RESEARCH NOTE

Cross-sectional study of IgG antibody levels to invasive nontyphoidal *Salmonella* LPS O-antigen with age in Uganda [version 1; peer review: 2 approved, 2 approved with reservations]

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v1 **First published:** 26 Jun 2019, **3**:1501
https://doi.org/10.12688/gatesopenres.13034.1
Latest published: 26 Jun 2019, **3**:1501
https://doi.org/10.12688/gatesopenres.13034.1

Abstract

Invasive nontyphoidal *Salmonella* (iNTS) disease is a major cause of deaths among children and HIV-infected individuals in sub-Saharan Africa. Acquisition of IgG to iNTS lipopolysaccharide (LPS) O-antigen in Malawi in early childhood corresponds with a fall in cases of iNTS disease suggesting that vaccines able to induce such antibodies could confer protection. To better understand the acquisition of IgG to iNTS in other African settings, we performed a cross-sectional seroepidemiological study using sera from 1090 Ugandan individuals aged from infancy to old age. Sera were analysed for IgG to LPS O-antigen of *S*. Typhimurium and *S*. Enteritidis using an in-house ELISA. Below 18 months of age, most children lacked IgG to both serovars. Thereafter, specific IgG levels increased with age, peaking in adulthood, and did not wane noticeably in old age. There was no clear difference in antibody levels between the sexes and the few HIV-infected individuals in the study did not have obviously different levels from uninfected subjects. While IgG to iNTS is acquired at a younger age in Malawian compared with Ugandan children, it is not clear whether this is due to differences in the populations themselves, their exposure to iNTS, or variations between assays used. In conclusion, there is a need to develop a harmonised method and standards for measuring antibodies to iNTS across studies and to investigate

Open Peer Review

Approval Status ✓ ✓ ? ?

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version 1 26 Jun 2019	✓ view	✓ view	? view	? view

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acquisition of such antibodies with age across different sites in sub-Saharan Africa.

Keywords
non-typhoidal salmonella, NTS, antibody, Uganda

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: Stockdale L: Formal Analysis, Investigation, Writing – Original Draft Preparation; **Nalwoga A**: Methodology, Writing – Review & Editing; **Nash S**: Formal Analysis, Visualization, Writing – Review & Editing; **Elias S**: Methodology, Resources, Writing – Review & Editing; **Asiki G**: Resources, Writing – Review & Editing; **Kusemererwa S**: Resources, Writing – Review & Editing; **Gilchrist JJ**: Data Curation, Writing – Review & Editing; **Newton R**: Resources, Supervision, Writing – Review & Editing; **MacLennan CA**: Conceptualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by the UK Medical Research Council (MRC): grant number MR/ J003999/1 to LS, grant number MR/K012126/1 to SN, and the Bill and Melinda Gates Foundation (BMGF): grant number OPP1148489 to CM. The Ugandan General Population Cohort study is jointly funded by the UKMRC and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Stockdale L, Nalwoga A, Nash S *et al.* **Cross-sectional study of IgG antibody levels to invasive nontyphoidal *Salmonella* LPS O-antigen with age in Uganda [version 1; peer review: 2 approved, 2 approved with reservations]** Gates Open Research 2019, **3**:1501 https://doi.org/10.12688/gatesopenres.13034.1

First published: 26 Jun 2019, **3**:1501 https://doi.org/10.12688/gatesopenres.13034.1

Introduction

Invasive non-typhoidal *Salmonella* (iNTS) disease is principally caused by serovars *S.* Typhimurium and *S.* Enteritidis and is thought to be responsible for up to 680,000 deaths annually, with Africa accounting for more than half of cases[1]. Much of this burden is in children under 5 years and HIV-infected adults. In view of this major global burden of disease, and rapid emergence of multidrug resistant iNTS strains[2], development of a vaccine is increasingly vital[3].

Studies in Malawian children indicate that anti-*S. Typhimurium* antibodies, notably IgG to O-antigen of LPS and flagellin, and serum bactericidal activity rises rapidly with age in the first few years of life corresponding with a fall in cases of iNTS disease[4,5]. One study found a positive correlation between serum bactericidal assay (SBA) killing and acquisition of anti-LPS IgG[5]. However, there is no standardised assay for measurement of iNTS-specific IgG, and the clinical significance of the iNTS SBA is unknown. Given that incidence of iNTS disease drops in children over 2 years, it has been suggested that a rise in specific antibodies and bactericidal activity correlates with protection. This hypothesis is complicated by the observation that among HIV-infected Malawian adults, high LPS-specific IgG was associated with a lack of *in vitro* bacterial killing[6].

Methods

In a cross-sectional study, we investigated NTS-specific antibody responses in the rural Ugandan General Population Cohort (GPC)[7]. Levels of IgG against serovars *S.* Typhimurium and *S.* Enteritidis LPS O-antigens were measured using a standardised in-house ELISA in stored sera from a cross-section of 1,090 Ugandans of all ages, 10 of whom were HIV-infected. Sera from adults (≥ 16 years) were collected from January 2014 to November 2015, and children (<16 years) from January 2016 to November 2017. Antibody units (AU) were calculated using Gen5 software (version 2.0) using a five-parameter logistic (5PL) curve generated with a standard serum from an iNTS-exposed individual. Sera were defined as seronegative if below the lower limit of detection (4 AU for *S.* Typhimurium and 5 AU for *S.* Enteritidis) at 1:100 serum dilution.

Written informed consent for the use of clinical records and biological samples for research purposes was obtained from all GPC participants following Uganda National Council of Science and Technology guidelines. Ethical approval for the use of samples for this study was obtained from The UVRI Research and Ethics Committee and from the Uganda Council for Science and Technology (Ref: GC/127/19/10/710).

Results and discussion

In this assay, overall O-antigen seropositivity was 82% for *S.* Typhimurium, and was 70% for *S.* Enteritidis. Levels of antibody were undetectable in at least 50% of children until 18 months for both serovars and a similar pattern of increasing IgG level was observed with increasing age (Figure 1A, B). There were no observable differences in antibody levels by sex (Figure 1C, D). HIV-infected individuals did not have notably high IgG antibody responses, although the study was not powered to demonstrate this.

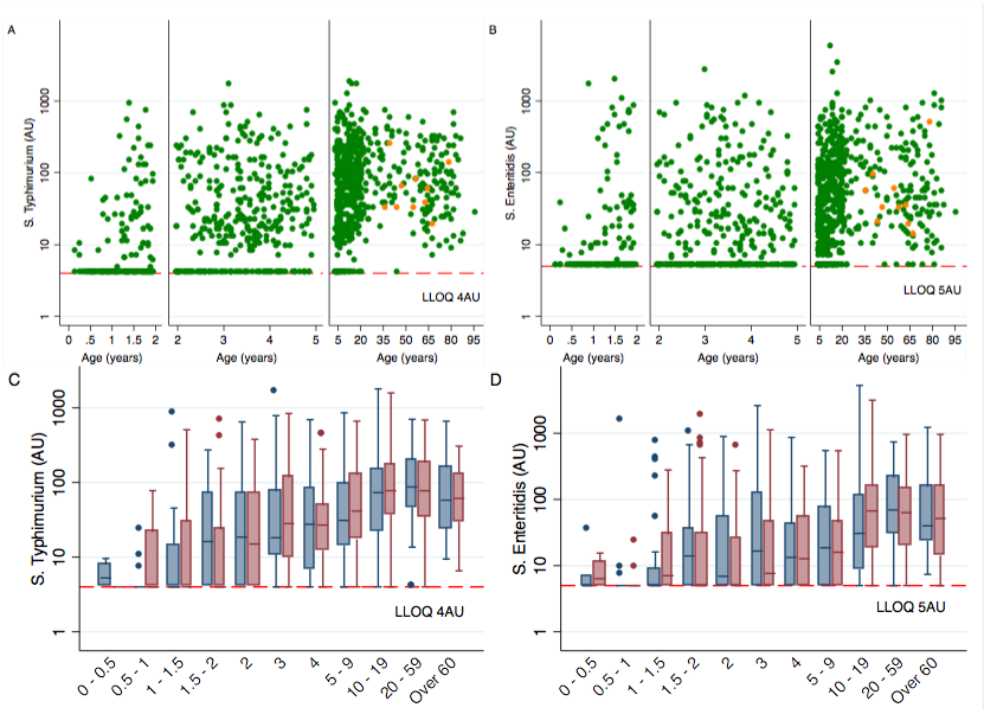

Figure 1. Plots showing antibody units (AU) for S. Typhimurium (**A** and **C**) and S. Enteritidis (**B** and **D**) by age. Orange dots indicate HIV infected individuals. (**A**, **B**). Females are indicated in red and males in blue (**C**, **D**). The box shows the interquartile range (IQR) with middle line representing the median. The whiskers represent the adjacent values, defined as 1.5 × IQR from the edge of the box, with values outside this range shown individually. LLOQ, lower limit of quantification.

Although performed using a flow cytometric assay, previously published data from Malawi suggest that NTS-specific IgG is present in the majority of children throughout infancy[4], contrasting with our results from Uganda. This could be due to variation in exposure to iNTS in Uganda compared to Malawi, or differences in assays. However, burden of, and exposure to, iNTS disease in Uganda is not well understood. A standardised assay is key to understanding variation in exposure across geographic locations to support vaccine development.

Data availability

Open Science Framework: Invasive Non-Typhoidal Salmonella serology in Uganda. https://doi.org/10.17605/OSF.IO/68BYT[8].

This project contains the age, sex, antibody levels, HIV status and *Salmonella* status of each participant.

Grant information

This work was supported by the UK Medical Research Council (MRC): grant number MR/ J003999/1 to LS, grant number MR/K012126/1 to SN, and the Bill and Melinda Gates Foundation (BMGF): grant number OPP1148489 to CM. The Ugandan General Population Cohort study is jointly funded by the UKMRC and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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PubMed Abstract | Publisher Full Text

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PubMed Abstract | Publisher Full Text

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PubMed Abstract | Publisher Full Text | Free Full Text

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PubMed Abstract | Publisher Full Text | Free Full Text

8. Stockdale L: **Invasive Non-Typhoidal *Salmonella* serology in Uganda.** 2019.
http://www.doi.org/10.17605/OSF.IO/68BYT

Open Peer Review

Current Peer Review Status: ✔ ✔ ? ?

Version 1

Reviewer Report 30 July 2019

https://doi.org/10.21956/gatesopenres.14147.r27466

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Michelo Simuyandi

Enteric Disease and Vaccine Research Unit, Centre for Infectious Disease Research in Zambia (CIDRZ), Lusaka, Zambia

The article describes an important aspect of research which needs more data. Knowing when children are exposed to salmonella infection can inform schedules for vaccination and other control interventions.

1. The aim of the study should be clear, given the limitation in the standardized method for immunological assessment (I suspect it was to determine the kinetics of antibody acquisition by age). The differences in assays could be discussed in the limitations and maybe how the difference can affect the date explained as well.

2. The assay is not described (just called in house) - reviewers could have advised how best to analyse or interpret the data. Did they try to do an EQA with another lab that used a different method to maybe determine concordance?

3. While the infants in this study have antibodies much later than Malawi, it is not clear if the mean concentrations increase with time or if it is just the number(%) (i.e. positivity rates) of participants with antibodies that increase over time. The authors could have compared the reported concentrations between the countries.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Enteric disease, vaccinology, immunology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 24 July 2019

https://doi.org/10.21956/gatesopenres.14147.r27468

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Scott M. Baliban
Center for Vaccine Development and Global Health (CVD), University of Maryland School of Medicine, Baltimore, MD, USA

This study aims to assess the presence of serum IgG to S. Enteritidis and Typhimurium by age and sex in a broad Ugandan cohort. It is of value because the sero-epidemiology of NTS in Uganda, as opposed to other African countries, is not well understood.

1. More detail is needed for the in-house ELISA. Presumably, it was conducted with purified OPS, as with other studies from this group (e.g. Fiorino *et al.*, 2017[1]). At the moment, it is not clear whether the serum IgG responses are directed against the OPS (and therefore serogroup B/D-specific) as opposed to the core oligosaccharide or lipid A (all other NTS). What is an Antibody Unit?

2. The authors mention that the lack of seroconversion to OAg-specific IgG in Ugandan children could be due to variation in exposure to NTS. To further support this hypothesis, it would be interesting to assess the kinetics of IgM since both anti-Typhimurium IgG and IgM can demonstrate bactericidal activity (Goh *et al.*, 2016[2]). If these titers are available, it would add a thought-provoking element to the data set.

3. Please state the statistical test used to compare groups.

4. Serovars do not need to be italicized.

5. Panels A and B are blurry, and the font size for the axes is small. Please add a label to the x-axis of panels C and D.

References

1. Fiorino F, Rondini S, Micoli F, Lanzilao L, et al.: Immunogenicity of a Bivalent Adjuvanted Glycoconjugate Vaccine against Salmonella Typhimurium and Salmonella Enteritidis.*Front Immunol* . 2017; **8**: 168 PubMed Abstract | Publisher Full Text
2. Goh YS, Necchi F, O'Shaughnessy CM, Micoli F, et al.: Bactericidal Immunity to Salmonella in Africans and Mechanisms Causing Its Failure in HIV Infection.*PLoS Negl Trop Dis*. 2016; **10** (4): e0004604 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious disease, bacterial vaccine development, humoral immunity

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 17 July 2019

https://doi.org/10.21956/gatesopenres.14147.r27467

Ruklanthi de Alwis
[1] Viral Research and Experimental Medicine Centre, SingHealth-Duke NUS, Singapore, Singapore
[2] Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore, Singapore

The study investigates the sero-prevalence of iNTS, serovar Typhimurium and Enteritidis in Uganda. The study further describes the sero-epidemiology by age and sex of these pathogens in Uganda. This is a very useful study since very little is known about the basic epidemiology of iNTS in the Uganda population.

- Since the authors' main explanation for the observed differences in sero-prevalence between Malawi and Uganda are due to differences in the experimental assays used to quantify O-antigen IgG, it would be good to give more details of this in-house ELISA assay.

- Since some of the authors from the Malawi study are also authors of the present study, is it not possible to run some of the Uganda sera (probably the sera in the less than 18 months age group) on the older flow-cytometry technique described in the MacLennan *et al.* (2008[1]) paper?

- I agree with the authors that very little is known about the most commonly circulating and disease-causing serovars of NTS in Uganda. However, it might be good to mention one or two current literatures, such as Afema *et al.* (2016[2]), where they show that neither *S.* Typhimurium nor *S.* Enteritidis are commonly isolated NTS serovars in Uganda (while in Malawi, Typhimurium and Enteritidis together make up over 90% of NTS cases (Feasey *et al.*, 2015[3])).

- It wouldn't hurt to show a graph of the O-antigen IgG data in HIV-infected and age-matched healthy individuals.

Minor comments:

- Please add x-axis titles to Figure 1C and 1D.

- Font labelling on panels 1A and 1B is small. Increase the font size on Figure panels 1A and 1B to match the font size of panels 1C and 1D.

- State the statistical test used to compare O-antigen IgG responses in females and males in Figure 1C and 1D.

References

1. MacLennan CA, Gondwe EN, Msefula CL, Kingsley RA, et al.: The neglected role of antibody in protection against bacteremia caused by nontyphoidal strains of Salmonella in African children.*J Clin Invest*. 2008; **118** (4): 1553-62 PubMed Abstract | Publisher Full Text
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Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
No source data required

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious diseases, antibody responses, epidemiology, vaccines

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 09 July 2019

https://doi.org/10.21956/gatesopenres.14147.r27470

Qingke Kong
University of Florida, Gainesville, FL, USA

This is an ELISA assay to measure the antibodies against O-antigen from S. Typhimurium and Enteritidis from clinical sera collected from patents in Uganda. I do not have scientific questions about this assay, but hope in the future, that the authors could establish a standardized method to figure out the reason for the controversy obtained from two locations.

Some minor grammar errors:

1. Please add a comma between “disease” and “suggesting” in: “...corresponds with a fall in cases of iNTS disease suggesting that vaccines able to...”.

2. A comma or conjunction words were missing in: “There was no clear difference in antibody

levels between the sexes and the few HIV-infected individuals in the study did not have obviously different levels from uninfected subjects".

3. "Typhimurium" does not need to be italic.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Salmonella infection and vaccine development, polysaccharide vaccine, bacteria-derived nanoparticle vaccine.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 09 Jul 2019

Sean Elias, University of Oxford, Oxford, UK

Thank you for your comments. I can confirm that we will be publishing the optimised standardised ELISA protocol in the near future and will fully address the issues that you have highlighted.

Competing Interests: No competing interests were disclosed.