Abstract Booklet 2017
Infection and Global Health Day
Title: Understanding the determinants of Group A Streptococcal pathogenicity

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Abstract

Introduction: *Streptococcus pyogenes* (GAS) is among the most diverse of any human pathogen, with a varied range of clinical manifestations, causing mild superficial infections to serious invasive infections. Between 2010 and 2012 there was an outbreak of invasive GAS infections in Liverpool, Merseyside that had a mortality rate of 29%. Following sequencing, clinical GAS isolates from the outbreak were determined as emm 32.2.

Aim: Develop clinically relevant in vivo models to assess virulence differences between emm 32.2 strains and other circulating sequence types (invasive and carriage), which could account for the different clinical outcomes.

Methods: We developed two models; a sepsis and a novel septic arthritis murine model. Bacterial CFU counts were enumerated from the blood and joints over time following infection. A tailor made highly sensitive ELISA based method was developed to measure the amount of streptolysin (SLO) released by each GAS isolate together with its haemolytic activity.

Results: Emm 32.2 isolates resulted in a more severe phenotype (sepsis) in comparison to other sequence types. Specific emm1.0 isolates were able to cause septic arthritis. The emm 32.2 strains in particular produced a much larger amount of (and more haemolytic) SLO toxin. When the toxin was removed or haemolytic activity reduced, it resulted in invasive isolates being cleared from the blood of the mouse.

Conclusion: Work suggests bacterial characteristics and in particular streptolysin is predictive of the clinical syndrome. Research is on-going to produce a streptolysin and super antigen deficient mutant to analyse further the mechanism of pathogenicity.
Title: Terazosin protects against experimental acute pancreatitis


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Abstract

Background: Circulating histones are elevated early in mouse models and correlate with disease severity. Terazosin, a marketed alpha1 adrenergic receptor agonist, could reduce intracellular calcium and recently has been shown to reduce cell death.

Objective: This study investigated whether terazosin alleviates acute pancreatitis

Methods: In vivo: intraperitoneal injection of caerulein (CER-AP) to induce mild acute pancreatitis and retro-infusion of sodium taurocholate into the biliopancreatic duct (TCL-AP) to induce severe acute pancreatitis mouse model were used. Pancreatic histopathology (H&E), serum organ injury markers (ELISA) and the levels of circulating histones (Western-blot) were assessed. In vitro: pancreatic acinar cells (AR42J) were used to investigate the underlying molecular mechanisms.

Results: In CER-AP models, terazosin treatment significantly reduced the overall pancreatic histopathological score, and also decreased the levels of serum amylase, ALT, BUN. In TLC-AP models, terazosin treatment has trend to reduce the overall histopathological score of the pancreas, and significantly decreased the levels of serum amylase, ALT, BUN, as well as cardiac troponin T. Terazosin treatment can markedly reduce the level of circulating histones in TCL-AP. The survival rate of terazosin treated mice was significantly higher than TCL-AP group. In vitro, terazosin significantly alleviated ROS production, increased AR42J cell viability upon caerulein analogue-cholecystokinin (CCK) induced. The alleviation of calcium overload by terazosin treatment is under examination.

Conclusion: These data demonstrate terazosin alleviates acute pancreatitis. This may be due to the reduction of calcium overload and ROS production in pancreatic acinar cells and lowering circulating histones, important mediators of multiple organ injury.
Title: Cellular Processing of Infectious Bronchitis Virus Spike, Membrane and Envelope Glycoproteins and Their Role in Virus Biology and as Targets for Antiviral Therapy

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Abstract

Infectious Bronchitis Virus (IBV) causes acute and highly contagious disease of chickens. The virus is a coronavirus and in the same family as SARS and MERS coronaviruses. These viruses have several structural proteins that form integral parts of the viral envelope – a requirement for viral entry. These include the Spike (S), Membrane (M) and Envelope (E) glycoproteins. The additions of the glycan groups are essential to their function and are thought to be a non-viral mediated process. Viruses are obligate intracellular parasites and increasingly viral proteins interact with host proteins in order to affect function and manipulate host cell processes. New strategies for anti-viral therapy, which circumvent the emergence of resistance, is to target host proteins that are essential for virus biology, with repurposed therapeutics. In order to identify cellular proteins that interact with the three IBV structural proteins, to mediate glycosylation, a quantitative label-free proteomic assay was used. This relied on overexpression of GFP-tagged versions of the proteins and LC-MS/MS. From the proteins identified a priority list of potential interactions was then assembled. Calnexin, which is an endoplasmic reticulum (ER) chaperon, was found as potentially a very significant protein for processing and maturation of the target IBV proteins. Biological investigation using a combination of siRNA ablation of calnexin and inhibition of function using small molecules in IBV infected cells demonstrated that this protein was involved in glycosylation of these viral proteins. The work shows how proteomics can be used to inform rapid functional analysis and identify therapeutic targets.
**Title:** *Ixodes ricinus* tick activity – microclimate influence and Borrelia prevalence.

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**Abstract**

*Ixodes ricinus* (the Sheep Tick) is a vector of many pathogens of human and veterinary importance. In the UK it is the primary vector of *Borrelia burgdorferi s.l.*, the causative agent of Lyme borreliosis - the most common vector-borne disease of humans in the temperate northern hemisphere. Three genospecies of this bacterial complex which are associated with Lyme borreliosis are known to circulate in the UK. Changing land use, human activity, wildlife distributions and weather patterns influence human exposure to this tick species.

The aim of this study was to investigate seasonal peak activity of *Ixodes ricinus*, specifically the microclimate factors which influence when peak activity commences and ends. In addition; the spatial and temporal prevalence of *Borrelia burgdorferi s.l.* was investigated across different land cover types and geographical regions.

Longitudinal surveying of twelve sites in England over two years, accompanied by repeated seasonal surveying of twenty four sites across six different land cover types, resulted in comprehensive data on *I. ricinus* activity, habitat and microclimate variables. A proportion of ticks collected from each of the survey sites were tested for the presence of pathogenic Borrelia genospecies.

Monitored field sites located in the south of England tended to exhibit a distinctive spring peak in *I. ricinus* activity, followed by much reduced activity. Northern field sites exhibited a spring peak, followed by more prolonged activity through summer. Borrelia prevalence was highest in broadleaf woodland. Borrelia was detected at all regional field sites, ranging from 2-6% prevalence.
Title: Using Big Data to improve animal disease surveillance: investigating the Enhanced Infectious Diseases (EID2) database and WAHIS

Authors: KM McIntyre, M Wardeh, M Baylis

Abstract

Few studies have examined global animal disease distributions. We investigate whether combining repositories of animal disease can improve occurrence understanding. The OIE’s WAHIS describes global distributions of confirmed infectious animal disease. The Enhanced Infectious Disease database (EID2) pools metadata on pathogens’ DNA/RNA sequences/scientific publications to explain where/when/in which hosts pathogens occur.

Data describing the global incidence of Foot and Mouth Disease (FMD), Leishmaniosis, Newcastle Disease (ND), and West Nile (WN) was obtained from WAHIS, and matched to EID2 spatial polygons defining pathogen presence/assumed absence. Quantitative comparisons were made of pathogen presence/assumed absence (using Cohen’s kappa coefficient, and diagnostic potential of sources).

The reported presence of FMD was similar at country/sub-country-level in WAHIS, compared to higher and much lower in EID2, respectively. Reporting rates were similar to FMD at country-level for Leishmaniosis, and low for both resources at sub-country-level. Country-level ND reporting was higher in WAHIS compared to EID2, and comparative to FMD at sub-country-level for both, respectively. Finally, WN at country-level was least reported in both, and reporting was low at sub-country-level.

In all cases except WN, agreement between sub-country-level presence was lower than country-level.

WAHIS only captured 24-60% of EID2 country-level presence, and 10-32% for sub-country-level except FMD (75%); EID2 captured higher-levels of WAHIS country-level presence except WN, but was less good at sub-country-level (except Leishmaniosis). Neither resource was obviously better at capturing absence.

These results suggest some agreement in identifying disease presence but neither resource overlap well. This suggests merit in their combination for mapping of infection.
Title: In vivo imaging of leucocyte migration in herpes simplex encephalitis

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Abstract

Introduction: Herpes simplex virus (HSV) encephalitis is a devastating condition of brain inflammation, characterised by infiltration of leucocytes and breakdown of the blood-brain barrier (BBB). Despite antiviral treatment 10-30% of patients die and most survivors suffer neurological sequelae.

In clinical samples I found that, despite antivirals, there continues to be marked up-regulation of host-inflammatory cytokines/chemokines, which were associated with clinical and neuroimaging severity and BBB permeability. However, our current understanding of the temporospatial kinetics of leucocyte migration into the brain, and the cytokines/chemokines driving this, remain poorly understood and are challenging to assess in clinical disease.

Methods: In a murine model of intrathecal HSV encephalitis, I imaged viral infection by confocal microscopy, assessed cytokine/chemokine time-course by bead array and leucocyte migration by flow cytometry.

I then interrogated the impact of ligand/receptor interaction on leucocyte migration in real-time in vivo using multiphoton intravital microscopy.

Results: I confirmed that this model induces encephalitis with a predilection for the temporal lobes reflecting clinical disease. I identified early significant increases in several mediators, particularly CXCL1, 9 and 10, and marked early neutrophil migration.

Neutrophils were found to crawl in the abluminal space after transmigration and lead to leakage of the blood vessel. I found that blockade of CXCL1 action using a monoclonal antibody and a CXCR2KO mouse significantly reduced neutrophil transmigration.

Conclusion: Chemokine signaling, particularly the CXCL1/CXCR2 axis, are non-redundant mediators of neutrophil migration and subsequent blood-brain-barrier leakage in this model of HSV encephalitis and may represent targets for future therapeutic research.
Title: Torix group Rickettsia are widespread in Culicoides biting midges (Diptera: Ceratopogonidae), reach high frequency and localise in the ovarian suspensory ligament

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Abstract

There is increasing interest in the heritable bacteria of invertebrate vectors of disease as they present novel targets for control initiatives. Previous studies on biting midges (Culicoides spp.), known to transmit several RNA viruses of veterinary importance, have revealed infections with the endosymbiotic bacteria, Wolbachia and Cardinium. However, rickettsial symbionts in these vectors are underexplored. Here, we describe a previously uncharacterized and overlooked association between Rickettsia and biting midges. Screening of 414 Culicoides individuals from 29 Palearctic or Afrotropical species revealed Rickettsia represent a widespread but previously overlooked association, reaching high frequencies in midge populations and present in 38% of the species tested. Sequence typing clusters the Rickettsia within the Torix group of the genus, a group known to infect several aquatic and hematophagous taxa. FISH analysis indicated the presence of Rickettsia bacteria in ovary tissue and crushed spermathecae, indicating a potential dual maternal/paternal inheritance. Localised infections are also observed in the ovarian suspensory ligament, offering a novel mode of endosymbiont horizontal and vertical transmission. Given the importance of biting midges as vectors, a key area of future research is to establish the impact of this endosymbiont on vector competence.
Title: Liposomes associated Mycobacterium glycolipids can modulate HIV-1 infection

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Abstract

Background: Tuberculosis (TB) is the leading cause of death among HIV-1-infected individuals and where co-infection with Mycobacterium tuberculosis (Mtb) exacerbates the progression of both diseases. We aimed to identify Mtb strains that differentially modulate HIV-1 infections (cis- and trans-infection) and immune skewing.

Materials and Methods: Glycolipids from pathogenic and non-pathogenic Mtb strains were integrated into liposomes. The resultant liposomes were tested for maturing dendritic cells (DCs) as well as modulating HIV-1 cis- and trans-infection of TZM-bl cells. Mutant Mtb strains were tested in the same assays.

Results: Liposomes differentially activated DCs with one pathogenic strain (HN878) showing the strongest effect in comparison to other strains (H37Rv, M. bovis, M. segmentis and EU127). None of the liposomes demonstrated modulation of HIV-1 cis-infection but did modulate trans-infection. H37Rv, M.bovis, EU127 all demonstrated inhibition of both R5 and X4 HIV-1 strains and with Raji-DC-SIGN cells as well as DCs being used to capture virus. Interestingly, sulfolipid 1 (SL1) knock out strain of H37Rv (PapA1Δ) lost the capacity to block DC mediated trans-infection. When PapA1Δ liposomes were complemented with SL-1, the block to trans-infection was restored to previous levels, indicating this to be the responsible glycolipid.

Conclusions: We demonstrate that Mtb glycolipids can differentially modulate DC maturation as well as reduce or block HIV-1 mediated trans-infection depending on the Mtb strain being tested. Both these mechanisms may help explain for variation between pathogenic and non-pathogenic strains and their influence on HIV-1 infection.
Title: Innate immune response to *Neospora caninum* infection in cattle

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Abstract

*Neospora caninum* is an intracellular protozoan parasite which causes abortion in cattle and neuromuscular disease in dogs. Bovine infection can be initiated in utero or after birth through ingestion of infectious material. Our previous studies have shown differences in young monocytes compared to adult cells in terms of cytokine responses and expression of CD80. In this study we investigated if there was an age dependent effect on invasion of monocytes and their subsequent interaction with NK cells during *N. caninum* infection.

NCLiv-1 isolate of *N. caninum* was maintained in a VERO cell line. Naïve CD14\textsuperscript{+} and NK-cell cells were isolated from peripheral blood mononuclear cells (PBMCs) from young and adult cattle by magnetic cell separation. Monocytes were sequentially infected with *N. caninum* CFSE labelled tachyzoites before co-culture with NK cells. The number of infected cells was determined post-culture and CD80 expression, as a marker of cellular activation, was determined by flow cytometry.

There was an age-related variation in infection of monocytes with cells derived from adult animals more likely to harbour greater numbers of parasites. A reduction in parasite numbers post culture with NK cells was observed in both young and adult cattle with increased CD80 expression also observed.

The greater uptake of parasites into monocytes in combination with NK-cell interaction inhibits parasite multiplication and may be one mechanism to explain the age-related differences in innate immune response to *N. caninum* infection.
Title: The variant antigen profile: how protein models can elucidate diversity of variant surface glycoproteins in natural and experimental infections of *Trypanosoma congolense*

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Abstract

*Trypanosoma congolense* is an extracellular haemoparasite transmitted by the tsetse fly that causes animal African trypanosomiasis in Africa. African trypanosomes establish long-lasting infections partly due to antigenic variation. The parasite surface is coated with a variant surface glycoprotein (VSG) monolayer, each cell expressing a single VSG from a genomic repertoire of many hundreds. Sequential replacement of VSG allows the parasite to evade immunity and survive long term. Population genetics of VSG leading to an understanding of antigenic diversity is vital to explaining variation in disease outcome and to developing effective vaccines. However, due to their number and complexity, we have lacked the tools to analyse VSG diversity from high-throughput data.

We have produced genomes for forty clinical isolates from six African countries and discovered that *T. congolense* VSGs segregate into defined, universal and exhaustive ‘phylotypes’. Exploiting this predictable repertoire, we developed variant antigen profiling, a novel bioinformatic approach to measure VSG abundance from deep sequencing data using amino acid models.

We have applied this approach to profile VSG expression in metacyclic parasites in the tsetse fly. Through transcriptomic analyses of 24 individual fly replicates, we show that the profile of metacyclic VSG expression is both reproducible and non-random, indicating that certain phylotypes are preferentially expressed in metacyclics.

Together, our results show that the VAP provides unprecedented ability to discriminate among variant antigens from high-throughput data. Ultimately, this will allow us to associate variant antigens with specific infection phenotypes leading to a better understanding of disease outcomes.
Title: Original antigenic sin in Japanese encephalitis virus vaccination

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Abstract

Japanese encephalitis (JE) virus (JEV) is a flavivirus transmitted by Culex mosquitoes. Despite the use of a live attenuated vaccine in endemic countries, there are approximately 70,000 JE cases occurring every year.

Dengue virus (DENV) is another flavivirus transmitted by Aedes mosquitoes. With an estimation of approximately 400 million DENV infections in all tropical and subtropical countries, DENV is the most important arbovirus.

Cross-reactivity among flavivirus is an important component of the immunological response to these viruses with relevant clinical implications.

We conducted a clinical trial in South India, which is endemic for JEV and DENV, analysing the T cell and antibody response to JEV and DENV in adult healthy volunteers vaccinated with the live attenuated JEV vaccine SA 14-14-2. We observed poor immunogenicity of this vaccine with low seroconversion rate and low level of JEV neutralizing antibodies among responders. Interesting, some volunteers responded to the vaccine by boosting the DENV neutralizing antibody titer. This is consistent with the phenomenon of original antigenic sin (OAS) which has previously been shown among different dengue serotypes.

Additionally, using a JEV peptide library, we identified 10 T cell epitopes. Most of them were present before vaccination and 7 out of 10 showed high degree of cross-reactivity with dengue variants.

In conclusion we confirmed the poor immunogenicity of the live attenuated JEV vaccine in adults and we described for the first time the phenomenon of OAS among different flavivirus serogroups.
Title: Development of a pen-side diagnostic test for liver fluke infection in cattle and sheep

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Abstract

*Fasciola hepatica* (liver fluke) is a common trematode parasite of cattle and sheep worldwide. It is highly pathogenic and therefore raises health and welfare concerns for infected individuals. Over recent years, the prevalence of liver fluke has increased significantly within the UK, which is linked to many factors such as climate change and increased evidence of resistance to commonly used drugs.

Current diagnostics are primarily based around faecal egg counts and ELISA based assays for antibody detection in serum and milk; however these tests can be slow and insensitive, and require samples to be sent to the laboratory for testing which adds time and cost to the diagnosis.

The aim of this project is to develop a novel lateral flow pen-side diagnostic test to allow farmers to quickly identify infected individuals for targeted treatment.

A recombinant Cathepsin L1 (CL1), the immunodominant fluke antigen, was produced in the yeast *Pichia pastoris*. Western blotting and ELISA showed that the recombinant antigen was more readily recognised by animals experimentally infected with *F. hepatica*. This was confirmed by peptide arrays, which identified 4 key immunogenic regions recognised by antibodies from animals experimentally infected. Proteomic identification of 2D gel-electrophoresis spots of fluke excretory-secretory (ES) antigen showed that naturally infected cattle recognised multiple targets within ES antigen. The results indicate that whole fluke ES antigen would perform much better in a diagnostic test and this was used to successfully produce a prototype lateral flow test to diagnose exposure to *F. hepatica* in cattle and sheep.
Poster Abstracts
Infection and Global Health Day
Title: Characterisation of convalescent plasma using an EBOV GP HIV-1 pseudo-typed assay

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Abstract

Studying survivors of the West African EBOV 2014-16 epidemic provides a unique opportunity to delineate immune responses controlling viral replication. These individuals provide a bank of convalescent plasmas (CP) that can be used to treat future outbreaks. Understanding immune responses associated with survival will indicate what an effective EBOV vaccine has to induce. We sought to develop an HIV-1 EBOV GP pseudo-typed assay which could analyse antibody (Ab) neutralisation.

Single-round infectious EBOV GP pseudo-typed virus were produced by co-transfecting a HIV-1 envelope deficient backbone with a plasmid expressing the 2015 GEBOV GP envelope into 293T cells. Produced virus was quantified via measuring HIV-1 p24 levels and infection monitored by measuring luciferase activity within infected cells. We utilised our pseudo-typed assay in inhibition assays where limiting dilutions of CP were tested for the capacity to restrict viral entry. Sixty-five CP samples were selected for analysis where total EBOV GP binding responses had been characterised using the DABA assay.

CP samples showed broad neutralisation potential against EBOV with an inhibitory trend ranging from low to high. EBOV GP Ab titres were used to select longitudinal CP samples for further characterisation; samples with high Ab titres were shown to neutralise EBOV to a greater capacity compared to lower titres (P<0.005).

We have developed a robust EBOV neutralisation assay that has shown to correlate with total EBOV GP Ab binding. This assay can be used in future characterisation of EBOV Ab responses in both survivors as well as vaccine recipients.
Title: Novel influenza vaccination strategy to enhance vaccine response in young children

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Abstract

Influenza remains a major killer disease despite the availability of several vaccine formulations. Current vaccines are based on surface HA protein, which varies between strains; hence, a new seasonal vaccine needs to be produced every year. Current efforts are focused on the development of vaccines to induce broad immunity against multiple subtypes of influenza.

We have previously shown that MVA-vectored vaccines efficiently express the target vaccine antigens, eliciting influenza-specific immune responses in human nasopharynx-associated lymphoid tissue (NALT). However, their immunogenicity in young children is generally lower as compared to that in adults. We hypothesise that, this poor response is due to insufficient priming for memory by natural exposure; and with appropriate immunological adjuvants, and/or through a prime-boost vaccination approach, the immune responses could be enhanced.

Currently, we are evaluating these novel flu vaccines in combination with various adjuvants; to induce mucosal immune responses in young children. We have measured the antibody responses to these vaccine candidates in combination with several commercially available adjuvants in a human ex vivo cell culture model using NALT immune tissue.

Vectored vaccines were shown to induce anti-HA antibody response in human NALT, suggesting the potential as intranasal vaccines. Addition of adjuvants potentiates antibody induction by MVA-mH5 vaccines in CD45RO-ve naïve cells. This response is more robust in adults; however, the addition of CPG and Endocine adjuvants appeared to enhance the response in young children. Further studies are focusing on optimising the best combination of adjuvants and the dosage for better vaccine immunogenicity for young children.
Title: Role of SPLUNC1 in the Natural Transmission of Influenza A Virus

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Abstract

The respiratory system epithelium has a critical role in the initial defence against microbes and secretes a number of proteins that function in innate immune defence. SPLUNC1 also called (Short Palate, Lung, and Nasal epithelium Clone1) is continuously expressed and secreted by the respiratory system epithelium. However, its precise biological functions remain unclear. To assess whether SPLUNC1 affected the transmission of influenza A (IAV) between infected and non-infected mice, we set up two experiments. The first experiment included 20 mice (10 wild type and 10 SPLUNC1 KO). At day 0 eight mice were infected (4x WT and 4x KO) each with 5x10^4 PFU Influenza A virus X31. Six hours later, the infected mice were introduced to naïve mice 2x WT index to 3x WT contact, and 2x KO index to 3x KO contact per cage (4 cages total). All mice were euthanized at 4 days post-infection, lung and nasal tissue samples were collected for analysis of virus titre. While the second experiment similar to the first experiment but the infected mice were introduced to naïve mice 2x WT index to 3x KO contact and vice versa per cage (4 cages total). The results showed that influenza transmitted more frequently and efficiently to SPLUNC1 KO mice, indicating a role for SPLUNC1 in the natural transmission of the virus.
Title: Latency associated nuclear antigen LANA play a pivotal role in MCF disease

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Abstract

Malignant catarrhal fever (MCF) is a generally infectious, lymphoroliferative multi-systemic fatal disease of cattle and many other species of Artiodactyl, characterized by low morbidity but high mortality and it occurs in different countries in worldwide. MCF is caused by Macavirus genus (previously known as Rhadinovirus) of the family Herpesviridae, subfamily Gammaherpesvirinae. The MCF group comprises 10 known members; two of these viruses are most important to describe the MCF infection in animals, alcelaphine herpesvirus-1 (AIHV-1) and ovine herpesvirus-2 (OvHV-2).

Disease caused by AIHV-1 is restricted to areas of Africa where wildebeest are present and to zoological collections elsewhere, and has been referred to wildebeest-associated MCF, while the OvHV-2 form occurs world-wide wherever sheep husbandry is practised and has been described as sheep-associated (SA) MCF. The mechanisms responsible for the lymphoproliferative and degenerative clinical and lesions observed in MCF are unknown.

Latency associated nuclear antigen (LANA) is OvHV-2 protein which play a fundamental role in the establishment and maintenance of virus latency and also in the pathogenesis of MCF. LANA protein interacts with a variety of cellular proteins, and it has multiple functional homologues to other DNA tumour virus replication or transcription proteins. No treatment has been found to provide any consistent benefit, supportive care to reduce the subclinical signs. No vaccine is currently available. The aim of this project is to study the proteins that interact with oLANA using proteomic techniques. This may reveal interactions that can be disrupted using existing compounds and lead to possible interventions in MCF.
Title: The First study of Chigger Mite Diversity on Rodents from the Asir Region of Saudi Arabia

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Abstract

Chigger mites are the larval stage of prostigmatid mites of the family Trombiculidae. They are important vectors of human disease including scrub typhus (Orientia spp.), bartonellosis (Bartonella tamiiae) and Hantaviruses. This study aimed to identify mites parasitizing wild rodents in Saudi Arabia, with a main focus on chiggers, as a prelude to microbiome sequencing of the mites to determine the prevalence of bacterial pathogens. In August 2016, an initial field survey of chigger mite diversity was conducted across six mountainous sites in the ‘Asir region of southwest Saudi Arabia. Rodents were trapped over three days (30 traps per day) using bread and peanut butter as bait. Chigger mites were collected from four predilection sites on the host (back, chin, ear, and anus) and fixed in absolute ethanol. A 10% subsample of chiggers was selected from each rodent for mounting in Berlese fluid and morphometric examination. A total of 3,146 of chigger mites were collected, of which the majority parasitized the back or anus of the host. Female mice exhibited a higher mean abundance of chiggers than did males. On the basis of the morphology of the scutum (or dorsal shield), sixteen chigger specie were assigned to subgenera, including a new and interesting observation that three putative new species of chigger mites were discovered on Acomys dimidiatus: Neotrombicula sp. n., Microtrombicula aff. machadoi, and, Schoutedenichia aff. Geckobia.
Title: Aedes aegypti mosquitoes are resistant to insecticides in Dengue-endemic regions of Saudi Arabia

Authors: Ashwaq Al Nazawi\textsuperscript{1,2}, Philip McCall\textsuperscript{1}, David Weetman\textsuperscript{1}

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Abstract

Dengue has been endemic in Saudi Arabia since 1994 and disease control programmes rely on using insecticides to control mosquito (Aedes aegypti) populations. Insecticide resistance is a growing concern that has yet to be well characterised in Saudi Arabia. As resistance mechanisms remained unknown, This study aimed to determine the prevalence of insecticide resistance in Ae. aegypti collected from Jeddah and Makkah and to investigate the role of two main mechanisms, target site mutations and metabolic resistance. Some kdr gene mutations were detected for the first time in Saudi Arabian mosquitoes. Microarray data was utilised to identify several differentially expressed genes. These Aedes mosquitoes are highly resistant to pyrethroids. Voltage-gated sodium channel (Vgsc) mutations are strongly associated with deltamethrin resistance and, though P450 enzymes seem to be the dominant metabolic mechanism, the data suggest a greater importance of target site mutations. We report the Vgsc mutations can be a valuable monitoring tool for monitoring developing insecticide resistance, however further investigations on other resistance mechanisms is still needed.
Title: Innate immune responses in head associated lymphoid tissues following IBV M41 inoculation in commercial broiler

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Abstract

Infectious bronchitis virus (IBV) primarily replicates in the epithelial tissues of the respiratory tract, particularly in the trachea. Little information is available on immune responses in the head associated lymphoid tissues (HALT) following IBV infection. For this reason, we examined the viral load, pro-inflammatory cytokines and host gene signatures in the HALT of chickens. Briefly, 21 day old broiler chickens were divided into two groups and infected via the intra-nasal route as follows; (i) virulent IBV M41 challenge (0.1 ml of 10^5.75 EID50/ml) and (ii) sham-infected with 0.1 ml of sterile water. Five birds from each group were humanely euthanised at five days post-infection (dpi) and the HG, turbinates, choanal cleft, pharyngeal and tracheal tissues were collected from each bird. RNA was extracted for virus detection and quantification, proinflammatory cytokine and host gene signatures. The IBV and gene expression assays were carried out using quantitative real-time RT-PCR (RT-qPCR). We found that the viral RNA load in the turbinates (2.67 log relative equivalent unit (REU)) and choanal cleft (2.62 log REU) was higher than those found in the HG, pharyngeal and tracheal tissues. Findings to date showed an upregulation of mRNA gene expression of the examined tissues in the infected compared to the uninfected group. We also saw up-regulation of the pro-inflammatory cytokine. Our preliminary findings demonstrate an innate immune response within the head associated lymphoid tissues at 5 days post M41 inoculation. One or more of these mRNAs in the HALT tissues could be included as a protection indicator in IBV vaccination-challenge model.
Title: Exploring a new surveillance platform for malaria: assessing the use of a finger prick blood for species specific detection of *Plasmodium* spp

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Abstract

Malaria affects 212 million people every year, causing 429000 deaths. It has been estimated that only 19% of malaria cases were detected by surveillance programmes in 2015, illustrating the need for innovative surveillance methods. Blood samples are collected as a part of surveillance programmes for other tropical diseases such as schistosomiasis, however these samples were utilised for the detection of a species-specific *Plasmodium* spp. This study aimed to establish whether qPCR using gDNA could be used effectively as a species specific surveillance method for *Plasmodium* spp. 247 blood samples were analysed using generic *Plasmodium* qPCR. Positive samples underwent qPCR using species specific *Plasmodium* primers. A sample of these samples underwent sequencing for species confirmation. Prevalence, sensitivity, specificity, positive and negative predictive values were calculated in order to assess the efficacy of the PCR as a diagnostic test compared to RDTs used in the field. Overall, singleplex PCR gave a prevalence of 74.90%, exceeding the prevalence obtained by RDT by 19.03% (RDT prevalence 55.87%). Pooling RDT and PCR results gave a prevalence of 83.81%. This was decided as a preferred method of determining prevalence, due to the ability to negate false negative results diagnosed by either method. qPCR using blood samples currently offers a superior surveillance method for species specific *Plasmodium*. 
Title: The role of immune tolerance pathways in bacterial pathogen colonisation

Authors: Hind Althagafi, Daniel Neill, Kadigolu Aras

Abstract

The human nasopharyngeal niche contains bacterial flora made up of commensal and potentially pathogenic species. Currently, the mechanisms by which the host tolerates colonisation of the airways by these pathogens are poorly understood. Aiming to determine whether a universal mechanism of immune tolerance operating in the nasopharynx enables colonisation by opportunistic bacterial pathogens, the study utilises both Gram-positive and Gram-negative bacterial species cultured with three different cell lines, Detroit 562 human nasopharyngeal epithelial cells, A549 human alveolar epithelial cells and the murine macrophage cell line J774.2. Levels of the key immunoregulatory cytokine transforming growth factor beta (TGF-β) were determined in culture supernatants using a luciferase reporter assay. Bacterial infection induced TGF-β production in all three cell lines, but particularly in Detroit pharyngeal epithelial cells. The dose-response curve for Detroit cells differed from those of A549 or J774. Detroit pharyngeal epithelial cell TGF-β production was induced by bacterial infection but inhibited at high densities of infection. By contrast, TGF-β production for A549 alveolar epithelial cells and J774 macrophages increased as bacterial density increased. J774 macrophages controlled S. pneumoniae and H. influenzae proliferation but were unable to limit proliferation of P. aeruginosa. Among the four species Streptococcus pneumonia strain has the ability to induce the highest level of TGF-b production. Then, Haemophilus influenza, Pseudomonas aeruginosa and Staphylococcus aureus respectively. TGF-β levels suggest that immunoregulation may play a role in bacterial colonisation of the nasopharynx and that the magnitude of this response might be linked to bacterial density.
Title: Haplotypes of the Human leukocyte antigen-G (HLA-G) different HIV-1 groups from the Netherlands

Authors: A Alyami, S Christmas, K Neeltje, G Pollakis, B Paxton, Z Al-Bayati, B Flanagan

Abstract

The Human leukocyte antigen-G (HLA-G) molecule plays an important role in immunomodulation. To date, 16 UTR HLA-G haplotypes have been previously defined by sequenced SNPs in the coding region. From these, UTR-1, UTR-2, UTR-3, UTR-4, UTR-5, UTR-6 and UTR-7 are the most frequent 3'UTR haplotypes at the global level. UTR-1 is associated with higher levels of soluble HLA-G and HLA-G expression, whereas UTR-5 and UTR-7 are linked with low levels of soluble HLA-G and HLA-G expression. Human immunodeficiency virus type 1 (HIV-1) infection results in the progressive loss of immune function in infected individuals. The virus escape mechanism typically includes T lymphocytes and NK cell recognition, and lyses by classical HLA-A and B down-regulation, which has been associated with non-classical HLA-G molecule up-regulation, respectively.

We evaluated the haplotypes of the HLA-G 3’ untranslated region frequencies observed in three HIV-1 groups from the Netherlands and their susceptibility to develop infection. The three groups are made up of mainly men who have sex with men (MSM), injection drug users (IDU) and a high-risk-seronegative (HRSN) group. DNA samples were amplified with published primers prior sequencing. According to our results, the low expresser frequencies show higher in HRSN compared to other groups. This indicating that 3'UTR polymorphisms may be identified as potential prognostic biomarkers to determine susceptibility to HIV.
Title: Estimating the tick burden in companion cats and dogs in Great Britain using electronic health records and household location of the animals

Authors: Elena Arsevska, Cyril Caminade, Alan Radford

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Abstract

We present the first results from an ongoing multi-disciplinary project in veterinary health informatics, which aims at estimating the tick burden among companion cats and dogs in Great Britain (GB). We used the clinical narratives of the electronic health records (EHR) of the Small Animal Surveillance Network (SAVSNET) to obtain information about animals infested with ticks (date of consultation, species and household location of the animal). By combining with bioclimatic and pet demographic data, as well as the behaviour of the animals (dog walks and cats wandering), and by using statistical learning we identify the key areas and months for vigilant monitoring of tick infestation in companion animals in GB.

From March 2014 to May 2017, from a total of 645,679 cat and 1,570,632 dog consultations; ticks were observed in 1,277 (0.19%) cat and 2,694 (0.17%) dog consultations. 61% of the cases were from urban areas. The tick infestation peaked from April to August, with cats being infested earlier than dogs. The highest number of tick cases was observed in Dorset, Somerset, and Cornwall (>34 cases per 10,000 consultations), compared to West Wales, Cheshire, Shropshire and Staffordshire (< 8 cases per 10,000 consultations).

Our spatio-temporal predictions showed highest suitability of tick presence in late spring and summer, with ticks being widespread, especially in the south and centre of the country. Winter and autumn were less suitable for ticks’ abundance, with lower probability of presence in the southern parts of the country and around the coast.
Title: Does the offer of an HIV test influence acceptability of HCV testing at point of care?

Authors: Harrison Austin, Giovanni Villa, Apostolos Beloukas, Andrew McCallum, Anna Maria Geretti

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Abstract

Eliminating Hepatitis C as a public health threat by 2030 has been set as a priority by the World Health Organisation (WHO). Half of HCV carriers in the UK remain undiagnosed, thus improving HCV testing uptake is crucial. Fingerprick blood collection and sensitive point-of-care tests (POCT) aid the rapid diagnosis of infection. This study assessed the feasibility and acceptability of the GeneXpert real-time system for detecting HCV RNA as a POCT in an emergency department and the influence of combined HIV and HCV screening. Between Feb and Jun 2017, all attendees of the Royal Liverpool University Hospital A&E minor injuries were given an anonymous questionnaire. Acceptability was a measure of participants consenting to undergo fingerprick capillary blood collection, then processed on GeneXpert. Two distinct and monthly alternating testing phases of HCV alone and HCV plus HIV were implemented. Post recruitment interviews with A&E staff were completed to determine service feasibility. 814/859 (94.7%) of questionnaires offered were completed with 324 tested. 211/324 (65.1%) were tested for HCV and 113/324 (34.8%) for HCV and HIV. HCV prevalence was 0.98% [95%CI, 0.5%-1.9%] with a HIV prevalence of 0.0% [95%CI 0.0%-0.5%]. Combined HCV and HIV testing, showed 50% decline (p<0.001) in uptake [OR 0.51, 95% CI (0.38–0.68)]. Thematic interview analysis gave positive opinions of routine implementation of HCV POCT by GeneXpert. Careful implementation of routine HCV POCT in A&E is feasible. The stigma associated with HIV may strongly dictate testing behaviour and decrease testing uptake. Combined offer of HCV and HIV could hamper HCV screening programs.
Title: Capturing urban household diversity – alternatives to the DHS Wealth Index

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Abstract

Socio-economic status (SES) influences the risk and outcomes of numerous diseases. The DHS (Demographic and Health Survey) wealth index is a widely-used SES measure, calculated using easy-to-collect data on assets, housing construction and water and sanitation access.

The Urban Zoo project is exploring microbial diversity in Nairobi using landscape genetics of E.coli from different urban niches, including humans, livestock, wildlife, food and environment. Seventeen neighbourhood types, characterised from satellite imagery, were stratified by average income, with 33 administrative districts selected and 3 households randomly chosen within the dominant housing type, further stratified by livestock-keeping status.

DHS index calculation demonstrated successful recruitment of families across the whole range of SES. However, investigating its link to bacterial diversity poses two problems: Livestock feature in the composite index, but we hypothesise they will independently influence bacterial diversity; secondly, wealth varies heterogeneously across housing types, suggesting the index does not adequately capture the diversity of urban environments.

Multivariate analyses suggest that two alternative methods may better describe this variation. Fuzzy clustering, which assesses a household’s affiliation to multiple pre-defined groups; or two indices each using subsets of household variables combined with measures of human capital, such as education. Whereas pig-keeping was associated with low SES, ruminants were found in more “rural-style” households independent of wealth, underscoring the need to incorporate SES and livestock variables separately. Urban household environments are highly variable and have different complexities to those in rural settings, and we anticipate this may be reflected in the diversity of their bacterial populations.
Title: Viral shedding and genetic stability of Rotarix® in a cohort of vaccinees in the UK

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Abstract

Sequential faecal samples from twelve infants vaccinated with Rotarix® were collected throughout their vaccination period. Vaccine RNA shedding was assessed in 17-45 samples from each. Genetic stability of vaccine virus has been assessed at 5-6 time points in two infants for viral segments VP3, VP4, VP6 and VP7.

Viral RNA was extracted from faecal suspensions, reverse-transcribed and quantified by vaccine-specific NSP2 qPCR. For NGS, vRNA was amplified by RT-PCR using segment-specific primers. Libraries were sequenced using Nextera® XT DNA kit v2 on an Illumina® MiSeq. Data were aligned to an in-house vaccine sequence (Mitchell et al., in preparation), calling mutations if ≥100 mean reads were present at a mean frequency ≥1% in at least 2/3 replicates.

Viral RNA in stool was detected at 103-109 copies/g. All infants shed detectable virus after dose 1, ranging from 107-109 copies/g, while eight shed lower loads after dose 2. VP4 presented the highest number of SNP loci, with a nucleotide region common to infants and in-house sequence, and a novel mutation in both infants.

While Rotarix® is reported to contain 106 CCID50/mL, vRNA from stocks had 2-3 log10 higher, suggesting it contains significant amounts of non-infectious virus. Viral loads in stool fluctuated with time of shedding and were within the range of wild-type infections (102-1010 copies/g), suggesting active replication in all infants. NGS data demonstrated the emergence of SNPs, a consequence of viral replication during shedding. The accumulation of mutations in the vaccine strain will be further investigated based on shedding load and duration.
Title: The Major Sources of High Levels of Circulating Histones in Sepsis

Authors: Zhenxing Cheng¹, Weiping Yu¹, Simon Abrams², Guozheng Wang², Cheng-Hock Toh²

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Abstract

Background: Sepsis is defined as an infection-induced "dysregulated host response" and annually claims over 10 million lives. Over 150 clinical trials examined the efficacy in blocking inflammatory mediators without success. In the early stage of sepsis, there is a large number of lymphocytes death and high levels of circulating histones, a group of damage-associated molecular patterns.

Objective: Investigate the major sources of circulating histones

Methods: In vivo, sepsis models were generated by three methods: cecum ligation and puncture, intraperitoneal injection of E.Coli or LPS; lymphocyte apoptosis were semi-quantified by immunohistochemical staining. Histones levels were measured. In vitro, apoptosis of lymphoma cell lines was induced by glucocorticoids; caspase-3 activation and histone release were monitored.

Results: In vivo, the extents of lymphocyte apoptosis in spleen and thymus positively correlated with the levels of circulating histones, but infusion of histones into mice did not lead to apoptosis in those lymphocytes, suggesting that histone toxicity is unlikely the cause of extensive lymphocyte apoptosis. Meanwhile, splenectomy in normal mice or nude mice significantly reduced circulating histones, suggesting lymphocyte apoptosis could be a major source of circulating histones. In vitro, apoptosis of lymphocytes was induced by steroids and extracellular histones were detected in media with strong correlation to the percentage of apoptotic cells. Z-VAD-FMK was able to inhibit the lymphocyte apoptosis and subsequently reduced histone levels. Those results strongly suggest that not only necrosis but extensive apoptosis could release histones.

Conclusions: Extensive lymphocyte apoptosis could release histones and is more likely a major source of circulating histones in sepsis.
Title: Genetic diversity and gene expression patterns of \textit{Plasmodium vivax} merozoite surface protein 7 (PvMSP-7) in Thai clinical isolates

Authors: Chew Weng Cheng$^{1,2}$, Andrew Jackson$^1$, Somchai Jongwutiwes$^2$, Chaturong Putaporntip$^2$

1. Department of Infection Biology, Institute of Infection and Global Health, University of Liverpool
2. Department of Parasitology, Faculty of Medicine, Chulalongkorn University

Abstract

PvMSP-7 is a multigene family consisting of 13 members that form a multiprotein complex during erythrocyte invasion. It has been suggested as a potential vaccine candidate against \textit{Plasmodium vivax} as there is evidence that it is immunogenic and vulnerable to antibodies. This study aimed to characterise patterns of genetic polymorphism and gene expression profiles among PvMSP-7 paralogs to examine any functional differences and evaluate their potential as vaccine candidates. Venous blood was collected from patients infected with \textit{P. vivax} malaria and parasite DNA/RNA was prepared. Sequencing was performed using high-throughput Illumina HiSeq4000 platform. PvMSP-7 genes displayed diverse patterns of sequence diversity. Six loci revealed high sequence variation whilst, seven others were rather conserved. Notably, most of the polymorphic regions were located within the central domain of the genes, where balancing selection seems to act indicating the effect of immune selection. Conversely, an excess of non-synonymous (dN) to synonymous (dS) at both end-terminals suggests purifying selection. These sites potentially serve as a starting point for vaccine design against the parasite. Transcript abundance varies between gene members, with PvMSP-7A, -7F, and -7M widely expressed. This may indicate that only certain PvMSP7 paralogs are constitutively expressed during red cell invasion and suitable for a comprehensive vaccine. The structural diversity and gene expression data described here provide further essential information in developing the PvMSP-7 multigene family as malaria vaccine candidates.
Title: Characterisation of the Role of the Deubiquitylase USP45 in Chikungunya Virus Infection

Authors: Naomi S Coombes¹, Neil W Blake¹, Judy M Coulson²

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Abstract

Chikungunya virus (CHIKV) is the cause of the extremely debilitating disease, Chikungunya fever, characterised by severe joint pain which can last for months or years. As a mosquito-borne alphavirus, CHIKV has been generating increasing concern as this re-emerging pathogen spreads worldwide. There are currently no vaccines or anti-virals available for CHIKV. With current outbreaks in the America’s escalating, identifying novel drug targets is of increasing importance. As obligate parasites, viruses depend on host-cell machinery to replicate. Identifying cellular pathways which are hijacked by viruses may provide the opportunity to develop therapeutics targeting host-factors. Ubiquitylation has been shown to be a key pathway targeted by viruses and the reverse reaction, deubiquitylation, has been generating interest for therapeutic intervention. We have been investigating the interaction of CHIKV with host deubiquitylases (DUBs) a family of ~100 enzymes. An initial siRNA screen using the model alphavirus Semliki Forest Virus (SFV) identified the DUB USP45 as playing a role in alphavirus infection, with an increase in cell viability post SFV infection observed after depletion of USP45. This work has been extended to CHIKV using a USP45 knockout cell line. USP45⁻/⁻ cells are less susceptible to CHIKV infection as reflected by a reduction in both viral RNA production and viral plaque formation compared to wild-type cells. To date little is known about the role of USP45. Our data imply a role of USP45 during CHIKV infection. Further analysis is on-going and suggests USP45 could be a potential target for anti-virals against CHIKV infection.
Title: Understanding and improving the outcome of encephalitis – preliminary results from ENCEPH UK

 Authors: S Defres, T Solomon, on behalf of ENCEPH UK Study and Steering Groups

1. Institute of Infection and Global Health, University of Liverpool
2. Tropical and infectious diseases Unit, Royal Liverpool University Hospital
3. Walton Centre for neurology and neurosurgery, Liverpool

Abstract

Background: Late diagnosis and treatment of encephalitis is associated with poorer outcomes. Previous research has illustrated that there are delays in recognition of encephalitis. ENCEPH UK, a NIHR funded programme of studies aims to improve the management of encephalitis including describing clinical features to predict the disease and its outcome.

Methods: ENCEPH-UK builds on the UK Health Protection Agency (now Public Health England) Aetiology of Encephalitis in England study. This study recruited over a two year period from 2005 to 2007. Potential cases of encephalitis were identified from 24 hospitals across three regions with predetermined inclusion and exclusion criteria. The same criteria were applied in the prospective cohort study which recruited adults from 30 hospital sites across the UK between 2012 and end of December 2015. Detailed clinical data was collected systematically.

Results: Early findings suggest some patients have multiple health care consultations prior to their ultimate hospitalisation. Autoimmune cases had the highest number of prior visits for example up to 6 different consultations whereas those with HSV had fewer prior health care visits, on average 2 visits. The presentation symptoms correlated with the speed of admission with those patients experiencing seizures being hospitalised quicker, compared to those whose presentation was related to confusion or personality changes. The prospective cohort study confirms similar findings.

Conclusions: To our knowledge no previous work has established the duration of illness prior to hospitalisation and what the triggers are for referral to secondary care and what factors affect the admission to hospital.
Title: Hormone interactions with *Pseudomonas aeruginosa* urinary tract infections

Authors: H Ebrahim¹, R Floyd², J Fothergill¹

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2. Department of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, UK

Abstract

Introduction: Urinary tract infections (UTIs) are the second most common infections around the world causing significant morbidity and mortality. These infections are classified into uncomplicated and complicated, with the latter associated with higher levels of multi-drug resistance, particularly in infections caused by *Pseudomonas aeruginosa*. Studies have shown that hormones such as estrogens exacerbate *P. aeruginosa* infections in cystic fibrosis patients and are involved in modulating exopolysaccharides. Thus, an investigation into the impact of hormones on UTI *P. aeruginosa* pathogenesis is warranted.

Methods: Gene expression of a panel of *P. aeruginosa* virulence factors was studied after exposure of bacteria to 10nM estradiol and testosterone In addition, 15 *P. aeruginosa* UTI isolates were examined by confocal microscopy to study the effects of hormones on biofilm architecture. The biofilms were grown statically for 48h with the addition of 10 nm estradiol, testosterone or progesterone. Biofilms were stained with Baclight® kit and 3D Images were produced using Imaris. Biofilm architecture was analysed using Comstat.

Results: These results suggest that the effect of hormones on expression of genes involved in biofilm formation, quorum sensing and pathogenicity is strain and hormone dependent. Analysis of the 3D images showed reduction of roughness-coefficient by progesterone.

Conclusions: Hormones modify virulence, gene expression and biofilm architecture of some populations of UTI *P. aeruginosa*. Due to the observed variations in isolates, some patients maybe more susceptible than others to recurrent and persistent infections. Understanding the factors that may increase bacterial persistence and pathogenesis might lead to alternative management and treatment strategies.
Title: Equine eosinophilic granuloma vs. cutaneous mast cell tumour: one, two or many entities?

Authors: Asma Elbahi

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Abstract

Introduction: Equine cutaneous mast cell tumours (MCT) and eosinophilic granuloma (EG) contain areas morphologically consistent with eosinophilic granuloma (EG) and mast cell aggregates (MCA) in different proportions. It is debated whether these are different entities. The aim of the study was to investigate MCT and EG to unravel similarities and differences and collect data that can explain their pathogenesis.

Materials and Methods: A total of 95 cutaneous lesions (EG, MCT) were re-examined to assess morphological criteria, including lesion size and tissue location, hair follicle association, proportion and distribution of infiltrating eosinophils, lymphocytes (T/B cells) and macrophages, and cell proliferation.

Results: Based on the presence and proportion of EG and MCT components, lesions fell into five categories: C-I (EG+ MCA-); C-II (EG>MCA); C-III (EG=MCA); C-IV (EG
Title: A novel blood test for meningitis: TRanscripts to Identify bacterial Meningitis (TRIM)

Authors: Janet Flatley¹, Petra Leidinger², Fiona McGill¹,³, Shona Moore¹,⁴, Tessa Prince¹, Małgorzata Wnek¹, Willaim Carman², Tom Solomon¹,⁵, Michael Griffiths¹,⁶

1. University of Liverpool
2. Fast Track Diagnostics
3. Royal Liverpool University Hospital
4. Liverpool School of Tropical Medicine
5. The Walton Centre
6. Alder Hey Childrens Hospital

Abstract

Background: Accurate and prompt diagnosis of bacterial meningitis is key for successful patient management and the determination of appropriate antibiotic use. Clinical features alone do not clearly distinguish bacterial meningitis from clinically similar syndromes, such as viral meningitis or meningism. Lumbar puncture followed by cerebrospinal fluid culture is the gold standard diagnostic test, but delays or problems are common with this procedure. A simple blood test, called the TRIM test (Transcripts to Identify bacterial Meningitis), has been developed in collaboration with Fast Track Diagnostics to improve the accuracy and fast track the diagnosis of bacterial meningitis.

Methods: Microarray analysis identified 5 host transcript markers in blood that distinguish bacterial meningitis patients from clinical mimics. The TRIM test, a routine RT-qPCR assay suitable for use in hospital laboratories, has been developed to detect this discriminatory RNA signature. The test has undergone blinded analysis in a set of 177 blood samples (bacterial n =26, viral and meningism n =151) collected from patients recruited to the UK Meningitis Study.

Results: In samples collected within a 5-day period following antibiotic initiation, the TRIM test exhibited 100% (13/13) sensitivity and 89% specificity (135/151) at classifying bacterial meningitis. Distinguishing between bacterial meningitis and viral meningitis the TRIM test exhibited 100% (13/13) sensitivity and 98% (51/52) specificity.

Conclusion: The TRIM test demonstrates high accuracy at classifying bacterial meningitis from clinical mimics. A test that is able to rule out bacterial meningitis will have significant clinical impact; a reduction of healthcare costs and the overuse of antibiotics.
Title: Diagnostic outcomes of ‘optico-spinal demyelination’: A 10 year prospective cohort study from the United Kingdom

Authors: Shahd Hamid, Jay Panicker, Kerry Much, Kumar Das, Tom Solomon, Mike Boggild, Anu Jacob

1. The Walton Centre NHS Foundation Trust
2. University of Liverpool

Abstract

Background: Patients with non-MS ‘demyelination’ can be challenging to classify. With the availability of Aquaporin-4 antibody testing, many patients with relapsing spinal cord and optic nerve syndromes can be classified as NMO spectrum disorders. Despite this, a number of patients remain who have ‘isolated optico-spinal demyelination’ whose natural history is uncertain.

Methods: This is a prospective longitudinal cohort study. From 2003 to 2005 cases of non-MS optico-spinal demyelination were reported via the British Neurological Surveillance Unit (n= 128). Some were typical for NMO and MS (n=61) as per criteria in 2005. After excluding these, 67 patients were classified as optico-spinal demyelination - unclassified (OSD-U) (52%). Patients and their physicians were contacted in 2011 and 2015 to ascertain most recent diagnosis. AQP4 antibodies were tested in all

Results: By 2015 outcomes of 64 of the 67 patients were obtained. A definite diagnosis was made based on clinical, radiological or serum AQP4 IgG in 50 (75%) patients. 39 were classified as NMOSD (58%), 11 as MS (17%) 14 remained as OSD-U (21 %). 3 (4%) patients were untraceable.

Conclusions: At 10 years from recruitment into study median of 12 years after onset of disease 75% of unclassifiable optico-spinal demyelinating disorders had a definite diagnosis. 17% developed MS and 58% developed NMOSD. 21% still remained unclassifiable. Whether the last group comprise a distinct disease entity or will turn out to be typical MS or NMOSD on longer follow up remains to be seen.
**Title:** Genomic and phenotypic insights into severe contact lens-associated eye infections caused by *Pseudomonas aeruginosa*

**Authors:** Yasmin Hilliam¹, Emily Richardson², Janet Wong¹, Joshua Quick², Carmen Martinez-Rodriguez¹, Timothy Neil³, Stephen Tuft⁴, Jo Fothergill¹, Stephen Kaye³, Nick Loman², Craig Winstanley¹

1. University of Liverpool
2. University of Birmingham
3. Royal Liverpool University Hospital
4. Moorfields Eye Hospital

**Abstract**

Bacterial infections of the cornea are a major cause of visual loss world-wide, with approximately 6000 cases of bacterial keratitis per year in the UK alone. Through the UK Microbiology Ophthalmic Group (MOG), we have collected 658 isolates of *Pseudomonas aeruginosa* associated with keratitis (2003-2012), and have linked clinical metadata, including contact lens use.

In this study, we used whole genome sequencing (WGS) of 411 these isolates in order to comprehensively characterize the UK *P. aeruginosa* keratitis-associated population. Genomic DNA was extracted from each of the *P. aeruginosa* isolates, and barcoded sequencing libraries were prepared and sequenced on the Illumina MiSeq platform.

Core genome SNP phylogeny of the keratitis isolates, alongside a reference set of a similar number of genomes from isolates obtained from a range of other infections, revealed two major clusters (Group 1, which includes PAO1 and Clone C; Group 2, which includes PA14). Amongst the wider *P. aeruginosa* population, Group 1 is dominant, but the keratitis isolates were distributed more evenly between the two major clusters, confirming a statistically significant association between keratitis isolates and Group 2. Group 1 and Group 2 were almost exclusively associated with carriage of exoS and exoU respectively, but there was no association between contact lens (CL) use and either Group 1 or Group 2. Further phenotypic analysis of the CL-associated isolates indicated variations in resistance to CL disinfection solutions and susceptibility to phages.

We gratefully acknowledge funding from Fight for Sight, UK.
Title: Can F. hepatica metacercariae survive ensiling and retain viability?

Authors: Bethan John¹, Jane Hodgkinson¹, Diana Williams¹, Nicola Beesley¹, Dave Davies²

1. The University of Liverpool
2. Silage Solutions Ltd.

Abstract

Fasciola hepatica, the liver fluke, is an important cause of morbidity and mortality in ruminants worldwide. Effects of sub-clinical infection on growth rate and milk yield is estimated to cost the UK cattle industry up to £40.4 million annually. It has been established that up to 50% infective metacercariae can overwinter on pasture and infect grazing livestock the following spring. However, the risk feeding silage poses to livestock infection is not well understood, hence our objective is to determine if F. hepatica metacercariae can survive ensiling and retain viability. We have optimised a PCR protocol to detect F. hepatica DNA originating from silage effluent. Effluent samples were inoculated with F. hepatica DNA and serially diluted (1ng – 0.1pg) to evaluate PCR sensitivity; detection levels of 1pg DNA were achieved. To ensure detection of metacercarial DNA from silage effluent, laboratory-maintained snails shed metacercariae onto silage samples which were washed and filtered. Subsequent e-DNA extraction from filters and PCR revealed as few as 6 metacercariae originating from silage can be detected, indicating that these washing and molecular protocols are applicable to larger scale ensiling scenarios. In preparation for laboratory-based ensiling, grass samples were dried to a variety of Dry Matter (DM) contents consistent with quality and spoiled silages. Small-scale ensiling tubes containing viable metacercariae and grass of differing DM contents have been established. For laboratory ensiling, survival of metacercariae will be conducted over a timeframe, a one month ensiling period is currently being assessed for metacercariae recovery, morphological analysis and diagnostic PCR.
Title: Host-derived markers of Lyme disease and their diagnostic potential

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1. University of Liverpool
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3. Public Health England

Abstract

Lyme disease (LD) is a multisystem infection caused by tick-borne spirochaetes of the *Borrelia burgdorferii* sensu lato group. UK laboratory diagnosis of LD involves the two-tier serological approach. The negative predictive value of the test has been challenged, particularly in early stage LD. There is considerable interest, therefore, in the development of improved diagnostic tests. The main aim of the project is to identify new markers that could form the basis for improved tests.

A mass spectrometry biomarker discovery study was undertaken on LD positive and negative residual diagnostic samples from UK LD testing by Public Health England. A control group of healthy subjects serum samples (from NHS blood transfusion service) were also included. To ensure differences were specific to LD rather than genetic to infection, a “related-disease control group” including serum samples from syphilis, leptospirosis and chronic fatigue syndrome were included. A total of 50 human samples were compared by label-free quantitative mass spectrometry. Lipocalin-2 was found at a significantly higher abundance in the LD-positive patients compared with those that were LD-negative. Lipocalin-2, a protein involved in immunity, has been found in mice exposed to *B. burgdorferi*. Further analysis by ELISA showed that Lipocalin-2 was significantly increased in LD positive patients.

The results of the mass spectrometry run have generated several proteins of interest that will be further investigated by ELISA on larger sample groups to further investigate their diagnostic potential.
Title: Characterisation Studies of Pathogenic Leptospira Outer Membrane Proteins

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2. Liverpool School of Tropical Medicine

Abstract

Leptospirosis is an emerging zoonotic disease, which affects a wide range of vertebrate hosts. Bovine leptospirosis has a substantial impact in terms of food security, animal welfare and public health which causes considerable economic losses through abortion, milk drop, reduced fertility and kidney failure. In the UK, estimated losses on cattle productivity were as much as £22.3 million per year with 70% of the unvaccinated dairy farms exposed to this bacteria. The pathogenesis of leptospirosis at both molecular and cellular level remain poorly understood due to limitation of knowledge and complexity of the disease. Vaccination is an important tool to control leptospirosis in cattle farms. Current available leptospiral vaccines which are bacterins have limitations such as only enabling short term immunity, failing to prevent pathogen transmission and reactogenicity. Several leptospire virulence factors have been identified thus far. Outer membrane proteins (OMPs) have been identified as important virulence factors and are also considered important vaccine candidates due to their ability to stimulate immune reaction during infection. In this study we have identified several genes encoding novel leptospiral OMPs and using a reverse vaccinology approach we have produced the recombinant proteins and characterised their function using functional binding assay with various types of extracellular matrix components and investigated their interaction with the host immune response. This research will help us to understand host bacteria interactions during pathogenesis, and underpin future diagnostics and vaccines.
Title: The burden of influenza-associated illness among hospitalised infants aged 8 days to 11 months at Queen Elizabeth Central Hospital (QECH), Blantyre, and Chikwawa District Hospital, Malawi. Updates from an ongoing prospective case control study.

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2. Malawi-Liverpool-Wellcome Trust Clinical Research Programme
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Abstract

Background: The burden of severe influenza-associated disease is likely underestimated due to healthcare access and limited testing.

Methods: March 2017-February 2020, we conduct a prospective case-control study enrolling hospitalized infants (not surgical or oncology) aged < 12 months at two South Malawian Hospitals. Healthy age-appropriate controls are enrolled from routine immunization clinics. Influenza is detected using real-time Reverse Transcription Polymerase Chain Reaction of nasopharyngeal and oropharyngeal swabs and acute and convalescent serology for influenza using hemagglutination inhibition assay. Influenza A is further sub-typed. Annual batched PCR testing using FTD 33-pathogens kit planned for other respiratory pathogens.

Results: During March 2017-July 2017, 260 cases and 111 controls have been enrolled. Among cases, 217 (83%) reported at least one respiratory symptom. Among the 43 cases with no respiratory symptom, most common symptoms were fever (34/43, 79%) and diarrhea (16/43, 37%). 51 cases reported HIV exposure and 5 were HIV-infected. Ten of 299 (3%) tested positive for flu (7/influenza A (6 H3N2, 1 H1N1sw), 3 influenza B). 9/260 (3.5%) were cases and 1/111 (0.9%) controls: all were from Blantyre. Comparison of flu positives among those with (8/217) and those without respiratory symptoms (1/43) did not show significant difference (odds ratio 1.61, p=0.99). Only 1 hospitalized influenza positive infant required oxygen. There were 4 deaths (1.5% of cases), none were positive for influenza.

Conclusion: In the initial 5 months of this study, most enrolled infants have respiratory symptoms. However, influenza prevalence is the same among infants without respiratory symptoms and those with respiratory symptoms.
Title: Variations in immunopathogenesis of infectious bronchitis virus Q1 in commercial broiler chicks with different growth rates

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Abstract

We aimed to investigate the immunopathogenesis of infectious bronchitis virus (IBV) Q1 in commercial broiler chicks with different growth rates and compare gene and cytokine expression.

Birds were monitored daily for clinical signs. At 3, 7, 9, 14, 21 and 28 days post infection (dpi), cloacal (CL) and oropharyngeal (OP) swabs were obtained for virus detection. Kidney, trachea and proventriculus tissue was collected at postmortem for qRT-PCR. Sera was taken at 7, 14, 21 and 28 dpi.

For line-A, swabs from the infected group were RT-PCR positive at all sampling days, whereas line-B were positive until 14 dpi. Significantly higher viral loads were found in the trachea, proventriculus and kidney of line-A compared to line-B at 7-9 dpi. At 21 and 28 dpi, antibody titres in line-B was higher than line-A by ELISA. Tracheal innate immune responses showed INFα up-regulation only in line-A, with INFβ upregulated in both lines. We saw significant up-regulation of TLR3 in line-A up to 7 dpi, and at all sampling points for line-B. Interestingly, MDA5 was only significantly up-regulated in line-A and down-regulated in line-B at 1 dpi. In the kidney tissues for line-A, INFα and INFβ were up-regulated at 1 and 1-3 dpi respectively. There was significant TLR3 up-regulation in line-B throughout the study period, but not line-A. MDA5 was significantly up-regulated in both lines at 7 and 9 dpi. It appears that the immunopathogenesis of IBV Q1 in slow growing chicks was somewhat milder, likely associated with the genetic make-up of this breed.
Title: HIV-1, HCV and Ebola Virus Envelope Trans-complementation can Modulate Virus Phenotypes

Authors: L McKay, A Ruggiero, G Pollakis, WA Paxton

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Abstract

Co-viral infections can lead to trans-complementation of virus envelopes onto viral particles. We studied the effects of different envelope expression (HIV-1 gp120, Ebola virus GP and HCV E1E2) on virus particles and the effects on modulating virus phenotypes.

Pseudo-typed viruses were produced by transfection of 293T cells with HIV-1 R5 and X4 gp120, Ebola GP and HCV E1E2 envelopes separately or in combination with an envelope deficient lentiviral backbone. Viral production was measured using p24-antigen ELISA and infection via luciferase quantification. A V5 tag was ligated into the JRFL and E1E2 expression plasmids and detected using a primary V5 antibody bound by an anti-mouse Goat PE conjugate. Trans-complemented virus was neutralised with either an anti-HIV-1 tri-Ab or Ebola survivor convalescent plasma.

Limiting dilutions of E1E2 plasmid caused a reduction in infectivity of the HIV-1 virion, however when the Ebola GP was incorporated into an R5 HIV-1 virion a significant increase in infectivity was seen on both susceptible and non-susceptible cell lines. Dual envelope expression was confirmed using the V5 tag. The presence of the Ebola GP on an X4 HIV-1 virion had a differential pattern of neutralisation than when HIV-1 or Ebola GP alone was used. A series of HIV-1 R5 envelopes with varying fusogenic properties were also tested.

HCV E1E2 envelope can interfere with HIV-1 virion production likely through trans-envelope complementation and potentially expands the HIV-1 reservoir. Trans-envelope complementation can also alter virus infectivity phenotype as well as the neutralisation profile of the virus.
Title: Immune-complex mimetics (ICMs): adjuvant free approach to vaccination and improved immune diagnostics for the control of flaviviruses.

Authors: Shona Moore¹, Pat Blundell², Lance Turtle¹, Richard Pleass²

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Abstract

Many important flavivirus diseases have no vaccine available. Zika virus caused hundreds of thousands of infections in 2015/16, as well as many thousands of cases of congenital Zika syndrome (CZS). Many vaccines are under development, but these require co-administration with adjuvants that are difficult to manufacture, are often unstable, and can have undesirable side effects. The use of adjuvants also greatly adds to the overall cost of vaccine development in low income settings.

Multimeric Fc-fusion proteins mimic immune-complexes by delivering antigens directly to antigen presenting cells, therefore ensuring that antigen processing is efficient as possible. This may allow delivery of effective vaccines that do not rely on adjuvants. The multimeric structure of the constructs allows for cross-linking and triggering of critical Fc-receptors that is not possible with monomeric Fc-antigen fusions or protein-in-adjuvant approaches.

We are developing multimeric Fc-fusion proteins containing the envelope protein domain III (EDIII) epitope from Zika virus, as well as from other major flaviviruses. EDIII have been cloned into a highly engineered Fc-fusion expression plasmid in order to form multimers. These have been successfully expressed by CHO cells and protein purified by affinity chromatography. The fusion proteins will next be screened for receptor interactions and for recognition by convalescent immune sera. Their interaction with immune cells and virus infected cells will be investigated initially while phase II of the project will allow further investigation of their immunogenicity. The outcome of this work is to develop novel vaccines, not only against Zika, but also other flaviviruses.
Title: Elucidation of the cellular interactome of ZEBOV VP35 for targeting the function of essential host factors with repurposed antiviral therapies.

Authors: Jordana Muñoz-Basagoiti\textsuperscript{1,2}, Stuart D Armstrong\textsuperscript{1,2}, Isabel García-Dorival\textsuperscript{1,2}, Miles W Carroll\textsuperscript{1,3}, Julian A Hiscox\textsuperscript{1,2}

1. NIHR HPRU EZI
2. IIGH
3. PHE

Abstract

Ebola virus disease (EVD) is a deadly disease in humans. With more than 11,000 deaths, the 2013-2016 West African Ebola virus (EBOV) outbreak highlighted the need to find efficient vaccines and therapeutics, which are currently unavailable. Viral genomes require the host cell machinery for RNA synthesis. The use of the specific virus-host protein-protein interactions (PPIs) as drug-targets is an attractive alternative to directly targeting viral proteins since RNA viruses are more prone to accumulate mutations. Our laboratory has developed a rapid screening method to detect these PPIs and to test the effect of their inhibition on the replication and transcription processes in EBOV.

The interactome of EBOV VP35, a viral component of the nucleocapsid complex that is required for the virus transcription, replication and antagonism of the type-I IFN response of the host, was elucidated by co-immunoprecipitation, LC-MS/MS and statistics analyses.

The cellular interactome of VP35 showed the viral protein to associate with 28 statistically significant host proteins, including nucleic acid binding proteins, transporter and cytoskeletal proteins, transfer/carrier proteins, enzyme modulators, hydrolases and membrane traffic proteins were the most abundant. As seen in other studies, the cytoplasmic dynein light chain 1 (DYNLL1/CD11L1) and ATP5I were found to have the highest potential association with VP35.

This makes them good candidates for drug-targeting antiviral therapies with siRNA and small molecule inhibitors in cells where a reverse genetics system that mimics the transcription and replication of Ebola virus can be used as part of high throughput screening assays.
**Title:** Phenotypic diversity within *Pseudomonas aeruginosa* populations causing urinary tract infections

**Authors:** J Newman, J Fothergill, R Floyd

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**Abstract**

Urinary tract infections (UTIs) are the most common healthcare acquired infection. A small but significant portion of UTIs, both healthcare acquired and community associated, are caused by *Pseudomonas aeruginosa*. *P. aeruginosa* is more frequently detected in complicated UTIs and shows a higher prevalence of resistance to carbapenems than other UTI-causing pathogens. The biofilm lifestyle of *P. aeruginosa* is believed to play a critical role in its antibiotic resistance. Studies of clinical *P. aeruginosa* in chronic lung infections have discovered remarkable within-patient phenotypic and genotypic diversity however bacterial populations during UTI have not been studied in detail. Therefore, the aim of this research was to study the heterogeneity within clinical samples collected from five patients suffering from uropathogenic *P. aeruginosa* infections.

A representative population of 40 isolates were collected from each of the five patient samples and subjected to phenotypic assays including pyocyanin assays, hypermutability assays, minimal growth assays and antimicrobial susceptibility tests (ASTs). Random amplification of polymorphic DNA (RAPD) PCR was conducted on all isolates to determine whether there was genotypic homogeneity within samples in spite of their phenotypic heterogeneity. Samples tested exhibited homogenous genotypes (as determined by RAPD PCR) while displaying heterogeneous phenotypes. Natural populations will be recreated in vitro using artificial urine medium to determine the contribution of co-existing phenotypic variants. Phenotypic variation within samples could play a crucial role aiding the adaptation of mature *P. aeruginosa* biofilms to the urinary tract. Increased understanding of UTI dynamics may lead to more effective treatment regimens for recurrent UTIs.
Title: The role of SIGLEC1 in Japanese Encephalitis

Authors: Tessa Prince¹, Raquel Medialdea Carrera¹, Lucille Rainbow², Tom Solomon³, Neil Blake¹, Mike Griffiths¹

1. IGH
2. ITM

Abstract

Background: Japanese Encephalitis Virus (JEV) is a major cause of viral encephalitis in Asia and with climate change and globalisation has spread to regions of the world where it has previously been unseen. As such an understanding of the pathogenesis of this neglected tropical disease should be promoted. Whole blood transcriptomics on the blood of Nepalese children with acute encephalitis syndrome (AES) has found up-regulation of several transcripts involved in the type I interferon response in patients diagnosed with Japanese encephalitis, in contrast to those with an unknown aetiology. Once such transcript was SIGLEC1. Little is known on the role of SIGLEC1 during flavivirus infection, therefore this study aims to examine the role of SIGLEC1 in Japanese encephalitis.

Methods: qRT-PCR was used to evaluate which cell types in the brain expressed SIGLEC1. Once this was determined, the cells were tested to assess whether they could be reverse-transfected with siRNA reliably. Specific siRNAs for SIGLEC1, RSAD2 (Viperin) and IFI27 were evaluated for their use in a knockdown model.

Results: qRT-PCR found SIGLEC1 RNA was expressed in human microglia, but not primary human astrocytes. Approximately 80% knockdown of SIGLEC1 RNA expression could be achieved within 72 hours at a concentration of 30nM siRNA.

Conclusions: Recent research has suggested that brain microglia may act as a reservoir for JEV virus during infection. JEV viral loads in control and SIGLEC1 knockdown microglia will be analysed to determine if SIGLEC1 plays a role in the pathogenesis of JEV infection in microglia.
Title: Identification of a novel specific cell-surface protein family as vaccine candidates in *Trypanosoma vivax*

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Abstract

Animal African trypanosomiasis (AAT), known as Nagana, is an endemic livestock disease in Africa and South America caused by the parasite *Trypanosoma vivax*. There is no vaccine available for AAT as since the parasite displays antigenic variation (VSG) in its cell-surface to evade the host immune response. However, *T. vivax* genome contains species-specific genes that are expressed in the bloodstream form indicating that the parasite surface coat consists of abundant, invariant proteins in addition to VSG. Using a reverse vaccinology approach, we present a novel family of *Trypanosoma vivax* specific cell surface proteins (TVCSP) expressed in the bloodstream form as vaccine candidates, and so offer a way to design an effective vaccine against the parasite. Our results show that the novel protein family has 44 members and they seem to be located in the cell-surface of the parasite according to our bioinformatics analysis. Moreover, phylogenetic analysis revealed 8 different clades within the family. All members of the novel family are expressed in South American as well as in East and West African populations. In order to identify TVCSP immunogenic epitopes, we also performed an overlapping peptide array analysis using sera from naturally infected cattle. This is the first report of a novel specific cell-surface protein family of *T. vivax* and are thus potential vaccine candidates to design a vaccine against AAT. Breaking through the antigenic variation impasse promises to revolutionize the fight against AAT and bring unprecedented benefits to animal health and agricultural productivity across the world.
**Title:** Replication of Mycobacterium Tuberculosis down-modulates HIV-1 expression in an in vitro co-culture system

**Authors:** A Ruggiero¹, M Pouget¹, S Donnellan², G Pollakis¹, G Biagini², WA Paxton¹

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**Abstract**

Background: Human immunodeficiency virus Type-1 (HIV-1) and Mycobacterium Tuberculosis (Mtb) co-infection represents a major public health concern. The understanding of the intracellular interactions between the two pathogens is still poorly understood due to the lack of in vitro co-infection systems.

Methods: THP-1 differentiated macrophages were used for infections with HIV-1 or VSV-G pseudo-typed virus (30 ng of p24 protein) and Mtb (5 MOI). Pseudo-typed viruses were produced by transfection of 293-T cells and expressed green fluorescent protein (GFP) or firefly luciferase (LUC). H37RV Mtb strain expressed the mCherry protein. Magnitude of infections were measured at 24-144 hours as follows: estimation of levels of GFP, mCherry or LUC; quantification of integrated HIV-1 DNA levels.

Results: Infection of THP1 cells was obtained for both Mtb and HIV-1 alone and in co-infection experiments. Interestingly, such reduction was observed both in cells pre-infected with Mtb or in cells that received Mtb after or with HIV-1. Similarly, Mtb infection levels were also reduced in the presence of HIV-1 co-infection (range of reduction: 1-3 fold). The reduction in pseudo-virus infectivity was observed for both HIV-1 and VSV-G viruses suggesting a post-entry block to infection.

Discussion: We showed in our in vitro system co-infection of cells with HIV-1 and Mtb. We demonstrated that either pre- or post-infection with Mtb lowers HIV-1 expression, indicating that Mtb antigens can modulate HIV-1 infectivity.
Title: A reverse vaccinology approach to producing vaccine candidates for bovine trichomoniasis

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Abstract

*Tritrichomonas foetus* is a unicellular protozoan parasite that causes the venereal disease bovine trichomoniasis. This disease is found throughout Europe and North America and is an emerging threat to UK cattle. Trichomoniasis increases rates of spontaneous abortion and foetal maceration in addition to causing infertility. This disease has economic implications as it can reduce calving rates by up to 35% and many infected animals are culled, leading to large financial losses. Currently there is no vaccine available that can provide long lasting immunity in cattle and prevent reinfection. This project aims to produce an annotated *T. foetus* genome sequence and use this to identify vaccine candidates using a reverse vaccinology approach. The *T. foetus* genome sequence was produced using PacBio RSII long DNA reads and assembled into 2700 contigs (N50=89,000). The sequence is being annotated with the use of gene predictive software and proteomic and transcriptomic data. Vaccine candidates will then be identified that are naturally immunogenic, parasite specific and cell-surface expressed. In silico methods will be used to identify cell-surface expressed proteins and predicted B-cell epitopes. A peptide array of the putative proteins found will be created and screened using infected cow blood sera in order to establish whether they are naturally immunogenic. This project is first step in producing a viable vaccine against this disease, producing validated vaccine candidates which could be further tested and then used in preliminary clinical trials.
Title: Comparative analysis of two HIV-1 DNA quantification assays for monitoring therapeutic vaccine trials

Authors: Jordan Thomas, Alessandra Ruggiero, William A Paxton, Georgios Pollakis

Institute of Infection and Global Health, Department of Clinical Infection, Microbiology, and Immunology, University of Liverpool

Abstract

Background: Despite the success of anti-retroviral therapy HIV-1 infection cannot be cured due to the maintenance of viral reservoirs. Therapeutic vaccines are being conducted where HIV-1 immune responses are induced with the aim of reducing or eliminating the reservoir pool. Measuring HIV-1 DNA in vaccinated individuals is required to monitor vaccine efficacy. Here we compared two DNA quantification assays to determine which assay to use in monitoring therapeutic vaccine success.

Materials and Methods: DNA was extracted from PBMCs of HIV-1 infected individuals and subjected to total HIV-1 and 2-LTR quantification using two previously described assays, targeting the 2-LTR region of the genome. Agreement between assays was determined using Bland-Altman analysis.

Results: Both assays demonstrated comparable performance in the quantification of total HIV-1 DNA. PCR efficiency of both assays was within the acceptable range (90-105%) and both assays can readily detect low copy numbers in virally suppressed patient samples. Although differences were observed in the quantification of some patient samples, generally there was strong correlation between assays (R²=0.8527) and Bland-Altman analysis revealed agreement between the quantification methods (Bias: -0.01±0.45 log10 and 95% Limits of Agreement, -0.8748 to 0.9049 log10).

Conclusions: Both assays have demonstrated comparable performance when quantifying low copy, virally suppressed patient samples and a combination of both methods may provide a comprehensive assay to quantify total and 2-LTR HIV-1 DNA in a therapeutic vaccine trial. Additionally, we are currently developing new and novel assays that can monitor for variation in HIV-1 mRNA expression within vaccine recipients.
Title: Safety, acceptability, and preliminary efficacy of vaginal probiotic and metronidazole maintenance therapy for the prevention of bacterial vaginosis recurrence in Rwandese women

Authors: MC Verwijs1,2, SK Agaba2, JHHM van de Wijger1,2 on behalf of the VMB Trial study team.

1. Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom.
2. Rinda Ubuzima, Kigali, Rwanda.

Abstract

Background: Bacterial vaginosis (BV) is associated with increased risk of HIV acquisition and pregnancy complications. Symptomatic BV is treated with metronidazole but recurrence is high.

Methods: Women with BV by Nugent/Amsel criteria were treated with 7-day oral metronidazole at the research clinic in Rwanda. After successful treatment, they were randomised into four groups (n=17 each): 1) Ecologic Femi+ vaginal probiotic; 2) Gynophilus LP vaginal probiotic; 3) oral metronidazole; and 4) behavioural counseling only (control group). Both vaginal probiotics contained lactobacilli but in different combinations and formulations; all interventions were self-administered 2-3 times per week for two months. Endpoints were measured at baseline, week 1, and months 1, 2, and 6.

Results: 64 women completed the study. Self-reported acceptability and adherence were high. The proportions of women in each group with structurally assessed self-reported urogenital symptoms (Fisher’s exact p=0.894) and spontaneously reported adverse events (p = 0.706) during product use were not statistically different. Structurally assessed pelvic exam findings were rare. None of the women developed vaginal candidiasis. During product use, compared to the control group, oral metronidazole reduced the incidence of BV (Nugent 7-10; per protocol incidence rate ratio 0.08, 95% confidence interval [0–0.57]), as did Ecologic Femi+ (0.28 [0.07–0.89]); the trend for Gynophilus LP was not significant (0.35 [0.08–1.22]). The protective effects disappeared after product cessation in all groups. By 6 months, 65% of the women had developed recurrent BV.

Conclusion: The interventions were feasible, acceptable, and safe, and further research is warranted to improve efficacy.
Title: Interactions of microbes with *P. aeruginosa* during chronic lung infection in cystic fibrosis patients.

Authors: LL Wright¹, L Chatterley¹, S Pottenger¹, N Loman², C Winstanley¹, J Fothergill¹

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Abstract

The Cystic Fibrosis (CF) lung facilitates the cohabitation of microbes and we are just beginning to understand the extent of this diversity. The bacteria in chronic lung infections can form complex, interacting communities, which adapt and diversify due to interactions with both the environment, including host responses, and each other. We aimed to study the interspecies interactions components using an in vitro model.

We have developed co-culture biofilm models in which microbial interactions can be investigated. Using an artificial sputum medium (ASM) model, which mimics CF sputum, *Pseudomonas aeruginosa* was co-cultured with pathogens, including *Staphylococcus aureus* and *Burkholderia cenocepacia*, and other common members of the CF lung microbiota, including *Streptococcus sp.*, to determine whether interspecies interactions facilitate changes in the virulence and diversification of *P. aeruginosa*. Virulence testing using the Galleria mellonella model showed significant changes in *P. aeruginosa* virulence after co-culture, and phenotypic differences between isolates were also apparent, including changes in antimicrobial susceptibility leading to the development of clinically resistant isolates. Whole genome sequencing revealed mutations in quorum sensing (QS) associated genes and two-component regulators. In addition, alterations in the gene expression of virulence-associated genes were detected including genes linked to QS, exopolysaccharide production and iron acquisition.

Interactions with other species can change the behaviour of *P. aeruginosa*, impacting on virulence and resistance. Understanding complex interactions between different bacterial species and in combination with immune components may uncover novel therapeutic targets and ultimately lead to altered CF patient management.
Title: An important role of IL-35 producing CD4 T cells in the regulation of superantigenic *Staphylococcus aureus* activated Th17 response in human NALT

Authors: Rong Xu¹, Xiaoqing Wei², Lualuaa Zaki³, Shamsher Ahmed Muhammad¹, Ravi Sharma³, Madhan Krishnan⁴, Sam Leong⁴, Aras Kadioglu³, Qibo Zhang¹

1. University of Liverpool
2. Cardiff University
3. Alder Hey Children’s Hospital
4. University Hospital of Liverpool

Abstract

Background: IL-35-producing CD4 T cell is a subset of Treg cells, which is critical in suppressing pathogenic Th17-mediated autoimmune diseases in mouse model, but it remains controversial in human. Nasal colonization with superantigenic *Staphylococcus aureus* (SAg-Sa) activates a potent pro-inflammatory cytokine response including Th17, which has the potential to cause autoimmune conditions if not controlled.

Methods: Adenotonsillar MNC were stimulated with SAg-Sa extracts in the presence or absence of recombinant IL-35 or IL-10. T cell responses and cytokine production were later measured in stimulated cells. CD4 T cells were isolated from SAg-Sa stimulated tonsillar MNC for the detection of IL-35 expression at both mRNA and protein levels.

Results: We found that SAg-Sa stimulated Th17 cell responses were positively associated with Foxp3+ CD4+ T cell activation. IL-17A and Foxp3 double positive CD4 T cells were also presented. Although the production of both IL-10 and TGFβ1 were increased following Sag-Sa stimulation, IL-10 pretreatment failed to suppress Sag-Sa activated Th17 response. More importantly, we detected both IL-35 subunits in human tonsil CD4 T cells for the first time. And in contrast to IL-10, IL-35 subunit p35 was decreased upon Sag-Sa stimulation, which may indicate IL-35 producing CD4 T cells played a role in the suppression of SAg-Sa activated Th17 response. Indeed, Prior treatment with IL-35 in MNC culture effectively inhibited Sag-Sa induced IL-17A production.

Conclusions: This study demonstrated that IL-35 producing CD4 T cells in human tonsil may be a key T cell population in the regulation of SAg-Sa associated pathogenic Th17 responses.