Appendix 17

Entebbe Mother and Baby Study

PROTOCOL AMENDMENT

Amendment title: Testing for human enteric viral infections (including human noroviruses and Hepatitis E virus) among children using the Entebbe Mother and Baby Study stored samples

29th September 2014

Investigators and collaborating institutions		
Name	**Institutional affiliation**	
Alison Elliott	MRC/UVRI Uganda Research Unit on AIDS London School of Hygiene & Tropical Medicine	Principal investigator
Ian Goodfellow	Division of Virology, Department of Pathology, University of Cambridge	Co-Investigator

Funding Organisations: **Alborada Trust**

Society for General Microbiology

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1. Background

Human Noroviruses (HuNVs) are now recognised as the major cause of viral gastroenteritis in the developed world, causing at least 2 million infections in the UK every year and 21 million in the USA with over 71,000 hospitalisations. The majority of infections occur in places with close living environments such as hospitals, military camps and schools. Hospital outbreaks result in ward closures and over 47,000 lost bed days per year in the UK at an annual cost to the NHS of over £115 million (1), making noroviruses economically significant pathogens. The majority of infections are acute and self-resolving but there are increasing reports of chronic infections in the immunocompromised, where the symptoms of severe diarrhoea and vomiting can persist for many years resulting in serious complications and increased risk of mortality. Although in developed countries the risk of mortality is largely confined to high risk populations, such as the very young, immunocompromised and elderly, HuNVs have recently been reported to be the second leading cause of death due to gastroenteritis in the USA.

The epidemiology of HuNV in developed countries is well documented; in contrast the prevalence in developing countries has not been well established. One study estimated that HuNVs are responsible for over 200,000 deaths in children under 5 each year (2), although this is likely to be a substantial underestimate due to difficulties in surveillance, sampling and conclusive diagnosis in some settings. A recently published study investigated the occurrence of HuNVs in infants and young children with acute diarrhoea in multiple countries across Asia, Africa and South America. In general norovirus was detected in 8-20% of cases with the exception of a few countries where sample size was limited to below 10 (3). However the samples were originally obtained by the WHO in 1976-1979 prior to the emergence of the pandemic GII.4 HuNV strains, which have since increased infection rates worldwide. Therefore more up-to-date surveillance data is required particularly with the progress of a vaccine into clinical trials. Whilst the utility of a vaccine may be limited in developed countries, where norovirus is largely an economic problem, increased surveillance of HuNV in developing countries may make the case for wider use and availability.

Hepatitis E virus (HEV) is an increasing problem in the UK and is thought to cause 60,000-100,000 infections per year. It typically causes an acute form of hepatitis but can also establish chronic infections in patients with underlying liver conditions. Infections during pregnancy result in high rates of mortality with approximately 30% lethality rates. HEV infection is known to cause large epidemics of liver disease in developing countries. In epidemic settings it is transmitted primarily by the fecal-oral route through contaminated drinking water. During 2007-2009 one of the largest epidemics ever reported in Africa and

worldwide occurred in Uganda, with over 10,000 infections and 160 deaths. Earlier this year the Ugandan Ministry of Health declared another on-going epidemic. Whilst surveillance is underway, the prevalence of HEV in children in Uganda has not been studied nor has the stage at which they may encounter the virus, which unlike HuNV generates protective immunity after the first exposure. A vaccine for HEV has licensed and is currently only used in China, therefore increased seroprevalence data could provide support for the vaccine to be made available for use in developing countries.

2. Objective

The overall objective of this project is to determine the seroprevalence of both HuNV and HEV in Ugandan children. The Entebbe Mother and Baby Study (EMaBS) centre has collected a large bank of samples from over 1,200 children in the population throughout the course of their first 5 years of life. This includes sampling of the mother during pregnancy, cord blood during birth as well as serum samples every year since birth.

2.1. Specific objectives

- To determine the prevalence of noroviruses and hepatitis E virus (HEV) in children in Uganda
- To determine the strains of norovirus most prevalent in Uganda
- To determine the impact of norovirus and/or hepatitis E virus infection in children in Uganda?
- To determine if there is a case to be made for the use of norovirus and/or hepatitis E virus vaccines in developing countries

In addition to providing novel information about these two important viruses, relevant to Uganda, the additional data will contribute to on-going aims of Entebbe Mother and Baby Study. The approved protocol includes investigation of age of infection with hepatitis A virus, as a measure of exposure to faeco-oral pathogens. The additional data from this study will be a useful complement to this information.

3. Methodology

3.1. Study design and study population

This longitudinal study will use stored samples from the Entebbe Mother and Baby Study (EMaBS). The Entebbe Mother and Baby Study (EMaBS) was designed to investigate the hypothesis that helminth infections modulate the host immune response both to themselves and to unrelated immunogens, pathogens and allergens. The study, which was a randomised, double-blind, placebo-controlled trial of

anthelminthic treatment during pregnancy and early childhood, has been completed, but continued follow up of the children to age 15 has been approved and is in progress. Children are currently aged between nine and eleven years.

To determine the exposure to HuNV and HEV in the cohort of Ugandan children (EMaBS), an enzyme-linked immunosorbent assay (ELISA) will be established for both viruses. The reagents required for this will first be generated in Professor Goodfellow's laboratory in Cambridge. A member of the laboratory team in Entebbe will visit Cambridge to learn the relevant technique. As HuNV cannot be grown in cell culture and the culture systems for the HEV are limited, recombinant expressed virus-like particles will be produced, which are structurally and antigenically similar to infectious virus. The methods for generating virus-like particles have been well established and are now routinely performed in our laboratory. The virus-like particles and other reagents will then be taken to the Uganda Virus Research Institute, where they will be used to set up an ELISA for each virus. Once the assays have been established, the blood samples obtained from the Ugandan children will be screened for antibodies against either virus beginning with those taken at 5 years. For any that test positive, we will then work back through the samples to determine at what age they became seropositive and first encountered each virus.

HuNVs are classified into a diverse range of genogroups and genotypes. The current prevailing strains belong to Genogroup II.4 and have been responsible for all epidemics in developed countries over the past decade. The prevalence of different strains in developing countries has not been fully established, with two studies from Brazil and Ethiopia suggesting that greater strain diversity is common (4, 5). This may influence the effectiveness of a vaccine generated against the predominant strain. Therefore the prevalence of norovirus genogroups will also be determined in the sample population by screening against pools of virus-like particles of different strains. If sufficient samples are available this could be narrowed down to assess exposure to individual strains and determine which strains of norovirus are the most predominant in Uganda. Although HEV exists as multiple genotypes, they fall within a single serotype which results in cross-protective immunity, therefore only a single virus-like particle will be used.

3.2. Laboratory investigations

We will use both ELISA and Luminex assays to determine the seroprevalence of these viruses.

Enzyme linked immunosorbant assay (ELISA) based approach

Children's plasma or serum samples, depending on availability of the samples, will be screened for seroconversion to HEV using genotype 3 VLPs, to noroviruses using two separate pools of genogroup I (GI) or genogroup II (GII) VLPs and to no viral like particle (VLP). Seropositivity will be scored against the background set using either no VLP or an unrelated animal calicivirus VLP (e.g. feline calicivirus or vesivirus 2117). A limited number of seropositive samples will then be screened using serial dilutions against the antigen to which they were positive, starting at dilutions of 1:50. If necessary (and sufficient material is available) we will deconvolute the GI and GII pools to determine seropravelence against individual genotypes.

Luminex based approach

If time allows, an enteric virus luminex assay will be developed and validated. The benefits of doing this would be novelty of the approach, using smaller sample volumes and the screening process would take less time. The drawback would be the timescale required to establish and validate the approach and that some samples would also then need to be screened using the ELISA method as this has been the widely used.

3.3. Sample size

Since this study aims to screen children to determine the seroprevalence of noroviruses and HEV and make inference about the whole Ugandan population, we will screen all available samples at year 5 followed by back testing of the seropositive children at their previous ages.

3.4. Ethical Approval

The Entebbe Mother and Baby Study (EMaBS) has ethical approval Uganda Virus Research Institute Research Ethics Committee (UVRI-REC) and Uganda National Council for Science and Technology (UNCST) and the London School of Hygiene & Tropical Medicine. This project will use stored EMaBS samples. During informed consent, participants have been informed that "Blood samples, including stored samples, will be used for tests for anaemia, malaria, and other infections, and for allergy tests. Some will be used for tests of immunity and some may be stored for other tests in future." Consent has been obtained for storage of samples for future use. Therefore, no additional consent will be requested for this study.

The results of the assays will be of no clinical relevance to the participants, since they will be performed on samples taken from clinically healthy children between three and six years ago and, even at the time, would have been indicative of a past infection from which the child had recovered. Results will not, therefore, be given to individual participants. Information on the project results, as a whole, will be made available to the participants at a participants meeting. These are generally held approximately once a year.

3.5. Data management

The new data generated from the laboratory assays will be held securely by MRC/UVRI. Data shared with Cambridge colleagues will be identified by a number only, for purposed of confidentiality.

4. Quality assurance and audit

To ensure quality and reliability of the data of this study Standard operating Procedures (SOPs) will be developed for laboratory procedures. The study will be regular monitored and conducted in accordance to the principles of Good Clinical laboratory Practice (GCLP).

5. Significance of the proposed work

This longitudinal study will be the first of its kind to follow the exposure of children in Uganda to these two viruses over their first 5 years of life. Therefore the results will be published in a peer-reviewed journal to contribute to discussions on the use of vaccines in developing countries. Having established the collaboration, it is expected to be maintained well beyond the duration of the reciprocal visits as it will benefit both of our laboratories. We hope to be able to subsequently expand the study to other enteric viral infections with the long-term aim of securing external funding to facilitate a prospective study that would also include the collection of faecal samples. This would facilitate genomics type studies that rely on new sequencing technologies to gain a better understanding of the prevalence and impact of enteric viruses in children in developing countries during the most critical stages of their life.

Results will also be shared with relevant colleagues in the Ministry of Health, and the Expanded Programme on Immunisation.

6. Timeframe

This project will commence in November 2014 with a visit from a lab technologist from the Uganda Virus Research Institute to Prof Goodfellow's laboratory at the University of Cambridge to learn how to

generate all of the necessary reagents. This will then be followed with a reciprocal visit of a post-doctoral researcher from Cambridge to Uganda in November 2014. This visit will be for the duration of approximately 6 weeks to establish and optimise the assays and complete an initial screen of the samples from the children at age5. For positive samples back-testing of the samples taken at earlier stages will then be performed and screening will continue beyond the duration of the visit for an expected 6 months in total (1 year at 0.5 FTE). During this time both labs will be involved in the data assembly and analysis.

7. Funding

Funding has been secured from the Cambridge-based Alborada Trust, and from the Society for General Microbiology, to enable us to undertake this serological study of two important human pathogens in Ugandan children. Details of the budget are shown below:

- £2000 will cover travel and accommodation for UVRI employee to visit Cambridge Society for General Microbiology
- £2000 for travel and accommodation for Cambridge employee to visit UVRI
- £1000 for a small amount of research consumables
- Additional funding may be available from Prof. Ian Goodfellow's Wellcome Trust Senior fellowship to cover the costs associated with supporting a lab technologist at UVRI

8. References

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