HEALTH PROTECTION RESEARCH UNIT
IN EMERGING AND ZOONOTIC INFECTIONS

NHS
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BOOK OF ABSTRACTS AND PRESENTATION OF KEYNOTE SPEAKERS

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KEYNOTE SPEAKERS

Keynote speakers for the 2016 National Institute for Health Research (NIHR) Health Protection Research Unit (HPRU) in Emerging and Zootnotic Infections (EZI) annual conference, on Zika and Other Emerging Infections: Dr Patricia Sequeira, Dr Alain Kohl and Professor Tom Solomon.

Dr Patricia Sequeira: The FioCuz response to the Zika outbreak

Patricia Sequeira was born in Rio de Janeiro, Brazil, where she studied Biomedical Sciences and obtained a masters degree in Microbiology and Immunology at Federal University of Rio de Janeiro. In 2002, Patricia moved to California to pursue a PhD degree in Infectious Diseases and Immunology at the University of California, Berkeley. Her PhD project focused on pathogenesis of tuberculosis and host immune response to *Mycobacterium tuberculosis* infection. She used cell culture and animal models to better understand the interaction between host and pathogen and the mechanisms of bacterial latency.

After graduating in 2007, Patricia took a post-doctoral position in Singapore, where she joined the Tuberculosis Drug Development team of Novartis Institute for Tropical Diseases. At Novartis, Patricia focused on identifying new drug targets for latent *Mycobacterium tuberculosis.* During her post-doctoral years at Novartis Patricia was involved in exchange programs and participated in laboratory training of technicians in Indonesia and Mozambique for the diagnosis of tuberculosis and identification of multi drug resistant bacterial strains.

In 2010, Patricia returned to Rio de Janeiro and joined the team of Research Scientists of the Reference Laboratory for Dengue, Zika, Chikungunya and Yellow Fever diagnosis at the Oswaldo Cruz Foundation (FIOCRUZ). Patricia is part of the group that first found the presence of Zika virus genome in the amniotic fluid of two foetuses diagnosed with microcephaly via ultrasound. Patricia is one of the leaders of Zika virus research at FIOCRUZ and has actively participated in the country's emergency response to the recent Zika virus outbreak.

Dr Alain Kohl: Zika virus interactions with host cells

Dr Alain Kohl obtained a "Diplom" in Biology from the University of Münster (Germany) and a doctoral degree in Microbiology from the University Paris 7-Denis Diderot (France). He carried out doctoral studies at the Institut Pasteur in Paris, focusing on Rift Valley fever virus. Following this he joined Richard Elliott's group at the University of Glasgow (which later moved to the University of St Andrews) in 2000 to continue my work on bunyaviruses. In 2006 Alain obtained a Wellcome Trust Research Career Development Fellowship to set up a group at the University of Edinburgh, and later also joined the Roslin Institute as Career Track Fellow. In 2011 he joined the MRC-University of Glasgow Centre for Virus Research as MRC Programme Leader.

In recent years Alain has become more involved in virus and mosquito ecology, which involves studies with partners overseas. He currently has overseas collaborations with partners in Brazil on Zika virus (MRC Newton Fund, UK-Brazil Neglected Infectious Diseases Partnership with FIOCRUZ Recife), and Uganda.

Professor Tom Solomon: The Health Protection Research Unit in Emerging and Zoonotic Infections - A Year of Challenges and Successes

Professor Tom Solomon studied medicine at Oxford, did a PhD in Vietnam and postgraduate virology in the United States, before training as a neurologist.

He is Director of the University of Liverpool's Institute of Infection and Global Health, Professor of Neurology at the Walton Centre NHS Foundation Trust and Director of the HPRU in EZI. He studies emerging viral infections, particularly those that affect the brain, has published more than 200 scientific papers, and was awarded the Royal College of Physicians Triennial Moxon medal in 2014.

ORAL ABSTRACTS

1

Did El Niño fuel the 2015-2016 south-American Zika outbreak?

Dr Cyril Caminade

Research Fellow

Vector Biology & Climate Modelling theme

Zika, a mosquito-borne viral disease that emerged in South America in 2015, has been declared a Public Health Emergency of International Concern by the World Health Organisation in February 2016. We developed a climate-driven R_0 mathematical model for the transmission risk of Zika virus (ZIKV) that explicitly includes two key mosquito vector species *Aedes aegypti* and *Aedes albopictus*. The model was parameterized using the most up to date information from the available literature. It was then globally driven by historical gridded temperature and rainfall datasets from the National Oceanic and Atmospheric Administration for the period 1950-2015. Sexual transmission of ZIKV was not considered in our model framework.

We find that ZIKV transmission risk in South America in 2015 was the highest since 1950. The R_0 maximum is related to favouring temperature conditions which caused simulated biting rates to be largest, mortality rates and extrinsic incubation periods to be smallest in 2015. This followed the suspected introduction of Zika virus in Brazil in 2013. In other words, the ZIKV outbreak in Latin America has very likely been fuelled by the 2015-2016 El Niño climate phenomenon affecting the region. The highest transmission risk globally is in South America and tropical countries where *Ae. aegypti* is abundant. Transmission risk is strongly seasonal in temperate regions where *Ae. albopictus* is present, with significant risk of ZIKV transmission in the south-eastern states of the USA, southern China and to a lesser extent over southern Europe during the boreal summer season.

However, some model parameters were approximated using published evidences for dengue virus. To refine our model parameters in future, mosquito infection experiments are currently under way in collaboration with the Liverpool School of Tropical Medicine.

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2

Developing a 'One Health' antibacterial prescription surveillance approach through the use of health informatics

Dr Fernando Sánchez-Vizcaíno

Research Fellow

Risk Assessment of Emerging and Zoonotic Threats theme

It is important to know how widely antibacterials are being used across both human and animal health. However, tools for integrating datasources contributed to by both human and veterinary healthcare have not been developed yet, nor has the extent to which small animals contribute to zoonotic antimicrobial resistant transmission to humans been investigated. The objective of this study is to demonstrate the feasibility of a novel "One Health Informatics" approach for comparing the antibacterials prescribing practices in human and small animals healthcare settings through the use of electronic health records (EHRs) obtained from a UK sentinel network of medical and veterinary practices.

Medical data were collected through NHS Liverpool Clinical Commissioning Group facilitation, from 26 general medical practices in Liverpool between June 2014 and May 2016. EHRs included patient information such as sex, age, residence, antibiotics prescribed and the consultation coding for both respiratory disease and gastrointestinal disease consultations. Veterinary data were gathered electronically in real-time by SAVSNET, the Small Animal Veterinary Surveillance Network, from 458 veterinary premises throughout the UK between April 2014 and March 2016. Each record included the animal signalment (including species, breed, sex, age, etc.), owner's postcode, syndrome information and treatments including antibiotics.

EHR were obtained from 4,121,340 (n= 157,274 patients) human consultations and 918,333 (n=413,870 dogs) canine and 352,730 (n=200,541 cats) feline veterinary consultations. In humans, total antibacterial-prescribing percentage of consultations was less common (4.51%, 95%CI: 4.49-4.53) than in dogs (18.8%, 18.2-19.4) and than in cats (17.5%, 16.9-18.1).

To understand these differences in prescribing between human and small animals healthcare settings it will be important to assess the different types of patient seen in medical and veterinary practice. These preliminary results demonstrate the feasibility of 'One Health' antibacterial prescription surveillance in a UK sentinel network of medical and veterinary practices.

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3

Metagenomic viral sequencing for non-targeted pathogen identification utilizing the MinION

Liana Kafetzopoulou

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Pathogen Discovery & Characterisation theme

Background

Viral pathogen identification and discovery is of significant importance to clinical virology and public health. The improvement of diagnostic methods for rare and emerging pathogens, both in speed and sensitivity, will provide an important advantage for disease control. Rapid and unbiased diagnostic methods are vital when developing a strategy for treatment and eradication of an emerging pathogen; more importantly in the containment of an outbreak. Advances in the field of sequencing over the last decade have allowed for pathogen identification without prior knowledge, introducing its potential and importance in diagnostic approaches. The development of the MinION, a portable palm-sized sequencing device run via a laptop computer has already introduced sequencing in real time in the field and overcome many limitations of its predecessors. We aim to develop a pipeline for non-targeted pathogen identification using a metagenomic approach.

Methods

The initial aim is to assess the feasibility and sensitivity of direct metagenomics on clinical samples both for detection and genome sequencing using the MinION. To achieve this, a range of clinical samples will be subject to metagenomic sequencing. The limitations and bottlenecks identified will help develop and optimise a pipeline that is currently being developed using a model system of Hazara virus spiked into human serum. Additionally this model system will be used to investigate various methods of background depletion and/or pathogen enrichment.

Results/Conclusions

We have demonstrated that direct metagenomic sequencing of clinical serum samples can elucidate full viral genomes from both Chikungunya and Dengue virus. Work to establish the cut-off limits for the current approach is underway. Using the Hazara virus spiked serum model we have also assessed the enrichment potential of numerous methods upon the viral:host RNA ratio. The most efficient approach identified for enrichment will be taken forward for further testing on clinical samples.

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4

Serum plasma proteins associated with acute Hantavirus infection in a pregnant woman

Sarah Bar-Yaacov

PhD Student

Clinical Surveillance theme

Hantaviruses form a group within the Bunyavirus genus. Rodent-borne hantaviruses cause two closely related syndromes in humans; haemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS). The endemic presence of hantavirus in the UK was confirmed for the first time in 2012 following investigation of 2 cases of acute kidney injury, a common complication of HFRS. In 2013 Public Health England launched a serosurveillance study that identified pet rat owners as a high risk group for exposure to hantavirus infection.

In July 2015 a female patient was hospitalised with acute kidney injury and diagnosed with hantavirus infection based on seroconversion. 4 serum samples were taken on sequential days of her hospitalisation. In addition serum was taken approximately 3 weeks after the patient was discharged, and a hanta-seronegative sample had been obtained 3 months beforehand for unrelated purposes. The patient was pregnant before, during and after illness. Label-free proteomics was used to investigate temporal changes in serum protein abundance during acute disease and convalescence using the seronegative specimen as a baseline comparator. The longitudinal data were compared to an analysis of serum proteomes derived from another cohort of 10 male and not-detectably pregnant female individuals. In this cohort, 1 patient had acute hantavirus infection at the time of sampling, 4 were seropositive for hantavirus antibodies indicating past resolved infections and 5 were seronegative. From the longitudinal data 5 protein clusters following distinct patterns of temporal abundance change were identified. Functional analysis revealed these clusters to contain proteins related to pregnancy, an activated immune response, oxidative stress and consumption of clotting factors. 11 proteins with elevated abundance during acute disease compared to seronegative and convalescence in the longitudinal study were shown to follow the same trend of abundance change in the non-pregnant cohort, marking them potential biomarker candidates for hantavirus infection.

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5

Characterisation of the increasing numbers of hepatitis E infections in England and Wales 2010-2015

Dr Clarissa Oeser

Research Fellow

Risk Assessment of Emerging and Zoonotic Threats theme

Hepatitis E virus (HEV), previously only known for causing acute infections in travellers returning from hyper-endemic countries, is now increasingly recognized as an emerging problem in industrialized countries. Autochthonous, food –borne transmission of HEV genotype 3 (GT3), although asymptomatic in most cases, can cause mild and self-limiting to severe acute hepatitis and also chronic hepatitis in immunocompromised patients. Enhanced surveillance of cases of acute HEV infections in England and Wales was established in 2005 to characterise non-travel associated cases of HEV and to identify potential risk factors. Health Protection Teams report and complete an enhanced surveillance questionnaire on each new case. Two Public Health England Reference laboratories undertake the majority of primary diagnostic testing for HEV in England and Wales. For case ascertainment, cases confirmed in these centres are compared with cases reported to the Second Generation Surveillance System (SGSS, a voluntary electronic reporting database for clinically significant pathogens by NHS hospital laboratory departments). Since 2010, a year on year increase of cases of acute HEV infection in humans has been reported in England and Wales and the number of HEV cases has tripled from 2010 to 2015. The majority (>70%) of cases of HEV GT3 infections are male, with a median age of > 60 years. As previously reported, the emergence of a novel group of the virus; HEV GT3 group 2 appears to be responsible for a large part of the increase in indigenous cases, causing >70% of all new cases. The increasing rates of HEV across England and Wales remain a concern, particularly as the natural history of this disease continues to be unclear. The emergence of a new phylotype largely responsible for the increase in cases warrants further research.

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6

The challenge of Zika virus diagnosis after acute infection - anti-Zika virus NS1 antibody ELISAs exhibit poor accuracy in Brazilian patients

Raquel Medialdea Carrera

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Clinical Surveillance theme

Zika virus (ZIKV) diagnostics have been an important public health challenge during this current outbreak. Accurate results are essential to guide appropriate clinical management particularly because among pregnant women, ZIKV can cause congenital disease. A false positive diagnosis may conduce to dreadful consequences, such as abortion. The WHO recommends serological testing for patients presenting seven or more days after disease onset (when ZIKV PCR becomes less sensitive). However, serological assays exhibit cross-reactivity against other flaviviruses.

The Euroimmun IgG and IgM ZIKV NS1 ELISAs were the first commercial serological diagnostics approved by the National Health Surveillance Agency of Brazil. In Brazil it has also been widely used the US Centers for Disease Control and Prevention (CDC) emergency use authorization protocol for IgM ZIKV ELISA testing. We report assay sensitivity and specificity among a Brazilian population.

Samples were referred for routine diagnostics to the reference Flavivirus laboratory at the Institute Oswaldo Cruz, Rio de Janeiro. Sensitivity was measured using paired sera collected in 2015-2016 from 57 ZIKV PCR positive cases. Specificity was assessed using sera from subjects with confirmed (PCR and IgM positive) exposure to Dengue (serotypes 1-4 [n=89]), yellow fever (n=19); collected in or before 2013 to exclude ZIKV exposure. ZIKV is estimated to have arrived in Rio in January 2015.[4]

The Euroimmun ZIKV ELISAs and the IgM ZIKV CDC ELISA exhibited poor accuracy among the Brazilian samples. The low IgG specificity is likely to reflect the local population's repeated and longer-term exposure to dengue and/or yellow-fever. The low IgM sensitivity may indicate that our local patients had experienced primary infection with another flavivirus.

Our findings exhibit the relevance of diagnostic assay evaluation using samples appropriate to the local population and emphazises the need for more accurate diagnostics, especially for ZIKV patients presenting after acute infection.

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7

National Leptospira Service improvements, introduction of typing and species identification direct on Clinical Specimens

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Leptospirosis is a worldwide zoonotic disease caused by Pathogenic *Leptospira*. In the UK, Leptospirosis disease and surveillance depends exclusively on laboratory data. Culture and Microscopic Agglutination Test (MAT) are the gold standard methods for detection of infection. Traditional *Leptospira* species identification requires an isolate, however culture is time-consuming taking several weeks and requires significant laboratory expertise to visualise live Leptospires, retain contaminant free cultures and determine the serovar and species type. PHE have recently made several improvements to the national Leptospira service including co-location of PCR testing with the imported fever service and relocation of reference testing to the Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU). The RVPBRU have recently developed a nested MLST method for use directly on clinical specimens which will allow for the first time the direct identification of the infecting species and simultaneous typing of pathogenic Leptospires without the requirement for prior culture. This enables rapid acquisition of typing data from patients that would not have been possible using traditional methodologies. Using these methods four hundred historical clinical DNA extracts from the UK specimens that were submitted to the *Leptospira* Reference Unit from 2012 to 2014 underwent 16s qPCR testing. qPCR confirmed the presence of pathogenic *Leptospira* species in 55/400 (13.8%) extracts. Historical clinical data for 23 samples was analysed. The panel tested included DNA extracts from serum and plasma. 17.4% were EIA positive and 8.7% were MAT positive or indeterminate. The reasons for referral ranged from occupational exposure to holiday acquired infection and common clinical symptoms included: flu like symptoms, fever, and kidney or liver symptoms. The DNA extracts are undergoing MLST to determine the prevalence of the species of *Leptospira* and MLST profiles of strains infecting patients in the UK from 2012 to 2014.

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8

Neurological disease associated with Zika infection

Dr Mark Ellul

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During 2015-16 Brazil experienced the largest epidemic of Zika virus ever reported. This arthropod-borne virus (arbovirus) has been linked to microcephaly and other congenital malformations in neonates and Guillain-Barré syndrome (GBS) in adults but other neurological associations are unclear. The risk factors leading to the development of these complications in some patients, but not others, are also unknown.

It is suspected that Zika virus may be associated with a wider range of neurological manifestations than previously recognized, for example preliminary reports have suggested an association with central nervous system disorders such as meningoencephalitis and myelitis.

We are currently undertaking a prospective case control study in Brazil to elucidate the spectrum of neurological disease associated with Zika infection, as well as risk factors for the development of complications such as co-infection with other circulating viruses. We will review the evidence of an association between Zika infection and neurological complications, both due to direct infection and postinfectious immune-mediated disease, and outline our current research strategy to study these neurological complications in adults in Brazil.

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9

Vector competence of British mosquitoes for ZIKV and other arboviruses

Dr Marcus Blagrove

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Vector Biology & Climate Modelling theme

Background
To date there has been no evidence of mosquito-borne virus transmission of public health concern in the UK, despite the occurrence of more than 30 species of mosquito, including putative vectors of arboviruses.

Methods
We assessed the competence of British mosquitoes for major arboviruses: dengue virus (DENV), chikungunya virus (CHIKV), West Nile virus (WNV), Japanese encephalitis virus (JEV) and Zika virus (ZIKV) using adult mosquitoes reared from wild, field-obtained immatures.

Results
We have so far demonstrated laboratory competence of Aedes detritus, Culex pipiens and Cx. modestus for JEV, and Ae. detritus, Cx. Pipiens, Cx. Modestus and Culiseta annulata for WNV. By contrast, there was no evidence of laboratory competence of Ae. detritus for either DENV or CHIKV. Results for ZIKV are pending with Ae. detritus, Cx. pipiens and Italian Ae. albopictus; results will be available in time for the conference…

Conclusions
To our knowledge, this is the first study to demonstrate competence of a UK mosquito for WNV and builds on our previous evidence that British mosquitoes are capable of transmitting JEV. We therefore confirm that British mosquitoes may present a potential risk for arbovirus transmission in the UK.

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10

Differential gene expression analysis of acute Ebola virus patients with fatal or non-fatal infections

Natasha Rickett

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Pathogen Discovery & Characterisation theme

The recent outbreak of *Zaire ebolavirus* (EBOV) in West Africa was unprecedented in scale and has sparked a great deal of interest into the pathogen. EBOV is the most pathogenic species in the family *Filoviridae*. Early symptoms are non-specific and flu-like, but these rapidly dissolve into systemic issues, frequently resulting in death. Fatal infections are associated with uncontrolled inflammation while survivors have an initial robust antiviral response followed by an effective antigen-specific response. However, there are a myriad of other factors influencing patient outcome, including viral load and co-infections. The elucidation of these factors could have major therapeutic implications.

RNA-sequencing was performed on 179 outbreak patient samples in order to assess which genes, if any, are differentially expressed in fatal verses non-fatal infections.

Here we demonstrate that higher viral load and malaria co-infection are correlated with poor patient outcome. The RNA-seq data allowed us to explore various avenues of research. One such path was the comparison of malaria testing methodology – i.e. co-infection confirmed by RNA-seq or rapid diagnostic test (RDT). The RDT was employed in the field, however, our RNA-seq data suggests this may have led to under-reporting of *Plasmodium spp*. Furthermore, there are a number of genes that are differentially expressed according to patient outcome – e.g. those associated with blood clotting and complement regulation. Our RNA-seq data allowed the employment of a machine learning technique in order to create a genetic profile for an average survivor. With the expression levels of ten determined genes we are able to predict patient outcome with 85% accuracy.

The identification of these differentially expressed genes could illuminate some of the factors influencing patient outcome. This, along with the formation of a "survivor profile", could have clinical implications in the future, both in terms of novel therapeutics and allocation of hospital resources.

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11

CD8+ T cell cross-reactivity between Zika virus and other flaviviruses

Dr Lance Turtle

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Invited HRPU Member

Zika virus (ZIKV) has recently emerged to cause large outbreaks in South America. ZIKV is a member of the genus *Flavivirus*, family *Flaviviridae* so is related to dengue virus (DENV), Japanese encephalitis (JE) virus (JEV) and yellow fever virus (YFV). Immune responses to flaviviruses are notoriously cross-reactive, with both beneficial and detrimental effects. In the case of DENV, cross-reactive T cells have been associated with both protection and pathology, but cross-reactive T cell responses between other flaviviruses have been relatively less studied.

We have recently identified several T cell epitopes of JEV using synthetic peptides to test subjects with natural JEV exposure. Highly flavivirus cross-reactive CD8+ T cell responses were associated with asymptomatic exposure to JEV.

Methods

Bioinformatic approaches (Clustal alignments of flavivirus polyproteins, BLAST and T cell epitope prediction programs) were used to assess the potential immunogenicity of previously identified T cell epitopes in ZIKV (and other flaviviruses). In selected cases cross-reactivity was tested using synthetic peptides of ZIKV corresponding to T cell epitopes previously identified in JEV and DENV by intracellular cytokine staining/flow cytometry.

Results

In our previous work, CD8+ T cell epitopes were concentrated in regions of the JEV polyprotein that are highly conserved between flaviviruses. Several of these epitopes are identical in ZIKV and are expected to be immunogenic. A number of other epitopes with limited amino acid differences (1-2) have also been identified. Preliminary proof-of-concept experimental data suggest that these epitopes are recognised.

Conclusions

CD8+ T cell responses are highly likely to be cross-reactive between ZIKV and other flaviviruses. The clinical significance of this is unknown at present, and requires testing in prospective studies. Given the disappointment of recent dengue vaccine trials, inclusion of T cell epitopes in flaviviruses vaccines is worthy of consideration.

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POSTER ABSTRACTS

1

Profiling the gene expression signatures of cells infected with a pathogenic ebolavirus

Andrew Bosworth

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Pathogen Discovery & Characterisation theme

Human disease caused by Ebola virus is characterized by high mortality rate, severe symptoms and some cases of haemorrhagic manifestations. Reston virus is also a member of the ebolavirus family, however unlike all other ebolaviruses Reston does not cause observable human disease. A possible reason for this could be differences in the way Ebola and Reston viruses interact with the host cell during infection. To investigate this hypothesis, human model cell lines were infected with the recently isolated Ebola virus variant circulating in West Africa or the non-pathogenic Reston virus. Using high resolution RNASeq and SILAC-Proteomic methods the cell biology changes induced by infection with these viruses was profiled and compared. Here we show patterns of gene expression and signaling pathway activity induced by infection with these two fundamentally different viruses.

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2

Quantitative analysis of the feeding and attraction behaviour of UK mosquito populations

Aislinn Currie-Jordan

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Vector Biology & Climate Modelling theme

Mosquito-borne viruses pose a global threat with increasing numbers of cases being recorded in Europe. Several mosquito species in the United Kingdom have the potential to act as vectors for viruses such as West Nile and Japanese encephalitis. However, despite the risk posed, our knowledge of their behaviour and feeding preferences is limited. Gaining such knowledge will improve our ability to quantify the risk posed to the UK by these mosquito-borne viruses. Additionally, it will provide important knowledge that could be used to develop appropriate control strategies against these mosquitoes. A combination of field and laboratory studies will be conducted with the aim of improving our understanding of the feeding and attraction behaviour of UK mosquito species.

A longitudinal study is currently underway to determine the seasonal abundance of mosquito species in the Wirral, UK. Six key sites have been selected and Mosquito Magnet traps are being used to collect adult mosquitoes. Traps are run for 72 hours each week, adults collected and returned to the laboratory for identification. Temperature and humidity are also being recorded.

Electric grids are a valuable research tool enabling a range of different behaviours to be observed: attraction to baits, alighting, feeding behaviour and interactions with traps. The standard design of E-grids is for use with tsetse flies. Laboratory studies have therefore been conducted to ensure their design is appropriate for use with mosquitoes. The E-grids will be used in a trap comparison study comparing the efficacy of different mosquito sampling methods. Catches from Mosquito Magnets, CDC light traps and BG sentinel traps will be compared to ascertain which method is most suited for monitoring UK mosquito species. In addition, PCR-based methods will be used to identify blood meals from field-collected mosquitoes. These results will give an indication of host preference of UK mosquito species.

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3

Overview of year one project aims for the assessment of the risk to the UK human population from tick-borne viruses

Maya Holding

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Vector Biology & Climate Modelling theme

The distribution of tick species and tick borne viruses varies across Europe and is continuously changing. In recent years, medically important viruses such as tick borne encephalitis virus (TBEV) and Crimean Congo hemorrhagic fever virus (CCHFV) have now been newly detected in countries. This project will explore a number of facets aiming to investigate the presence and prevalence of key tick borne viruses currently in the UK and explore the potential for new tick species and tick borne viruses to enter the country.

Louping ill virus (LIV) is also the only tick borne virus in the UK known to cause disease in vertebrates. While LIV is endemic in the UK, the closely related TBEV seems to be restricted to Scandinavia and Central and Western European countries. This study aims to develop an understanding of the incidence of LIV in questing ticks within a wide variety of habitats, across areas of the south west of England, with sampled ticks additionally being tested for the presence of TBEV. Preliminary Year 1 survey work has been conducted, collecting ticks in both the Spring and Autumn period. Virus testing will take place once essential seasonal field work has been completed

Migratory birds could be a potential source of both tick and pathogen transmission into the UK. Potential tick borne viruses that could be transported to the UK by ticks on migratory birds include TBEV and CCHFV. The second main focus of Year 1 work is on the study of ticks which have been imported on migratory birds. This will include a screening programme for the presence of viruses in these ticks. *Ixodes ricinus* collected from autumn migratory birds will be tested for TBEV and *Hyalomma marginatum* imported by spring migrants will be tested for (CCHFV).

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4

Host derived markers of Lyme disease and their diagnostic potential

Greg Joyner

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Clinical Surveillance theme

Lyme disease (LD) or Lyme borreliosis (LB), caused by *Borellia burgdorferi* (*B.b*), is the most commonly reported tick-borne disease in Europe and the United States. Human cases have increased steadily in the UK in recent years with around 1000 laboratory-confirmed cases being reported annually since 2007[1]. LD can be diagnosed based only on clinical presentation such as the presence of an erythema migrans (EM) rash and a recent history of tick-bite; therefore, the true incidence of the disease in the UK is unknown.

Serology tests are the mainstay of laboratory diagnosis for Lyme disease. In the UK, LD diagnostic testing at the PHE specialist Lyme referral lab at Porton Down, follows the internationally-accepted 2-tier serological approach. It is acknowledged that serological testing is less effective in diagnosing the earliest stages of LD when the level of circulating antibodies to borrelial antigens is low.

The aim of this study is to apply mass spectrometry-based proteomics to identify serum biomarker signatures that differ between patients with acute Lyme disease and (1) normal, healthy controls and (2) patients with other related diseases. A minimal biomarker panel will be identified. Study samples are currently being prepared for mass spectrometry. This process includes serum depletion, a technology that reduces the protein dynamic range of serum allowing low abundance proteins to be detected.

Primary Objectives

- To identify serum biomarkers that are differentially expressed in acute seropositive Lyme borreliosis compared to normal, healthy controls and patients with other related diseases (syphilis, leptospirosis and chronic fatigue syndrome).
- To define a minimum set of differentially expressed serum biomarkers that theoretically distinguishes acute Lyme borreliosis from normal, healthy controls and patients with other related diseases.
- To screen sera from control groups (normal healthy controls and disease controls) for Lyme seroreactivity. Sufficient samples will be screened for this discovery study and the follow-on validation study.
- From serological screening of normal human donor sera, obtain a preliminary estimate of the seroprevalence of Lyme borreliosis in the NHSBT donor population.

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5

Gene signatures mark survival from the 2014-2015 Ebola virus outbreak

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Pathogen Discovery & Characterisation theme

In 2014 Western Africa experienced an unanticipated explosion of infections with Ebola virus (EBOV). This highly fatal disease afflicted more than 28,000 people and killed an estimated 11,000. The outlook for patients infected with EBOV is bleak. Approximately half will die and those who survive are at risk for long-term health consequences. What discriminates fatal from non-fatal outcomes is unknown. Here we use transcriptome data from infected and convalescent patients to identify host factors that are associated with patients that survived or succumbed to disease. Our data show that individuals who succumb to the disease show stronger upregulation of interferon signalling and acute phase responses compared to survivors during acute infection. Using genes that showed differential expression when fatal cases were compared to survivors we were able to identify a small set of genes that acted as a strong predictor of survival.

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6

Determining the viral life cycle of Enterovirus 71 Infection in Human Rhabdomyosar-coma Cells

George Lock

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Clinical Surveillance theme

Enterovirus 71 (EV71) of the *Picornaviridae* family is the leading cause of hand, foot and mouth disease (HFMD) globally and coined as the next pathogen to supersede poliovirus after its eradication. Virus replication occurs in 4 distinct stages; eclipse, latent, maturation and release. The timings at which these occur vary greatly amongst viruses. One-step growth curves allow each stage of the virus life cycle to be determined.

Cells were infected at MOI's 1, 5 and 10 with samples taken at every six hours for 36 hours. The supernatant was aspirated and saved for subsequent virus titration and the cells were lysed and supernatant recovered for virus western blot analysis. Immunofluorescence (IF) was used to enable visualization of virus replication inside the cell at each time point in infection.

At 2 hours post infection, no virus protein could be visualized within the cells via IF or noted in western blot, indicating that the virus was in the eclipse phase of replication. At 6 hours post infection, virus protein could be visualized in the cells via IF with minimal virus protein detected in western blot. A low virus titre was released from the cells. This indicates that a small amount of virus is released at 6 hours post infection (p.i.) and any virus released at a later dated could be a result of progeny virus infecting neighbouring cells. Hours 18 to 24 p.i. show an exponential increase in the virus released from the cells. Hour 24 p.i. showed lower concentrations of virus protein in western blot and fewer infected cells in IF. This is most likely due to a mass release of the mature virions from the cells. Hours 30 and 36 p.i. the virus titre of the supernatant plateaus with all cells having been infected and lysed halting virus production.

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7

The influence of climate and ecological variables on *Ixodes ricinus* activity in England and Wales

Liz McGinley

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Vector Biology & Climate Modelling theme

The Sheep tick (*Ixodes ricinus*) is the primary vector of *Borrelia burgdorferi s.l.*, the causative bacterial agent of Lyme borreliosis in Europe. Lyme borreliosis is thought to be the most common vector borne disease of humans in the temperate Northern hemisphere but this tick is also known to act as a vector for several pathogens of human and veterinary significance, including the bacterial pathogen; *Anaplasma phagocytophilum*, protozoan parasite; *Babesia divergens*, and viral pathogen; Tick Borne Encephalitis virus. Its feeding behavior and three stage life cycle help facilitate the transfer of pathogens to and between its hosts - however a range of complex ecological, climate and land-use factors determine the prevalence of infection within tick populations as well as human exposure to tick bites

This specific tick species is believed to have a wide distribution across the UK and anecdotal evidence suggests that its range is expanding. Such expansion could have serious implications for human and veterinary health in relation to tick-borne disease. Indeed, advanced surveillance over the past decade has highlighted an increase in the number of reported cases of Lyme borreliosis in the UK.

My PhD research is focused on understanding the dynamics driving *I. ricinus* activity – its seasonal patterns, spatial distribution and how climate variables influence its host seeking activity. By monitoring *I. ricinus* activity and related environmental variables at multiple sites across England and Wales, I hope to improve our understanding of factors which influence its seasonality – allowing for the development of robust models for human tick bite risk. In addition I hope to investigate the prevalence of *Borrelia burgdorferi s.l.* in spatially and temporally distinct *I. ricinus* specimens which have been collected over the course of this project.

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8

Country-specific risk of infection with chikungunya and dengue among travellers returning to the United Kingdom

Margaux Mesle

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Epidemiological Approaches theme

International travel plays a key role in the large-scale spread of many novel and emerging infectious diseases (NEIDs), such as MERS, Ebola, and pandemic influenza. Assessing the public health risk posed by international travel is an important aspect of preparedness and response planning to NEIDs. Improving the understanding the range of risks related to travellers' behaviour may, therefore, increase accuracy in predictions of future importation risks. Here, we estimate the relative risk of infection by two different vector-borne viral pathogens among travellers returning to the United Kingdom.

We used information on returning UK travellers diagnosed with dengue or chikungunya between 2010 and 2014 in combination with contemporary detailed passenger itinerary information. Incidence records for destination countries were gathered to estimate country-specific prevalence. We modelled the annual number of imported cases as a Poisson random variable with the mean given by a function of within country prevalence, the number of passenger bookings, and a country-level fixed effect. Separate analyses were performed for dengue and chikungunya cases.

After accounting for annual variation in country-specific prevalence, we found significant differences in the risk of infection associated with visiting different countries and regions. The Caribbean, and more specifically Barbados, was associated with the lowest region and country-level risk. Little variations were seen in the region-level risks between the viruses.

While we cannot exclude country-level effects resulting systematic reporting biases, country-level similarities for two biologically distinct infections suggests our analysis may provide insight into the risk behaviour of travellers visiting different countries. We anticipate that this information will be useful in parameterising future models of importation risk, and may serve to improve remote surveillance of emerging outbreaks through monitoring of cases among returning travellers.

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9

A mechanistic climate-driven model for *Aedes albopictus* and dengue in Europe

Soeren Metelmann

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Vector Biology & Climate Modelling theme

Dengue is a vector-borne viral disease that affects several hundred million people each year. While most people get infected in tropical regions, autochthonous transmissions of dengue viruses have recently been reported in European countries such as Croatia and France. *Aedes albopictus*, an invasive and competent vector originating from South East Asia, has adapted to colder and more temperate climate conditions and has widely spread in Europe since its introduction in Italy in 1990. Still, most of the modelling effort has been carried out based on assumptions related to the mosquito's original tropical strain. Here we present a compartmental, climate-driven model for *Ae. albopictus* which allows to explore both its potential biological envelope and its capability to transmit the dengue virus in Europe. The model explicitly considers the mosquito's adaptation to temperate climates such as a diapausing stage and is driven by gridded daily climate data for Europe (*E-OBS*) and particularly the UK (*UKCP09*). Findings suggest that *Ae. albopictus* might spread to regions where it has not yet been observed, especially northward. While some parts of southern England might be suitable for the establishment of this mosquito species, the environmental conditions do not appear to be yet suitable for dengue viral transmission. However, this might change in the future, with expected warmer temperature conditions due to global climate change. If these findings prove true, this potential threat is not solely an issue for the dengue virus but also for other viruses such as Zika and chikungunya.

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10

Elucidation of the cellular interactome of the EBOV RNA dependent RNA polymerase

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Pathogen Discovery & Characterisation theme

With a fatality rate up to 90%, the 2014 Ebola virus (EBOV) outbreak in West Africa highlighted the absence of an available treatment and an effective vaccine. Little is known about what the mechanisms that control the cell fate in EBOV-infected cells are. By gaining knowledge on the interactions between the host and the virus proteins, the finding of small molecule inhibitors able to block them and prevent EBOV from using the host working machinery for its own benefit might be feasible.
This project focuses on the study of the host proteins that play a significant role in the replication and transcription of EBOV by using a proteomic approach to elucidate the cellular interactome of one of the nucleoprotein complex proteins, the viral RNA polymerase.

A mini replicon system is used in this study to mimic the replication of EBOV to be able to work in a BSL-2 lab. So far, the minigenome and support plasmids of the mini replicon system have been made. The construction encoding the recombinant protein L-mCherry has been expressed in BRST7 cells only when VP35 is cotransfected showing functionality but less efficiency that the wild type L.

The next step will be the immune-precipitation of the recombinant protein in order to get pull-downs of the complexes “L-host proteins” and analyse the interactions by mass spectrometry. A bioinformatics analysis will be carried out on the statistically significant protein-protein interactions and they will be validated by western blot. The use of this proteomic approach will generate the cellular interactome of the EBOV RNA dependent RNA polymerase.

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11

Detection of a hantavirus, Tatenale virus, in field voles from North Wales during hantavirus surveillance

Ellen Murphy

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Risk Assessment of Emerging and Zoonotic Threats theme

Hantaviruses are a genus of *Bunyaviridae*, which include rodent viruses that are known to cause mild to severe illness in humans, making them a significant concern to public health. In the UK, there are two hantaviruses which have been reported in wild rodents; namely Seoul virus (SEOV), which was detected in 2 wild Brown rats (*Rattus Norvegicus*) in Yorkshire in 2012 and Tatenale virus (TATV) which was detected in a single field vole (*Microtus Agrestis*) in Cheshire in 2013. In the UK, the field vole is believed to be the most abundant mammalian wildlife species, with an estimated population of 75 million. To further understand the prevalence of TATV in UK voles, lung and kidney material from 23 field voles from 3 different sites across the UK were screened using a pan hantavirus nested RT-PCR (targeted at the polymerase (L) gene). Two field voles from a site in North Wales were shown to be positive for hantavirus RNA. Sanger sequencing of the partial L gene amplicon confirmed the presence of TATV RNA. Material from a further three voles from the same site were also shown to be positive by RT-PCR and sequencing is currently under way. The repeated detection of this virus in a field vole population, which is geographically distinct from the original 2013 site, indicates that this hantavirus could be more widespread throughout the UK field vole population than previously thought. There is little information known about TATV in terms of its zoonotic potential and pathogenicity in humans, so further research is needed to accurately determine if it represents a public health risk.

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12

Characterising Post Ebola Syndrome, Sierra Leone

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Background: The Ebola Virus Disease (EVD) outbreak in West Africa (2014-16) left over 17000 survivors. Many survivors had medical complaints that continued after discharge or started soon after.

Methods: Presenting complaints from an entire cohort of survivors from a single Ebola Treatment Unit at MH34, were collated (1). This informed development of a documentation system for clinical encounters. Using this data, patients were recruited to Ophthalmology, Neurology, Psychiatry and Disability studies.

Results: Over 500 patients have attended. Three weeks after discharge, the main complaints were musculoskeletal pain (70%), headache (48%) and ocular problems (14%) (44 patients). Patients with headache had lower admission plasma PCR cycle threshold values (higher plasma viral load) than those without headache ($p<0.03$).

Ocular problems in 81 EVD survivors and 106 unaffected controls have been investigated. A range of retinal lesions were observed including one that is more common in EVD survivors 14.8% of cases, and not seen in controls (OR=38.2, 95% CI 2.2-657.6, $p<0.01$). White cataracts were also more common in EVD survivors 7.4% vs 0% (OR=18.3 95% CI 1.0-330.5 $P<0{\cdot}05$). Aqueous fluid from two EVD survivors with cataract and no anterior chamber inflammation were PCR negative for Ebola virus. (2)

Nineteen patients with neurological complaints underwent specialist evaluation at a neuro-psychiatric clinic. Headache was the most common complaint 47% (9/19). 29% (5/17) had abnormal cranial computer tomography. 63% (12/19) had psychiatric symptoms requiring mental health follow-up. 26% (5/19) met criteria for mental disorder, most commonly Major Depressive Disorder. No significant cognitive deficit was found. Median WHODAS score was 8.33% (0-89%). Patients attributed their disabilities to physical and psychological causes.

Discussion: A pragmatic approach has provided a timely response to the needs of survivors of the outbreak for modest cost. The project has provided clinical care, successful research studies, and strengthened research capacity.

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13

Temporal and spatial surveillance of ticks in the UK using companion animal electronic health records

John Tulloch

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Epidemiological Approaches theme

Ticks represent a large reservoir of zoonotic disease and are a source of increasing public concern. We propose that surveillance of electronic health records (EHRs) of companion animals, via the Small Animal Surveillance Network (SAVSNET), could provide a method of describing tick activity temporally and spatially across the UK.

EHRs from volunteer veterinary premises were collected in real time from 30th March 2014 to 29th May 2016. EHR's identified via free text analysis, were read by domain experts identifying consults where a tick was visually confirmed by a veterinary surgeon or nurse on the animal during the consult.

The weekly rate of tick identification was 15.28 ticks identified per 10,000 consults (weekly minimum and maximum of 0 and 52.2). The peak tick activity each year was reported in June, with lowest levels in January. Dogs matched this temporal pattern, with mean tick activity of 14.84 (minimum 0, maximum 65.5). Cats followed a different temporal pattern, with a large peak in May and a secondary smaller peak in the autumn, and lowest levels in February. Cat's had a mean tick activity of 18.25 (minimum 0, maximum 87.2). The relative risk of a dog presenting with a tick compared to a cat was 0.73 (95% CI: 0.67-0.80, $p<0.005$).

Our data confirms that the southern central counties of England are tick activity hotspots, but also suggest that North and Mid-Wales, Northern counties of England and Southern counties of Scotland are tick activity hotspots. Throughout most of the UK ticks were still being found on companion animals during the middle of winter.

We believe that these results could be utilised to form the basis of risk models of tick borne diseases (TBDs) of humans and animals. We anticipate that our results from SAVSNET will help inform the communication of public health messages and increase the awareness of ticks and TBDs in the general population.

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14

Does network centrality indicate the risk of transmitting and contracting infectious diseases, and how does this differ according to pathogen transmission routes?

Dr Maya Wardeh

Postdoctoral Researcher

Risk Assessment of Emerging and Zoonotic Threats theme

We focused on 47 mammalian and avian hosts, including humans and animals commonly used in Europe as food or kept as pets. We identified 157 pathogens which cause significant clinical disease in these species, and determined possible transmission routes for each pathogen. Interactions between hosts and pathogens were automatically mined from the NCBI Taxonomy database, the NCBI Nucleotide database, the NCBI Medical Subject Headings library and PubMed. We interpreted hosts as those connected via the pathogens they share. We applied network analysis tools to investigate the risk of humans contracting diseases from their domesticated animals via each of the identified transmission routes. In each network, hosts were linked if they shared at least one of the high impact pathogens. Links were weighted by the number of pathogens shared as well as the Cohen-Kappa agreement measure. We studied the characteristics and architecture of each transmission route network, and used a suite of eleven centrality measures to identify the most central hosts. We investigated whether central hosts are at greatest risk of infection. Subsequent to this, do they also pose the highest risk of transmitting disease in each of the networks? Finally, we expanded the analyses to include all vertebrates and explored whether high centrality hosts identified in the initial networks, maintained similar levels of centrality in the expanded networks.

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15

Characterisation of the Public Heath England Lyme Borreliosis Service sample dataset from 2012-2016 and evaluation of its potential for undertaking predictive analytics

Dr Jenny Warner

Postdoctoral Researcher

Clinical Surveillance theme

Clinical data mining is a growing research area which aims to look for additional knowledge contained within patterns of data recorded in clinical databases, in order to improve patient outcomes. In Lyme disease, there are a number of different clinical outcomes of disease, varying in severity, for which there may be predictors hidden within the clinical data.

The Rare & Imported Pathogens Laboratory (RIPL) at Public Health England provides diagnostic testing for Lyme disease to the NHS. RIPL took over the service in 2012 and now holds 4 full years of Lyme disease sample data within the laboratory's sample database. Detailed understating of the dataset is a critical first step in data mining to determine whether the data quality and quantity is sufficient to be able to draw conclusions of any real-word value. Within each sample record should be basic patient demographic data, clinical history, diagnostic test results and final clinical interpretation. However, the records are frequently incomplete as they rely on the quality of the information received on the sample referral form. Here we present the first descriptive study of the RIPL Lyme dataset, using a variety of analysis tools to examine the quantity and quality of data contained within it.

Two recent changes have been made to the recording of Lyme disease test results in the database. The first is the recording of density values for each of the bands on the immunoblots, including subthreshold bands. The second is the addition of a codified final diagnosis field, primarily for reporting disease incidence. We consider how this extra data adds richness to the dataset and increases its potential for research use, whilst considering what could be done in the future to address the issue of records with a high proportion of missing data values.

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