis of staphylococcal infections.

CM 39 Reduced susceptibility to lincosamides and type A streptogramins is conferred by the enterococcal gene *lsa* in *Staphylococcus aureus*

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A growing number of 'incomplete' ABC transporters that confer resistance to antibiotics have been identified in Gram-positive pathogens. The group all possess two fused ATP-binding domains but characteristically lack any identifiable membrane-spanning domains. Members of this family include the *msr*(A), *msrC*, and *msr*(D) genes that confer resistance to macrolides and type B streptogramins (MS_B) in staphylococcal, enterococcal and streptococcal species respectively, *vga* genes that confer resistance to type A streptogramins (S_A) in *Staphylococcus aureus*, and *lsa*, a lincosamide and S_A (LS_A) resistance determinant in *Enterococcus faecalis*.

We demonstrated previously that the chromosomally-encoded *msrC* determinant of *Enterococcus faecium* confers high-level resistance to MS_B antibiotics in *S. aureus*. We report here the cloning of *lsa* from the chromosome of *E. faecalis* on to a staphylococcal plasmid. Transformation of sensitive *S. aureus* with *lsa* gave rise to increased MICs of clindamycin (from 0.06 to 4 mg/L), lincomycin (from 0.5 to 32 mg/L), and pristinamycin IIA (S_A) (from 2 to 64 mg/L).

It is known that *msr*(A) and *msr*(D) can be transferred between clinical isolates via plasmids and transposable elements respectively. Since the levels of resistance to MLS antibiotics conferred by *msrC* and *lsa* are greatly enhanced in *S. aureus*, it is a cause for concern that these determinants could be mobilised in a similar manner.

CM 40 Development of low density clinical diagnostic array platforms: *in silico* probe design and molecular validation

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In vitro diagnostic molecular biorecognition approaches are emerging as a powerful alternative to micro-organism identification methods based on culturing. Molecular diagnosis of bacterial pathogens enables rapid and accurate results for timely prescription of effective antimicrobial chemotherapies.

This paper describes the in silico design of a panel of 30 nucleic acid probes for the identification of 96% of culture positive tests. These probes will be used in solid phase array formats for the recognition of 16S ribosomal sequences to enable bacterial identification in a single test. Probe design requires the analysis of multiple factors and an e-science approach can be used to coordinate and manage this parallel data processing. EMBOSS provides server-based applications that can be linked via hypertext transfer protocols with the aid of Taverna to allow high throughput sequence processing. Probe design entailed sequence collection from the Ribosomal Database Project II, followed by sequence processing using 'emma' for multiple sequence alignment, 'cons' for consensus sequence determination and 'diffseq' for the identification of variable regions at genus and species level. Finally, candidate probe hybridisation thermodynamics are predicted and used to select a probe panel suitable for use under common hybridisation conditions.

The thermodynamics of the probe panel will be determined using thermal melt analysis and assessed for specificity and sensitivity using the reverse line blot (RLB) method, a macro-scale multiplexed platform. To date, the RLB method has been successfully used for the identification of Clostridia and Streptococci species. Ultimately the probes will be transferred to a microarray platform and integrated with sample preparation methods within a micro Total Analysis System for point of care testing.

CM 41 Development of reversed line blots to detect lactic acid bacteria and gut microbiota in pigs

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Specific oligonucleotide probes were obtained from a phylogenetic analysis of 16S ribosomal DNA (rDNA) sequences of lactic acid bacteria (LAB) and other gut bacteria previously isolated from pigs. The LAB and other gut bacteria were obtained from the faeces of nursery pigs using MRS agar. Genomic DNA of bacteria was isolated from single cloned colonies and the 16S rDNA was amplified by PCR using universal bacterial primers. The acquired sequences were compared with the DNA sequences in Genbank. Genotypic examination demonstrated that the bacteria isolated 16S rDNA sequences were similar to those of *Lactobacillus acidophilus, L. animalis, L. gallinarum, L. kitasatonis, L. salivarius, Streptococcus alactolyticus, Strep. hyointestinalis* and *Sarcina ventriculi*. The 16S rDNA sequences of the isolates were aligned and investigated for potential specific oligonucleotide probes for each bacterial species. A reverse line blot assay has shown that the designed probes can specifically detect the LAB and other bacterial DNA. These probes can be used to monitor gut microbiota of pigs in response to different diets and patterns of management.

CM 42 Clonal population of serovar 1A-2 isolates of *Neisseria gonorrhoeae* in Scotland revealed by *opa*-typing

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Gonococcal isolates which require arginine, hypoxanthine and uracil (AHU–), mostly belong to serovar IA-2; they share a wide variety of unusual phenotypic markers; and comprise a very homogeneous group. The results of some studies indicated that *Neisseria gonorrhoeae* has a non-clonal population structure. However, there are some cases where evidence provides strong support for the existence of gonococcal clones.

In this study, fifty-six isolates of *N. gonorrhoeae* (all serovar 1A-2), originating from the different regions of Scotland, were further characterised using a simplified PCR-RFLP (*opa*-typing) technique. Two enzymes *Taq* I and *Alu* I were used to analysis of the banding patterns; the 56 gonococcal isolates gave 9 and 10 *opa*-types, respectively. The majority of isolates belonged to 2 (79%) *Taq* I *opa*-types or 3 (76.7%) *Alu* I *opa*-types. The present study, which is so far the only one of its type in Scotland, clearly demonstrate the potential of *opa*-typing as a highly discriminatory tool, as compared to phenotypic-based techniques for the epidemiological investigation of gonorrhoea. Furthermore, these findings were used as a further evidence to show that a clone (1A-2/ AHU–) of *Neisseria gonorrhoeae* was circulating in Scotland during the study period, 1992–1994.

CM 43 Molecular characterisation of pigmented and non-pigmented isolates of *Mycobacterium avium* subspecies *paratuberculosis*

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Five pigmented isolates of *Mycobacterium avium* subsp. *paratuberculosis* were examined by pulsed-field gel electrophoresis (PFGE), IS900 restriction fragment length polymorphism (IS900-RFLP) and by IS1311 polymorphism analysis using PCR. All of the pigmented isolates exhibited one of three distinct PFGE profiles with *Sna*B I designated 9, 10 and 11 and with *Spe* I designated 7, 8 and 9, which generated three multiplex profiles designated [9–7], [10–8] and [11–9]. All of the pigmented isolates had the same IS900-RFLP *Bst*E II and *Pvu* II profiles. The IS900-RFLP BstE II profile was new but the IS900-RFLP *Pvu* II profile corresponded to *Pvu* II type 6 of a sheep strain described by Cousins et al. (*Aust Vet J* 2000 78, 184–190). IS1311-PCR analysis typed all of the pigmented isolates as sheep (S) strains. The genetic relationship between pigmented and non-pigmented isolates was investigated using multiplex PFGE data from the analysis of both the five pigmented isolates and 88 non-pigmented isolates of *M.avium* subsp. *paratuberculosis* from a variety of host species and geographic locations. It was possible to classify the isolates into two distinct types designated Type I, comprising the pigmented isolates, and Type II comprising the non-pigmented isolates that exhibit a very broad host range.

CM 44 Molecular characterisation of *Mycobacterium avium* subsp. *paratuberculosis* strains isolated from goats

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Paratuberculosis in goats occurs worldwide causing considerable economic losses mainly due to reduced milk production. Nowadays, there is still relatively little knowledge about the epidemiology of this disease in goats, and only a few epidemiological studies have been carried out in goats naturally infected with *Mycobacterium avium* subspecies paratuberculosis *(M.a.paratuberculosis*). The objective of this study was to characterize forty four clinical caprine isolates of *M.a.paratuberculosis* by different molecular techniques (Pulsed-Field Gel Electrophoresis [PFGE], Restriction Fragment Length Polymorphism analysis coupled with hybridization to IS*900*, and IS*1311* Polymerase Chain Reaction-Restriction Enzyme Analysis) to determine the most useful technique for molecular typing of caprine isolates, as well as to disclose the genetic variation amongst caprine isolates and the relationship with strains isolated from other animal species. PFGE was found to be the most discriminative technique identifying a total of 13 'multiplex' PFGE profiles, ten of which were novel profiles found only in caprine isolates to date. All isolates were genotyped as Type II strains, except two isolates that resembled the intermediate group referred as Type III.

CM 45 Survival of *Mycobacterium bovis* in rumen contents

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Despite dramatically increasing rates of bovine tuberculosis (TB) in the UK the transmission mechanism of *Mycobacterium bovis* to cattle is still unclear. It has been proven, experimentally, that a large proportion of eructated gases circulate around the respiratory tract and that during this process infectious aerosols are generated from the rumen. This may be a mechanism for transmission of bovine tuberculosis (TB) from contaminated pasture to the respiratory tract of cattle. This study assessed the survival of *Mycobacterium bovis* in cattle rumen contents to determine the feasibility of this transmission mechanism. *M. bovis* was exposed to rumen contents across a permeable membrane. The rumen contents were collected directly from cattle at slaughter and placed in a sterile plastic bag. Twenty-five 'exposure units' containing *M. bovis* suspension were placed in the bag, which was incubated at 37°C inside a rotating drum. Viable cell counts were performed on five units a day for five days, which is the normal maximum retention time of food in the rumen. There was a negligible drop in numbers of viable *M. bovis* in rumen contents over this period, demonstrating that eructation could be a transmission route for bovine pulmonary TB.

CM 46 Chromosomal location of β-lactam resistance genes in *Serratia fonticola* UTAD54

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The members of *Serratia fonticola* express both a chromosomally class A beta-lactamase (FON-A) and a species specific AmpC beta-

lactamase. An environmental isolate, UTAD54 displays an unusual resistance to carbapenems and contains two additional beta-lactamases: a class B metallo-enzyme (SFH-I) and a class A beta-lactamase (SFC-1).

To compare the genomic organization of strain UTAD54 and three reference strains of *S. fonticola* we used pulsed-field gel electrophoresis (PFGE) to separate macrorestriction fragments; the location of the beta-lactamase genes was established by DNA hybridisations. The presence of extrachromosomal elements was also investigated.

Macrorestriction analysis using *Xba*I revealed a large number of fragments and a considerable variation among the different strains. PFGE of I-*Ceu*I digestions revealed six intense bands, totalizing 3.3 Mb, and a faint band of approximately 2 Mb. A high degree of conservation in number and size of I-*Ceu*I fragments was observed. With the exception of the large faint band, all bands gave good hybridisation signals to an rDNA probe indicating their chromosomal origin. By hybridising with specific probes, it was possible to undoubtedly allocate the genes $bla_{\text{Fon-A}}$, $bla_{\text{SFC-1}}$ and $bla_{\text{SFH-I}}$ to chromosomal DNA.

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CM 47 Overexpression and biochemical characterization of a carbapenem-hydrolyzing class A β-lactamase from an unusual strain of *Serratia fonticola*

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An unusual bacterial strain, *S. fonticola* UTAD54, was isolated from untreated drinking waters in the Northeast of Portugal and showed to be resistant to carbapenems. The genome of this bacterium encodes a metallo-enzyme (Sfh-I) and a novel class A carbapenem-hydrolyzing β-lactamase, SFC-1. The genes encoding both enzymes are absent from other *S. fonticola* strains. β-lactamases capable of hydrolysing carbapenems are known, although comparatively rare. Carbapenems are an important class of β-lactams owing to their stability to most β-lactamases and are of particular use in treating infections associated with ESBL and AmpC producers. In order to understand the extraordinary pattern of antibiotic resistance displayed by *S. fonticola* UTAD54, we started the characterization of SFC-1. PCR specific primers were designed to contain the appropriate restriction sites for cloning the bla_{SFC-1} gene in the expression vector pET-26(+). The construction was transformed into the expression host *E. coli* BL21 (DE3). The accuracy of the DNA construction was confirmed by restriction analysis and DNA sequencing. Crude cell extracts of the *E. coli* clone expressing the recombinant enzyme were prepared. Activity assays with aztreonam and SDS-PAGE gels confirmed the expression of the SFC-1 enzyme. Susceptibility tests showed a high-level resistance to imipenem and meropenem with MICs of 32 µg/ml for both drugs. Protein purification was made by ion-exchange chromatography and gel filtration. The protein concentration of each sample was determined with the Pierce BCA protein assay. The purity of the SFC-1 preparation was determined by SDS-PAGE. According to the results the purified protein migrates as a single band of approximately 30 kDa. Isoelectric focusing revealed two forms with pIs of 7.6 and 8.2. Hydrolysis studies showed that the purified SFC-1 hydrolysed carbapenems, penicillins, cephalosporins and monobactams.

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CM 48 Demographic survey for oesophagostomum infection in communities in Bawku-East district of Northern Ghana

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Oesophagostomiasis is a little known infection of man caused by the parasitic nematode worms Oesophagostomum bifurcum (*O. bifurcum*).

Until some ten years ago it was considered a rare zoonotic infection and monkeys were thought to be the normal host. Only small numbers of cases have been registered in the world's medical literature, yet the infection is clinically important and very common in northern Ghana and Togo. As high cases are not known somewhere else in the world, human oesophagostomum escaped medical attention.

The study was therefore aimed at identifying the rate of *O. bifurcum* infection in the Bawku-East district of the Upper East region of Ghana in preparation for a mass treatment to explore the organism's susceptibility to the drug Albendazole.

Stool samples were collected and cultured, and examined microscopically to determine the presence of the larvae adult worms.

Co-infection with other worms was common especially hookworm and strongyloides. Women and Children were the most infected.

Treatment with 400mg Alendazole is feasible and could be used to start the mass treatment exercise.

CM 49 Smear negative culture positive in the programme

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Smear microscopy is the sheet anchor of the DOTS programme. Therefore it is essential that the sensitivity and specificity of smear microscopy are very high.

Objectives a) To quantify the proportion of smear negative cases that are culture positive in patients with symptoms of pulmonary tuberculosis (b) To see whether concentration techniques improve the rate of smear positivity among culture positives.

Methods Sputum from smear positive patients and a sample of smear negative patients attending a tertiary care centre was put up for culture and sensitivity. Of 385 smear positive patients 81 were culture negative. Of 77 smear negative patients 55 were culture positive.

To improve the yield, the patients were subjected to concentration method for smear. Of those who were smear negative by the concentration method, none were culture positive. Thus the routine smear microscopy is likely to miss culture positive cases while the concentration method does not miss cases.

Posters

Environmental Microbiology Group

EM 01 Molecular methods to assess the diversity of rock fungal communities

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A significant barrier to our understanding of the impact of fungi on geological processes is the lack of accurate means to estimate and characterize their diversity. To gain a better understanding of the organisms that constitute fungal cryptoendolithic communities, we conducted culture-independent analyses of the phylogenetic composition of such communities by examining the 18S rRNA gene as well as the internal transcribed spacer region (ITS). The application of culture-independent molecular techniques based on total DNA extraction overcame some of the problems associated with traditional cultivation bias. After extraction of amplifiable DNA from a selection of rock types, a suite of molecular techniques was used to characterize fungal communities including automated ribosomal intergenic spacer analysis (ARISA) in combination with the non-automated RISA technique. Denaturing gradient gel electrophoresis (DGGE) based on the 18S region of the rRNA gene was also used for community analysis. Our results demonstrated that rock-inhabiting fungal communities are relatively diverse for such apparently inhospitable environments. Furthermore, community composition varied between different geographical locations and rock type, revealing that rock substrates support a richer fungal diversity than previously thought. We can conclude that molecular methods provide a powerful technique for analyzing rock-dwelling fungal communities.

EM 02 Fungal leaching of important metal oxides

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Metal oxides have a wide variety of commercial applications. Many are used as dyes in paints and specialist coloured building materials, including concrete and mortar. Fungal metabolites, such as H^+ and organic acids, have been shown to be capable of metal complexation and dissolution of a variety of metal/mineral compounds, but their effects on oxides have not been widely explored. The objective of this work was to investigate the ability of two organic acid-producing fungi (*Aspergillus niger* and *Penicillium simplicissimum*) to transform zinc oxide (ZnO), hematite and magnetite (Fe_2O_3 and Fe_3O_4), copper oxide (CuO) and calcium oxide (CaO). The results show that zinc and calcium oxides were rapidly solubilized by both fungi, with relatively high concentrations of zinc and calcium being accumulated in the biomass. Copper and the two iron oxides were transformed less effectively but appreciable amounts of the metals were still found in the fungal colony. These results highlight the adverse effects of fungal growth on pigmented and dyed building materials.

EM 03 Warfare growth strategies of mycelial systems in toxic metal-containing environments

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As a defence against toxic metal stress, mycelial systems can adopt various growth strategies analogous to those adopted in human warfare. Fungal negative tropism to toxic metals and growth cessation can represent a 'retreat' strategy aimed at avoiding toxic metal-contaminated areas. Fungi penetrating metal-containing domains can form dense aggregated mycelium representing a 'phalanx' state, single explorative unbranched hyphae can represent a 'guerrilla' strategy (multiple 'phalanx'-'guerrilla' transitions may occur), while the formation of mycelial cords and synnemata can represent a 'reallocation' strategy. Growth of intrahyphal hypha observed for the extraradical mycelium of ectomycorrhizas in toxic metal medium can represent the employment of 'armour'. In metal-polluted domains, fungi often produce high local concentrations of chelating and sequestering agents which precipitate metals and ameliorate their microenvironment. The remote targeting of extracellular metal sequestration could be regarded as a kind of 'bombardment' tactic. A combination of morphological responses can therefore contribute to fungal survival in toxic metal-containing domains.

EM 04 Biosensor analysis of the soil solid phase to investigate contaminant bioavailability and bioaccessibility

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The majority of biosensor tests involve extracting contaminants into an aqueous phase, or for organic pollutants into an organic solvent. Rigorous organic solvents can over-estimate the fraction of the compound that is bioavailable/accessible to biosensors, whereas aqueous extracts overlook the toxic interactions in the solid phase (Shaw *et al.*, 2000). Solid phase analysis involves bringing biosensors into direct contact on the surface, and in the pore spaces, of soils.

The main focus of work to date has been to assess the effect of varying the bioavailability of a heavy metal (Zn) on microbial respiration, and to develop a solid phase toxicity assay by adjusting soil matric potential and introducing bioluminescent bacterial biosensors to ensure solid phase contact. Solid phase work has involved the design of equipment compatible with a Tension table (the means for adjusting matric potential), and with adjustment of the various assay factors such as soil volume, matric potential and biosensor inoculum size, which will enable the biosensor being used (*Escherichia coli* HB101 pUCD607 IMS, a robust metabolic sensor which carries the full *lux* cassette (*lux* CDABE) downstream of a strong constitutive promoter on a multi-copy plasmid) to give an optimal indication of toxicity in contaminated soils.

EM 05 Influence of abiotic soil factors on PAH extraction by NEETs

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Release of organic pollutants into the environment still remains a global environmental problem. Polycyclic Aromatic Hydrocarbons (PAHs) are ubiquitous pollutants and some have carcinogenic and/or mutagenic properties. Historically contaminated sites often prove difficult to remediate as the contaminants are said to have aged and may be rendered unavailable. Exhaustive chemical extractions that have been used previously remove total concentrations of the pollutant and fail to reflect bioavailability. The availability of PAHs for organism uptake is intricately linked to soil physico-chemical parameters as well as soil texture.

The Non Exhaustive Extractive Techniques (NEETs) used in this work (hydroxypropyl-beta-cyclodextrin (HPCD) and Amberlite XAD-4), involve a more passive approach and thus are indicators of bioavailability. Preliminary research on one soil type with phenanthrene has shown that HPCD extractions have a linear correlation with mineralisation.

This work compares the effects of different soil types on the efficiency of NEETs at the laboratory scale for various PAHs. HPCD and XAD-4 were used to extract naphthalene from five different soils. The HPCD extractable fraction correlated closely with the portion that was mineralised, but the XAD-4 extractable fraction did not. Further experiments are ongoing using historically aged soils that have been extracted using HPCD prior to and after inoculation with a PAH degrader.

EM 06 Development of a soil and sediment extraction technique compatible for biosensor testing of hydrocarbons

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The assessment of contaminated sites necessitates the design and refinement of environmentally compatible extraction procedures for the target pollutants.

The extracts can be quantified using chemical and biological methods. Bacterial biosensors are increasingly being used to assess the toxicity of the bioavailable portion of these extracts. The regulatory authorities understand the power of the sensors but highlight the need for environmentally relevant procedures. Currently the extraction of hydrocarbon compounds from soil in a form that can be tested by the biosensor *Vibrio fischeri* involves the use of methanol. However a change of phase to hexane is necessary before confirmatory chemical analysis by gas chromatography. The behaviour of a range of aliphatic organic chemicals involved in this partitioning has been investigated. The method has been refined and optimised and by the use of appropriate internal standards has shown that when using 5ml methanol: 10ml hexane the partitioning is approximately 1: 0.56 for all of the hydrocarbons investigated.

EM 07 Monitoring of DNAPL remediation using microbial biosensors

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Remediation and monitoring of DNAPLs (particularly Chlorinated Aliphatic Hydrocarbons) can be problematic.Rapid inexpensive detection of these contaminants in soil and groundwaters is essential for the management of the clean up operation. Luminescence based biosensors have been shown to have good quantatative correlation with single DNAPL pollutants such as TCE. The aim of this study was to attempt to determine performance in a more realistic scenario where mixed contaminants were present. Environmental samples were tested for a whole range of volatile organic pollutants using GC-ECD and biosensor response to these mixtures was determined. GC-ECD detection, although highly sensitive is not field compatible. By contrast the speed and resilience of the biosensors make this an ideal application. Continual monitoring of remediation progress is key to environmental improvement:biosensors may prove to be of high value in such a strategy.

EM 08 Heavy fraction of crude oils: a risk assessment

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During the First Gulf war (1990–1991), destruction of oil fields in Kuwait resulted in a large environmental catastrophe. The amount of soil contaminated is in the range of 40–50 million metric tonnes which are either in oil lakes or at their peripheries. Additionally, about 1000 km^2 of desert soil was contaminated by air-borne oily material. As a result of extended exposure to the harsh atmospheric conditions of Kuwait, the crude oil in these lakes lost the volatile fraction of hydrocarbons and experienced other physical and chemical changes. A remediation trial was carried out. Results indicated that there are some hydrocarbons degradation occurring in the samples. Increase of CO_2 production in samples indicated that the indigenous population utilized the hydrocarbons. TPH levels decreased indicating active degradation. MPN analysis showed that microbial communities are in continuous change and the samples are active. The toxicity test showed that remediation caused a reduction in toxicity. Bioremediation is a valid option for these soils if a cost effective management strategy is implemented.

EM 09 Evaluation of a range of treatments in the management of a hydrocarbon contaminated biopile

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Hydrocarbons are ubiquitous environmental contaminants, which may be both toxic and recalcitrant. Biopiling techniques are used increasingly to remediate such contaminants. In this study a range of treatments were tested including regular turning, bioaugmentation as well as nutrient and a surfactant amendment. The addition of surfactant increased the hydrocarbon bioavailability and the rate of degradation. Turning treatment improved the hydrocarbon bioavailability as well as reducing the toxicity level. Even the control treatment was effective for biopiles where no active treatment was carried out. In this study, the addition of inocula was unsuccessful indicating the importance of assisting the indigenous degrading community. The economic costs of a remediation strategy must be considered relative to the time constraints of the project.

EM 10 A toolkit for the rapid assessment of microbiologically influenced corrosion

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Microbiologically Influenced Corrosion (MIC) is a form of steel

corrosion that is of increasing concern in harbour environments. This project aims to create a toolkit that will quantify the risk of MIC occurrence at Aberdeen Harbour. Results will be incorporated into Aberdeen Harbour Boards' Corrosion Asset Management Tool, a risk-based model that will prioritise berths for maintenance and repair. MIC is predominantly carried out by the Sulphate Reducing Bacteria that can directly attack steel to form iron sulphides. SRB occupy anaerobic niches in biofilms that are attached to steel structures.

The toolkit comprises ATP and protein quantification of harbour biofilm samples, traditional cultivation and molecular probing methods for the quantification detection of Sulphate Reducing Bacteria and Iron Oxidising Bacteria, coupled with the use of laboratory microcosms to mimic corrosion processes *in vivo*, and various analytical seawater measurements including BOD, DOM and salinity.

The biological component of biofilms was found to be variable throughout the Harbour and was positively correlated with the severity of corrosion underneath the biofilm. Areas showing high bacterial activity have high concentrations of DOM in the seawater. There was also a positive relationship between salinity and MIC causing bacteria.

EM 11 Evaluation of strategies for the bioremediation of benzene in an anaerobic aquifer

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An anaerobic aquifer had been historically contaminated with high levels of benzene (up to 200 mg/l). Field (collaborators) and laboratory (this study) investigations were conducted to study anaerobic bioremediation potential and options for biostimulation. To achieve this, standard anaerobic enrichment cultures were employed and columns for the simulation of field condition were developed. No clear evidence for anaerobic benzene degradation was found in enrichment cultures after 35 weeks, though signs of microbial activity were observed. Stimulation with different levels of hydrogen peroxide proved to be most effective with occurrence of sequential aerobic and anaerobic degradation. Laboratory studies were not directly transferable to the field, where evidence of anaerobic degradation had been found. Hydrogen peroxide would accelerate the process but would have to be adapted to be applicable in field conditions.

EM 12 Integrated physico-chemical, microbial and ecotoxicological assessment of remediation potential at a hydrocarbon impacted inter-tidal site

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Migration of hydrocarbons from a former coal tar site on the banks of the River Tyne in Newcastle has led to contamination of the saline sediments on the foreshore and its adjacent waters. Remediation plans have commonly been based solely on chemical analysis. However, for *in-situ* remediation, it is important to consider the role of indigenous micro-organisms to an effective attenuation of contaminants. A baseline study utilising 192 samples detailing physico-chemical, toxicity assessments by luminescent bacterial biosensors has been undertaken. In addition, microbial characterisation (CLPP, DGGE, FISH) and remediative test microcosms from selected areas have been carried out to interpret and assess the remediation potential by the indigenous microbial community. A fully integrated understanding of the remediation potential can be used to prioritise areas within contaminated sites and to predict *in-situ* remediation outcomes.

EM 13 Plant assisted volatilisation of semi-volatile persistent organic pollutants (POP's)

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Bioremediation of contaminated sites using plants offers considerable potential, but the mechanistics of such 'phytoremediation' is not well understood. By studying the fate of chlorinated benzene using specifically designed microcosms in a controlled laboratory setting it has been determined that volatilisation, not degradation, is the primary mechanism for enhanced loss from the rhizosphere.

POP's have a low mobility within plant tissues. This study hypothesised that plant-assisted volatilisation occurs directly from the soil, due to changes in soil structure and moisture content, not via transport of POP's within plant tissues.

Studies show increased volatilisation in planted systems. Soil moisture content is thought to be a main factor in volatilisation rates. The combination of detailed 2D studies of drying effects with chemical fate studies is hoped to show more clearly this link between moisture content of soil (altered by presence of plant roots) and removal of dichlorobenzene via volatilisation.

Plant assisted volatilisation may be beneficial in remediating POP contaminated soils and has major implications for previous studies investigating the fate of POP's in the rhizosphere, which rarely consider volatilisation losses.

EM 14 Predictive ecotoxicity assessment of potentially toxic elements using luminescence-based biosensors

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Microbial biosensors based on luminescence reporter genes are often used to complement chemical analysis in order to determine the bioavailability of contaminants. This study focuses on the response of two such sensor types to investigate the toxicity of metal ions and relates the response to physiochemical characteristics. A metabolic sensor responsive to total pollutant burden was compared with a resistance-based sensor that luminesced in the presence of known contaminants.

Quantitative structure-activity relationships (QSARs) have been applied to model the toxicity of organic compounds but there are few examples of models for predicting elemental toxicity. Despite a focus on organic compound based QSARs, ion characteristics such as the covalent index, the log of the first hydrolysis constant and softness parameter have been used to predict quantitative ion character-activity relationships (QICARs) for metal ions.

The aim of this study was to consider the response of a) a metal-inducible sensor and b) a toxicity sensor to metals. The toxicity of 8 metal ions to *Escherichia coli* HB101 pUCD607 was correlated with ion specific parameters, and the corresponding induction of *Vibrio anguillarum* pRB28 by 13 metal ions was also investigated.

The resulting EC50 values for *E. coli* HB101 pUCD607 and bioluminescence values for *V. anguillum* pRB28 displayed similar patterns of toxicity as predicted by ionic characteristics. The relative toxicity of metal ion to *E. coli* HB101 pUCD607 was predicted using stepwise multiple linear regressions against ionic characteristics to give a response of Pb, Zn, Ni, Co, Cd, Cu, La, and Fe. A similar order of response was seen for *V. anguillarum* pRB28 induction. The results indicated that QSAR trends in relative toxicities could be predicted from ion characteristics reflecting differences in metal-ligand interactions.

The role of luminescent-based bacteria in environmental monitoring can only be extended through a comprehensive understanding of the mechanisms that cause a response. In this study we acknowledge the response in aqueous systems and future work will extend this to soil and sediment approaches.

EM 15 Application of a bioluminescence-based fungal bioassay to contaminated soils

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Bioluminescence-based microbial biosensors provide a biological tool for the assessment of metal bioavailability. Most of the biosensors used are bacterial and as such fail to represent wider soil ecological niches. The development of a bioluminescence-based fungal bioassay has extended the existing suite of biosensors available for ecotoxicity testing. The naturally bioluminescent fungi *Armillaria mellea* and *Mycena citricolor* were found to be sensitive to Cu and Hg, as well as to Zn and Cd, respectively. The fungal bioassay assessed the toxicity of pollutants in both laboratory-amended and historically contaminated soils. Chemical analysis quantified the pollutants and this was translated into a bioavailability model using contaminant partitioning (K_d) within the soil. The K_d values explained the bioavailability of Cu and hence the bioluminescence response in historically contaminated soils. This study demonstrates the importance of integrated chemical and biological assessments in soil toxicity testing, and the value of bioluminescent fungi as biosensors in contaminated sites.

EM 16 Formation of DNA adducts in polycyclic aromatic hydrocarbon (PAH) contaminated soils

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Soil pollution by mutagenic compounds such as polycyclic aromatic hydrocarbons (PAHs) is a significant problem. One of the more abundant environmental PAHs is benzo[a]pyrene (BaP), which is a common by-product of combustion processes and is a suspected mutagen. The mutagenic activity of BaP most likely stems from the potential of BAP transfromation products to form DNA adducts. Given the significance of BaP as a soil pollutant there is significant interest in determining the availability of the compound in soils to assess the risk and potential to biologically treat (bioremediation) contaminated soils. The objective of this study was to isolate and quantify benzo[a]pyrene-derived DNA adducts in aged contaminated soil samples to gain fundamental understanding of PAH bioavailability insuch environments.

The study involved DNA isolation and quantification of DNA adducts from aged contaminated soil samples. The isolated DNA represents a wide range of soil microbes and therefore should give an estimate of the availability of BaP (and transformation products) to a wide range of soil organisms. Three different soil types (Cruden Bay, Boyndie and Insch: of varying soil textures) with differing organic matter levels were incubated at 20^{o}C, 60% water holding capacity with benzo[a]pyrene (BaP), 300 mg per kg dry soil. At time points 0, 50, 100 and 220 days, BaP was extracted and quantified by HPLC or synchronous fluorescence spectroscopy (SFS); DNA was isolated and measured by using Bio101 kits and PicoGreen Reagent-Fluorescence, respectively; DNA adducts were assayed by ^{32}P-labelling. The results demonstrated large differences in both BaP biodegradation and DNA adducts levels measured in the three soils. After 220 days incubation BaP residues were remaining 77.0% for Cruden Bay; 85.1% for Boyndie and 94.8% for Insch. DNA adducts were produced in different amounts in the three soils: Cruden Bay (high organic matter and high clay): 826.7±124.6 DNA adducts per 10^8 undamaged nucleotides; Boyndie (high sand and low organic matter): 35.2±22.3 DNA adducts per 10^8 undamaged nucleotides; and Insch (moderate organic matter and clay): no adducts. The formation of DNA adduct appears to be related to the biodegraddation of BaP during incubation.

The presence of DNA adducts caused by PAH metabolites indicates that PAHs are bioavailable to microbes in soils as BaP has been metabolised and the study of soil DNA adducts could provide fundamental information on BaP availability in soils.

EM 17 The effects of a non toxic surfactant on pentachlorophenol bioavailability and bacterial community structure in soil microcosms

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Current estimates reveal that there are approximately 10,000 sites in England and Wales that may be classified as contaminated. *In situ* bioremediation of contaminated soil is often limited by low bioavailability due to the poor aqueous solubility of many contaminants. The ability of surfactants to increase the mass flux of low solubility compounds means that these compounds can potentially be used to enhance the aqueous solubility of non aqueous phase soil contaminants, and thus increase their bioavailability to microbial degraders.

Although surfactants have been successfully used to increase the mass transfer of soil contaminants to the aqueous phase, surfactant addition often results in a reduction in the rate and extent of contaminant biodegradation. This highlights the need to understand the effects of surfactant addition in order to optimise their use and to assess their ecological impact, particularly with respect to microbial communities. The effects of a non toxic surfactant on pentachlorophenol toxicity and biodegradation will be discussed. Changes in bacterial community structure and composition in pentachlorophenol / surfactant treated soil microcosms will also be presented.

EM 18 Effects of sheep dip pesticides on biofilms involved in secondary sewage treatment

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Spills of sheep dips into rivers continue to be a problem. At Lancaster we have investigated what would happen if sheep dip,

formulated using either synthetic pyrethroids or organophosphate pesticides, reached a sewage treatment works.

We added sheep dips to biofilms taken from submerged biological contactors, activated sludge and trickle filter stones and examined the effects on faecal indicator numbers, protozoal health and biofilm integrity. All but the lowest concentrations of sheep dip inhibited (killed?) protozoa and nematodes within minutes and resulted in disintegration of the biofilms. Protozoal and nematode death was faster and at lower concentrations when the two types of pesticide were added together compared with the individual pesticides. Faecal coliform numbers rose by 2 to 3 logs in submerged biological contactor effluent, by between 3 and 4 logs in activated sludge liquor and by 3 logs in trickle filter effluent, with little difference between the sheep dips. The increase in bacterial numbers is caused by the pesticides removing the grazing pressure exerted by the protozoa and nematodes. These results show that a sheep dip spill reaching a sewage treatment works would severely disrupt secondary treatment and result in a highly contaminated sewage effluent. It would take a considerable time for the biofilm to redevelop and for the plant to become operational again.

EM 19 The influence of transformer oil on the bioavailability and chemical extractability of PAHs in soil

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Polycyclic aromatic hydrocarbons (PAHs) contamination is often associated with light non-aqueous phase liquids (LNAPLs) in soil that can function as sorbents for hydrophobic contaminants. This study characterized the fate of phenanthrene, initially dissolved in transformer oil (a LNAPL), in soil with increasing contact time. Additionally, the affects of the presence of transformer oil on the biodegradability of aged PAH residues in a former coke-plant soil was assessed. The relationship between cyclodextrin extractability and microbial degradation of the PAHs was also investigated. Total ^{14}C-phenanthrene-associated activity, the fraction available for chemical extraction and the microbially degradable fraction all displayed biphasic declines over 54 weeks. Cyclodextrin extractability, previously shown to accurately measure the total PAH fraction available for microbial degradation, correlated linearly and significantly in both the phenanthrene ageing experiment and the coke-plant soil biodegradation study. HPCD extractions slightly under-estimated total contaminant fractions available for biodegradation in the presence of transformer oil in the coke plant study; possibly related to interference of the oil with the HPCD-extraction. An effect of transformer oil concentration on HPCD extractions was not observed. Overall, the presence of transformer oil had little effect on phenanthrene ageing in the soil at the concentrations investigated in this study.

Keywords LNAPL, cyclodextrin, contaminated soil.

EM 20 Linking indigenous catabolic activity to cyclodextrin extractability: determination of the availability of PAHs to soil microbes

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Hydrophobic organic contaminants (HOCs) are ubiquitous environmental contaminants that accumulate in soils; often associated with non-aqueous phase liquids (NAPL). HOCs, often display long-term persistence and may be a cause for concern with regard to the risk assessment and remediation of contaminated land. Risk estimations of contaminated land currently centre on total contaminant loadings as determined by exhaustive chemical extractions. Recent evidence suggests that such extractions overestimate the fraction available to biota and more realistic determinations are currently sought. This study illustrated that the microbially available fraction of PAHs in both laboratory-spiked and field-contaminated soils may be determined directly by measuring the catabolic activity of the indigenous microflora. A simple and well established soil-slurry microcosm technique was employed. This approach removes the need for the addition of catabolically active inocula. Further, the technique was tested in soils amended with 0.05, 0.01 or 0.005% transformer oil (a light NAPL) and compared to a no-oil control. Significant linear correlation with gradients (m) approximating to 1 (one) showed that the amount of PAHs removed by the indigenous microflora was directly comparable to that removed either by a HPCD extraction or by an enriched bacterial inoculum.

Keywords Bioavailability, LNAPL, coke-plant soil.

EM 21 Relationship between cyclodextrin extraction and microbial mineralisation of phenanthrene

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A number of soil extraction techniques have been proposed to determine the putative bioavailability of organic contaminants in soil. Exhaustive methods using organic solvents have little relevance to the concentration of contaminants, which are actually bioavailable. A study was conducted to investigate the relationship between hydroxypropyl-β-cyclodextrin (HPCD) and microbial mineralisation of phenanthrene in soils with differing characteristics. This study aimed to investigate the relationship between sequential HPCD extraction and microbial mineralisation of phenanthrene in three soils. Phenanthrene extractability was assessed at 24 h intervals over 10 d and compared to cumulative microbial mineralisation using an enriched Pseudomonad inoculum. The cumulative total of phenanthrene extracted by HPCD exceeded the mineralisation asymptote. However, the overall total extents mineralised were statistically similar to that extracted after the first 24 h by HPCD, demonstrating that a single HPCD extraction accurately and reproducibly predicts the total fraction of phenanthrene available for microbial mineralisation in different soils.

EM 22 The effect of slurrying on phenanthrene mineralisation in soils

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Soil slurries are routinely used to determine the total contaminant that is bioavailable for microbial degradation. However, slurrying destroys the aggregated structure of the soil and may significantly alter the distribution of target compounds between the soil and the aqueous phase, thus giving an overestimation of the bioavailable fraction. The aim of this study was to investigate the effect of slurrying on the biodegradation of phenanthrene in four soils with differing clay and organic matter content. The mineralisation of phenanthrene was compared in soils re-hydrated to field moisture content (30%) and in soils, which had been slurried using a soil to water ratio of 1:3. Total mineralisation was also compared to the HPCD extractable fraction for slurried and non-slurried soils. The total extents and rates of mineralisation were significantly greater in slurried than non-slurried soils. Similarly, residual contaminant availability for microbial mineralisation, following a HPCD

extraction was assessed. Mineralisation of phenanthrene in soils pre-extraced with HPCD was negligible suggesting that the microbially available fraction had been wholly removed.

EM 23 Determining the microbial bioavailability of hydrocarbons in soils

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Bioavailability limitations are often reported to limit the biodegradation (a key removal process) of hydrocarbon contaminants in soils. Bioavailability is frequently investigated using the PAH phenanthrene as a model contaminant. However, aromatic hydrocarbons have wide ranging physico-chemical characteristics and aliphatic hydrocarbon bioavailability is largely unexplored. The aim of this study was to investigate the bioavailability of 5 model hydrocarbons with broad physico-chemical properties and monitor the changes over time. A pasture soil was spiked at 50mg kg^{-1} with ^{14}C-labelled naphthalene, phenanthrene, pyrene, Benzo[a]pyrene or hexadecane and was aged under both sterile soil and non-sterile soil microcosms. Both biological (mineralisation, biomass uptake) and chemical (mild shake extractions) measurements were employed and correlations between the sets of data were investigated. The results highlight that physico-chemical characteristics are key to microbial bioavailability and strongly affect the success at extracting a bioavailable fraction. A trend in the speed and extent of sequestration depending on physico-chemical characteristics is also apparent. Additionally, the results suggest that the model aliphatic hydrocarbon sequesters in soil.

EM 24 Microbial populations of mineral spoils and their potential roles in biomining

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Mineral tailings and spoil heaps, are unavoidable legacies of mining. Wherever sulfide-rich mine wastes are left exposed and uncontained, microbially-mediated mineral dissolution results in the chronic and widespread release of (usually) highly acidic, heavy metal-laden water into drainage streams and eventually other watercourses. However, the same microbial activities are utilised industrially to extract metals from ore and in contrast 'biomining' involves the contained management and irrigation of ore materials, and extracting metals from the mineral leachate. To allow prediction of long-term pollution from mine wastes and to assess their potential as a resource that may be exploited, the microflora of both managed and unmanaged sites across the U.K., Europe and the U.S.A. have been investigated via direct isolation using selective solid media and by an array of culture-independent methods. Such analyses have shown that, while the more familiar acidophiles are found in these spoils and heaps, they are often dominated by microbes that are rarely described as inhabitants of acidic environments. This study has not only increased the understanding of the microbial populations in mineral heaps and spoils, it has also highlighted the fact that newly discovered microbes isolated from the sites may be important in the extraction of metals from mineral spoils.

EM 25 Sulfide production and metal removal by mixed populations of bacteria in low pH bioreactors

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Sulfidogenesis at extremely low pH was investigated in a bench-scale bioreactor, set at pH values between 3.0 and 4.2. This sulfidogenic bioreactor contained a defined mixed culture of a heterotrophic acidophile (*Acidocella* isolate PFBC) and a moderately acidophilic sulfate-reducing bacterium (*Desulfosporosinus* isolate M1). This partnership is key to sulfidogenesis at low pH, since isolate M1 is an incomplete oxidizer of glycerol and excretes stoichiometric amounts of acetic acid, which is highly toxic to most acidophiles (including *Desulfosporosinus)* at relatively low concentrations in acidic media. The acetic acid is oxidized to CO_2 by isolate PFBC. The pH of the sulfidogenic bioreactor was varied to examine the efficiency of sulfidogenesis and the selective precipitation of dissolved heavy metals from liquors containing a mixture of zinc, copper and iron. Changes in microbial populations in the bioreactor culture were monitored using fluorescent *in situ* hybridisation (FISH). In addition, the performance of the mixed culture was evaluated by measuring concentrations of glycerol, acetic acid, soluble metals and sulfate. Sulfidogenesis was demonstrated to occur in cultures maintained at pH values as low as 3.0. In these cultures of varying pH, phase-partitioning of copper, zinc and iron occurred, facilitating the selective recovery of these metals from synthetic mixed-metal waste streams.

EM 26 Microbial ecology and biogeochemical cycling of metals in experimental wetland mesocosms

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Wetlands have been used as potential 'passive' remediation solutions for acidic mine water pollution since the 1980's, having the attraction of being relatively low cost/maintenance systems that utilize natural biological processes. This paper describes the microbiology and geochemistry of experimental wetland mesocosms developed to study passive remediation of synthetic acid mine drainage (AMD). The mesocosms constituted twelve cores taken from a natural wetland impacted by AMD at the former Parys copper mine, North Wales. Each core was contained in a Perspex column with a working volume of ca, 19,500 cm^3. These were divided into four sets of triplicates, including NPK fertilization; inoculation with sulfidogenic sediment; fertilized and inoculated; and non-amended controls. The mesocosms were irrigated with synthetic AMD for 22 weeks, and the physico-chemistry and microbiology of the drainage water was monitored. Cores that had been amended with sulfidogenic sediment exhibited the greatest ameliorative effect in terms of raising water pH and lowering concentrations of copper and zinc in the synthetic AMD. Iron- and sulfur-oxidizing, as well as iron-reducing acidophiles were detected in water draining all cores using cultivation-dependent approaches. PCR-based techniques detected archaea, sulfate-reducing bacteria and iron-reducing *Geobacter* spp. within the drainage from all mesocosms. Microbial community diversity was elucidated using terminal restriction fragment length polymorphism (T-RFLP) analysis. In addition to the microflora in the mesocosms, the rate-limiting factor for AMD remediation was shown to be the availability of organic carbon to fuel sulfidogenesis.

EM 27 Mecoprop degrading soil community diversity and functional analysis

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Chlorophenoxyalkanoic acid herbicides are a group of structurally related compounds used extensively to kill broad leaved weeds in cereal crops. Accumulation in the environment presents a serious threat due to their carcinogenic and tetratogenic effects. Degradation of these herbicides occurs by the natural microbial communities in

the environment. However mecoprop [2-(2-methyl-4-chlorophenoxy) propionic acid] persists in the environment due to its resistance to microbial degradation. Seven soil samples were collected from fields previously treated with different herbicides. Enhancement of mecoprop degradation was achieved by successive subculture of these soil samples and degradation was monitored using chlorometric and HPLC analysis. DNA was extracted from each subculture and PCR amplified using 16S rRNA and *tfdA* (the functional gene responsible for the first step of mecoprop degradation) gene primers. PCR products were analysed using denaturant gradient gel electrophoresis (DGGE). DGGE analysis of 16S rDNA PCR products showed gross shifts in bacterial communities over successive subcultures. 16S rDNA clone libraries were generated from PCR products obtained from one of the soil samples. 150 clones were screened by DGGE analysis and clones of interest were selected for full 16S rDNA gene sequencing. A phylogenetic analysis of mecoprop degrading clones using DNA distance methods was performed. 16S rDNA DGGE and phylogenetic analysis of the soil samples resulted in identification of diverse community members from different soil samples. In contrast *tfdA* DGGE analysis indicated a high degree of sequence conservation between soil samples. It is therefore proposed that mecoprop degradation community structure is divergent though *tfdA* sequence homology remains conserved between these communities.

EM 28 A molecular characterisation of the community structure and function of PAH-degrading bacteria present in chronic petroleum-polluted sites

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Petroleum oil pollution is a severe global environmental problem and impacts on human health and the environment. Many of these pollutants include recalcitrant polyaromatic hydrocarbons (PAHs) (e.g.napthalene, anthracene). Napthalene has been used as a model to determine the principles of PAH metabolic pathways in bacteria. However, this may not be representative of the microbial biodegradation of more complex PAHs and this is now beginning to receive the attention it deserves.

The application of molecular techniques in microbial ecological studies has allowed the rapid screening of environmental samples and circumvents the biases incurred from culture-based methods. The aims of this research were to apply molecular techniques to characterise the microbial population diversity and metabolic function in PAH-contaminated sediments. Microcosm experiments were established and enriched in media containing different concentrations of PAHs. Degradation of PAHs was monitored over time and quantified by High Purity Liquid Chromatography (HPLC). Reductions in individual PAHs were as great as 60% after 14 days enrichment. Denaturing Gradient Gel Electrophoresis (DGGE) of the 16S rRNA genes demonstrated the predominance of key microbial groups involved in PAH degradation and sequence analysis confirmed their identity. PCR amplification of the functional genes demonstrated the potential for biodegradation at these sites.

EM 29 Molecular characterisation of 2,4-D degrading communities

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Phenoxyalkanoic acid is a group of structurally related herbicides extensively applied to the environment. Microbial degradation is undertaken by complex natural microbial communities within soil. Catabolism of 2,4-dichlorophenoxy-acetic acid (2,4-D) is encoded by 2,4-D degradation genes (*tfdA-F+K*), often located on conjugative plasmids. The aims of this study were isolation, enhancement and identification of 2,4-D degrading bacteria from soil and investigation of community structure dynamics following herbicide enrichment.

Bacterial enrichments were performed using agricultural soils with 2,4-D as sole carbon source. Enhancement of 2,4-D degradation was achieved by subculturing; degradation was monitored by chloride ion release and HPLC. Molecular identification of isolates was undertaken. Bacterial community analyses were performed by denaturant gradient gel electrophoresis (DGGE) after subcultures with 2,4-D. PCR amplification of the 16S rRNA and *tfdA, B* and *C* genes were performed followed by DGGE.

Molecular identification of the isolates indicated high homology to *Burkholderia hospita*. DGGE analysis of the 16S rRNA demonstrated bacterial community changes during successive subcultures. Sequence analysis identified species within the 2,4-D degradation communities, which proved undetectable in successive subculture. This suggests these organisms are not significant in initial 2,4-D degradation. Plasmid profiling has indicated the presence of *tfd* genes within isolates and borne on the plasmid.

EM 30 Characterisation of a broad-spectrum halophilic amidohydrolase (amidase) enzyme system from a haloarchaeal strain, *Halorubrum* sp. E4

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A novel haloarchaeal strain, *Halorubrum* sp. E4, was isolated and found to hydrolyse a range of aromatic, aliphatic and heterocyclic amides and *N*-benzoyl amino-acids to yield their corresponding carboxylic acids. The amidohydrolase (EC.3.5.1) activity was constitutively expressed, and both growth and non-growth substrates were hydrolysed. Cell-free extracts from *Halorubrum* sp. E4 rapidly hydrolysed butyramide and benzamide, with optimum activity in buffer containing 3–4 M KCl. No hydrolysis of aromatic or aliphatic nitriles was detected, although cell extracts did catalyse acyl-transfer reactions to yield hydroxamic acids, suggesting that the strain expresses a classical amidase (EC 3.5.1.4), possibly related to the 'AS signature group' of enzymes. Amidase and *N*-benzoyl amino-acid hydrolysis (hippuricase) activities were readily separated by fractionation of cell-extracts using column chromatography, suggesting that *Halorubrum* sp. E4 in fact expresses at least two distinct amidohydrolases. However, butyramidase and benzamidase activities co-purified, indicating that a single broad-spectrum amidase enzyme is responsible for hydrolysis of these substrates. To our knowledge, this is the first report of a halophilic amidase enzyme, and the first example of an amidase in the kingdom Euryarchaeota. Work to purify and characterise this novel extremophilic enzyme, which may find applications as a biocatalyst, is ongoing.

EM 31 Biodegradation of aromatic compounds by extremely halophilic micro-organisms

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Extremophiles offer new opportunities for the isolation of novel biological molecules that have the potential to promote various fields

of biotechnology. They are a source of highly stable enzymes, which have the possibility to benefit biocatalysis. The extremely halophilic archaea are of particular interest because of the stability of their proteins in high salt systems (effectively a low water environment). We have isolated and characterised novel aromatic and amide degrading haloarchaea, particularly those that possess oxygenase-mediated degradation pathways. Ongoing aims are to explore the evolution of such biodegradative routes within the haloarchaea, determine the pathways by which they metabolise these compounds and characterise the enzymes and genes responsible. We present here data on recently isolated strains capable of degrading a number of aromatic compounds, and compare these prokaryotes to previously isolated haloarchaeal strains. We also provide evidence for aromatic degradation via CoA intermediates within this group, and present a novel method for synthesizing aromatic CoA thioesters for use as substrates in enzyme assays.

EM 32 Low pH-inducible polyphosphate accumulation: pilot-scale application to wastewater treatment of a previously unrecognised aspect of microbial phosphorus metabolism

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We have identified a hitherto-unreported aspect of microbial phosphorus (P) metabolism *i.e.* uptake of 'luxury' levels of P under mildly acidic conditions, and its accumulation as insoluble intracellular polyphosphate. The phenomenon was initially discovered using pure cultures and under laboratory conditions but appears widespread amongst soil and aquatic micro-organisms. Thus it is likely to have far-ranging implications for P-cycling in the environment. To investigate its possible application to wastewater treatment we constructed a 2000-litre activated sludge pilot plant at a municipal sewage treatment works. This removed more than 60% of influent phosphate from primary settled sewage at pH 6.0, but less than 30% at the typical operational pH for the works of 7.0 – yet there were no deleterious effects on other treatment parameters. At these two pH values the P content of the sludge was, respectively, 4.2% and 2.0%. At pH 6.0, 35% of sludge microbial cells contained visible polyphosphate inclusions; the corresponding value at pH 7.0 was less than 10%. The novel process does not require extensive retrofitting of existing treatment works or high influent volatile fatty acid concentrations, and hence may provide an attractive alternative to existing biological and chemical options for phosphate removal from wastewater.

EM 33 The naphthalene dioxygenase from *Rhodococcus* sp., NCIMB12038

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The biodegradation of naphthalene has been extensively studied in *Pseudomonas sp.*, the naphthalene dioxygenase system present in this organism becoming the biochemical and genetic paradigm for naphthalene catabolism. The naphthalene dioxygenase terminal component from *Rhodococcus sp.* NCIMB12038 has been isolated and characterized and its crystal structure has been elucidated. We present here work on the isolation of the other components of this naphthalene dioxygenase enzyme system. We also present work on the regulation and control of naphthalene dioxygenase gene expression and contrast it with that of the *Pseudomonas putida* paradigm.

EM 34 Biodegradation of volatile pollutants in the unsaturated zones of contaminated soils

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Natural attenuation of a polluted site is an attractive means to manage remediation if microbial degradation can be validated and the presence and activity of biodegradative micro-organisms demonstrated. Although there has been a considerable amount of research on the biodegradation of common volatile compounds, there has been little focus on the mediating microbial populations and their biodegradative capacity in the unsaturated zones of contaminated soils. The ongoing aims of this project are to determine the extent to which these common volatile pollutants are biodegraded by micro-organisms as they migrate through the unsaturated zones of soils, with optimal conditions determined for the microbial populations. Also of interest will be the robustness to changing conditions with respect to temporary dehydration and selection of a biodegradative population, survival and recovery when water is present, providing considerable insight into the degree to which the microbial populations can respond to volatile pollutants. To date we have collected data on micro-organism population types in the samples used with a number of BTEX degraders isolated, their biodegradative capacity and ability to survive dehydration and re-wetting conditions.

EM 35 The microbial aspects of site assessment, bench scale treatability, and subsequent monitoring of a SEquenced REactive BARrier at a former gaswork site

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The need for the remediation of Manufactured Gas Plants and brownfield sites within the UK is immense. Within the UK alone up to 1500 former gaswork sites still pose major environmental problems for site owners in relation to onsite contaminants and redevelopment. A multidisciplinary field study of a former gas production facility was carried out prior to the installation of an engineered SEquenced REactive BARrier (SEREBAR). Physical, chemical and biological processes are covered within the remit of natural attenuation, however, research has indicated that biological degradation imparts the major influence in contaminant reduction of coal tar derivatives.

Numerous soil cores, obtained at various depths, and groundwater samples from 12 multilevel boreholes were characterized for microbial ecology. The microbial structure across the site was monitored by DGGE analysis and revealed a similarity between, both, the free-living (planktonic) and attached (biofilm) microbial community. Further research indicated the potential for biotic mineralization of the priority pollutants (PAHs), at rates feasible

for the design and installation of a SEREBAR based treatment system. Evidence for the implementation of a biological Permeable Reactive Barrier (PRB) is provided.

EM 36 Function and diversity of PAH dioxygenase enzymes at a former gasworks site

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Polycyclic aromatic hyrdrocarbon (PAH) catabolic pathways in bacteria, combined with engineered bioremediation technologies can offer a versatile and cost-effective alternative to more traditional physiochemical methods of site remediation. Assessment of the PAH-biodegradation potential of the indigenous microbial population at contaminated sites has been traditionally determined using culture-based techniques. However, PCR based methods which target genes encoding the PAH degradation enzymes offer a more rapid approach to such characterisation. PAH degrading bacteria can be detected by targeting the large alpha subunit gene of the initial dioxygenase enzyme in the PAH degradation pathway. However, a true indication of this potential may not be obtained due to the diversity of these genes. This project seeks to determine the diversity of the naphthalene dioxygenase alpha subunit gene and bring to the fore, any new sequences at former gasworks site where naphthalene is the principal pollutant, prior to, and after installation of an engineered permeable reactive barrier (PRB). To date, the biodegradation potential of the indigenous population distributed throughout the site and within the PRB has been assessed revealing heterogeneous numbers of naphthalene degraders.

EM 37 Fate of ammonia and heterocyclic aromatic compound production and degradation at a contaminated former gasworks site

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Heterocyclic aromatic compounds may comprise 5% to 13% of coal tar products. Also several heterocyclic compounds have been shown to be generated *in vitro* by bacteria through the action of oxidative ring fission and nitrogen incorporation into the benzene ring using ammonia as a nitrogen source. It is our aim to determine if such a process can occur during remediation of coal tar waste. We have produced picolinic acid *in vitro* from catechol using cell extracts from *Pseudomonas Putida* strain G7 and aim to reproduce this using conditions that mimic those at a contaminated site. We have also isolated a range of heterocyclic compound degraders from contaminated groundwater samples and intend to identify them on the basis of their 16S RNA sequence. Potential modes of ring fission for a range of aromatic compounds and possible heterocyclic compound formations are also discussed.

EM 38 Microbial degradation of the synthetic pyrethroid cypermethrin

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The agri-food sector contributes 10% of Irish GNP and 11% of employment, but it is also the main producer of organic and chemical wastes in Ireland. In upland and some lowland grassland areas, sheep production is significant and sheep dips are widely used to control a wide range of animal parasites. Until recently, most sheep dips have been based upon a range of organophosphate chemicals but these have been banned because of their high mammalian toxicity and health problems associated with their use. Sheep dips are now largely formulated from synthetic pyrethroids (pyrethrin), an insecticidal ester originally derived from the flower heads of certain *Chrysanthemum* species. Although these compounds exhibit a much lower mammalian toxicity than their organophosphate counterparts concentrations as low as 10ng/L have the potential to eradicate invertebrate life in rivers and lakes. The causes of this pollution include loss from the dipping tank, run-off from recently dipped sheep and/or leachate from land farming. The wash from woollen mills in the textile industry is also a potential source of this toxic waste. At present information regarding the biodegradation of synthetic pyrethroids in the environment is incomplete. The focus of this project is to identify and isolate, using both conventional and molecular approaches, micro-organisms which break down synthetic pyrethroids. Several bacteria were isolated from both uncontaminated soil and soil previously exposed to synthetic pyrethroid sheep dip using submerged fermentation. Extraction and analytical methods were developed to detect this compound in aqeous based systems. Isolates have been screened for their ablility to degrade the synthetic pyrethroid cypermethrin in submerged fermentation.

EM 39 Effect of polycyclic aromatic hydrocarbons on microbial community profiles in soil

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Creosote is a persistent chemical mixture composed of approximately 85% Polycyclic Aromatic Hydrocarbons (PAHs), 10% phenolic compounds and 5% N-, S- and O-heterocyclics. It has been extensively used as a commercial timber preservative in wood-treatment plants, leading to widespread contamination of soils and groundwaters underneath wood treatment plants and in adjacent areas. Conventional clean-up procedures for PAH contaminated sites normally involve incineration and require transport of all soils and sediments from the site to an incinerator. Bioremediation is a potentially cost effective and safe alternative for the rehabilitation of contaminated sites. However, it remains an unpredictable technology due to a lack of understanding of the microbial communities involved in bioremediation and the factors affecting these communities. In soils, and in most other environments, it is clear that a very low percentage (<1%) of microbial diversity present can be cultured and assessed using conventional culture-based microbial methods. This study is concerned with assessing the factors affecting microbial community dynamics in soil from a creosote-contaminated site. The bacterial community present was examined using conventional and molecular techniques, including total cell numbers and terminal restriction fragment length polymorphism (TRFLP). Results indicate that the nature of the PAH substrate and time have the most significant effect on degradation rates and bacterial community profile.

EM 40 Microbial degradation of petroleum hydrocarbons in soil

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Crude oil is a vital component of modern industry with over $3.5x10^9$ tonnes p.a. consumed globally. Petrochemicals are transported worldwide and spillages occur frequently. When spillages occur a number of cleanup options are available which include bioremediation. Bioremediation has considerable potential but is still an unpredictable technology as our ability to investigate microbial populations implicated in biodegradatory processes has until recently been limited to the minority of culturable soil micro-organisms. However, with the application of molecular biological techniques to studies of soil microbiology we may now view biodegradatory processes without the constraints of organism culturability. This study is concerned with the microecological shifts associated with bioremediation of tetradecane, a component of crude oil, in a soil slurry system. Soil microbial community activity was assayed by dehydrogenase measurements and community structure was assessed using tRFLP. The microbial population in the soil removed the added tetradecane within 28 days. Multivariate analysis indicated that certain ribotypes within the soil community were affected by the presence of tetradecane.

EM 41 Effects of inoculum pre-treatment on the survival and catabolic gene expression of inoculated *Sphingobium yanoikuyae* B1 in an aged PAH-contaminated soil

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Many sphingomonads have been isolated for their ability to degrade pollutants from a diverse range of environments. Polycyclic aromatic hydrocarbons (PAHs) are persistent environmental pollutants which can be degraded by sphingomonad species and are the focus of widespread concern because of their proven mutagenic properties. *Sphingobium yanoikuyae* B1 is able to utilise a broad range of aromatic hydrocarbons and co-oxidise others. We have previously shown that strain B1 is still active after 30 days when inoculated into an aged PAH-contaminated soil microcosm. This study aimed to establish whether strain B1 is still actively utilising PAHs in the soil after this time.

Carbon starved or phenanthrene pre-treated B1 cells were inoculated into aged PAH-contaminated soil microcosms. At suitable time points (0, 2, 5, 10, 20, 30, 40 days) RNA was extracted from the soil and expression of two ring cleavage dioxygenase genes (*bphC* and *xylE*) was assessed by RT-PCR. 16SrRNA DGGE was used to follow strain presence and general activity in the soil community. Gene expression was correlated with PAH concentration in the microcosms by endpoint analysis using GC. This yielded an overall picture in which strain B1 remained active and had little overall impact on composition of the soil bacterial community.

EM 42 Impact of pollution on the dissemination of quaternary ammonium compound (QAC) resistance genes and evidence for co-selection of drug resistance genes in environmental bacteria

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QACs are widely used in hospitals and industry as biocides and fabric softeners. QAC resistance genes are commonly situated on integrons, which may also carry antibiotic resistance genes. Samples were taken from a QAC contaminated reed bed system. Control samples were also taken from various agricultural soils. Incidence of class 1 integrons was significantly higher in strains isolated from QAC contaminated samples. *Vibrio, Serratia, Pseudomonas* and *Enterobacteriaceae* sp. were identified as carrying integrons. The variable region of integrons was characterised using PCR and sequencing. The integron in 18/19 isolates was identical, carrying genes with high identities to a class1 integrase, a gene of unknown function and *qacE*. The integron appears to be highly mobile with a broad host range. PCR probing of total community DNA illustrated the presence of class 1 integrons in unculturable bacteria, particularly in agricultural soil amended with sewage sludge.

EM 43 Observations on the application of mushroom compost and bioaugmentation on the bioremediation of phenols and aromatic hydrocarbons

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The relative contribution of bioaugmentation and environmental amelioration to a successful bioremediation strategy is an area open to debate. We report here on the application of phenol degrading bacteria and mushroom compost in field trials that were conducted at two former chemical facilities for the purpose of developing a remedial design.

In the first trial, five treatments were established consisting of various permutations of mushroom compost (approx. 20% by volume), nutrients, an inoculum of commercially available bacteria ('Phenobac'), together with a control. Two of the treatments were established as 'biopiles', with forced aeration, two were subjected to periodic mechanical aeration. From starting concentrations in the order of 600 to 700 mg/kg, the three treatments receiving mushroom compost achieved mean reductions in total phenols of 99% over an eight week period. These compared to reductions of 30 to 48% in treatments receiving simply nutrients, with or without bioaugmentation and less than 1% reductions in the control (although the latter showed significant variability).

Bioaugmentation appeared to increase the rate, but not the extent of bioremediation: the mean concentration of total phenols in the compost-amended soil was reduced from 600 mg/kg to less than 20mg/kg after two weeks, whereas in the non-inoculated, compost-amended soil, the mean concentration was reduced from 710mg/kg to 320mg/kg. After 8 weeks however, mean concentrations ranged from 1 to 2 mg/kg in both treatments.

The second trial evaluated the effects of mushroom compost addition on the degradation of several alkyl benzenes and naphthalene. In this study, the degree of reduction in contaminant concentrations was mostly less in mushroom compost-amended treatments than in those without. For mechanically-aerated treatments the respective differences in contaminant reduction between compost-amended and without, over approximately 3 months were 94% versus 99% for 1,3,5-trimethylbenzene, 80% versus 98% for ethyl benzene, 90 versus 99% for xylenes and 93 versus 99% for naphthalene. For the biopiles, the respective reductions were 80% versus 79% for 1,3,5-trimethylbenzene, 25% versus 76% for ethyl benzene, 53 versus 64% for xylenes and 64 versus 73% for naphthalene.

The reduction in the degree of contaminant loss could possibly reflect a reduction in bioavailability in compost-amended treatments. (This may not have even a significant issue in the first trial due to the more polar nature of the phenolics reducing the degree of absorption by the compost).

Careful consideration as to the benefits of both bioaugmentation and compost addition in developing a bioremediation strategy. Geotechnical issues relating to compost addition and the potential for methane generation from reinstated material may also be significant issues.

EM 44 The source and distribution of thermophilic campylobacters in village pond water

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Aims*:* To investigate the sources of thermophilic campylobacters in village ponds water

Methods A total of 90 faecal samples of ducks and 36 samples of run-off water were collected from three ponds. The faecal samples were examined by means of selective enrichment. Different volumes of run-off water were examined by membrane filtration followed by selective enrichment. Presumptive isolates were confirmed by Gram stain, cell morphology, presence of catalase and oxidase, growth under microaerobic but not aerobic conditions and PCR. Typing of confirmed isolates was achieved by DNA sequencing of PCR products.

Results Confirmed *Campylobacter* species were recovered from 11% of the faeces and 17% of the run-off water. Sequence data showed that *Campylobacter jejuni* is predominant in duck faeces, while *Campylobacter coli* was the only strain found in run-off water.

Conclusion The faeces of waterfowl and rain-related run-off water are both sources of thermophilic campylobacters to village ponds. Different *Campylobacter* species may, however, have different origin; *C. jejuni* from ducks, *C. coli* from rain-related run-off.

EM 45 The spatial impact of mercury pollution on microbial communities in an artificial soil system

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Bacteria have been shown to have a fundamental role in soil processes, such as biogeochemical cycling, decontamination of toxic materials and the maintenance of soil structure. Soil is the most complex of all environmental matrices and provides a highly diverse range of microenvironments for soil bacteria. There is also often significant variation in temporal and spatial distribution of chemicals, such as nutrients and pollutants, through the soil substrate. The evaluation of detrimental effects of pollution on bacterial diversity and ecosystem function in soil has recently garnered much interest. The aim of the current work is to assess the effect of spatial heterogeneity of pollutants on bacterial communities in soil.

An artificial soil system is being used to assess the impact of mercury pollution distributed at even distributions or localised foci using populations of *Pseudomonas fluorescens* SBW25, with and without the mercury resistance plasmid pQBR103.

It is expected that different spatial distributions of pollutants will affect relative population numbers. Preliminary data will be presented.

This research has been funded by the Natural Environment Research Council.

EM 46 Comparative inactivation of faecal indicator bacteria in traditional metal and earthernware water storage vessels

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The effectiveness of storing water contaminated with faecal indicator bacteria in brass or earthern vessels of the type used in rural India have been investigated. Suspensions of *Escherichia coli* in sterile distilled water were maintained for up to 48 h in each vessel and enumerated by surface plate counts on non-selective nutrient agar and several selective coliform media at 37°C either under standard aerobic conditions, or under conditions designed to neutralise reactive oxygen species (ROS). The counts obtained for *E. coli* decreased on storage for 6–24 h in the brass vessel; counts for the selective media were consistently lower than for equivalent counts for non-selective medium, with ROS-neutralised conditions giving consistently higher counts than aerobic incubation. However, after 48 h, no bacteria were cultivable under any conditions. Similar results were obtained using water from environmental sources in the Panjab, and from rural households where brass and earthern vessels are used for storage of drinking water. In all cases, storage of water in a brass vessel resulted in the inactivation of *E. coli* and coliforms over a 48 h period.

EM 47 The study of a lab-scale contaminant plume

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A novel imaging system using the fluorescent oxygen tracer Ruthenium (II)-dichlorotris(1,10-phenanthroline) ($Ru(phen)_3Cl_2$) was developed to investigate the biodegradation of acetate and phenol by a consortium grown from an environmental sample from borehole MW7 from a contaminated site in St Albans, England.

A flow cell containing quartz sand (212–300 µm) covered with a biofilm was set up to allow a degrading plume of acetate or phenol to be formed from a point source within the walls of the flow cell. Oxygen distribution was monitored using UV excitation of the dye $Ru(phen)_3Cl_2$ and imaged using a CCD camera. Carbon source concentration was measured using ion chromatography (IC) analysis (for acetate) or high performance liquid chromatography (HPLC) analysis (for phenol) by sampling through septa ports within the wall of the flow cell. Microbial analysis was carried out by destructive sampling across transects of the flow cell followed by fluorescence microscopy for total cell counts using DAPI, and active cell counts using the tetrazolium salt, CTC. Changes to the microbial population across a transect of the plume was monitored with PCR-TGGE.

EM 48 Characterisation of the gut microflora of the Goeldi's monkey, *Callimico goeldi*

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The study of mammalian insectivory has been neglected in contrast to folivory, frugivory and grazing mammals. A major component of insects is their chitinous exoskeleton but there is little information about how mammals may break down large structural carbohydrates, such as those found in the chitin. Nutritional problems including wasting disease, zinc and calcium deficiencies have been experienced in captive callitrichids (marmosets and tamarins). In the case of wasting marmoset syndrome there are indications that diet and intestinal microflora may play a role. Anecdotal evidence would suggest that insectivory is crucial for good health in these primates and such information is vital to correct captive management and hence conservation of callitrichids.

Chitin is a polymer of unbranched chains of β1,4-linked *N*-acetylglucosamine residues, considered difficult to digest by

non-specialised mammals. Information regarding digestion and absorption of chitin in the digestive tract of mammals is limited. Chitinolytic bacteria have been found in a number of organisms including the minke whale. Enzymatic chitinases in the digestive tracts of animals have been the subject of much research and have been isolated from the gastric mucosa of a primate, *Perodicticus potto*, but chitinolytic micro-organisms have not been investigated thoroughly. Using culture-based techniques the intestinal microflora of the Goeldi's monkey, *Callimico goeldi* is being characterised and screened for chitinolytic activity. Further work is underway to apply molecular techniques to the characterisation of microbial diversity of the gut microflora of marmosets.

EM 49 The use of Multi-Locus Sequence Typing (MLST) to study genetic variation within *Ralstonia solanacearum*

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Multilocus sequence typing (MLST) is a nucleotide sequence based approach for the characterisation of isolates of bacteria and other micro-organisms. With MLST, isolates of micro-organisms are characterised using the nucleotide sequences of the internal fragments (approximately 450–500 base pair) of selected housekeeping genes. For each housekeeping gene, sequence differences between isolates are assigned as distinct alleles, and for each isolate the alleles at each of the loci define the allelic profile or sequence type. The sequence types identified for each species can be stored in a database to allow Internet access and easy identification of species and strains by other MLST users. MLST provides an accurate and sensitive molecular typing system that can be used to track different strains of important pathogens in epidemiological studies.

Thus far, MLST has mainly been applied to human pathogens. SASA is the first to use this particular technique for the genetic characterisation of a plant pathogen, namely the bacterium *Ralstonia solanacearum*, the cause of potato brown rot. The aim of this study is to identify possible genetic differences within *R. solanacearum* biovar 2, race 3, which has not been possible with other techniques. It is hoped that any differences found can be related to differences in virulence and used to clarify other aspects of brown rot epidemiology.

To create a MLST database for *R. solanacearum*, a number of candidate housekeeping genes were selected by mining the complete genome sequence of the strain GMI1000, a race 1 isolate of *R. solanacearum* from tomato. On the basis of the chosen housekeeping genes, nested primers were designed which were tested against a selection of *R. solanacearum* isolates. After this initial screening, seven housekeeping genes involved in small molecule metabolism were selected for further testing. The external primers were used as PCR primers and the nested primers as sequencing primers. Initially, 110 isolates of *R. solanacearum* are being sequenced, which includes different biovars from around the world. The preliminary results indicate genetic variation between the biovars but not within biovar 2.

EM 50 The impact of *o*-cresol and 2,4, dimethylphenol on anaerobic microbial associations

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The anaerobic bacterial toxicity and the fate of cresols and xylenols, which readily enter the groundwater environment from different sources, e.g. leaks at oil and gas works, are not fully understood. Continuous flow multi-stage (CFMS) systems and anaerobic batch cultures were employed to analyse the anaerobic microbial toxicity of two phenolic compounds, o-cresol and 2,4-dimethylphenol (DMP). Anaerobic batch cultures were inoculated in triplicate with return activated sewage sludge in growth medium that was supplemented with cellobiose (1720mg/l) and sulphate (480mg/l), and either DMP (40–150mg/l) or *o*-cresol (100–1200mg/l). With DMP, initial inhibition of sulphate reduction was observed, followed by complete reduction, within 20 days. In all DMP-supplemented cultures, neither Volatile Fatty Acid (VFA) production and utilisation nor methanogenesis was apparently inhibited, with the possible exception of propionate turnover at the highest DMP concentration. With *o*-cresol, sulphate reduction was completely inhibited at concentrations >500mg/l, and temporarily inhibited at all other concentrations tested. Differences in VFA production and utilisation were observed at concentrations greater than 200mg/l. Inhibition of methanogenesis was recorded at DMP concentrations ≥500 mg/l. By use of ongoing CFMS system studies, in which the physiologically-different bacterial groups are spatially segregated, the effects of each phenolic compound on Sulphate Reducing Bacteria, acidogens, acetogens and methanogens will be elucidated.

EM 51 Potential of *Trichoderma harzianum* (Strain T22) for use in bioremediation, phytoremediation and land stabilization strategies

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Trichoderma harzianum (Strain T22) is a rhizosphere competent, plant growth promoting soil fungus sold commercially to improve plant health and plant growth. The mechanisms by which T22 improves plant production are manifold including production of specific enzymes, ability to parasitize plant pathogens, production of surfactants and the induction of physiological changes in plants that lead to increased uptake of nutrients by the plant. We have investigated the use of T22 to enhance bioremediation and phytostabilisation of contaminated land by looking at the potential of the fungus to degrade organic pollutants, enhance uptake of pollutants such as PAHs and heavy metals, and enhance plant growth in contaminated soil. We found that the fungus increased the growth of crack willow by more than 30%. The fungus can degrade pollutants such as cyanide and phenols, but as yet we have no evidence to suggest that the fungus can degrade polycyclic aromatic hydrocarbons. Growth stimulation of crack willow by T22 lead to an increased uptake of metals from contaminated soil, and the ability of the fungus to enhance plant growth could be used in soil stabilisation programmes. Currently trials are underway to investigate the potential of T22 under typical field conditions in the UK.

EM 52 The presence of earthworms; their effects upon hydrocarbon degrading micro-organisms

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Earthworms have considerable beneficial influence upon the structure of soils and are known to promote microbial numbers in a number of ways. These can include the fragmentation of soil organic matter, favourable intestinal conditions, and aided dispersal with increased numbers of micorooganisms being found in earthworm burrows and casts.

Vermicomposting of contaminated soils is an area currently under consideration. It is expected that the positive influence of earthworm presence in such a system will promote the intrinsic hydrocarbon degrading microbial populations. Research is currently focusing upon

the application of earthworm gut flora, in the form of worm cast, in which already established hydrocarbon degrading organisms will be present.

A two stage study is currently investigating this hypothesis using both spiked soils and actual field contaminated soils.

EM 53 Acid resistance and the effect of pH control on the growth of *Azospirillum brasilense* Sp245

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Bacteria of the genius *Azospirillum* are a well characterized plant growth promoting rhizobacteria. Several authors have reported the enhancement of plant growth and crop yield upon its inoculation on plant roots. The observed plant response to *Azospirillum* inoculation has been attributed to the production of indole-3-acetic acid (IAA) by these bacteria. A first prerequisite for a successful *Azospirillum*–plant interaction is that the bacteria survive and proliferate to significant numbers on the host root system. Since their hosts species are mostly annuals they need to multiply in the absence of their host plant and survive from one season to the next. *Azospirillum* do display a number of very efficient physiological mechanisms that may enable them survive the hazards associated with their environment. These include poly-B-hydrobutyrate synthesis, flock and cyst formation, production of melanin and polysaccharides and protection inside ectomycorrhzal fungal spores. An important stress condition that must be faced by *Azospirillum* is the extensive variation in H+ ion concentrations in the soil. Soil pH presents a crucial environmental factor which could undermine the overall plant growth promoting capabilities of *Azospirillum brasilense*. An understanding of the level of acid resistance by *A. brasilense* should be regarded as a priority in research on this plant growth promoting rhizobacteria. This supposedly important factor is neglected and nothing is known about how *Azospirillum* surmounts this hurdle. In this work, we have investigated acid resistance and analysed the influence of pH on growth of *Azospirillum brasilense* Sp245. Using a controlled fermentor, micro-aerobic batch cultures of *Azospirillum* were conducted at different pH values (in the range of common soil pH) in minimal medium with L-malate as the sole carbon source. *Azospirillum* could not grow at pH 5.5. Growth at pH 6.3 proved most efficient resulting in the best coupling between time, energy and biomass. The lethal pH was determined to be 2.5. A sub-lethal acid shock at pH 5 increased the acid resistance of cells several folds and enabled the culture of this bacterium at pH 5.5. The response of the bacterium to acid shock as determined by an SDS PAGE eletrophoresis revealed changes in protein synthesis in response to acid shock. This study shows that *A. brasilense* can adapt to survive in severe acid environments. This adaptation is concomitant to the synthesis of new proteins.

EM 54 Pathogen survival in industrial waste generated from metal finishing industries in Delhi, India

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The small scale industries have been regularly dumping hazardous waste in 28 authorized industrial estates and several non-confirming area in Delhi (NCT[1], of Delhi, 2001). Approximate 70% of total hazardous waste generated in Delhi is from metal finishing industries (DPCB[2], 2000). Wazirpur industrial area is a first major industrial estate located on north-west part of Delhi. There are more than 1000 smallscale industries and more than 80% are metal finishing industries. These industries are released a large volume of solid and liquid waste which are directly dumped in the roadside and affecting the local environment. The results revealed that this wastes was found to be very acidic (pH, 2.0–3.0) and contained toxic heavy metals apart from other microbial nutrients.

Keeping in mind the human health the analysis of medically important micro-organisms were done in this waste. 100 samples were collected from Wazirpur industrial area in three seasons monsoon, winter and summer during 2000–2001. Helminthes and protozoans were detected in 12.23% and 18.89% samples. Bacterial infections were reported due to presence of *Staphylococcus, Streptococcus, E.coli, Klebsiella, Enterobacter, Pseudomonas* and *Proteus* etc. Environmental Mycobacterium is a new threat associated with this industrial waste and 6.67% samples were positive.

These pathogens are transmitting via intestinal discharge of human and animal activities and mixing of area sewage with said waste. This waste become a reservoir of cysts, eggs of parasites and other pathogens and causes establishment of microbial infection in area workers though different routes viz. direct contact, common vehicle, air and many vectors. During last 10 years there was an increase in absolute terms in the incidence rate of both intestinal and respiratory diseases in these industrial estates (GOI[3], 2001). These industries are polluting the river Yamuna and also transmitting the pathogens in river water in 22 km stretch between Wazirabad and Okhla (CPCB[4], 2000).

In this paper, we summarize existing information on [1]the presence of microbial pathogens in this waste as protozoans, nematodes and bacteria; [2]isolation and identification of environmental Mycobacterium; [3]sources of these pathogens and routes of transmission of these pathogens.

[1]NCT- National Capital Territory; [2]DPCC – Delhi Pollution Control Board; [3]GOI – Government of India; [4]CPCB – Central Pollution Control Board

EM 55 Fungal diversity in the contaminated soil of Wazirpur industrial area, Delhi, India

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Industrialization, urbanization and increased vehicular traffic have resulted in increased contamination of our environment by heavy metals. Wazirpur industrial area is a first major industrial estate located on north-west part of Delhi. There are more than 1000 small scale industries and more than 80% are metal finishing industries. These industries are released a large volume of solid and liquid waste which are directly dumped in the roadside and affecting the local environment. The results revealed that this wastes was found to be very acidic (pH, 2.0–3.0) and contained toxic heavy metals (Cu, Cr, Ni, Fe, Mn) apart from other microbial nutrients.

Due to long persistance of this waste in the environment without any treatment many of fungal isolates from the sorrounding environment are settled down on the upper surface of waste and few of them are capable of growing in the toxic conditions and long nature has in turn resulted in development of metal resistant fungal strains. These strains are minimizing the heavy metals toxicity either by metal complexation or precipitation and/or other mechanisms.

However exploitation of fungal diversity was done in contaminated soil of Wazirpur industrial area through out the year 2000–2001.

More than twenty strains were isolated and maximum were belongs to *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Mucur* sp., *Absidia* sp., *Alternaria* sp., *Cladosporium* sp., *Curvularia* sp., *Humicola* sp., *Memnoniella* sp., *Myrothecium* sp., *Neocosmospora* sp., *Paecilomyces* sp., *Papulaspora* sp., etc.

Significant seasonal variation were found in fungal diversity. After the detailed screening of these fungal isolates four fungal isolates were found to be resistant for chromium (VI) and nickel (II) salts . These fungal isolates showed higher level (100–10000 ppm) of resistance for these salts and quantity of metal uptake varies with different species. The toxicity is also influenced by different factors like pH and composition of growth medium. The objectives of present investigation are (1) Isolation and Identification of fungal strains from contaminated soil (2) Study of seasonal variation in fungal diversity (3) Screening of metal salts resistant fungi from industrial sludge.

EM 56 Do hormones play a role in the seasonal shedding of *Escherichia coli* O157:H7 in ruminants?

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Seasonal shedding of *E. coli* O157:H7 (EC) in ruminants is well documented, however reasons for this phenomenon are unknown. We hypothesize that seasonal shedding of EC is a result of physiological responses by the animal to changing daylength. In a series of experiments utilizing naturally-infected beef cattle and experimentally-infected sheep, we examined the effect of exogenous melatonin administration and chemical inhibition of the thyroid, on fecal shedding and gastro-intestinal (GIT) populations of EC. Animals treated daily with a low oral dose of melatonin (mg) had shedding patterns and GIT populations of EC similar to controls. However, when a high dose of melatonin (g) was orally administered to naturally-infected cattle, the number of cattle shedding EC was lower ($P = 0.05$) compared to control steers. Chemical inhibition of the thyroid, via administration of propyl-thiouracil (PTU), had no effect on fecal shedding or GIT populations in naturally- or experimentally-infected cattle and sheep. However, following termination of the PTU treatment in naturally-infected cattle, a greater percentage of PTU treated animals shed EC compared to control steers. Early results indicate that hormones known to respond to changing daylength may play a role in the seasonal shedding of EC in ruminants.

EM 57 How common are bacteriophage in feces of US feedlot cattle?

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Escherichia coli O157:H7 is a food-borne pathogen of critical importance that often colonizes cattle. *E. coli* O157:H7 can be specifically killed by bacteriophage (bacterial viruses); and bacteriophage treatment has been suggested as a pre-harvest intervention strategy to reduce food-borne pathogens in cattle. No systematic approach to determine the incidence of anti-*E. coli* O157:H7 phage (AO157P) has been previously performed. Therefore the current study was designed to determine 1) the incidence of *E. coli* O157:H7 and *Salmonella* and 2) the incidence of AO157P in the feces of feedlot steers in commercial feedlots in the United States. Fecal samples (n = 60) were collected from four feedlots in two Southern Great Plains states (total n = 240 fecal samples). *Salmonella* and *E. coli* O157:H7 were found in 3.8% and 11.7% of the fecal samples, respectively. AO157P were found in 15% of the fecal samples. AO157P were present in all four feedlots and were found in 55% of the cattle pens. Our results indicate that anti-*E. coli* O157:H7 is very widespread in feedlot cattle, indicating that further research into the ecological role of bacteriophage in the gastrointestinal tract is needed.

EM 58 Near-surface structure of iron minerals influences the rate of dissimilatory iron reduction

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Dissimilatory iron reduction is one of the most significant redox processes in nature, of particular interest to contaminant degradation and immobilisation. At circum-neutral pH, most Fe(III) is insoluble, thus cells must transfer electrons to solid mineral species *via* outer membrane cytochromes and intimate association of bacteria with a surface appears necessary. Modeling of the electron transfer (ET) process to three iron mineral surfaces suggests clear differences in rate exist between the surfaces. Measurement of forces generated between minerals and *Shewanella oneidensis* using AFM indicate that surfaces to which ET is predicted to be fastest, *i.e.* hematite (001), support greatest adhesive force. Surfaces to which ET is slowest, *i.e.* magnetite (111) support least adhesive force. Cell accumulation studies indicate that hematite (001) also supports greater cells densities than magnetite, the latter surfaces releasing relatively more daughter cells into the aqueous phase. There is therefore, compelling preliminary evidence to suggest that atomic/electronic structures of mineral surfaces may directly influence attachment and activity of iron reducing bacteria by influencing the final metabolic step, transfer of electrons from cytochromes to mineral surfaces.

EM 59 Phage to treat furunculosis (*Aeromonas salmonicida*) in salmonids

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Aeromonas salmonicida is a Gram-negative bacterium that causes furunculosis, a disease of salmonids that often results in significant mortality in hatchery salmon and trout. Currently the disease is controlled by preventative measures or actively with chemotherapy. The aim of this project is to examine bacteriophage therapy as an alternative to antibiotics. Several bacteriophages specific to *A. salmonicida* were isolated from Puget Sound (Washington, USA), with the four most active selected for further characterization. All four were *Myoviridae*, with genome sizes of approximately 140 kb (phages Her98 and Lott1) or 45 kb (phages AEV1 and Kcrk). These phages kill all 23 strains of our *A. salmonicida* collection. Seventy percent of these strains possess the S-layer generally associated with pathogenicity, and 17% were found to be resistant to two (oxytetracycline and sulfadimethoxine-ormetoprine) out of the three antibiotics (sulfamerazine), approved to treat furunculosis in US hatcheries. Both nonpathogenic and pathogenic hosts lyse around 100 minutes post phage infection, with over a four-log drop in bacterial survivors. Our results suggest that phage therapy could be a viable alternative to antibiotics in the treatment of furunculosis in hatcheries, substantially reducing the amount of antibiotics released into the environment.

EM 60 The use of ε-caprolactam as an inducer of an aromatic nitrilase in the filamentous fungus, *Fusarium solani* IMI 196840

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It has been previously shown that benzonitrile at a concentration of 0.1% (v/v) induces a nitrilase enzyme in *Fusarium solani* IMI 196840. This nitrilase had a subunit size of 76 KDa and a native size of 620 KDa. (*1*).

The use of ε-caprolactam as an inducer of nitrile degrading enzymes has also been reported in a number of bacterial strains, namely, a nitrilase of *Rhodococcus rhodochrous* J1 (*2*), a nitrile hydratase of both *R. erythropolis* strains JCM6823 & JCM2892 (*3*) and a nitrile hydratase of *Agrobacterium tumefaciens* (*4*).

Addition of this cyclic amide at a concentration of 0.5% (w/v) in a nutrient rich broth and incubation under normal growth conditions leads to the expression of a nitrilase enzyme. The enzyme has a subunit size of 41 KDa and the level of expression is approximately 25 times greater than that with benzonitrile. Its presence does not greatly affect growth and it is not a substrate to the enzyme.

This presentation will describe the substrate specificity on a number of nitriles and introduces the hypothesis of the presence of two nitrilase enzymes under induction from two different compounds, one of which, ε-caprolactam, is a novel inducer of a fungal aromatic nitrilase.

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EM 61 Can 15-N stable isotope probing be used to identify microbes involved in crop residue degradation in soil?

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Release of mineral nitrogen (N) from crop residues incorporated into soil is dependent upon the soil's resident microbiota and the quality of the input. However, the characteristics of organisms contributing to crop residue breakdown, and the role of community structure in controlling the release of mineral-N, are poorly understood. Stable-isotope probing (SIP) of DNA using ^{13}C labelled substrates is a culture-independent technique that provides a link between function and phylogeny of micro-organisms. We investigated the feasibility and limitations of ^{15}N-DNA SIP using labelled and unlabelled pure microbial cultures and soil microcosms.

DNA was extracted from *Pseudomonas putida* grown in a mineral salts medium to which 99 at% ^{14}N- and 99 at% ^{15}N- labelled $^{15}NH_4$ $^{15}NO_3$ were added as sole N source, providing ^{15}N enrichment increments between 0 and 99 at% ^{15}N. Using isopycnic centrifugation, the minimum ^{15}N DNA enrichment required to obtain a clear separation between labelled and unlabelled DNA was 50 at% ^{154}N.

Rye grass was grown in sand and fed with nutrient solution containing 99 at% $^{15}NH_4{}^{15}NO_3$ as sole N source. After 8 weeks rye grass shoots were incorporated into sandy-loam soil and incubated under controlled conditions. At weekly intervals DNA was extracted from the amended soil and subjected to isopycnic centrifugation. After visualization, fractions were removed from the bottom to the top of the gradient and ^{15}N analysis of the DNA fractions, using mass spectrometry, showed that enrichment declined with increasing distance from the bottom sample. 16S rRNA PCR-denaturing gradient gel electrophoresis analysis of DNA fractions showed differences in banding between ^{14}N and ^{15}N labelled DNA fractions, with the community profile remaining stable between 7 and 28 days following rye grass incorporation into soil.

EM 62 The study of microfungal flora of air in Gheshm iseland – the largest Iseland in Khalige Fars

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The fungal spores of air in Gheshm iseland were isolate and identified by air traping technique and traditional plate culture. The isolates were identified whith macroscopy and microscopical morphotyping. A total of 615 colony were identified. 80% of fungal spores identified belongs to Dematiaceous fungi and others known as Hyalinehyphomycetes.

The frequency of fungal spores isolate were as follows:

Alternaria sp. 70.93%, Penicillium sp.8.45%, Cladosporium sp.6.9%, Aspergillus sp 3.43%, Drechslera sp. 1.2% and others which consists of : *Fusarium* sp., *Helminthosporium* sp., *Ulocladium* sp., *Stemphilium* sp.

This research showed a significant diferrence in Dimatiaceous fungal spore distribiution in warm climate such as Gheshm iseland with colder climates such as Hamedan and Isfahan and Tehran.

Posters

Food & Beverages Group

FdBev 01 The application of an internal positive control to a real-time 5' nuclease PCR assay for the rapid detection of *Listeria monocytogenes* DNA extracted directly from foods and clinical samples

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Food matrices and clinical samples contain a variety of components inhibitory to PCR. Although many inhibitors are removed during DNA extraction, the efficiency of removal cannot be guaranteed and often depends upon the extraction procedure used. The use of an internal positive control (IPC), a non-target DNA sequence that is co-amplified with target DNA, is a useful method for identifying PCR inhibition and thus indicating potentially false negative results.

A commercially available IPC assay was duplexed with existing 5'nuclease PCR (TaqMan) assays and applied to DNA isolated from bacterial cultures, foods and clinical specimens using a variety of extraction techniques.

PCR inhibition was detected using the IPC when assays were performed with high concentrations of DNA from bacterial cultures and in DNA extracted from some food samples. No inhibition was detected in assays performed on DNA extracts from serum or cerebral spinal fluid.

Using this universally applicable IPC system it is possible to detect amplification inhibition in 5'nuclease PCR (TaqMan) assays. The IPC is an important tool for improving the interpretation of PCR assays by identifying false negative results. The use of an IPC is an essential step in the standardisation of PCR techniques for food and clinical microbiology laboratories.

FdBev 02 DNA sequence conservation of pCC31, a large conjugative R plasmid in *Campylobacter coli*

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With the increasing incidence of *Campylobacter* derived illness, a need for more effective control measures to prevent infection has arisen. Plasmid carriage by *Campylobacter* and the expression of antibiotic resistance traits have previously been well documented (Bradbury and Munroe, 1985; Cabrita, *et al.*, 1992; Taylor, *et al.*, 1981). However, large (>40kb) plasmids remained genetically uncharacterised until a recent report on pVir (Bacon, *et al.*, 2000) highlighted the possibility of these plasmids affecting virulence. The recently sequenced 45kb conjugative R plasmid from *Campylobacter coli* strain CC31 has been investigated by mutational and phenotypic characterisation in order to gain a further insight into the function of *Campylobacter* plasmids.

The development of a *Campylobacter* plasmid gene microarray specific for individual open reading frames present in pVir and pCC31 was used to compare plasmids from several laboratory strains and clinical isolates and has highlighted the extent of the distribution of this promiscuous, tetracycline resistant plasmid. The breadth of pCC31 host range is presently unknown, but our findings indicate that plasmids play an important role in maintaining genome plasticity in *Campylobacter* species and may also enable horizontal transfer of virulence, antibiotic and conjugative genes between *Campylobacter* and other pathogens.

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FdBev 03 Identification of the mechanisms responsible for the initiation of growth in *Salmonella enterica* serovar typhimurium using DNA microarrays

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Lag phase is an important stage of bacterial growth with particular relevance for food spoilage and food-borne disease. The length of lag phase is a major factor in determining when food becomes unsafe to consume; unfortunately one of the current weaknesses of predictive microbiology is the prediction of lag time. The physiology of lag phase remains poorly understood; it was not known whether the initiation of bacterial growth would involve large-scale changes at the transcriptional level. This study aims to provide an insight into the physiology of growth initiation and should allow refinement of current predictive microbiology models. We have developed a protocol which allows isolation of RNA from lag phase cells in sufficient quantities for microarray analysis. We have identified some key genes and pathways which are activated during the earliest stages of growth. In particular, genes involved in phosphate transport and iron transport both showed characteristic induction patterns. This work provides a basis from which we will probe the mechanisms involved in the initiation of growth.

FdBev 04 Rhamnose fermentation capabilities of *Escherichia coli* O26 and increased isolation of *Escherichia coli* O26 from bovine faeces using Rhamnose MacConkey agar supplemented with cefixime and tellurite

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Earlier studies have shown that *Escherichia coli* serogroup O26 vary in the ability to ferment rhamnose and this appears to be correlated to the presence of Verocytotoxin genes. In our first study 249 *E. coli* O26 isolated from Scottish cattle between 2002 and 2004 were examined for their ability to ferment rhamnose. (Screened using rhamnose peptone water.) 115 of 122 VTEC strains were unable to ferment rhamnose after incubation at 37ºC for up to 4 days whereas 92 of 127 non-VTEC strains fermented rhamnose in that time.

In our second study, a longitudinal study of 41 Scottish beef calves, pre-enrichment and immunomagnetic separation of faeces were used to compare CT-RMac with Merck Chromocult TBX agar for the isolation of *E. coli* O26 from 767 rectal faeces samples. *E. coli* O26 was isolated from 162 samples using both CT-RMac and TBX agar, 148 from CT-RMac alone and 9 from TBX alone.

Our studies show that VT positive O26 are more likely to ferment rhamnose than VT negative strains and there is an increased isolation rate for *E. coli* O26 using CT-RMac compared to TBX agar.

FdBev 05 Functional analysis of *Bifidobacterium*: why does *Bifidobacterium bifidum* require *N*-acetylglucosamine for growth?

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Bifidobacterium bifidum, a prevalent member of the gastrointestinal microflora, is believed to play an essential role in the initiation of the immune system in neonates and the maintenance of a healthy gut microflora. In contrast to other bifidobacterial species, *B. bifidum* is unable to synthesize N-acetylglucosamine (NAG) from saccharide precursors despite the presence of saccharide transport pathways. However in the gut, mucin-hydrolysing enzymes of *B. bifidum* presumably provide N-acetylhexosamines required for growth. Bioinformatic analysis of the *B. longum* genome sequence revealed a putative *glmS* gene coding for an amidotransferase required for NAG biosynthesis and an essential enzyme in peptidoglycan biosynthesis. The *glmS* gene of *B. longum* was cloned and sequenced, confirming a coding potential for a 630 aa protein with 42% homology to the *E. coli* glucosamine-fructose-6-phosphate transaminase. This purified enzyme catalyses transfer of ammonium from glutamine to fructose-6-phosphate resulting in glutamate and glucosamine-6-phosphate. PCR analyses to compare the *glmS* genetic locus of *B. longum* and *B. bifidum* revealed a deletion in the 5' end of the *B. bifidum glmS*. This may account for the organisms requirement for NAG for growth. Further work is currently underway to analyse the transport mechanism for N-acetylglucosamine in *B. longum* and to further characterise the NAG auxotrophy in *B. bifidum*.

FdBev 06 Isolation and characterization of bacteriophages against *Escherichia coli* O157:H7 isolated from commercial feedlot stock pens across the Southern Plains States

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Escherichia coli O157:H7, one of today's leading public health concerns, is a food-borne pathogen that lives commensally in the guts of ruminants. Cattle are a major reservoir leading to human exposure; a survey published in 2001 estimated as many as 80% of feedlot cattle shed O157:H7 in the summer months. The focus of this research is to isolate and characterize bacteriophages that infect O157:H7, estimate their geographic distribution and develop a pre-harvest intervention strategy to control the entry of O157:H7 into the human food chain. Forty-nine phages active on *E. coli* O157:H7 were isolated from 120 samples of cattle feces collected from commercial feedlot pens across the southern plains states (USA). Screening for phages was done by enrichment on *E. coli* B and on a non-pathogenic *E. coli* O157:H7 strain NCTC 12900. The most efficient phages were single-plaque purified and characterized by pulse-field gel electrophoresis, host range, PCR, infection profile and electron microscopy. All are exclusively lytic, have a range of genome size, and distinct host ranges on the ECOR collection. Under aerobic conditions, most lyse 12900 cells in 30 min in rich media. Further experiments are being carried out to evaluate their effectiveness in cocktails and under anaerobic conditions.

FdBev 07 Detection of *Vibrio parahaemolyticus* in seafood

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Vibrio parahaemolyticus has been implicated in outbreaks of seafood-associated gastroenteritis in several countries. Seafood normally implicated include shellfish like oysters and clams, in addition, shrimps and crabs can act as carriers for *V. parahaemoltyicus*. A majority of the cases remain undiagnosed because of the self-limiting condition of the infection. However, mortality can result when the organism enters the blood stream.

V. parahaemoltyicus infections normally occur on as large-scale outbreaks and therefore a rapid method is required to trace the organism to the source of infection (usually seafood). Current rapid detection methods are based on PCR, which gives false positive results with closely related Vibrio spp. Also, PCR can often be inhibited by enzymes present in the food sample.

A novel method to the detection of this organism involves the use of phage antibodies. Previously (1), we have shown that phage display can be used successfully to differentiate between cultures of *V. parahaemolyticus* and non-parahaemolyticus spp. Four *V. parahaemolyticus*-specific phage antibody clones were isolated after analysis by ELISA, FACS, Western Blotting and CDR3 sequencing. To test if the clones retained their binding capacity in seafood, oyster (*Crassostrea gigas*) homogenate was seeded with *V. parahaemolyticus*. The clones were then tested for binding *V. parahaemolyticus in vitro* using ELISA and chemiluminescent assay. In addition, different pre-enrichment times and culture densities of *V. parahaemolyticus* were tested. It was found that phage clones still retained binding capacity in the presence of oyster tissue and a short enrichment period enhanced the binding of phage to *V. parahaemolyticus*.

FdBev 08 Isolation, characterisation and virulence of *Enterobacter sakazakii* from Korean infant foods

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Background *Enterobacter sakazakii* is a recognised contaminant of powdered infant foods that is associated with neonatal infections such as necrotising enterocolitis, bacteraemia and meningitis.

Methods Enterobacteriaceae were isolated from Korean infant food products. The phenotypes and 16S rDNA sequences of *E. sakazakii* isolates were compared to other food, clinical and environmental strains. Isolates were also assessed for attachment and invasion of CaCo2 human epithelial cells and the effects of bacterial extracts

on the reduction of methyl blue tetrazolium (MTT) by N2a murine neuroblastoma cells.

Results Enterobacteriaceae were detected in 44.4% of infant food products (n=36) after enrichment, with 11.1% samples containing *E. sakazakii*. All strains were identified as *E. sakazakii* by biochemical profiles. 16S rDNA sequence comparison indicated all isolates clustered with the type strain with 0.09–0.38% divergence. One isolate produced unusually large amounts of capsule when grown on Infant Formula Milk agar and showed a higher propensity for attachment to CaCo2 cells. Inhibition of MTT reduction by N2a cells indicated all isolates produced cytotoxins.

Conclusion *E. sakazakii* isolates from Korean infant food products were biochemically and genetically typical of this species. All strains produced cytotoxins and all were able to attach to and invade CaCo2 cells.

FdBev 09 Involvement of lipopolysaccharide in killing of *Salmonella* by egg albumen

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Transmission of *Salmonella* to egg albumen can occur during the passage of the egg through an infected oviduct. In this environment, *Salmonella* cells are exposed to antimicrobial proteins such as lysozyme at a temperature of around 41°C. Although *Salmonella enterica* serovars *Enteritidis* and *Typhimurium* can both infect hen reproductive tissues, usually only Enteritidis is found in the egg contents after lay. We examined the survival of these serovars and others associated with hen or egg infection in egg albumen at 41°C over 24h.

Strains of group D_1 *Salmonella*, including *Enteritidis*, survived in significantly greater numbers than group B strains, such as *Typhimurium*. The survival of mutants with altered lipopolysaccharide (LPS) structure was examined, as was the binding of lysozyme to LPS. Lysozyme was found to bind to O-chain subunits of LPS and was bound in higher amounts to group B compared to D_1 strains. Rough strains, with no O-chain, exhibited the lowest binding.

Albumen proteins are probable involved in the bacterial killing at avian body temperature. The fact that *S. typhimurium* is more sensitive to this process than *S. enteritidis* may explain why this serovar is only rarely found in the contents of freshly laid eggs.

FdBev 10 Genetic analysis of factors affecting acquisition of foreign DNA by *Staphylococcus aureus*

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S. aureus have mechanisms that inhibit their ability to acquire foreign DNA. This interferes with the transfer of antibiotic-resistance genes and makes them difficult to genetically manipulate in the laboratory; only one available strain can accept *E. coli* plasmids by electroporation. This strain, RN4220, also accepts *E. faecalis* conjugative plasmids at a higher frequency than its parent 8325-4. In an attempt to identify the mutation(s) in RN4220 responsible for these differences its restriction modification genes (*hsdM*, *hsdS*, *hsdR*) were sequenced. HsdM and HsdS are thought to form a complex that modifies DNA at short specific sequences. They can also form a complex with HsdR that digests the same sequences if unmodified, as they are in foreign DNA. Sequencing of RN4220 revealed a point mutation in the *hsdR* gene that introduces a stop codon, resulting in a truncated predicted product. A lack of functional HsdR would render cells unable to digest foreign DNA, providing a likely explanation for RN4220's phenotype. The intact *hsdR* gene was subsequently cloned and introduced into RN4220 in order to complement the mutation. Results of this study will further our understanding of horizontal gene transfer and allow the generation of molecular tools to facilitate the study of *S. aureus* genetics.

FdBev 11 Bacterial contamination of fruit and vegetables at point of sale

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A range of fruit and vegetables (some pre-packed) was purchased at point of sale from supermarkets, market stalls and an organic shop and tested for contamination with *Aeromonas, Campylobacter, Escherichia coli* (lettuce only), *Listeria* and *Salmonella*.

The following plant foods were tested: lettuce, spinach, broccoli, apples, potatoes, strawberries, bean sprouts, frozen peas, tomatoes, raspberries, spring onions, beetroot, cabbage, cucumber, cress and carrots. Triplicate samples (25g) were suspended in 225 ml 0.01% buffered peptone water, stirred electronically for 20 minutes, added to selective broths and agars and incubated at appropriate temperatures and atmospheres.

Natural (non-spiked) controls were set up using farm slurry, freshly made silage and bird faeces to check that the media and incubation conditions were capable of isolating the desired bacterium from environmental samples.

Isolates of the bacteria were also tested for their ability to survive on vegetables at room temperature and in the refrigerator.

Aeromonas was found on a wide range of vegetables and was able to survive on vegetable surfaces at room temperature for at least 9 days.

Campylobacter was not found in any fruit or vegetables but was present in the controls. Spiked samples survived for only 24h on lettuce, bean sprouts and spring onions at room temperature and for 48 h in the refrigerator.

Listeria was found in spring onions and the controls. It survived on spring onions for at least 5 days both at room temperature and in the fridge.

Salmonella was found in mushrooms and bean sprouts as well as in the controls. It survived for at least 10 days on lettuce, bean sprouts and spring onions at both room and refrigerator temperatures.

E. coli was present in low numbers in lettuces bought from several of the shops. It was entirely absent from pre-packed and pre-cut lettuce but was found in numbers well above the EU recommended guidelines in lettuce from an 'organic' shop.

FdBev 12 Role of phage shock proteins in *Salmonella typhimurium*

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Salmonella species can cause wide-ranging disease from mild food-poisoning (enteritis) to a systemic, sometimes fatal typhoid infection.

These bacteria have evolved to survive in different environments with-in the host and do so through the regulation of differential gene expression.

RpoE is an alternative sigma factor involved in the virulence and extracytoplasmic stress response of the *salmonella* species. We performed micro-array analysis to identify putative rpoE regulated genes.

The genes responsible for the phage shock proteins (PSP) constitute one such set of genes up-regulated when rpoE was over expressed in *Salmonella typhimurium*.

Using the RED mutagenesis system, two psp mutants were constructed. A psp mutant was constructed which lacked the entire PSP operon and a pspC mutant was constructed which lacking only the PSP C gene. Following mutagenesis, extensive phenotypic characterisation was performed on both mutants to allow comparisons to be made between the Wild type *Salmonella typhimurium* strains and also the rpoE mutant. The phenotypic data obtained will be presented alongside in-vivo data to illustrate the effect of the psp mutants on growth and virulence in *Salmonella typhimurium*.

FdBev 13 Effect of population density and growth phase on pulsed light inactivation of temperature stressed *Listeria monocytogenes*

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Pulsed UV-rich light is an emerging non-thermal treatment technology that is receiving attention for use in the inactivation of problematic micro-organisms. A pulsed light disinfection system has been developed, which employs the use of intense, short duration light pulses to kill microbial populations within very short timescales. However, little is known about the effects of pulsed light treatment on temperature-stressed cells grown to different population density at various stages of growth. This study compared the effects of different population densities (10^5, 10^6, 10^7 and 10^8 CFU/ml) and growth phases (middle log, late log and stationary phase) of *L. monocytogenes* (serotype 4b) grown under three different temperature conditions (20°C, 37°C and 45°C) when subjected to pulsed light treatment. *L. monocytogenes* was exposed to PUV light at 2, 4, 8, 12 and 16 pulses and the degree of motility was observed using hanging-drop microscopy and viability via CFU counting. The results show that different population densities and growth phases of *L. monocytogenes* demonstrate variations in their susceptibility to pulsed light inactivation.

MI 01 Impact of nitrate and anaerobic growth on suceptibility of *Pseudomonas aeruginosa* biofilms

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Pseudomonas aeruginosa pulmonary infection is the leading cause of morbidity and mortality in Cystic Fibrosis (CF) patients. The reduced oxygen concentration observed in sputa coupled with respiration of *P. aeruginosa* is believed to create anoxic zones within the CF lung. Microscopic and biochemical evidence also suggest that *P. aeruginosa* within the CF lung are growing as biofilms. Examination of the influence of nitrate and anaerobic growth on the susceptibility of biofilms of the laboratory strain NCIMB 10548 to tobramycin demonstrated a slight increase in the susceptibility of biofilms grown under anaerobic conditions or aerobically in the presence of nitrate. Susceptibility of three CF clinical *P. aeruginosa* isolates to tobramycin demonstrated strain dependent alterations in biofilm killing under aerobic and anaerobic conditions. However, tobramycin at concentrations up to 128mg/L was unable to eradicate biofilms of either clinical or laboratory *P. aeruginosa* strains under any of the conditions tested.

These results show that oxygen limitation alters, in a strain dependent manner, the susceptibility to tobramycin of *P. aeruginosa* grown as biofilms.

MI 02 *Burkholderia cepacia* complex organisms accumulate inorganic polyphosphate at the acidic pH of the cystic fibrosis lung

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Bacteria belonging to the *Burkholderia cepacia* complex (Bcc) are important opportunistic human pathogens in the cystic fibrosis (CF) lung. The airways in CF patients are chronically acidified and during exacerbations of pulmonary symptoms the pH of airway condensate has been reported to be reduced to 5.4. Environmental Bcc has shown enhanced uptake of inorganic phosphate and its intracellular accumulation as polyphosphate (polyP) under reduced pH conditions. Polyp has been shown to have a regulatory role in the physiological adaptation of some microbial cells during alterations in their environment and growth phase.

In this study clinical Bcc isolate FMB15 was examined for alterations in phosphate metabolism under increasingly acidic conditions to determine if the acidic environment of the CF lung alters the accumulation of polyP. The formation of polyphosphate by Bcc FMB15 was tested at pH 7.5 and at pH 5.5. PolyP was present at 2 µmol phosphate/mg protein at pH 5.5 and at 0.5 µmol phosphate/mg protein at pH 7.5.

Clinical isolates of Bcc show enhanced intracellular accumulation of polyP in response to an acid environment. This may have some significance in relation to the pathogenesis of Bcc in the acidic environment of the CF lung during an acute exacerbation.

MI 03 The absence of the plasmid encoding *finB* invertase does not affect antigenic variation

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The genome of *Bacteroides fragilis* contains a number of invertible promoter regions that control, for example, polysaccharide biosynthesis and are related to antigenic variation. The inversion of these DNA regions is thought to be mediated by a mechanism similar to the Hin-system, which controls the expression of two antigenically different flagella in *Salmonella*. The DNA inversion is mediated via the association of the Hin invertase enzyme, with 30–32bp regions of dyad symmetry that bound the invertible regions of DNA. The *B. fragilis* NCTC9343 genome sequencing project has revealed two Salmonella *hin* homologues, *finA*, located on the chromosome, and *finB*, located on a 36.5kb plasmid. The aim of the current study was to determine whether the absence of *finB* impairs antigenic variation. The results indicate that in the absence of the *finB* gene, isolates are still capable of antigenic variation. When using monoclonal antibodies raised against *B. fragilis* NCTC9343 surface antigens, one isolate, *B. fragilis* VH, did not express any of the variable polysaccharides probed for.

MI 04 Efficacy of fluoroquinolones against experimental *Francisella tularensis* systemic infection in mice

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Human tularemia infection occurs throughout the Northern hemisphere, most frequently in Scandinavia, North America, Japan and Russia, however there have been reports suggesting that it may be even more widely distributed stretching as far as Europe. Traditionally tularemia has been treated using streptomycin, however quinolones such as ciprofloxacin have shown promising results in both experimental and clinical infection.

This study assessed the *in vivo* efficacy of two new generation fluoroquinolones gatifloxacin and moxifloxacin alongside ciprofloxacin in an experimental *Francisella tularensis* Schu S4 infection in Balb/c mice.

Following a sub-cutaneous challenge with $1x10^6$ cfu/ml *F. tularensis* Schu S4, mice were given antibiotic by oral administration twice daily continuing for 14 days. Antibiotic treatment was initiated at 6, 24 and 48 hours post exposure.

Gatifloxacin, moxifloxacin and ciprofloxacin were most effective when given 6 hours post-exposure. When treatment was delayed to 24 and 48 hours, survival rates decreased. However, both the gatifloxacin and moxifloxacin treated groups gave significantly higher survival rates compared to the ciprofloxacin treated group.

This study shows both gatifloxacin and moxifloxacin show promise for the treatment of experimental tularemia and they could be considered as alternatives to traditional treatments.

MI 05 Intracellular localization and modification of the pseudomonal type III secreted toxin ExoU

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ExoU is a toxin secreted by the type III secretion system of *Pseudomonas aeruginosa* into eukaryotic cells. ExoU acts as a potent phospholipase that contributes to increased bacterial virulence. In common with other type III secreted Pseudomonal toxins, ExoU requires an unknown eukaryotic cofactor for activity. The aim of this study was to characterise the intracellular target of ExoU, and determine how the eukaryotic cell activates the toxin. Immuno-fluorescence and western blot analysis of fractionated cells revealed that ExoU is localised at the plasma membrane after transfection and infection. The region required for this localisation is within the extreme C-terminal residues. We found that ExoU is modified to a higher molecular weight form following transfection and infection, which was exclusively found in membrane fractions and required the same extreme C-terminal residues needed for membrane localization. Full toxicity of ExoU also required a similar region of the molecule. We conclude that the C-terminus of ExoU is required for membrane localization and modification to a higher molecular weight modified form, which seems to be necessary for toxicity.

MI 06 The effect of antimicrobials on clinically important multi-drug resistant bacteria

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The indiscriminate use of antibiotics in recent years has led to the emergence of multi-drug resistant microbes, and an alarming rise in hospital-acquired infections. Methicillin resistant *Staphylococcus aureus* (MRSA) causes invasive infections in hospitals, particularly among immunocompromised individuals and those with open wounds. *Pseudomonas aeruginosa* has developed multi-drug resistance and is a major cause of serious infection and lung damage in Cystic Fibrosis sufferers. MRSA and *P. aeruginosa* have been isolated from disinfected locations in hospitals such as catheters, and disinfectant soap dispensers. There is concern that, as for antibiotics, intensive exposure of hospital pathogens to residual amounts of disinfectants may result in resistance to these agents. The minimum bactericidal concentration (MBC) of a range of biocides commonly used in hospitals, including chlorhexidine, triclosan and benzalkonium chloride, was determined for a number of strains of MRSA and *P. aeruginosa*. The effect of continuous exposure of the bacteria to biocides was established, and the molecular mechanisms of resistance to antimicrobials were investigated using PCR. This study produced clinically relevant information on the effect of biocides on multi-drug resistant bacteria, which is vital for disinfection practices within the hospital environment.

MI 07 Investigation of the uncharacterised collagen-like repeat protein, PclA from *Streptococcus pneumoniae*

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The Gram-positive bacterium, *Streptococcus pneumoniae* (the pneumococcus) is an important cause of death and disease worldwide. Two pneumococcal strains, R6 and TIGR4, have been fully sequenced and annotated and have provided an invaluable resource to investigate the pathogenesis of pneumococcal disease. Examination of these genome sequences, revealed a predicted open reading frame, *spr1403*, encoding a large protein (265 kD) in the genome of R6 that is absent TIGR4. A striking feature of this protein is a large number of GXY repeats characteristic of collagen. This is also a feature of the *Streptococcus pyogenes* proteins, streptococcal collagen-like proteins A and B (SclA and B) and we have therefore named Spr1403 PclA for pneumococcal collagen-like protein A. We have investigated this protein with regards; strain distribution, transcriptional regulation, its role in virulence, colonisation and adherence.

MI 08 Effects of adenylate cyclase toxin of *Bordetella pertussis* on different mammalian cells

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The adenylate cyclase toxin (CyaA) secreted by *Bordetella pertussis* is able to enter eukaryotic cells and cause a dramatic increase in cAMP levels by its adenylate cyclase enzymatic activity (AC) which is stimulated by host calmodulin. In addition, the CyaA toxin exhibits an intrinsic haemolytic activity that is independent of AC activity. In this work, native CyaA and enzymatically inactive CyaA*, leaking AC activity were expressed as recombinant proteins from *E. coli* and purified by Q Sepharose followed by butyl Sepharose column chromatography. The purified preparations had very low LPS levels, 126 & 67 IU/mg respectively. The cytotoxicity and the capacity to induce apoptosis of these CyaA preparations were compared on different mammalian cells: J774.2 and U937 macrophage cell lines, Vero cells, rat basophilic leukemia cells, and sheep bone marrow mast cells. It was shown for example that the CyaA induced apoptosis in J774.2 macrophages at 0.078µg/ml whereas CyaA* was unable to do this. The end point for the cytotoxic effect (50% killing) of CyaA and CyaA* on J774.2 were of 0.025 and 2.5 µg/ml, respectively. In contrast, the cytotoxicity effect of these two toxins were similar on Vero cells at >2.5µg/ml. The ability of native CyaA to inhibit the repiratory burst and phagocytic activities of J774.2 and U937 macrophage cell lines was also investigated.

MI 09 Adjuvant and protective properties of *Bordetella pertussis* adenylate cyclase toxin

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The adenylate cyclase toxin (CyaA) of *Bordetella pertussis* is an important virulence factor and belongs to the pore-forming Repeats in ToXin (RTX) family. CyaA is unique among these toxins in that it has an adenylate cyclase (AC) enzymic moiety. Recombinant detoxified CyaA*, lacking AC enzymic activity, was produced in *E. coli* and purified extensively. Endotoxin levels in the final preparations were <1EU/µg protein. Mice were immunised twice at a 4 week interval with 25 µg of CyaA* plus a sub-protective dose of a commercially-available acellular pertussis vaccine (ACV). Two weeks after the final immunisation, mice were challenged intra-nasally with *B. pertussis*. A significant reduction in bacterial numbers in the lungs at 7 days post-challenge was seen in mice that had received CyaA* plus ACV compared to control groups. The immunomodulatory role of CyaA* was further investigated by immunoglobulin and cytokine analysis. The availability of different forms of recombinant CyaA means that it will be possible to investigate the role of the AC moiety and other CyaA domains in adjuvanticity and protection. The findings from this study may contribute to the further development of acellular pertussis vaccines.

MI 10 Identification of nucleotide sequences specific to cystic fibrosis epidemic strains of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is the most common pathogen associated with morbidity and mortality in cystic fibrosis (CF) patients. In 1996 we reported the spread of an epidemic strain of *P. aeruginosa* (Liverpool epidemic strain ([LES]) among CF patients in Liverpool. A recent survey to determine the distribution of *P. aeruginosa* genotypes in England and Wales found LES to be the most prevalent genotype and identified other transmissible strains, including a Midlands epidemic strain (Mid1), which was the second most common genotype found. We previously used suppression subtractive hybridisation (SSH) to identify a small number of DNA sequences present in the LES but absent from a reference strain (PA01). One sequence (PS21) is currently targeted in a diagnostic PCR test for the LES. Using SSH, a substantial database of subtracted sequences has now been obtained for the LES (>100) and Mid1 (>50) strains. PCR amplification has been used to screen a panel of *P. aeruginosa* CF isolates to determine the prevalence of a number of these sequences, with a view to identifying genes that may contribute to their transmissibility, and to facilitate the development of a multiplex-PCR test for the two most prevalent CF epidemic strains in the UK.

This work was supported by the UK Cystic Fibrosis Trust.

MI 11 The nematode *Caenorhabditis elegans* as a model for *Burkholderia cepacia* complex infection

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The *Burkholderia cepacia* complex (BCC) is recognised as an important opportunistic pathogen in patients with cystic fibrosis (CF) and is associated with increased mortality. The BCC comprises a family of at least ten different species. Marked variability is observed in the clinical outcome among the different species of the complex, with *B. cenocepacia* and *B. multivorans* widely considered to be the most prevalent and virulent in CF patients. Recently non-mammalian hosts have been used as models to study human bacterial pathogens and host-pathogen interactions. The soil-borne nematode *Caenorhabditis elegans* has emerged as versatile alternative model to study BCC infection. We have utilised both fast-killing and slow-killing solid media-based *C. elegans* assays, and a liquid based feeding inhibition assay, to demonstrate variation in the pathogenicity of the different species comprising BCC. By comparing wild-type strains with specific knockout mutants, we have also shown that type III secretion genes play a role in virulence in the *C. elegans* model. Furthermore, mutation of a gene involved in the production of dinucleoside polyphosphate hydrolase was found to have an adverse effect on nematode killing.

MI 12 Effects of human serum on virulence properties of *Acanthamoeba* (encephalitis isolate)

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Acanthamoeba are the causative agents of fatal Acanthamoebic granulomatous encephalitis (AGE). AGE is limited to immunocompromised patients suggesting that host natural immune response is sufficient to control and/or eradicate these pathogens, but the molecular mechanisms remain unclear. In this study we determined the effects of serum from healthy individuals on the virulence properties of *Acanthamoeba castellanii* (isolated from an AGE patient).

We determined that serum exhibited amoebastatic activity over 72 h, however the viability of *Acanthamoeba* remained unaffected. Adhesion assays revealed that serum inhibited >50% binding to human brain microvascular endothelial cells (HBMEC). In addition, *Acanthamoeba* mediated HBMEC cytotoxicity was significantly inhibited in the presence of serum (>40%). Zymography assays revealed that, in the presence of serum, *Acanthamoeba* exhibited decreased extracellular protease activities. Interestingly, serum enhanced the phagocytic activity of *Acanthamoeba*, as measured by bacterial uptake.

In conclusion, we have shown that human serum has inhibitory effects on *Acanthamoeba* growth, protease secretion, binding to and subsequent cytotoxicity of HBMEC. Conversely, *Acanthamoeba* phagocytosis was stimulated by serum.

MI 13 *Acanthamoeba cysts*: characteristics and functional properties

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Acanthamoeba is an opportunistic protozoan that is widely distributed in the environment and can cause human infections. The life cycle of *Acanthamoeba* consists of a vegetative infective, trophozoites form. However, under harsh environmental conditions trophozoites differentiate into a double-walled, metabolically inactive and resistant cyst form. Research in *Acanthamoeba* has been mostly focussed on the infective trophozoite form and its pathogenic mechanisms. In this study, we determined the properties of *Acanthamoeba* cysts belonging to different genotypes. We determined that food deprivation stimulate encystment in *Acanthamoeba* isolates belonging to T1, T2, T3, T4 and T7 genotypes. In addition, increase in osmolarity triggered encystment in T2, T3, T4 isolates but T1 and T7 failed to encyst. Adhesion assays revealed that *Acanthamoeba* cysts belonging to T1, T2, T3, T4, and T7 genotypes exhibited no and/or minimal binding (<5%) to the host cells. Fluorescein-labelled lectins showed that all *Acanthamoeba* isolates tested, exhibited binding to concanavalin A, indicating the expression of mannosyl- and/or glucosyl-residues. Future studies will make use of osmolarity to understand the molecular mechanisms of *Acanthamoeba* encystment.

MI 14 Molecular strain typing of *Mycobacterium tuberculosis* isolates from two cases of suspected cross contamination

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Here we report two examples of molecular strain typing being used to investigate potential cross contamination by *M. tuberculosis*. In the first, four patients each had *M. tuberculosis* isolated from bronchaveolar lavage specimens collected by a bronchoscope which was subsequently found to be faulty. MIRU typing was used to investigate the possibility of transmission of *M. tuberculosis* between the patients or cross-contamination of the specimens. MIRU patterns for all patients involved were different, indicating that the four

patient isolates were different strains and not the result of cross contamination. The second example involved a clinician with smear positive pulmonary tuberculosis. A specimen of cerebral spinal fluid that was collected under the supervision of this clinician was culture positive for *M. tuberculosis* although the diagnosis of tuberculosis meningitis was thought to be unlikely and was later ruled out. A second clinician presented with pulmonary tuberculosis, who had worked at our hospital briefly and was an associate of the first clinician. The MIRU patterns generated for the isolates from the two clinicians and also from the CSF specimen were distinct, indicating that contamination at sampling had not occurred.

MIRU typing provided a timely response to these incidents; had the current gold-standard method of IS6110 RFLP been used, these results would have taken a matter of weeks rather than the one week turnaround for MIRU. Currently there is no extensive database of MIRU typing as there is for IS6110, so the wider applications of the latter method have obvious benefits. However, in small suspected outbreaks, such as the ones described, MIRU typing is invaluable in providing a definitive, rapid result.

MI 15 Differential gene and protein expression in *Streptococcus uberis* biofilm cells

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Despite previous and on-going attempts to control bovine intra-mammary infections, *Streptococcus uberis* remains one of the major causes of mastitis in UK dairy herds. Failure to control infection by *S. uberis* is largely due to a lack of knowledge regarding the mechanisms by which it causes disease. A better understanding of these mechanisms will ultimately aid in the control of *S. uberis* infection.

In vivo, the dominant mode of growth for the majority of micro-organisms is as biofilms. Gene expression and growth rates of biofilm cells differ markedly to that of planktonic cells. Biofilms also show increased resistance to both antimicrobial agents and the hosts' immune response.

Previous studies on *S. uberis* pathogenicity have focussed on planktonic cells and thus may not accurately reflect the true situation *in vivo*.

We are currently assessing the ability of *S. uberis* and defined mutants for the ability to form biofilms and investigating differential gene and protein expression of wild type and mutant.

MI 16 Genotype changes in invasive *Haemophilus influenzae* type b in the UK between the pre-vaccine and post-vaccine populations

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Haemophilus influenzae type b (Hib) invasive disease in UK children nearly disappeared following implementation of Hib vaccine in 1993. Between 1999 and 2003 the incidence of Hib disease in vaccinated children increased. This was associated with implementation of an acellular pertussis combination Hib vaccine with lower immunogenicity. A Hib specific bacterial property was an alternative or additional factor that may have contributed to this rise in incidence. To investigate this possibility a change in the population structure between the pre-vaccine and post-vaccine populations was studied. 138 isolates from the pre-vaccine era and 138 isolates from 2002 and 2003 were characterised by MLST. 20 MLST types were observed in the pre-vaccine population, of which 88 (64%) and 20 (15%) were ST6 and ST44 respectively. 17 MLST types were observed in the post-vaccine population, of which 120 (87%) were ST6 and no other ST occurred frequently. These populations were significantly different: $\chi^2_{12} = 31.9$; $p = 0.001$. This change in the population of sequence types between the pre- and post-vaccine eras and the significant increase of ST 6, raises the possibility that a Hib variant contributed to the rise in Hib disease among vaccinated children.

MI 17 A hyperinvasive *Haemophilus influenzae* type b genotype in The Gambia

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Haemophilus influenzae type b (Hib) caused severe disease, mainly meningitis in children before the implementation of Hib conjugate vaccine. The major virulence factor is the type b capsule. The extent to which other virulence factors contribute to the invasive potential of Hib is not known. The aim of this study was to compare invasive and carried populations of Hib for differences in population structure as determine by multi-locus sequence typing (MLST). 124 invasive Hib and 191 carried Hib isolated from children in the Gambia were analysed using MLST. 36 MLST were found, of which 18 occurred among the invasive isolates and 27 occurred among carried isolates. One sequence type, ST53 occurred more frequently among invasive (30% of isolates) than among carried isolates (16% of isolates). The invasive ratio of this sequence type was 2.3 (95% CI: 1.3 – 3.9; $p = 0.003$). All other sequence types occurred among carried and invasive isolates in similar proportions. This result indicates that ST53 is associated with a greater invasive potential when compared to other Hib causing invasive disease in The Gambia. This variation in the invasive potential of Hib is consistent with factors other than just capsule contributing to the virulence.

MI 18 Development of a reproducible model of ovine caseous lymphadenitis, and a diagnostic assay to monitor disease status

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Caseous lymphadenitis (CLA) is a chronic, suppurative disease, primarily of sheep and goats, caused by the organism *Corynebacterium pseudotuberculosis*. Prior to 1990, CLA was absent from the UK; however, following the importation of infected goats the incidence of disease has increased steadily. Very little is known about the pathogenesis of CLA in this country, especially with respect to the virulence mechanisms of *C. pseudotuberculosis* and the host response to infection. Despite the availability of CLA vaccines in other countries, there is currently no licensed vaccine or serodiagnostic test available in the UK. We have developed a reproducible ovine model of infection to allow the study of the UK form of CLA. Significantly, this can be achieved using an inoculum of as few as 10^2 organisms. An ELISA, based on a purified, recombinant form of phospholipase D (an important virulence factor), has been used to monitor serum antibody responses in experimentally infected and vaccinated sheep. It has also been used to successfully eradicate disease from an infected commercial sheep flock. Currently, the test is under development to be offered on a commercial basis to the sheep-farming industry.

MI 19 Analysis of translocated *Escherichia coli* isolates from patients with Crohn's Disease and bowel cancer

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Many cases of sepsis following gastrointestinal surgery are assumed to result from enteric bacteria translocating across the intestinal barrier, then causing infection at extraintestinal sites. It is unclear if it is bacterial or host attributes that facilitate translocation. Crohn's disease (CD) and bowel cancer patients have been reported to carry unusual adherent-invasive *E. coli* (AIEC). This study aimed to determine if it is AIEC that translocate in these patients.

Faeces was obtained from CD or bowel cancer patients at the time of surgery. During surgery mesenteric lymph nodes (MLN) were harvested and sent for bacterial culture. Nine cases (4 CD, 5 cancer) were identified where MLN yielded *E. coli* isolates, these were then compared to isolates from the patients' own faeces. ERIC-PCR typing demonstrated translocated isolates matched strains predominating in the faeces in all cases. DNA hybridisation showed the translocated isolates did not belong to common pathotypes of *E. coli,* apart from 1 DAEC strain. 8/9 (89%) adhered in a HEp-2 adherence assay, but only one with a pattern similar to an AIEC control. Two of the 9 isolates invaded HEp-2 cells.

Conclusion Translocating strains originated from the commensal gut flora of the patients but the majority (78%) did not have invasive properties as seen with AIEC, to explain their passage to the MLNs. This suggests host intestinal barrier function may be more important in determining when translocation occurs.

MI 20 Functional studies of a eukaryotic-like tyrosine protein kinase expressed by the Shiga toxin-encoding prophage 933W

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Shiga toxin(Stx)-producing *Escherichia coli* (STEC) are human-specific enteric pathogens. The Stx-encoding prophage 933W, isolated from the STEC clinical strain EDL933, encodes a functional tyrosine protein kinase, Stk, that on the amino acid level is similar to eukaryotic protein kinases. We previously found that *stk* is conserved in the genomes of nine of thirty clinically-isolated STEC strains, suggesting that there is selective pressure for this gene to be maintained. However, a biological role for Stk has not as yet been determined.

The biochemical activity of Stk suggests that, like other bacterial encoded effector proteins that contribute to virulence, Stk may function to modulate the phosphorylation of eukaryotic cell proteins. To assess this idea we have studied the effect of Stk in *Sacchromyces cerevisiae*. Overexpression of Stk in *S. cerevisiae* is toxic, whereas, overexpression of a mutant version of Stk, functionally inactive because it lacks a key residue in the catalytic domain, is not toxic. This Stk-induced lethality is specifically suppressed by a multi-copy plasmid that includes a ~6.8 kilobase sequence from yeast chromosome XII. Studies are underway to identify the ORF that, when overexpressed, specifically suppresses the Stk-induced lethality in yeast.

Alternatively, Stk could phosphorylate a bacterial protein(s), modulating its activity. To this end, we are comparing the phosphorylated protein profiles of EDL933 lysogens carrying either the wild-type 933W prophage or one deleted for *stk*.

MI 21 Enhancement of immune response against *Orientia tsutsugamushi* in C3H/HeN mice by synthetic oligodeoxynucleotides (ODNs) encoding CpG motif as an adjuvant

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Background and objective of investigation In this study, CpG ODNs were evaluated for their immunostimulatory activity on mice vaccinated with recombinant outer membrane protein P56 (rP56Δ) of *Orientia tsutsugamushi*.

Methods Three doses of rP56Δ plus Freund's Adjuvant or rP56Δ plus CpG ODNs Ag were used to immunize on 6- to 8-wk-old female C3H/HeN mice. ELISA was used to measure general antibodies responses; Isotype-specific Ig ELISA assays were used for the measurement of antibodies subtypes from mice sera. Cytokine ELISAs was used to measure levels of IL-4, IL-12, and IFN-γ in culture supernatants.

Results CpG ODNs could improve the immunization efficiency of rP56Δ by shortening the immunization schedule. Both rP56Δ plus Freund's Adjuvant and rP56Δ plus CpG ODNs enhanced humoral immunity. Among these, IgG1, IgG2a, IgG2b and κ light chain were induced significantly. Further, rP56Δ stimulated spleen cells of vaccinated BALB/c mice to produce more IL-12 and gamma interferon than IL-4.

Conclusion Recombinant protein rP56Δ could stimulate C3H/HeN mice to produce immune response; and CpG ODNs adjuvant enhanced its immunization effect and shortened the immunization time.

Keywords *Orientia tsutsugamushi*, CpG Motif, recombinant outer membrane protein, adjuvant, immunization

MI 22 Control of coccidiosis in poultry

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An egg adapted gametocytes, *E. tenella,* vaccine(s) were used against coccidiosis in chickens. On day five chicks were divided into four groups and administered vaccine(s) orally viz; Vaccine I (gametocytes), Vaccine II (gametocytes inactivated), Vaccine III (gametocytes sonicated inactivated) and fourth group was served as control given normal saline. On day fifteen immunized chicks were challenged with 60,000–70,000 sporulated oocysts of mixed species of Eimeria. A total of one hundred and twenty six birds were used in this study out of them ninety four birds were scarified and thirty two chicks died during challenge response. On day 21 post vaccination birds were subjected to postmortem and their lesions score were recorded. A maximum of 46 birds having lesions in intestine and caeca of Group-IV birds were observed while a minimum of 17 birds having lesions in intestine and caeca were observed in Group-III. There was non-significant difference ($P>0.05$) in lesions score of Group-I, II and IV. Lesions scores in Group-III were significantly different ($P>0.05$) from Group-I, II and IV.

Posters

Physiology, Biochemistry & Molecular Genetics Group

PBMG 01 Mutational analysis of the *attB* locus in the phage ϕC31 site-specific recombination system

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The *Streptomyces* temperate bacteriophage, ϕC31, encodes an integrase required for site-specific integration and excision of its genome with the host chromosome. ϕC31 integrase is a member of the serine recombinase family of site-specific recombinases and its mechanism is poorly understood.*In vitro* ϕC31 integrase has been shown to catalyse only the integration reaction between the phage and host attachment sites, *attP* and *attB,* generating the recombinant products, *attL* and *attR*. Recombination between *attL* and *attR* or other combinations of sites i.e. *attB*x*attB*, *attP*x*attL* etc have never been observed *in vitro* and it has recently been shown that the block in recombination between these 'non-permissive' sites is a failure to form stable synapses. We believe that certain regions/bases must be present on *attB* and *attP* that allow the integrase to recognise them, and once bound form a specific synapse. To investigate this we carried out a mutational dissection of the minimal *attB* site. Certain base pairs in *attB* appear to be essential for efficient recombination. As these sites still bind integrase the block in recombination must be lie in the events that occur downstream of DNA binding, such as activation of DNA cleavage or ability to form a synapse.

PBMG 02 The phage growth limitation system in *Streptomyces coelicolor* A3(2)

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The phage growth limitation (Pgl) system of *Streptomyces coelicolor* A3(2), confers protection against the phage, ϕC31, and its homoimmune relatives. The Pgl phenotype is characterised by the ability of Pgl^+ hosts to support a phage burst on initial infection but subsequent cycles are severely attenuated. It has been proposed that phage are modified during the first burst, and modified phage are restricted in the second and subsequent infectious cycles. Four genes are required for a functional Pgl system, a plasmid encoding *pglWXYZ* is able to confer the Pgl^+ phenotype to *S. lividans* implying that these four genes constitute the whole system. Expression studies indicate that all these genes are expressed even in the absence of phage infection implying that regulation may be post-translational.

The pgl system consists of; *pglX,* encodes a putative DNA adenine methyltransferase, it also has a closely related paralogue, *pglS,* which when overexpressed in a Pgl^+ strain confers partial sensitivity to ϕC31 i.e. it interferes with the Pgl system; *pglY,* encodes a possible ATPase; PglZ shows no homology to known proteins; and we propose that PglW, a putative serine-threonine protein kinase is a regulator of the system. We are currently investigating the conserved motifs within the Pgl proteins, with a view to establishing the mechanism of phage resistance in *S. coelicolor*. BLAST searches with the Pgl proteins against the protein databases have indicated the presence of homologues in a wide range of bacteria.

PBMG 03 Mutational analysis of ϕC31 integrase

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The *Streptomyces* temperate bacteriophage ϕC31 encodes an integrase required for site-specific recombination with the host chromosome. The ϕC31 integrase is a member of the serine recombinase family and has, at its N-terminus, a resolvase/invertase-like catalytic domain. The structure-function relationships of the extended C-terminal region of ϕC31 integrase are largely uncharacterised and therefore the mechanism of recombination by this recombinase is poorly understood.

Control of integration versus excision is essential for efficient progression through the lytic and lysogenic life cycles. The observation that integrase can only recombine *attB* and *attP* (i.e. integration) *in vitro* to form *attL* and *attR* (the substrates for excision) indicates that the recombinase itself is highly regulated. Excision does not occur *in vitro* because integrase fails to form a synapse with *attL* and *attR*. This project aims to test the hypothesis that a putative synaptic interface is formed between ϕC31 subunits bound to *attP* and *attB* and whether we can obtain gain of function mutants of integrase that recombine *attL* and *attR in vitro*. We have isolated recombination defective mutants. Two of the mutants have substitutions in positions equivalent to γδ/Tn3 residues that are known to take part in synapsis. Other mutants lie in the uncharacterised C-terminal region of integrase.

PBMG 04 The effect of cyanophage S-PM2 infection on phycoerythrin gene expression, phycoerythrin synthesis and breakdown in *Synechococcus* sp. WH7803

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Marine members of the genus *Synechococcus* are photosynthetic cyanobacteria, which are of great ecological significance. Unlike higher plants, light harvesting for photosynthesis depends on a specific structure, the phycobilisome, composed mainly of phycobiliproteins together with non-pigmented linker polypeptides. Due to the abundance of marine cyanophages these cyanobacteria are often subject to infection. The phage S-PM2 has been extensively characterized in this laboratory. Spectrophotometry has revealed that phycoerythrin, the dominant phycobiliprotein in *Synechococcus*, is progressively degraded during the process of S-PM2 infection, presumably to serve as a source of amino acids for the synthesis of virion proteins. We have identified an S-PM2 gene encoding a homologue of the cyanobacterial CpeT protein which may have a role in regulating the expression of the phycoerythrin genes. Using quantitative real-time PCR, we found that phage *cpeT* gene expression was greatest at the early stage of infection. We are currently investigating how phage S-PM2 controls the synthesis of phycoerythrin and the degradation of the phycobilisome.

PBMG 05 The complete DNA sequence of *Lactococcus lactis* bacteriophage Tϕ712

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The bacteriophages of lactic acid bacteria have long been the subject of major research efforts, because of the financial impact of fermentation failures caused by phages phage attack. The temperate bacteriophage Tϕ712 was originally isolated from *Lactococcus lactis* NCDO712. It integrates into the chromosome of *L. lactis* NCDO712 and its derivatives in a site specific manner. The removal and reintegration of Tϕ712 was the original demonstration of classical lysogeny in *Lactococcus*. A special feature of Tϕ712 is its ability to promote transduction of plasmids that replicate via a rolling circle mechanism, giving it scope to be used as a tool for the genetic manipulation of otherwise inaccessible lactococcal strains. We have sequenced the complete genome of Tϕ712. It has a size of 42,073 bp with a GC content of 35.22%, which is similar to its host *L. lactis*. Comparative genome analysis of Tϕ712 with genome sequences of lactococcal and streptococcal phages revealed that Tϕ712 shows sequence identity ranging from 17.6–9.9% with other lactoccocal phages from the P335 species. The genome shows the typical modular structure of phages from the λ super group of *Siphoviridae*. Upon bioinformatic analysis 58 open reading frames representing 90.6% of the total genome sequence could be identified.

PBMG 06 Recombination of lambda phage genomes: implications for bacteriophage-mediated DNA vaccination

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Whole bacteriophage lambda particles have been demonstrated to be an efficient DNA vaccine vector and offer several advantages over traditional plasmid-mediated DNA vaccination. Eukaryotic expression cassettes containing a vaccine gene were excised from expression plasmids and cloned into the lambda gt11 (λgt11) genome. λgt11 was chosen as the vaccine vector because it is well characterised and commercially available.

The stability of phage vaccines grown over several generations was assessed by DNA restriction endonuclease profiling. Genomic deletions, mapping to the right end of the cloned inserts and the right arm of λgt11, accumulated over several passages and ultimately replaced the parental genotype population with the deleted genotype.

Comparison of DNA sequences obtained from the parental and deleted genotypes suggest that deletion was the result of homologous recombination between plasmid-derived *lac*Z sequences found flanking the expression cassette, and the λgt11 *lac*Z gene, which flanks the cloning site.

Evidence suggests that if a DNA sequence containing inverted and/or direct repeats is cloned into a phage genome, it is likely to be subject to genomic recombination and possibly deletion. This has important implications for vaccine design, particularly when constructing phage vaccines containing multiple copies of the same gene or several different vaccine genes.

PBMG 07 Expression of *katG* and *katE* and catalase activity of *Escherichia coli* in batch and continuous cultures

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It has previously been demonstrated that *Escherichia coli* can be detected in the range $1x10^2$ to $1x10^8$ cfu/ml, using cell capture by lateral flow immuno-assay, combined with electrochemical detection of the decomposition of hydrogen peroxide (H_2O_2) by bacterial catalase activity. This method may be applicable for the detection of bacteria directly from environmental, clinical or industrial samples.

The genes for *E. coli* catalases have been shown to be induced in the presence of a number of compounds, indicating that total catalase activity may be altered during the assay, leading to inaccuracy in determining cell numbers. We have further examined the induction of catalase gene expression using strains of *E.coli* DH5α transformed with plasmids containing the *lux* operon of *Photorhabdus luminescens* downstream of *katG* or *katE* promoters, and simultaneously measured total catalase activity using the electrochemical method.

Cells were challenged with inducers of *katG* and *katE* in log phase and stationary phase batch cultures, and in continuous cultures at different dilution rates. The results show differences in gene expression between the growth methods, phases and rates, and the effect of changes in *katG* and *katE* expression on total catalase activity of *E. coli*.

PBMG 08 Integrases of Stx phages

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The key virulence factor in STEC is the expression of Shiga toxin encoded in the late gene region of temperate lambdoid phages. However the use of differentially labelled recombinant Stx phage ($\phi24_B$) demonstrated the production of multiple lysogens. We have identified the $\phi24_B$ integrase gene and discovered at least a portion of a homologous integrase gene in the *E. coli* K-12 strain MC1061, the presence of which may explain the integration of the second phage. Deletion of this gene and determination of its affect on the ability to form double lysogens will be described. The integrase encoded by $\phi24_B$ is different from all other previously described Stx phage integrases, though it has been found in other lambdoid phages. There are examples of Stx phages for which an integrase gene has not been identified, but only genes identical to those found in other Stx phage were sought. We are using a bank of 11 primer sets to identify integrase types from Stx phage isolates with the aim of examining the diversity from these populations. The presence of different integrases within a population of Stx phages would enable multiple different integration events to occur in a susceptible host bacterium, leading to the carriage of more than one prophage.

PBMG 09 Characterisation of the right operator region in an Stx Bacteriophage, $\Phi24_B$

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Shiga toxin producing *E. coli* (STEC) are a major public health concern. *E. coli* 0157:H7, a notorious member of this group requires a very low infective dose of organisms to cause a potentially life threatening disease. Shiga toxin genes are carried and spread by a heterogeneous group of lambdoid bacteriophages, the Stx-phages. It had been assumed that these phages would behave similarly to λ phage, but there is evidence that both the infection processes and the immunity mechanisms are distinct and have important implications for pathogenicity. A major difference is the ability of the Stx2 phage, $\Phi24_B$, to produce double lysogens in *E. coli*. It has been reported that Stx phages conform to the λ immunity model as they possess sequences homologous to the λ phage immunity region. However, with the discovery of double lysogens it is clear that Stx phages deviate from the rules of λ phage immunity. The sequences of the Stx immunity region are arranged differently to λ phage with the DNA binding site, O_R3, lying within the *c1* ORF. Our hypothesis is that the proteins recognising this area will behave differently from those controlling λ phage. Using band shift and DNA footprint analysis we can determine how the three areas of O_R (O_R1, O_R2, O_R3) are used by $\Phi24_B$ compared to λ phage. Reporter gene technology will also be

employed to determine how these binding patterns influence expression of *cI* and *cro*.

PBMG 10 Functional analysis of the *hemP* homologue (*ydiE*) of *Escherichia coli*

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In the bacterium *Bradyrhizobium*, haem production depends on iron availability and is regulated by the Irr protein (a homologue of the'ferric uptake regulation' protein, Fur). In other bacteria, very little is known about the mechanism of haem biosynthesis control in response to iron (Andrews, 2003). Recently, an Fe^{2+}-Fur repressed gene (*ydiE*) was identified by DNA-array-based transcriptional profiling (McHugh *et al*., 2003). YdiE (63 residues) is homologous to the HemP protein (81 residues) of the pathogen *Yersinia enterocolitica*. *hemP* is the first gene in the haem-uptake operon (*hemPRSTUV*) conferring the ability to utilise haem as an iron source within the host environment. However, the precise role of HemP in haem uptake is unclear since it is unrelated to other transport proteins and would appear to be cytoplasmically located, possibly a peripheral membrane protein. HemP homologues are also found associated with haemin uptake systems in *Pseudomonas* and *Rhizobium*. However, although the *ydiE* gene is well conserved in *E. coli* and close relatives, it appears to be mono-cistronic and not associated with any other genes potentially involved in haem uptake. Based on its potential role in haem utilisation and its iron-dependent expression, we speculate that it may function in the control of haem biosynthesis in response to iron availability. However, no effect of *ydiE* inactivation on protoporphyrin accumulation has been detected by us so far.

YdiE has been overproduced as a His_6-tagged protein and purified, and found to be monomeric. The protein has an unusually high predicted pI (9.8), suggesting that it interacts with nucleic acid, the membrane or another protein. NMR spectroscopy is being employed to resolve the YdiE structure (expected to be novel) and has indicated that the protein is mainly composed of β-strands. Interestingly, the translational-initiation region of *ydiE* has no consensus ribosome-binding site but the gene is still translated according to data obtained from translational *ydiE-lacZ* fusions. This indicates that either an auxiliary ribosomal protein, non-coding RNA or a translation factor might be involved in *ydiE* translation. Studies with strains containing *ydiE-lacZ* transcriptional or translational fusions have confirmed the iron dependence of *ydiE* expression and shown that expression of *ydiE* is growth-phase dependent, with maximal expression in the exponential phase. Expression would also appear to be Crp repressed (induced by glucose). The *ydiE* promoter fragment is retarded by the Fur complex and contains a predicted Fur box centred at -42 with respect to its start codon, consistent with the Fur dependence of *ydiE* expression. Currently, microarrays are being employed to determine whether there are any expression changes associated with *ydiE* inactivation or overexpression.

PBMG 11 Characterisation of polysaccharide biosynthesis genes in *Bordetella bronchiseptica*

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Introduction *Bordetella bronchiseptica* colonises the respiratory tract of many mammals, and causes acute tracheobronchitis in dogs (kennel cough). Lipopolysaccharide (LPS) is the major component of the outer membrane of Gram-negative bacteria and is essential for viability and virulence. *B. bronchiseptica* LPS contains a homopolymer known as O-antigen, composed of repeating units of a rare sugar derivative. The *wbm* locus is required for O-antigen expression and, based upon homology, encodes many functions necessary for biosynthesis and transport of this molecule. This work aims to characterise the functions of *wbm* genes.

Results Δ*wbmD* and Δ*wbmE* mutants produce O-antigen structurally identical to wild-type, but with altered modal chain lengths. Mutants of the putative ABC O-antigen export transporter genes, *wbmM* and *wbmN*, do not express O-antigen and exhibit a slow growth phenotype. Three putative NAD-dependent oxidoreductases/epimerases, WbmF, WbmG and WbmH, have been purified and their ability to reduce NAD+ in the presence of various sugar nucleotides is being characterised. WbmG has been crystallised and X-ray diffraction data collected to 2.5Å resolution.

Conclusions These data support the various hypothesised roles of *wbm* genes in O-antigen expression and shed light upon the molecular mechanisms of biosynthesis, chain length determination and export of this molecule.

PBMG 12 Identification and preliminary characterisation of temperate bacteriophages in *Salmonella enterica* and *Salmonella bongori*

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Salmonella species are responsible for a wide variety of infections in a diverse range of organisms. Non-typhoidal salmonellae are among the leading causes of food-borne disease in man. Some serovars are host-restricted whereas others have a wide host range. It is becoming increasingly clear that temperate bacteriophages play an important role in the evolution of bacteria including the salmonellae, and in the emergence of new epidemic strains. In this study lysogenic bacteriophages were chemically induced from a broad range of clinical and environmental *Salmonella* isolates. Initially liquid cultures were assayed for the presence of phages by measuring the degree of lysis of the bacterial cells. Lysates were then examined by electron microscopy to determine the morphology of the induced bacteriophages. Phages belonging to the Myoviridae and Siphoviridae morphology families were identified. Cross-infection experiments were also carried out to elucidate the direction of possible horizontal transfers of bacteriophage-encoded genes between *Salmonella* isolates. *Salmonella* bacteriophages have been shown to be infective in closely related as well as distantly related *Salmonella* serovars, thus supporting the theory that bacteriophages are likely to play a significant role in the evolution of *Salmonella*.

PBMG 13 Molecular relationships between dsRNAs of phi-6 bacteriophage, *Pseudomonas syringae tolasii* and mushrooms virus X browning related dsRNAs – an update

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A newly recognised infectious disorder on cultivated mushrooms (*Agaricus bisporus*) referred to as 'patch disease' was first noted in 1996 on British farms (Warwick HRI, www.hri.ac.uk UK), with crop losses and economic implications to the industry. The farms associated with Mushroom Virus X (MVX) are often observed with barren patches beside healthy looking mushrooms, arrested pins, premature opening, brown, off-colour and distortions in shape. Diseased mushrooms carried at least 26 characteristic (MVX) dsRNA bands (Grogan *et al.* 2003, *Mycol Res* 107, 147–154) ranging from 640 bp to 20.2 kbp and 4 low molecular weight dsRNAs (2.0, 1.8, 0.8 and 0.6 kb) among them are diagnostic MVX bands associated with mushroom browning syndrome. Sonnenberg and Lavrijssen 2004 (www.americanmushroom.org) showed that the 4 diagnostic MVX dsRNA segments did not share sequence homology between themselves or with larger segments, indicating that the presence of multiple viruses. Nevertheless, they observed that as the 4 bands occured together, they may also be representing one virus. The origin of these unusual dsRNAs or the virus(es) are yet to be deciphered and it is of concern to the British Isles as MVX symptoms were reported in Irish farms over the last few years. We recently reported (Rao et al 2004, www.americanmushroom.org) upon samples obtained from Republic of Ireland farms that mushroom browning syndrome in *A bisporus* is linked to phi-6 bacteriophage related dsRNAs and surrogate bacterial host *Pseudomonas syringae* pv. tolasii. The sequence comparisons of the plaque RNA obtained from MVX-mushrooms showed highest homology to tri-segmented phi-6 bacteriophage whose host, a phytopathogen *Pseudomonas syringae* pv phaseolicola is found in compost straw. The sequences from dsRNAs isolated fromVLPs in CsCl density gradient fractions of diseased mushrooms concurred with S-segmented phi-6 dsRNA sequences, with deletions in *pac* sequences in short hairpin regions crucial for packaging. Hybridisation experiments indicated that the plaque RNA sequences shared homology with the MVX (browning) dsRNA bands (2.0–0.6bp). We now report 'multiplex nested' RT-PCR tests which confirmed the presence of *Ps syringae* tolasii or phi-6 and homologous viruses [mushroom bacilliform virus (MBV)] in spawned compost or diseased mushroom samples. This raises the possibility that homologous virions may act as 'helpers' to the phage dsRNAs, culminating as 'defective' VLPs in an 'incompatible' mushroom host. We suggest that during mushroom cultivation, the phage from naturally occurring straw phytopathogen *Ps syringae* could have been surrogated by susceptible *Ps syringae* tolasii, a causal agent for mushroom browning. Interestingly, BLAST alignments of deduced RdRP (RNA dependent RNA Polymerase) sequences of MVX-mushroom dsRNAs exhibited highest similarity also with RdRPs of PRD1 phage (host: *Salmonella typhimurium*), fowl plague virus and to those of dsRNA carrying *Cryptosporidium baileyi* (poultry biotype), some of which are known to occur in chicken litter, an ingredient of the compost for mushroom cultivation. Expression analyses of viral sequences, host transfection, transformation experiments and the determination of the source(s) for MushroomVirus X related dsRNAs are among the core objectives of our future investigations.

PBMG 14 Cloning of *murC* Gene to study its role in attachment of lactobacilli to poultry GI tract epithelium

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The GI tract is an open flow system thus the ability to adhere to the GI tract is an important prerequisite for selecting probiotic strains. It has been proposed that bacteria such as Lactobacilli exclude the attachment of pathogenic organisms through competition for receptors in the intestinal wall. Adhesion of lactic acid bacteria (LAB) ensures that they persist in the gut, long enough to exert their additional beneficial effects.

Experiments using differential display technology have shown that in the presence of mucin, *murC*, a gene involved in cell wall biosynthesis, appears to be upregulated in a strain of *Lb. thermotolerans*. This is consistent with the notion that *murC* plays a role in the attachment of LAB to the intestinal epithelium.

In order to study the expression of the *murC* gene at the molecular level it was necessary to obtain a cloned gene fragment. Degenerate primers based on known DNA sequences were synthesised. Using total DNA from *Lactobacillus thermotolerans* an amplicon of the predicted size was generated. Cloning and sequencing revealed a 697bp *murC* gene fragment. The isolated *murC* gene fragment showed sequence overlap with a fragment previously identified by differential display thus confirming its identity.

This clone will permit the confirmation of the role for *murC* in adhesion by Northern blot analysis of gene expression.

PBMG 15 Investigation of the DNA replication stage of a lactococcal bacteriophage

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Tuc2009 is a lysogenic bacteriophage isolated from *Lactococcus lactis* subsp. *cremoris* UC509, a bacterium that is used for the production of Cheddar cheese and is a typical representative of a bacteriophage infecting a Gram-positive bacterium. The complete sequence of this phage has been determined and bioinformatic analysis revealed that a specific fragment of the genome of Tuc2009 contains several open reading frames (ORF's), whose deduced protein products exhibit similarities to proteins known to be involved in DNA replication.

This project aims to identify and characterise genes essential for Tuc2009 DNA replication (and consequently Tuc2009 propagation). For this purpose, a bank of random mutant Tuc2009 phage will be generated by incubating phage particles with hydroxylamine and selecting for nonsense mutants on a host strain harbouring a suppressor plasmid. Temperature sensitive mutants will also be generated in a manner similar to that described for λ. Following an initial classification of the random mutants by propagation on the wild-type host, suspected DNA replication mutants will be analysed by an established intracellular phage DNA replication assay. DNA replication deficient mutants confirmed in this manner will be further studied using complementation assays. The molecular mechanisms employed by proteins identified in this manner will be scrutinised using protein-DNA interaction analyses and/or protein-protein interaction analyses.

PBMG 16 Preliminary characterisation of a symbiont-encoded bacteriophage

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Sodalis glossinidius is a bacterial endosymbiont of tsetse flies (*Glossina* spp.), medically and agriculturally important vectors of African trypanosomiasis. Observations of bacteriophage-like particles

within *S. glossinidius* in tsetse led to an investigation of the origin of such phages. *Sodalis* cultures treated with mitomycin C fail to produce phage particles, though late log phase cultures sporadically display plaques when plated on solid media. Filtered material isolated from plaques has no discernible effect upon *Sodalis* but causes a propagation of plaques with *Escherichia coli*, verifying the presence of a lytic bacteriophage. Purified bacteriophage particles show morphology typical of the *Siphoviridae* (long-tailed) group of phages, encompassing a circularly permuted 45, 169 bp double stranded DNA molecule, apparently lacking in cohesive ends. Predicted coding sequence homologies indicate a modular nature to the genome with a region of putative tail genes predominantly comparable in order and sequence to bacteriophages of the lambda-like supergroup. The apparent absence of an integrase-like sequence and presence of a RepA homologue may suggest an extrachromosomal plasmid-like state for the phage in *S. glossinidius*. In conjunction with other studies of functional bacteriophages in insect symbionts, this research raises interesting questions about the nature and evolution of such symbioses.

PBMG 17 Disruption of bacterial genes using retargeted group II introns

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The TargeTron™ Gene Knockout System is a novel functional genomics tool based on a group II intron from *Lactococcus lactis*. Like conventional transposon mutagenesis methods, group II introns inactivate genes by insertion, however, recent advances in understanding group II intron biology have enabled insertion to be site-specific, allowing knocout of virtually any desired DNA target. Features of the group II retrohoming mechanism make it attractive for genetic manipulation. First, retrohoming is highly efficient and specific. Retrohoming frequencies can approach 100% and are six orders of magnitude higher than the frequency of ectopic integration. Second, the introns are minimally dependent on host factors, making them applicable to a broad range of bacteria. To date, the TargeTron™ system has been validated in *E. coli, Staphylococcus aureus, Clostridium, Shigella flexneri, Salmonella typherium*, and *Lactococcus lactis*.

The TargeTron™ system has many features that make it attractive for metabolic engineering and systems biology research. In addition to site-specific knockouts, random chromosomal insertion libraries can be created. Using this approach, essential and non-essential genes can be rapidly identified by PCR and sequencing. More importantly, once a desired random insertion is identified by PCR, the gene specific intron sequence can be cloned and used to re-create the site-specific knockout in hosts with alternative genetic backgrounds. This eliminates the need for maintaining large clonal libraries to isolate random insertion events and the need for phage-based transfer of mutations between strains.

In this study, we demonstrate the use of the TargeTron™ method to rapidly disrupt several *E. coli* genes using a retrotransposition-activated kanamycin marker. In a particular application, we have inactivated the *endA* gene in *E. coli* BL21(DE3) in an attempt to improve the quality of plasmid DNA isolated from this protein expression strain. We also show the use of random chromosomal insertion libraries to rapidly characterize gene essentiality by PCR. Characterization of a PCR-isolated RNase E insertional mutant will be discussed.

PBMG 18 Flagellar, motility and chemotaxis genes of *Rhizobium leguminosarum*

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Rhizobium leguminosarum is a Gram negative bacterium which is able to form nitrogen fixing nodules on the roots of various legumes. During the processes of nodulation and infection of plants, chemotaxis and motility have been shown to play a role in directing the bacterium to appropriate infection sites on plant roots. However, after infection, chemotaxis and motility are presumably no longer required, and the genes involved appear to be down-regulated. We are studying the role of various components in the motility and chemotaxis pathways of *R. leguminosarum* in order to confirm that the genes are down-regulated, and to identify the signalling pathways involved in this regulation. We have shown using gene fusions to several chemoreceptor (*mcp*) genes that down-regulation occurs and is not dependent on known symbiotic regulatory genes. We have used a genetic screen to identify two genes involved in down-regulation.

Antiflagellar antibodies were also used to demonstrate that flagellar proteins were present in much lower abundance in bacteroids than in free-living cells. Since *R. leguminosarum* has five flagellin genes, one of which is plasmid-encoded, we are currently investigating the role of each gene in motility, and studying their regulation. We have established that *flaA* mutants still make flagella, though their swimming is impaired to some extent. Three potential regulatory genes which are located in the flagellar, motility and chemotaxis gene cluster are also being investigated for their role in regulating motility and chemotaxis in both free-living and symbiotic conditions.

PBMG 19 Phylogenetic analysis of condensation domain in nonribosomal peptide synthetases suggests the evolutionary relationship in the peptide donor molecules

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Nonribosomal peptide synthetases (NRPS) are modular multifunctional enzymes that synthesize an incredibly diverse set of biological active peptides. The condensation (C) domain of NRPS catalyses peptide bond formation of two consecutively bound amino acids onto the growing peptide chain during nonribosomal peptide synthesis. In this study, a phylogenetic approach was used to investigate the functional and structural diversity of bacterial C-domains. We demonstrate that C-domains are classified into two major functional subdomains, N-acylation and peptide bond forming subdomains. The peptide bond forming subdomains are able to be further categorized into L-peptidyl donor and D-peptidyl donor groups. L-peptidyl donor group is composed of L-specific peptidyl donor/ aminoacyl acceptor ($^{L}C_{L}$), and L-specific peptidyl donor/ D-specific aminoacyl acceptor ($^{L}C_{D}$) types. Whereas $^{D}C_{D}$ and $^{D}C_{L}$ conforms single D-peptidyl donor group. This study suggests the evolutionary relationships of C-domain are recorded in the peptidyl donor molecules.

PBMG 20 Subcellular distribution of nutrients in arbuscular mycorrhizal fungi associated with cucumber using X-ray microanalysis

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The elemental distribution (P, Ca, K, Mg) within inter- and intracellular structure of arbuscular mycorrhizal (AM) cucumber root was determined using energy dispersive X-ray spectroscopy (EDAX). Cucumber was inoculated with the AM fungus, *Glomus mosseae* (BEG 107) and grown using compartmentalised pots with separate zones for hyphal growth. X-Ray microanalytical investigations revealed P contents was mainly detected in the arbuscular cytoplasm, and accumulated in poly phosphate granules, while the content in the arbuscular interface was significantly lower compared to those of the other cell compartments. High Ca content was detected in interfacial apoplast and cell wall compartments, while cytoplasmic contents in cells were lower. Interestingly, Ca was mostly accumulated in poly-P granules. In conclusion, poly-P granules in AM fungal vacuole and interfacial apoplast between fungal and root cells could be regulating points for transfer of cations between symbionts, that is, from AM fungus to plant roots. Supported by grants from KOSEF (R03-2002-000-20029-0).

PBMG 21 Identification of two genes responsible for the production of signal molecules in *Pseudomonas aeruginosa*

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We have shown previously that the PA4205-PA4208 genes form an operon coding for the components of an efflux pump (MexGHI-OpmD) contributing to the resistance of *P. aeruginosa* to vanadium (Aendekerk *et al*., 2002). Mutants in the *mexI* or *opmD* genes have a decreased fitness, and fail to produce the quorum sensing signal molecules 3-oxo-C12-homoserine lactone and C4-homoserine lactone, as well as the 2-heptyl-3-hydroxy-4(1*H*)-quinolone (PQS). Here, we show that the two genes, upstream of the operon, PA4203 and PA4204, are needed for the expression of the *mexGHI-opmD* genes as well as for the genes for the biosynthesis of PQS (as shown for *pqsA* and *phnA*). Conversely, mutants in *mexI* and *opmD* still express the PA4203 and PA4204 genes, indicating that these two genes are regulating the pump operon. PA4203 encodes a LysR regulator and PA4204 a putative periplasmic protein. Inactivation of PA4203 and PA4204 also results in the inability to produce the quorum sensing *N*-acyl homoserine lactones as well as PQS. Similarly to *mexI* and *opmD* mutants, both mutants in PA4203 and PA4204 show an important growth delay. Interestingly, inactivation of PA4204 also results in the loss of production of the siderophore pyoverdine. The same mutant was however still in state to utilize externally provided pyoverdine for its growth in the presence of the iron (III) chelator EDDHA. These two genes were therefore designated as *pspR* (PA4203) and *pspA* (PA4204), which stands for Pseudomonas signal production. These two genes were also inactivated in the PA14 strain, which is virulent in plants, and the resulting mutants were found to have lost their virulence. We conclude that PspR is a regulator for the *mexGHI-opmD* pump genes and that PspR, and especially PspA, are needed for the fitness of *P. aeruginosa*, including its capacity to scavenge iron. Future research will focus on the function of these two genes by combining transcriptome and proteome analysis.

Reference Aendekerk, S., Ghysels, B., Cornelis, P., & Baysse, C. (2002). Characterization of a new efflux pump, MexGHI-OpmD, from *Pseudomonas aeruginosa* that confers resistance to vanadium. *Microbiology* **148**, 2371–2381.

PBMG 22 An integrated computational approach to predict non-coding RNAs in bacterial genomes

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Small, non-coding RNAs (sRNAs) have been shown to play important regulatory roles in both prokaryotes and eukaryotes. In bacteria, nearly all sRNAs identified to date are located in regions between annotated ORFs, are associated with predicted rho-independent terminators and/or putative promoters, and are conserved among related bacterial species. While these common characteristics have been utilized to predict novel sRNAs in a number of studies, a unified bioinformatic approach that efficiently incorporates them into a predictive algorithm has yet to be developed. To this end we have created a program that integrates the coordinate positions of any or all of these features and, within a few minutes, predicts the positions and strand orientations of sRNA candidates. Using *V. cholerae* as a test subject, a total of 34 sRNAs were predicated by the program. These included 8 of the 9 known or putative *V. cholerae* sRNAs and 26 candidates for novel sRNAs. The expression of 10 of these candidates will be tested by Northern analysis both to test the efficacy of our predictive algorithm and to identify novel sRNAs for future study.

PBMG 23 Evaluation of chickpea germplasm for resistance to six pathotypes of ascochyta blight in Iran

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Ascochyta blight is the most important limitative disease reducing production of chickpea in many areas. The resistance lines were considered as the best way to control the disease. So identification of suitable resistance sources against population of pathogen in each area is important. In this study chickpea germplasm of Ferdowsi University was evaluated against six pathotypes of the pathogen. Five hundred-seventy accessions were cultivated in an augmented design with ten blocks and sixty-seven rows. Plants were inoculated with mixture suspension spores of six pathotypes in five to seven leaves stage. Suitable humidity and temperature was provided using mist irrigation system. When the susceptible lines (ILC1929 and ILC263) died completely, disease severity was scored on a scale of 1 to 9, where 2 and 3 =resistance, 4 and 5 = moderately resistance, 6 and 7 = moderately susceptible and 8 and 9 = highly susceptible. 2, 25, 32 and 41% of accessions were divided in those groups respectively. Percentage of Kabuli type in resistance groups was more than Desi type, but in susceptible groups Desi type was more than Kabuli type. Only one Desi (MCC523) and five Kabuli accessions (MCC54, MCC133, MCC3.11, MCC142 and MCC331) were resistant in different growing stages against six pathotypes of ascochyta blight.

Keywords chickpea – Ascochyta Blight – screening – resistance – germplasm

PBMG 24 Interactions of *Streptococcus pneumoniae* with glycoproteins

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Streptococcus pneumoniae, an important pathogen, is constantly exposed to host glycoproteins both at colonisation and infection sites. Genome sequencing of *S. pneumoniae* (Tettelin *et al.*, 2001) provided data that suggested that this organism has many systems for the degradation of host macromolecules, resulting in damage to host tissues and release of potential sources of nutrient. The aim of the study presented here was to characterise functional pathways involved in the degradation and utilisation of glycoproteins by *S. pneumoniae*. Cellular fractionation and proteomic analysis of glycoprotein-grown bacteria demonstrated the presence of surface-associated adhesins and glycosidases (including the virulence factor, neuraminidase), these latter proteins acting to degrade oligosaccharide sidechains of glycoproteins. Systems with potential roles in the transport of these released sugars were also up-regulated by growth in the presence of glycoproteins. We also present data that demonstrate that *S. pneumoniae* has catabolic pathways that mediate the concerted degradation of glycoprotein-derived sugars, including sialic (N-acetylneuraminic) acid, galactose and N-acetylhexosamines. Collectively, these data demonstrate that *S. pneumoniae* can effectively degrade the oligosaccharide sidechains of glycoproteins, releasing sugars that are transported and used to support growth. These sugars may also provide intermediates that can channel into capsule formation, an important factor in the virulence of this organism.

Posters

Plenary session

PS 01 Involvement of cellular actin filaments in egress of budded virus from *Autographa californica* nucleopolyhedrovirus-infected insect cells

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Budded viruses (BV) of the baculovirus *Autographa californica* nucleopolyhedrovirus (AcNPV) are produced during egress of nucleocapsids from infected insect cells, acquiring cellular membranes enriched with the viral GP64 attachment and fusion glycoprotein. The cytoskeleton is known to be critical for transport of nucleocapsids to the nucleus during early stages of infection, and a similar association is thought to be involved in the egress of subsequent progeny virus. Lantrunculin B (LB) prevents actin polymerisation, a key event in baculovirus replication. BV released from LB-treated cells was reduced compared to controls, suggesting a role for actin in virus budding. Removal of LB from cells at various times post infection was accompanied by a return of the budding process, evidenced by restoration of BV detection in extracellular medium. Western analyses indicated elevated levels of viral proteins in the membrane of treated cells, suggesting the absence of BV titre in culture medium was a consequence of virus budding, and not an inhibition of viral protein synthesis, or of transportation of virus proteins to the plasma membrane. We propose that cellular actin is involved in the terminal stages of BV egress, following assembly and transport of viral nucleocapsids to GP64-enriched regions of the cell membrane.

PS 02 Tissue tropisms of the canine coronaviruses

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Two coronaviruses of dogs are known to date: canine enteric coronavirus (CECoV), belonging to group 1 and the recently detected canine respiratory coronavirus (CRCoV), which is a member of group 2. CRCoV has frequently been detected in dogs suffering from canine infectious respiratory disease and may be involved in this disease complex.

This study aimed to determine the tissue tropisms of these viruses during natural infections.

We performed a post-mortem examination on twenty dogs from a re-homing centre and collected a range of tissue samples, which were analysed by RT-PCR for CRCoV and CECoV.

CRCoV was predominantly detected in the upper respiratory tract and only rarely in the colon and the mesenteric lymph node. CECoV was most frequently identified in intestinal tissues and faeces and was not detected in any respiratory tissues. The mesenteric lymph node of one dog was positive for both, CRCoV and CECoV.

The respiratory and enteric coronaviruses of dogs therefore show distinct tropisms possibly due to interaction with different receptors. Further investigations using *in situ* hybridisation or immunohistochemistry will be required to determine the specific target cells of CRCoV.

PS 03 Bovine noroviruses: inter- and intra-genotype relationships of complete genomes and immune responses in experimental calves

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Noroviruses, members of the family *Caliciviridae*, are economically important viruses that cause outbreaks of gastroenteritis in man and are associated with diarrhoea in cattle. Previous studies using partial (41%) genome sequence established that the bovine noroviruses, represented by Bo/Jena/80/DE (genotype-1) and Bo/Newbury2/76/UK (genotype-2), formed a 3rd genogroup distinct from the human noroviruses. We confirmed the inter- and intra-genotype relationships determined by sequence analysis of the complete genome sequences (7311 nucleotides) of two genotype-2 bovine noroviruses (Newbury2 and Dumfries), and examine immune responses in experimental calves by ELISA using Newbury2 virus-like particles (VLPs). All 6 gnotobiotic calves infected with Newbury2 seroconverted (mean serum IgG titre ($\log^{10}$), 3.9 [σ = 0.387]). Sera from Dumfries infected calves (n = 2) showed a high antigenic cross-reactivity to Newbury2 VLPs (IgG titre, 4.0 [σ = 0.173]). This reflected the close genomic relationship (97% amino acid identity in the capsid protein) between the genotype-2 viruses. Calves infected with Newbury1, rotavirus, coronavirus, astrovirus and parvovirus did not seroconvert to Newbury2 VLPs. The distinct genomic relationship between genotype-1 and -2 of the bovine noroviruses and antigenic cross-reactivity within genotype-2 form the basis to study immune responses to Newbury2- and Jena-like viruses in experimentally and naturally infected cattle.

PS 04 The effect of disruption of the vaccinia virus A26L gene on maturation of IMV particles

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Vaccinia virus (VACV) produces two types of mature virion: enveloped virus (EV) and intracellular mature virus (IMV). It has previously been shown that EV is produced first in the replication cycle, and it has been suggested that IMV production occurs by default when the supply of cellular membranes that wrap EV is exhausted. This study examines the hypothesis that IMV production is not a default, but is an active process mediated by the VACV A26L gene product, which has previously been shown to be an IMV specific protein. A recombinant virus has been constructed in which the A26L gene is deleted. Analysis of IMV and EV production in recombinant and wild-type virus using CsCl gradients shows a significant reduction in the quantity of IMV produced and the ratio of IMV/EV, suggesting that the A26L gene plays a role in maturation of IMV.

PS 05 Evidence of reassortment in Crimean-Congo haemorrhagic fever virus

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The complete nucleotide sequences of the small (S) and medium (M) segments of three independent strains of Crimean-Congo haemorrhagic fever (CCHF) virus isolated in Uzbekistan, Iraq and Pakistan have been determined. Partial S and M segment sequences from two additional strains including partial L segment sequences from five strains of CCHF virus have also been obtained. These data have been compiled and compared with published full-length and partial sequences of other CCHF virus strains. Analysis of virus strains for which complete and partial S and M segment sequences are available reveals that the phylogenetic grouping of some strains differs between these two segments. We provide data suggesting that this discrepancy is not the result of recombination, but rather the consequence of reassortment events that have occurred in some virus lineages. Although described in other genera of the *Bunyaviridae* family, this is the first report of segment reassortment occurring in the Nairovirus genus.

PS 06 Estimation of selective pressure and evolutionary rates in HCV genotypes 1–6

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Envelope glycoproteins E1 and E2 are hypothesised to be exposed the selective environment of the host in HCV infection, making them ideal candidates for measuring adaptive evolution. In this study, sequence data generated from a cross-genotype panel, coupled with sequence data available via the LANL HCV database, were used to address a number of questions.

Previous studies on HIV have shown a tendency for positively selected sites to be located at the same positions in different HIV-1 groups and subtypes. Elevated d_N/d_S ratios along terminal branches within the HIV phylogeny have also been reported. Estimation of the d_N/d_S ratio along internal and terminal branches for HCV genotype specific phylogenies, in conjunction with identification of specific codons under positive selection, have allowed the assessment of patterns of adaptive evolution in HCV at the genotypic level.

Although the presence of GORS in the HCV genome makes the estimation of MRCA dates from deep with the HCV phylogeny unreliable, temporally spaced sequence data were also used in an attempt to estimate underlying mutation rates for the different HCV genotypes.

PS 07 The secreted form of the G protein is a virulence determinant of bovine respiratory syncytial virus (BRSV)

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BRSV, which is closely related to human (H)RSV, is a major cause of lower respiratory tract disease in young calves. The RSV surface glycoproteins F and G are attachment proteins. However, viruses lacking the G protein replicate efficiently *in vitro*, but are highly attenuated *in vivo*. The G protein is produced in two forms, a transmembrane form (Gm) and a truncated, secreted form (Gs). Studies in mice infected with recombinant (r)HRSV expressing only Gm or only Gs have shown conflicting results. We have therefore investigated the pathogenesis of rBRSV expressing only Gm or only Gs in young calves.

Whereas the replication of rBRSVGm and wild-type (WT) rBRSV in the nasopharynx was similar, replication of rBRSVGm in the lung was reduced 1,000-fold. In contrast, peak titres of rBRSVGs in the nasopharynx were 100-fold less than those of WT rBRSV and rBRSVGs could not be isolated from the lungs. Pneumonic lesions were only seen in calves infected with WT rBRSV. These findings suggest that Gs is important in establishing lower respiratory tract infection and that it can provide some of the functions of G in the upper respiratory tract.

Although infection with rBRSVGm was confined largely to the upper respiratory tract, BRSV-specific IgG2 serum antibody responses were greater than those induced by WT virus and protection against challenge with virulent BRSV was similar to that induced by prior infection with WT rBRSV.

PS 08 Withdrawn

PS 09 Modulation of cell division and interferon responses by an African swine fever virus gene

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Most modifiers of cell biology and immune responses have been identified through bioinformatic analysis. Large DNA viruses, however, contain 20–30% of 'unassigned' ORF's which lack obvious mammalian homologs in the database and which are not either virus structural proteins or enzymes. It is possible that many of these have evolved strategies for host cell manipulation. In order to test this hypothesis, a number of ORF's of the African Swine Fever Virus (ASFV) have been cloned and screened in functional assays.

One of these, when expressed in fibroblast cells, mediated (1) An inhibition of gene expression controlled by the IFN beta promoter and (2) An activation of NF-kB activity. Cells expressing this viral gene through recombinant retroviral infection were also able to overcome growth arrest in G0/G1 induced by culture in 0.5% serum. Thus, this viral gene has indeed evolved for host cell manipulation, and thus plays a role in virus pathogenesis. Deletion of this gene would be a rational approach towards the construction of an ASFV vaccine.

PS 10 Modulation of interferon response by an African swine fever virus gene

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African swine fever (ASF) is one of the most important virus diseases of swine. There is no treatment and an effective vaccine has still not been developed. Control, therefore, is based on a rapid and efficient diagnosis and subsequent adoption of strict sanitary measures.

Viruses have evolved mechanisms to evade and modify host immune responses. Identification of ASFV 'evasion' genes may have immediate practical input, as in general such genes, whilst giving the

virus an advantage over the host, are non-essential for virus growth *in vivo*. Thus deletion of virus 'evasion' genes offers a rational strategy for the development of non-pathogenic, deletion mutant vaccines.

In the past, the majority of such 'evasion' genes have been identified through their homologies. In order to identify 'evasion' molecules without known structural homologues, unassigned ASFV genes were screened in functional assays for modulation of interferon (IFN) responses.

We have identified an ASFV gene able to inhibit both the induction of IFN beta promoter by a dsRNA analogue and the response of the host cells to IFN alpha and IFN gamma. To further investigate the mechanisms of this inhibition, the gene was screened in the Yeast Two Hybrid system in order to identify cellular target(s) of the protein. One of the positive clones identified in this screening was STAT2, suggesting that inhibition of the IFN response is through modification of STAT function.

PS 11 Late viral interference between endogenous and exogenous retrovirus

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The protection of the host from infection by pathogenic exogenous retroviruses is one of the roles hypothesized for endogenous retroviruses (ERVs). The sheep genome contains 20 copies of ERVs highly related to the exogenous and pathogenic Jaagsiekte Sheep Retrovirus (JSRV). One endogenous locus, enJS56A1, is defective for viral exit, although it contains intact open reading frames in its genome. Interestingly, enJS56A1 blocks the release of JSRV viral particles (VPs) but doesn't interfere with other retroviruses (e.g. MPMV, Mo-MuLV).

By electron microscopy, JSRV-expressing cells displayed intracellular and budding VPs. Instead, in enJS56A1 or enJS56A1+ JSRV transfected cells only intracellular particles were detected, mostly aggregated at the perinuclear region.

By constructing JSRV/enJS56A1 chimeras/mutants, we established that three residues in Gag determine the defect in viral exit (R98C and V102L) and the interference ability of enJS56A1 (R21W). Single mutant JSRVR21W blocks the wild type analogously to enJS56A1. Interestingly, R21 is conserved among all betaretroviral matrix proteins.

By RT-PCR we determined that enJS56A1 (or similar loci) is expressed *in vivo* in the sheep endometrium. enJS56A1 is the first example of a naturally occurring ERV expressing a dominant negative Gag acting at a late step of the retroviral replication cycle.

PS 12 Internal recognition of the TBE virus RNA-dependent RNA polymerase, NS5 by a PDZ domain in the mammalian LAP protein, Scribble

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Human Scribble (hScrib) is a member of the LAP protein family, containing 16 leucine rich repeats (LRRs) and four PDZ domains. Structural studies on LAP proteins suggest localization to restricted regions of the cytocortex corresponding to arthropod and metazoan cell-cell junctions. hScrib has been reported to partially localize at presynaptic compartments suggesting a role in neuronal transmission. The LRRs of hScrib are the primary determinants of basolateral membrane targeting whereas PDZ domains function as protein interaction modules.

PDZ modules have been shown to preferentially recognise short, C-terminal motifs, 3–5 residues, in their target proteins. A PDZ-ligand sharing this motif specificity is the RNA-dependent RNA polymerase, NS5, from tick-borne encephalitis (TBE) virus. Since one of the features of encephalitis involves neuronal cell morphology defects we investigated the engagement of hScrib to flaviviral NS5 proteins.

Interestingly, a yeast-two hybrid screen with NS5TBE as bait against a human brain cDNA library identifies a novel hScrib interaction. Surprisingly, binding seems to bypass the generic C-terminal NS5 motif. Genomic mapping using site-directed mutagenesis pinpoint instead an internal motif within the N-terminal methyltransferase region. The interaction was confirmed in mammalian cells by coimmunoprecipitation and western blot analysis. Our data reveal the existence of a tight hScrib/NS5 interaction and propose a potential role in TBE pathogenesis.

PS 13 The SARS coronavirus nucleocapsid (N) protein is phosphorylated and localizes in the cytoplasm by 14-3-3 mediated translocation

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The SARS-CoV nucleocapsid protein (N) is one of the four structural proteins of the virus and is predicted to be a 46kD phosphoprotein. Our *in silico* analysis predicted N to be heavily phosphorylated at multiple residues. Experimentally, we have shown in this report that the N protein of the SARS-CoV gets serine-phosphorylated by multiple kinases, both in the cytoplasm and the nucleus. The phosphoprotein is stable and localizes in the cytoplasm and coprecipitates with the membrane fraction. Also, using specific inhibitors of phosphorylation and a coimmunoprecipitation assay, we show that the nucleocapsid protein is a substrate of cyclin dependent kinase (CDK), glycogen synthase kinase (GSK3) and casein kinase II (CKII). Further, we show that the phosphorylated protein is translocated to the cytoplasm by binding to 14-3-3 (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein). 14-3-3 proteins are a family of highly conserved ubiquitously expressed eukaryotic proteins that function primarily as adapters that modulate interactions between components of various cellular signaling and cell cycle regulatory pathways through phosphorylation-dependent protein-protein interactions. Coincidentally, the N protein was also found to downregulate the expression of the theta isoform of 14-3-3 (14-3-3θ) leading to the accumulation of phosphorylated N protein in the nucleus, in the absence of growth factors. Thus data presented here provides a possible mechanism for phosphorylation dependent nucleo-cytoplasmic shuttling of the N protein. This 14-3-3 mediated transport of the phosphorlyated N protein and its possible implications in interfering with the cellular machinery are discussed.

PS 14 CNS gene therapy applications of the Semliki Forest virus 1 vector are limited by vector toxicity *in vivo*

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In athymic mice with no T-lymphocyte responses some strains of Semliki Forest virus (SFV) are able to establish a life-long persistent infection of the CNS. The SFV1 vector system has been shown to be highly efficient in gene transduction of a broad range of host cells. To determine transgene expression and the outcome of infection for SFV1 based vectors in mature murine CNS cells we have studied the SFV1 based, non-replicating, virus-like particle (VLP) system and the time course of its enhanced green fluorescent protein (d1EGFP) expression in substantia nigra neurons in the mouse brain. Precise stereotaxic inoculation of EGFP expressing VLPs into the substantia nigra resulted in a fast onset of transgene expression. Activation of microglia and a mononuclear cell inflammatory response were apparent from four days post inoculation. EGFP expression was evident in healthy cell bodies and neurites, including dopaminergic neurones, for up to twelve weeks post inoculation. However over this time, numbers of EGFP-positive cells declined and an axonopathy, as evidenced by Bielschowsky's silver staining, was increasingly apparent. These findings are consistent with a slow loss of EGFP expressing neurons. We conclude that the SFV1 vector system is currently limited in its application for CNS gene therapy by its neurotoxicity. The use of this system in gene therapeutic applications will require the development of less cytotoxic vectors.

Posters

Systematics & Evolution Group

SE 01 Multilocus sequence typing of *Campylobacter jejuni* from animal and environmental sources

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Campylobacter gastroenteritis is a major zoonotic infection. However, the association between *Campylobacter jejuni* and isolates of animal and environmental origin remains ill-defined.

MLST was used to analyse *C. jejuni* isolates from a cross-sectional study of zoonotic infection based upon an area of the Cheshire countryside in NorthWest England.

Isolates (172) from various animal and environmental sources were analysed by MLST resulting in a total of 65 sequence types (STs). Isolates from cattle faeces were significantly over represented in the ST-61 complex. The greatest diversity of sequence types were identified for organisms isolated from water. The majority of bird isolates had STs belonging to the ST-45 complex. *C. jejuni* isolates from cattle faeces belonged to clonal complexes established in human disease and this study suggests the association between ST-61 complex and cattle. Isolates of the ST-45 complex appear to be more common in association with the environment and wildlife which may represent an important source of transmission to humans. There appears to be greater diversity of MLST types in water and wildlife many of which have not yet been identified in human infections.

SE 02 Suppression subtractive hybridisation reveals genomic variations between strains of *Burkholderia pseudomallei*

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Burkholderia pseudomallei is the causative agent of melioidosis, an often fatal disease endemic in South-East Asia and Northern Australia. *B. thailandensis* is a closely-related species that is avirulent in animal models. A number of studies have suggested considerable variation between the genomes of *B. pseudomallei* and *B. thailandensis*. However, there is little known about genomic variation within *B. pseudomallei*.

Using suppressive subtractive hybridization (SSH), we compared the genomes of strain 338, associated with a chronic infection, and strain 520, associated with an acute infection. SSH is designed to identify sequences present in one strain (tester) but absent from another (driver). Subtractions between the two strains were carried out using strain 338 as tester and using strain 520 as tester. Using a combination of SSH and PCR assays, a number of putative genes / genomic islands that vary between strains of *B. pseudomallei* were identified. The subtracted sequences fell into one of two categories: (i) unique to a single strain of *B. pseudomallei*, or (ii) present in some strains but absent from others. A genomic island containing putative transcriptional regulator, transporter and restriction modification genes, and exhibiting variable prevalence amongst strains of *B. pseudomallei* from diverse geographical locations was identified.

SE 03 Comparative genomics using a seven-strain *Staphylococcus aureus* microarray: the ultimate typing tool

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We have built a microarray containing 3626 *S. aureus* genes, including all those from each of the seven sequenced strains. The microarray has been validated for comparative genomics and is 96% accurate in identifying the presence or absence of approx. 1500 accessory genes and gene variants in any given strain. A comparison of 161 community strains of carriage and invasive *S. aureus* shows the bacterial population is extremely clonal. Each clonal group of strains matches the 'clonal clusters' described by MLST typing, and we have identified approx 250 'core-variable' genes characteristic of each group. Within clonal clusters, there is an enormous amount of variation in the carriage of mobile genetic elements that carry virulence and resistance genes, including bacteriophages, *S. aureus* pathogenicity islands, plasmids and transposons. Somewhat surprisingly, we found no strong evidence of virulence genes that were particularly associated with invasive isolates, suggesting that in the community *S. aureus* are opportunistic pathogens.

SE 04 WebACT: A simple online implementation of the Artemis Comparison Tool

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The number of completed and available small genomes has increased substantially in recent years and, with the decreasing cost of sequencing, these numbers will rapidly increase further. Large-scale comparative sequencing projects covering extensive sections of chromosomal DNA from many strains within an individual species are also becoming more routinely feasible. With the increasing amounts of data there is a concurrent need for user-friendly, easily accessible, tools for comparative sequence analysis for the laboratory researcher.

The Artemis Comparison Tool (ACT) is a DNA sequence viewer based on Artemis[1] that allows a user to view BLAST comparisons between two or more sequences. ACT is available free as a downloadable JAVA™ application from the Sanger Institute[2]. In order for a user to view comparisons, ACT requires that a 'comparison file' be loaded, containing the results of a BLAST alignment between the sequences of interest, which is then displayed within ACT to show regions of homology between the two sequences. Presently, the generation of sequence comparison files and their subsequent loading into ACT is the responsibility of the user. This process requires a UNIX machine and some knowledge of computing and can be daunting and time-consuming for the laboratory scientist. Here we describe a website that provides a simple online tool for the generation of comparison files from user inputted data, or from public database searches, based on sequence identifiers, with subsequent viewing through a version

of ACT that runs at the website as a JAVA™ applet. Furthermore, webACT stores pre-computed up-to-date comparison files that allow any published bacterial genomes to be selected from drop-down lists and to be compared and viewed on-line. Individual gene names can also be searched for (e.g. *recA*), and the homologies within regions of the genome flanking the gene of interest can be viewed. All comparison files computed on-line and pre-computed bacterial genome comparison files are available to download for viewing using the stand-alone version of ACT. Availability – webACT will be available at http://www.webact.org

References http://www.sanger.ac.uk/Software/Artemis/ – Artemis homepage / http://www.sanger.ac.uk/Software/ACT/ – ACT homepage

SE 05 Strain- and species-characteristic markers in the ribosomal DNA of three species of anthropophilic dermatophyte fungi

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Trichophyton violaceum, T. rubrum and *T. mentagrophytes* var. *interdigitale* are highly human-adapted species of dermatophyte fungi, with a wide geographic distribution. We have characterised a novel type I intron in the 25S gene of *T. mentagrophytes* var. *interdigitale* which appears to have been deleted from many of the rDNA repeats of this fungus. Excision of similar introns has not been observed in other dermatophyte species, and represents a new species-characteristic marker in this taxon. We have also identified four homopolymeric microsatellites in the nontranscribed spacer (NTS) region adjacent to the 25S gene in *T. rubrum* and *T. violaceum*. Variation in these simple repeats is shown to be either species- or strain-characteristic. Intrastrain microsatellite genotypes of random isolates of *T. rubrum* and *T. violaceum* are compared with polymorphisms identified at an unlinked, non-ribosomal poly GT microsatellite locus, and with RFLP patterns generated by a variable sub-repeat element (TRS1) also in the ribosomal NTS region. The utility of microsatellite fingerprinting for epidemiological investigations and for assessing evolutionary diversification in dermatophyte fungi is discussed.

SE 06 Determining genomic divergence and species delineation in *Streptomyces* using AFLP DNA fingerprinting

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fAFLP was evaluated in comparison with DNA:DNA relatedness for high G+C *Streptomyces* using the best represented and defined species groups, the *S. violaceoruber* and *S. albidoflavus* from the International Collaborative Study on Streptomycete Biology (ICSSB) as a model. AFLP data showed close agreement with DNA:DNA relatedness. Within the *S. violaceoruber* or *S. albidoflavus* species groups, strains exhibiting levels of DNA similarity higher than 72% exhibited levels of AFLP similarity higher than 50%. The combined data from AFLP analysis and the DNA:DNA relatedness showed that *S. caesius*, *S. coelescens*, '*S. coelicolor*' A3(2), '*S. coelicolor*' Waksman 3443, *S. humiferus*, *S. lividans*, *S. lividans* 66, *S. violaceolatus*, *S. violaceoruber* and *S. lazureus* showed >60.9% AFLP similarity and >73.3% DNA relatedness were members of the *S. violaceoruber* species. While seven out of the ten *S. albidoflavus* subjective synonyms, namely *S. felleus*, *S. canescens*, *S. odorifer*, *S. albidoflavus*, *S. coelicolor* Müller, *S. limosus* and *S. sampsonii* showed >49.2% AFLP similarity and >85.0% DNA relatedness were classified as members of the *S. albidoflavus* species group. However, three subjective synonyms, *S. rutgersensis* subsp. *rutgersensis*, *S. gougerotii* and *S. Intermedius* showed <68% DNA relatedness to the *S. albidoflavus* species group and represented a separate genus, but may be seen as indicative of closely related species. This finding suggests that AFLP fingerprinting could be used as a supplementary tool to DNA:DNA pairing for determining the taxonomic position and defining bacterial species within the streptomycetes, in the post genomic era.

SE 07 Molecular genotyping of multinational *Corynebacterium pseudotuberculosis* isolates using pulsed-field gel electrophoresis

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Caseous lymphadenitis (CLA) is a chronic, suppurative disease caused by *Corynebacterium pseudotuberculosis*, and is a problem globally. Variation in the clinical manifestation of CLA in different countries prompted speculation regarding biotype diversity, suggesting that there might be a European biotype which was distinctly different to those from other sources. Forty-five sheep, goat and horse isolates of *C. pseudotuberculosis* from the Netherlands, Australia, Canada, Northern Ireland and Eire, were characterized by pulsed-field gel electrophoresis and compared against a previously published panel of United Kingdom isolates. Digestion with *Sfi*I generated 16–18 bands in the 48.5 to 290 kb range, and differentiated eight distinct pulsotypes. While the ovine and caprine strains displayed a high degree of clonality, the equine isolates were distinctly different and could be further differentiated on the basis of their ability to reduce nitrate. With respect to the sheep and goat industries, our finding that the genome of *C. pseudotuberculosis* is highly conserved internationally, suggests that a common approach to disease control measures may be successful.

Authors

Special symposium

Emerging diseases of wildlife and farmed animals

Wildlife infections and emerging diseases

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Recent decades have seen increased interest in infections of wild animals: partly because some of these infections spill over into domestic animal populations; partly because some affect human beings; but also because of increasing recognition that infection and disease can affect wildlife populations themselves, and thereby may contribute to declining biodiversity. Approaches to controlling these infections have developed more slowly, but have benefited from a growth in multidisciplinary approaches to understanding the ecology of infections. Rather than the old knee-jerk reaction of eradicating the wildlife host, vaccination programmes for wildlife and targeted interventions based on predictive modelling of likely spill-over into human or domestic animal populations, have become more common. Huge problems remain, however, in surveillance and in implementing control methods in the field, particularly at the global level. Furthermore, with an increasingly ecological approach to understanding such diseases, has come an appreciation that these infectious agents and the diseases they cause can themselves contribute to biodiversity, raising further potential dilemmas for those designing control programmes.

Education & Training Group session

Virology: is it practical?

Virology teaching: how safe is it?

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Practical virology for students may seem a contradiction in terms. Practical should provide hands-on experience but the Advisory Committee on Dangerous Pathogens places human viruses in Containment Levels which all identify a risk to the laboratory worker of exposure to infection. Health and Safety legislation requires these risks to be assessed and controlled as far as is reasonably practicable. One method of control is to avoid any infection risks and, partly as a consequence, virology practicals are now more likely to be molecular biological procedures such as PCR or immunohistochemical techniques or computer-based exercises.

However, viruses are much more than 'interesting macromolecules'. Student interest in the biology of viruses could more effectively be engaged by practical work with live virus. Safety concerns for practical virology could be addressed by use of a former smallpox vaccine, modified vaccinia Ankara (MVA). This attenuated virus was used without complications for vaccination of more than 120,000 humans, including immunocompromised individuals. MVA is unable to grow productively in many cells of mammalian origin but can be grown in continuous cell lines in the laboratory under conditions of Containment Level 1, that is, without risk of infection.

Physiology, Biochemistry & Molecular Genetics / Cells & Cell Surfaces / Microbial Infection / Education & Training Groups joint session

Bacteriophage evolution, ecology and applications

Phages and virulence

Integration of the filamentous CTX bacteriophage in *Vibrio cholerae* by the host recombinases XerC and XerD

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CTXϕ is a filamentous bacteriophage that encodes cholera toxin, the primary virulence factor of *Vibrio cholerae*. CTXϕ integrates site-specifically into the larger of the two *V. cholerae* chromosomes, rendering non-pathogenic isolates toxigenic. The CTXϕ genome lacks an integrase; instead, integration depends on the host-encoded tyrosine recombinases XerC and XerD. Integration of CTXϕ occurs near the chromosome mid-point within the *dif* site, which is the XerC and XerD substrate for resolution of chromosome dimers. The elements on the bacterial chromosome (*attB*) and the phage genome (*attP*) required for CTXϕ integration have been defined. *attB* is comprised of a binding site for XerC and XerD. *attP* is a comparatively longer sequence that contains two binding sites, each occupied by a XerC/D pair. One of these XerC/D binding sites spans the core recombination region, while the other site is approximately 80 bp away. Although integration occurs at the core XerC/D binding site in *attP*, the second site is required for CTXϕ integration. In vitro cleavage reactions showed that XerC and XerD cleave *attB* and *attP* DNA; however these data suggest additional cellular factors such as DNA replication or Holliday junction resolvases are required for productive integration *in vivo*.