An investigation into the role of HIV-exposure in infectious disease mortality in children from the Western Cape

by

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PART 0: PREAMBLE

DECLARATION

I, *Robert Anthony Reed*, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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ABSTRACT

Background

Globally over 2 million children are born to HIV positive mothers annually (WHO, 2010). In the Western Cape there are approximately 90,000 births per year (Elley, 2006; Statistics South Africa, 2012). HIV prevalence among antenatal attenders in the Western Cape is 17 per cent, below the national average of 30 per cent (NDOH, 2012). The implementation of HIV testing and prevention of mother-to-child transmission (PMTCT) programmes in low and middle income nations has risen sharply over the last decade. In 2012 the Western Cape reduced vertical transmission rates in infants under 2 months of age to 2 per cent (Sherman et al, 2014). Resulting increases in life expectancy and reduced MTCT have led to an increase in the population of HIV exposed uninfected children (HEU).

Studies across Africa have found HEU children experience a greater risk of mortality than HIV unexposed uninfected (HUU) children (Brahmbhatt et al, 2006; Shapiro et al, 2007; Landes et al, 2012). HEU children also appear to be more susceptible to infectious disease compared to HUU (Liu et al, 2000; Reikie et al, 2011). Awareness of this vulnerable population is growing (UNAIDS, 2010; Mugwaneza et al, 2011). As the population of HEU children increases so too does the need for a better local understanding of risks they face. Research investigating the factors influencing mortality amongst HEU children could aid the identification of high risk children from an earlier point in life.

Objective

The primary objective of this study was to establish whether there is an association between a child being HIV-exposed and a child dying from an infectious disease. The study looked to identify the odds of HIV exposure in cases (those who died from an infectious disease) compared to odds of HIV exposure in controls (those who did not die). The study also looked to identify other risk factors associated with mortality and determine the prevalence of HIV exposure in the sample.

Method

A case control design was used to collect data via medical record review at three hospitals in the Western Cape. Study sites included two central tertiary hospitals namely Red Cross Hospital (RCCH) and Groote Schuur and the regional hospital New Somerset Hospital which also serves as a relief hospital for RCCH. These hospitals account for the majority of paediatric admissions in the sub-district (Westwood et al, 2012a; Westwood et al, 2012b).

The HIV exposure status of 93 cases admitted with an infectious disease who died was compared with 93 controls admitted with an infectious disease who did not die. Controls comprised children under 13 years of age who survived an infectious disease. Cases comprised children under 13 years of age who died of an infectious disease whilst admitted to hospital. Both cases and controls were assessed to exposure to HIV and exposure to other factors.

Analysis

Exploratory analysis was used to establish the prevalence of patient characteristics and inform the methods used in later phases of regression analysis and model selection. Univariate and multivariate regression analysis was used to answer four questions: (1) Was there an association between infectious disease mortality and HEU children? (2) Was there an association between infectious disease mortality and other significant risk factors? (3) Were there any variables that confounded the association between infectious disease mortality and HEU children? (4) Were there any variables which modified the association between infectious disease mortality and HEU children?

Univariate logistic regression analysis was applied to all independent variables and was used to identify variables showing an association with mortality. Independent variables with a $p< 0.15$ were eligible for inclusion in further model selection. Multivariate regression analysis was used to identify the variables associated with mortality controlling for other variables. Model selection used stepwise addition of variables into a regression model. Final model selection was guided by Akaike Information Criterion (AIC) to determine the combination of variables that could best account for mortality.

Results

38.71% (36/93) of cases were HIV exposed whilst 22.58% (21/93) of controls were HIV exposed (p=0.017). In terms of HIV exposure-infection status, 32.36% (30/93) of cases were HEU versus 18.28% (17/93) of controls (p=0.06).

Cases and controls only differed significantly according to their main infection (p<0.001). Gastroenteritis was more common amongst controls (47.31%; 44/93) than in cases (18.28%; 17/93). LRTI was more common amongst cases (44.09%; 41/93) than controls (38.71%; 36/93). Levels of septicaemia and meningitis were also higher amongst cases than controls.

Univariate regression analysis found a statistically significant odds of death from an infectious disease in HEU children twice that of HUU (OR: 2.23, 95% CI: 1.12-4.44). LRTI (OR: 2.95, 95% CI: 1.44-6.04), septicaemia (OR: 13.80, 95% CI: 3.56-53.47), and meningitis (OR: 6.47, 95% CI: 2.15-19.44) were all significantly associated with increased odds of death. Univariate regression analysis identified feeding practice a confounder of the association between HIV exposure-infection status and mortality. Age and sex were also identified as confounders.

The final model allowed us to conclude that being HEU was a risk factor for mortality after adjusting for age, sex, feeding practice, and main infection. The odds of death for HEU children was 2.29 times greater than for HUU (aOR: 2.29, 95% CI: 1.06-5.0). LRTI, septicaemia, and meningitis were all significantly associated with mortality at the 5% level. A child admitted with a LRTI had a 3.12 increased odds of dying versus a gastroenteritis admission (aOR: 3.12, 95% CI: 1.47; 6.61). Children admitted with meningitis had an 8.59 times increased odds of death (aOR: 8.59, 95% CI: 2.58-28.60). The largest association was found in children admitted with septicaemia who demonstrated a 13.44 times increased odds of death (aOR: 13.44, 95% CI: 3.35-53.99).

Conclusion

Considering past research there was reason to believe that HEU children are at increased risk of death when admitted to hospital with an infectious disease. After controlling for confounders and the type of infectious disease, HEU children demonstrated an increased odds of death from an infectious disease compared to HUU (aOR: 2.29, 95% CI 1.06-4.97). This finding indicates an

inherent risk associated with being HEU. This finding agrees with studies from across Africa (Zaba et al, 2005; Brahmbhatt et al. 2006; Chilongozi et al, 2008; Kurewa et al, 2010; Mugwaneza, 2011). Our study also indicated that septicaemia and meningitis present a challenge in critical care. Children with septicaemia had over 13 times increased odds of death (aOR: 13.44, 95% CI 3.35-52.99). Children with meningitis had almost a nine times increased odds of death (aOR: 8.59, 95% CI 1.58-28.60).

We can conclude that children born to HIV positive mothers should be considered a vulnerable group even if vertical transmission has been prevented. Determination of a child's exposure status could be a valuable tool in identifying vulnerable children and reducing child mortality. Effort should be focussed upon the retention of HIV-exposed children in care in the Western Cape.

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PART A: PROTOCOL

Abbreviations

AIC: Akaike information criterion

AIDS: Acquired Immune Deficiency Syndrome

ARV: Anti-Retroviral

Child PIP: Child Healthcare Problem Identification Programme

CI: Confidence interval

DoH: Department of Health

ELISA: Enzyme-linked immunosorbent assay

HAART: Highly Active Anti-Retroviral Treatment

HIV: Human Immunodeficiency Virus

HR: Hazard Ratio

ICD-10: 10th revision of the International Statistical Classification of Diseases and Related Health Problems

LRTI: Lower respiratory tract infection

MK: Marasmus-Kwashiorkor

MTCT: Mother-To-Child Transmission

NDOH: National Department of Health

OR: Odds Ratio

OWFA: Overweight for age

PCR: Polymerase Chain Reaction

PMTCT: Prevention of Mother-To-Child Transmission

RCCH: Red Cross Children's Hospital (Cape Town)

RR: Risk Ratio

TB: Tuberculosis

UNAIDS: The Joint United Nations Programme on HIV and AIDS

UNICEF: United Nations Children's Fund

UWFA: Underweight for age

WHO: World Health Organisation

Introduction

One and a half million women infected with human immunodeficiency virus (HIV) become pregnant in low and middle income countries annually (UNICEF, 2012). In South Africa, over 30% of women attending public antenatal facilities are HIV-positive (NDOH, 2011). Despite this, South Africa's National Plan for reducing mother-to-child HIV transmission (MTCT) has achieved great success, bringing MTCT rates below 5% (CDC, 2011).

In the Western Cape in 2012 an estimated 17% of all children were born to HIV positive mothers (NDOH, 2013). With the continued success of prevention of mother to child transmission (PMTCT) the population of HIV exposed uninfected children (HEU) is only likely to increase (Sloegrove et al, 2010). Studies of HEU children have shown a higher risk of mortality when compared to unexposed children (Zaba et al, 2005; Brahmbhatt et al, 2006; Marinda et al, 2007; Shapiro et al, 2007; Kuhn et al, 2008). Data for the Western Cape reveals that over one third of under five deaths were due to infectious disease (excluding those living with HIV) (Groenewald et al, 2013). A review of past research reveals that whilst HEU children are vulnerable to infectious disease the strength of association can vary significantly by context (Brahmbhatt et al, 2006; Kuhn et al, 2008; Afrean et al, 2014). With this in mind the need for locally focussed research to direct further investigation and planning of interventions should not be underestimated.

The aim of this study is to investigate the association between HIV exposure and infectious disease mortality in children in the Western Cape. The role of other biological and social risk factors shall also be investigated.

Objectives

- Determine the prevalence of HIV exposure within cases and controls.

- Establish whether there is an association between infectious disease mortality and HIV exposure in children admitted to hospital in the Western Cape.
- Identify other significant risk factors associated with mortality.

Methodology

A case-control design will be used to compare HIV exposure status of 93 patients who died from infectious disease with 93 controls who did not die. A retrospective medical record review of children admitted to hospitals in Cape Town with infectious disease will be undertaken. The minimum sample size was calculated based upon a design providing 80% power, a confidence level of 95%, an assumed exposure prevalence of 17% (NDOH, 2013), with the ability to detect an odds ratio of 2.7 (Gichuhi et al, 2005; Marinda et al, 2007; Nielsen-Saines et al, 2011). Data will be analysed using regression analysis to identify factors associated with death.

The individual Child Healthcare Problem Identification Programme (Child PIP) data and medical records of children and mothers will be reviewed. The data collected will include medical history and related information such as HIV status, treatments received, feeding practice, and nutritional status. Socio-demographic information such as age, sex, and address will also be collected. Data retrieved from records shall be recorded in such a way to ensure that it is not personally identifiable. The study is not anticipated to affect normal operations of the health facilities.

Inclusion/Exclusion Criteria

Two groups will be studied using this protocol:

1. Controls: Children aged under 13 years of age who survived an infectious disease in the Western Cape between January 2008 to December 2014

2. Cases: Children under 13 years of age who died of an infectious disease in the Western Cape between January 2008 to December 2014

   Separate inclusion and exclusion criteria shall be used for each of the two groups. The criteria for each are listed below:

   Children aged 13 or less with an infectious disease in the Western Cape who survived

   Inclusion Criteria

- Aged 0 to 13 years of age
- Diagnosed with an infectious disease
- Survived condition

   Exclusion Criteria

- Aged over 13 years of age
- Diagnosed with an infectious disease
- Died from condition

   Children under 13 years of age with an infectious disease in the Western Cape who died

   Inclusion Criteria

- Aged 0 to 13 years of age
- Diagnosed with an infectious disease
- Died from condition

   Exclusion Criteria

- Aged over 13 years of age
- Not diagnosed with an infectious disease
- Survived condition

Data Collection

Data will be collected between November 2014 and April 2015. Cases and controls will be identified using the aforementioned inclusion criteria. Cases will be collected using records held on the Child Health Care Problem Identification Programme (Child PIP) which serves as a child mortality audit tool. Controls will be collected from the three sites by first accessing ward records to identify eligible controls. The patient numbers of controls will be used to collect the full range of variables from medical records hospital (see Table 1, Appendix D for the full list of variables). For each of the three sites all eligible cases and controls will be numbered sequentially. A random number generator will then be used to allow a simple random sample to be drawn for both cases and controls.

The medical records for cases are available on the Child PIP database. Patient folder numbers will be collected from Child PIP and written medical records obtained when further detail is required. Medical records of controls will be accessed through hospital record departments.

Data Entry and Management

Data for both cases and controls will be recorded manually on data collection forms (see Fig. 7, Appendix D) and then manually re-coded as per the codes shown in Table 1, Appendix D. This data will then be entered onto a Libre Office Data Spreadsheet (The Document Foundation. 2015. *LibreOffice: Version 4.3.7*. Berlin, Germany: The Document Foundation) and exported to STATA 12 (StataCorp. 2011. *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP) for data management and statistical analysis. Data will be validated through the use of double entry for both the raw data collection and recoding of variables. Data will be

transcribed from the same patients records on separate days and collection sheets compared. Any conflicting data will be confirmed through rechecking of medical records.

Data and Safety Monitoring

All data will be accessed and stored using password protected software and hard drives. All data will be retrospectively collected from clinical records. There will be no risk to anyone.

Statistical Analysis

Data will be analysed using STATA 12 (StataCorp. 2011. *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP). A range of statistical methods shall be used. Those used will include but not be limited to measures of central tendency (mean, median, and mode) and measures of dispersion such as variance and standard deviation. Significance levels will be used to inform analysis and varied depending upon the stage of analysis. Tests of significance will be used such as Chi-square and t-tests. Logistic regression analysis will be used to establish the association between HIV exposure and death and the association between other independent variables and death. Logistic regression analysis will also be used to identify confounders and effect modifiers. When interpreting p-values from univariate analysis a $p < 0.15$ will be considered sufficient for inclusion in model selection. A significance level of 0.05 will be used for all other statistical tests and interpretation of the final multivariate model.

Univariate logistic regression

Univariate logistic regression analysis will be applied to all independent variables and be used to identify variables showing an association with the dependent variable (mortality). Independent variables with a $p < 0.15$ will be eligible for inclusion in further model selection. Categorical variables will be treated as blocks when adding to regression models. As such only one category in each categorical variable will be required to display a $p < 0.15$ to qualify an entire variable

block for inclusion in the next step (see Table 1, Appendix D containing binary and categorical variables).

Potential confounders will be identified through the use of box plots, Pearson's chi-square test, and logistic regression to establish whether they satisfy the three criteria of potential confounders:

1. Variable was associated with the mortality in HUU
2. Variable was associated with being HIV exposed
3. Variable was not in the causal pathway

Any variable satisfying the criteria will be considered a potential confounder. Logistic regression analysis will then used to confirm such variables as confounders. A variable will be considered a confounder if its inclusion results in a >10 per cent in the estimate of association with the main exposures of interest.

Multivariate logistic regression and model selection process

All confounders will be forced into the base model and qualifying variables added one at a time. The Akaike Information Criterion (AIC) of each successive model will be compared to that of the base model. The model with the lowest AIC will be selected and taken forward to the next step until all eligible variables have been added. In the penultimate step all variables discarded during the model selection process will be readmitted to the model one at a time to ensure important variables are not omitted. The final step will involve the creation of interaction terms representing interaction between HIV exposure-infection status and other variable in the final model. Retainment of interactions in the model will be dependent upon their ability to produce a lower AIC than the model omitting that interaction.

Akaike Information Criterion (AIC)

The AIC measures how well a model fits the dataset. As detailed above model selection will utilise the AIC to determine the best model and identify the variables with significant influence over mortality. The AIC will inform the selection of a model which fits the data well whilst containing the minimum number of variables needed to account accurately for the outcome (Tu and Gilthorpe, 2011; Woodward, 2013).

Variables of Interest

Data will be collected on the following variables: age, sex, child ART, child HIV Status, feeding practice, HIV exposure, HIV exposure-infection status, maternal HIV status, maternal ART, mother's health status, primary carer, main infection, nutritional status, other infections, underlying conditions.

Age in months (Continuous)- Age of child recorded in months and validated through the use of the child's date of birth and date of death. Recorded as: 0-155 months.

Child ART received (Binary)- Whether the child had ever been in receipt of ART other than PMTCT before admission. Recorded as: Yes; No

Child HIV Status (Binary)- According to the results of ELISA or PCR as recorded on medical record or Child PIP record. Recorded as: HIV negative: HIV positive.

Feeding Practice (Categorical)- The feeding practice used by the mother or other care giver during the first 12 months of the child's life. Classification depends upon the use of breast feeding and formula feeding during the first 6 months of life. Recorded as: Never breastfed; Exclusively breastfed; Mixed feeding

Sex (Binary)- Whether a child was male or female. Recorded as: Male; Female

HIV exposure status (Binary)- Whether a child was or was not HIV exposed through birth to a HIV positive mother. Recorded as: Exposed; Unexposed.

HIV exposure-infection status (Categorical)- Whether a child was:

- HIV unexposed-uninfected (HUU): HIV negative child born to a HIV negative mother

- HIV exposed-uninfected (HEU): HIV negative child born to HIV positive mother

- HIV exposed uninfected (HEI): HIV positive child born to HIV positive mother.

Recorded as: HUU; HEU; HEI

Main Infection (Categorical)- The main cause of admission or death as recorded on medical records. Infectious diseases will be grouped according to categories of disease common in the Western Cape (Groenewald et al, 2013). Recorded as: Gastroenteritis; TB; LRTI (bronchiolitis, bronchitis, pneumonia, croup); Septicaemia; Meningitis; Other.

Maternal ART received (Binary)- Whether the child had ever received ART other than PMTCT before admission. Recorded as: Yes/No

Maternal HIV Status (Binary)- According to the results of ELISA or PCR as recorded on medical record or Child PIP record. Recorded as: Negative; Positive

Mother's health status (Binary)- Whether the child's mother was 'Alive and well' and able to care for the child or 'Dead/Sick' implying the mother was unable to care for child. Recorded as: Alive and well; Dead/Sick

Other infection (Categorical)- Whether an additional infection contributed to admission or death. Recorded as: None; Gastroenteritis; TB; LRTI (bronchiolitis, bronchitis, pneumonia, croup); Septicaemia; Meningitis; Other.

Primary Carer (Binary)- Whether the carer was the mother or 'other' (father, grandparent, foster parents, orphanage etc.) Recorded as: Mother; Other

Nutritional Status (Categorical)- Established through clinical observations by physicians and use of WHO z-score weight-for-age charts noted on medical records as:

- Overweight for Age (OWFA)

- Normal

- Underweight for Age (UWFA)

- Marasmus: A form of serious protein-energy malnutrition (PEM) with protein malnutrition predominant. Characterised by severe wasting due to the breakdown of fat and muscle for energy.

- Kwashiorkor: A form of serious PEM due to insufficient calorie intake. Characterised by too much fluid in body tissues leading to oedema.

- Marasmus-Kwashiorkor (MK): A combination of marasmus and kwashiorkor leading to a combination of wasting and oedema.

Recorded as: OWFA; Normal; UWFA; Marasmus, Kwashiorkor; MK

Underlying Condition (Binary)- Whether a child had an underlying condition such as congenital malformation, condition arising in perinatal period, disease of nervous system. Recorded as: Present; Not present

Risks

There is a minimal risk of not maintaining anonymity of patient data. To minimise the risk patient names or identification numbers shall not be used as identifiers on study records. Patient data shall be anonymised at the first stage of data collection. There is a minimal risk that unauthorised access to online patient medical records may occur. To minimise this risk patient medical records and Child PIP data shall be accessed via secure servers, utilising passwords known only to the researcher.

Benefits

There will be no direct benefits to participants in this study. This study will contribute to a greater understanding of risk factors for mortality in patients with HIV exposure. This study may contribute to the direction of future research and the provision of more appropriate treatment.

Privacy and Confidentiality

Data retrieved from Child PIP and medical records shall be recorded in such a way to ensure that the information is not personally identifiable. Data collected from online medical records shall be anonymised and retained for research and record keeping purposes. Data shall be destroyed upon conclusion of the study. All data shall be stored on password protected computers and servers.

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PART B: LITERATURE REVIEW

Search Strategy

Searches took place between November 2014-April 2015 through the following online bibliographic databases and electronic search engines:

- Medline (PubMed)
- Google Scholar

Searches were limited to English language articles published between 1990 to 2015. Key words relating to HIV, infectious disease, morbidity, mortality, and Africa were combined in pubmed searches (see details below):

- (((HIV) OR hiv exposed) OR hiv exposed but uninfected) AND "africa"

- (((HIV) OR hiv exposed) OR hiv exposed but uninfected) AND "infectious disease"

- (((HIV) OR hiv exposed) OR hiv exposed but uninfected) AND "mortality"

- (((HIV) OR hiv exposed) OR hiv exposed but uninfected) AND "morbidity"

Various articles were also sourced using google search (google.com) utilising terms similar to those listed above, and by accessing organisational websites including:

- Avert (www.avert.org/hiv-aids-south-africa.htm)
- Centers for Disease Control and Prevention (www.cdc.gov)
- Health-e (www.health-e.org.za)
- Health Systems Trust (www.hst.org.za)
- Western Cape (www.westerncape.gov.za)
- World Health Organisation (www.who.int)

The reference lists of all articles were also reviewed in order to find additional articles not identified via online and database searches.

Inclusion Criteria

Below are the types of research and publication eligible for inclusion

- Quantitative studies (with or without control group)
- Systematic reviews
- Descriptive studies
- Organisation/Governmental Reports

Study Selection

All articles were vetted according to the title and abstract. This was followed by a review of the full content of the article to establish its relevance. All relevant articles were synthesised into the literature review.

An Introduction to the issue of HIV-exposed uninfected children (HEU) in Africa and South Africa

Globally over 2 million children are born to HIV positive mothers annually (WHO, 2010). Mother to child transmission (MTCT) accounts for over 90 per cent of paediatric HIV/AIDS cases (Koye and Zeleke, 2013). Two hundred and sixty thousand children were infected with HIV in low and middle income nations in 2012 (UNAIDS, 2013) with the majority infected via MTCT. In the Western Cape there are approximately 90,000 births annually (Elley, 2006; Statistics South Africa, 2012). Approximately thirty per cent of women attending antenatal services in the public sector are HIV infected (NDOH, 2012). However HIV prevalence among antenatal attenders in the Western Cape is below the national average at 17 per cent (NDOH, 2012). A study by Karpelowsky et al (2011) provided support for this estimate. In a prospective cohort study, performed at a paediatric centre in Cape Town, Karpelowsky et al (2011) found similar annual HIV-exposure levels of 15 per cent.

The implementation of HIV testing and prevention of mother-to-child transmission (PMTCT) programmes in low and middle income nations has risen sharply over the last decade. As a result the annual number of children infected with HIV globally began to decline in 2004 (UNAIDS, 2010). South Africa initiated nationwide single dose nevirapine in 2003 (Meyer, 2007) and polymerase chain reaction (PCR) testing for infants at 6 weeks in 2004 (Stevens et al, 2006). A national HIV counselling and testing campaign in 2010 saw HIV testing coverage rise from 25 per cent in 2008 to 46 per cent in 2012 (Shisana et al, 2014). In 2010 South Africa also implemented antiretroviral therapy (ART) as part of PMTCT from the fourteenth week of pregnancy. This marked a significant departure from the former practice of starting ART during the final trimester (NDOH, 2010; Barron et al, 2012). The above measures resulted in 95 per cent of pregnant women being tested for HIV as of 2011 (WHO, 2011) and more than 95 per cent of HIV positive pregnant women receiving ARV treatment as part of PMTCT (UNICEF, 2011; JUNP-HIV/AIDS 2012). As a result national PMTCT transmission rates declined from 16.4 per cent in 2006 to 2.4 per cent in 2012. In 2012 the Western Cape reduced vertical transmission rates in infants under 2 months of age to 2 per cent (Sherman et al, 2014). The resulting increases in life expectancy and reduced MTCT have led to a rise in the number of HIV-exposed uninfected children (HEU).

Slogrove et al (2011) and Slogrove et al (2012) estimate that 25 per cent of South African newborns

can be classified as HEU. Studies from a range of contexts have found HEU children experience greater mortality rates than their HIV unexposed uninfected (HUU) counterparts (Brahmbhatt et al, 2006; Shapiro et al, 2007; Landes et al, 2012). HEU children are also more susceptible to infectious disease when compared to HUU children (Liu et al, 2000; Reikie et al, 2011). Recognition of this potentially vulnerable population is growing and efforts to further reduce under five mortality in sub-Saharan Africa will demand greater focus upon HIV exposed children (Mugwaneza et al, 2011). Considering what is currently known, calls for more attention to be focussed upon HIV exposed children at both an individual and population level appear justified and necessary (WHO, 2009; Shapiro and Lockman, 2010; Slogrove et al, 2010; Sugandhi et al, 2013). Furthermore, if efforts to reduce mortality amongst HEU children are to be successful then investigation of risk factors should be considered a research priority (Reikie et al, 2011; Slogrove et al, 2011). Gaining a better local understanding of risk factors could greatly aid the identification of at risk children from an earlier point in life.

HEU Children and Mortality

HIV infection is a recognised risk factor for child mortality in southern Africa (Mann et al, 1986). Mortality in HIV-infected children is up to 18 times higher than in HIV-uninfected children (Ryder et al, 1994; Taha et al, 1999; Dabis et al, 2001; Mbori-Ngacha et al, 2001; Obimbo et al, 2004). The influence of HIV-exposure upon mortality has received comparatively little attention and studies have tended to focus upon its association with morbidity rather than mortality. Despite this a detailed review of the literature identified studies in a range of contexts investigating the association between HIV-exposure and mortality. This body of research will be summarised and reviewed in the remainder of this section.

Studies from a range of countries in Sub-Saharan and Southern Africa highlight the excess mortality experienced by HEU children compared to HUU. Chilongozi et al (2008) investigated the mortality of infants by HIV exposure status in a pooled analysis of three Sub-Saharan African countries (Malawi, Tanzania, and Zambia) prior to the availability of ART. Infants born to HIV-positive mothers were more likely to die over the 12 month follow-up period than infants born to HIV-negative mothers (HR: 3.95, 95% CI: 2.3-6.9). This study failed to account for the role of wider

determinants (such as breastfeeding and nutrition) which may confound the association between exposure and mortality. An additional limitation is the fact that the study excluded those with pre-existing or chronic illnesses. As a result the sample in this study was unlikely to be truly representative of populations in Sub-Saharan Africa. A study in rural Uganda by Brahmbhatt et al (2006) found that by 18 months of age the cumulative mortality rate in HEU children was significantly higher than HUU after adjustment for a mother's socio-economic status, age, and underlying conditions (RR:1.16. $p < 0.05$). However, the extent to which this study can be used to identify the role of feeding practice is limited given the failure to distinguish between exclusive and mixed breasfeeding practices. Another pooled analysis combined data from three prospective cohort studies in Malawi, Tanzania, and Uganda with children classified according to maternal HIV status. Zaba et al (2005) utilised a three year follow up, substantially longer than Cholingozi et al, (2008) and Brahmbatt et al (2006). Interestingly the study still found an additional risk of mortality in HEU children after controlling for age and sex (RR: 2.9, 95% CI: 2.3-3.6). These pooled analyses clearly indicate an association between HIV exposure and mortality at a regional level. However, despite the increased power pooled analysis offer one must be aware that they combine data from multiple populations with diverse standards of care and service delivery. As such, pooled analysis can make it difficult to identify variations in mortality by nation and district. Therefore, applying the conclusions to our population in the Western Cape should be done with caution. Another limitation of the above studies is the impact of attrition bias on the results.

Zijenah et al (1998) investigated mortality in infants born to women attending antenatal clinic in Harare, Zimbabwe. Zijenah et al (1998) found that of infants born to HIV-positive mothers, 19.6 per cent died compared to 5.4 per cent infants born to uninfected women ($p = 0.01$). Another Zimbabwean study by Marinda et al (2007) found mortality by 24 months was 9.2 per cent for HEU children versus 2.9 per cent for HUU ($p < 0.05$). Nathoo et al (2005) went further in addressing this question. Utilising data from Zimbabwe between 1991 and 1995 they conducted a multivariate analysis of factors associated with infant mortality. Being born to an HIV-infected mother was found to be an independent risk factor for infant mortality (HR: 3.23, 95% CI: 3.17–15.85). Low birth weight (HR: 2.80, 95% CI: 1.52-5.13) and maternal death (HR: 2.77, 95% CI: 1.09-7.06) were also found to be significantly associated with infant death. Whilst mortality was associated with early signs of HIV infection in infants, these results highlight the excess vulnerability of HEU infants in Zimbabwe

All three Zimbabwean studies discussed (Zijenah et al, 1998 Nathoo et al, 2005; Marinda et al, 2007) used data from a period when PMTCT was not part of national policy. Kurewa et al (2010) on the other hand recruited mothers enrolled on Zimbabwe's national PMTCT programme. The case-control study aimed to identify infant mortality trends and risk factors associated with HIV exposure. Differences in mortality risk between HIV-exposed and HIV-unexposed children emerged at 6 weeks of age (RR: 9.71, 95% CI: 1.22-77.32). This difference was greatest at 4 months, with infants born to HIV-positive mothers 22 times more likely to die at this stage (RR: 21.84, 95% CI: 2.93-162.98) (Kurewa et al, 2010). Despite the wide confidence intervals this trend of increasing mortality through time suggests an important role for social and environmental factors. Other studies have focused upon trends in mortality through time by utilising longer follow up periods. In Botswana, Shapiro et al (2007) found that at 6 months 3.6 per cent of HEU infants had died versus 0.8 per cent of HUU infants (p= 0.01). This difference widened further by 24 months of age to 6.7 per cent versus 1.6 per cent (p= 0.002). In a west African study, Hong et al (2007) used a cohort of 3600 children born between 1999–2003 to measure the association between being born to a HIV positive mother and child mortality in Ghana. After controlling for factors influencing infant mortality (sex, nutritional status, absence of antenatal care, low birth weight, and household pollution) the hazard ratio for HIV exposure and death increased from HR 3.01 (95% CI 1.64-5.50) to HR: 3.51 (95% CI 1.87-6.61). The large sample and time frame of this study, plus its use of nationally representative data (Ghana national heath survey) means it lends robust support to the linking of HIV-exposure to child mortality. Mugwaneza (2011) utilised a 2 year follow up and found that HEU children had a 3.5 times greater risk of mortality (HR:3.5, 95% CI: 1.8-6.9). Mugwaneza et al called for more studies investigating longer term survival in contexts like South Africa where PMTCT, ART, and promotion of breastfeeding is widespread.

In contrast to the above findings which demonstrate an association between HIV-exposure and childhood mortality, examples of contrary conclusions are widespread. In Malawi, Taha et al (1999) found no significant difference between the mortality rates, birth weight, nor immunologic characteristics of HEU and HUU children. This study also discounted any role for social factors such as education and socio-economic status. Similarly, Sutcliffe et al (2008) failed to find a significant increase in mortality between HEU and HUU children in Zambia after adjusting for biological factors such as haemoglobin and lymphocyte levels. It should be noted however that

Sutcliffe and colleagues studied a population with no access to ART. They also recruited children attending a vaccination clinic which may have led to selection bias. A Gambian cohort study considered longer term survival of 700 children up to 8 years post HIV-exposure (van der Loeff et al, 2003). Overall the mortality rate of children born to HIV-positive mothers was not significantly different from children born to HIV-uninfected mothers. Maternal death emerged as major risk factor for child death, irrespective of maternal or child HIV status. A prospective cohort study at a maternal clinic in Kigali by Spira et al (1999) also failed to identify any significant increase in mortality risk for HEU children versus HUU after controlling for age and parity (HR: 0.4, 95% CI: 0.1–1.6). Similarly, a study by Chopra (2008) assessing the effectiveness of South Africa's PMTCT programme at three locations found no variation in mortality between HEU children and HUU (HR: 0.7, 95% CI 0.3-1.5). Socio-economic status, birth weight, and number of clinic visits also failed to emerge as risk factors for mortality in HEU children. More recently Zash et al (2014) found no significant difference in mortality by HIV-exposure status nor feeding practice in neonatal intensive care units (ICU) in Botswana. This study has limitations however, such as its use of a short 28 day follow up. The highly controlled nature of ICU may also have moderated the impact of risk factors faced by children in the study.

The HIV-exposed uninfected child has received increased attention over the past decade (Filteua 2009). The role of HIV-exposure in mortality has been investigated across Africa through a variety of methods. A recent systematic review by Afran et al (2014) found ‘compelling evidence’ that HEU children faced increased risk of mortality compared to HUU. This literature review identified many studies supporting the conclusions of Afran et al. However there is evidence to the contrary and also various limitations in the current body of research. Most studies used samples from small clinics as opposed to better resourced tertiary hospitals. Otherstudies were done where PMTCT coverage was either low or non-existent.As a consequence results from such studies have limited applicabilty to a Cape Metro setting. The maximum period of follow for most studies was 24 months which limits their the ability to account for the role of environmental and caregiver factors. Many studies also combined HEU and HIV-exposed infected (HEI) children into one group. As such the interpretation of such studies is difficult as it is not possible to establish the proportions of infected and uninfected children in the HIV exposed category (Ota et al, 2002; Nakiyingi et al, 2003; Ng’weshemi et al, 2003; van der Loef et al, 2003). There is a definite need for more research into the influence of HIV-exposure on mortality in areas with high coverage of both ARV and PMTCT such as in the Western Cape.

The association between HIV exposure and infant mortality from infectious disease

Over 6 million children die due to infectious diseases annually and the majority of deaths occur in low income countries with high HIV prevalence (Lopez, et al 2006; Black et al, 2008; WHOGHO, 2015). Diarrhoea and pneumonia are the two major causes of child mortality, accounting for 29 per cent of all child deaths under the age of five (WHO-UNICEF, 2013). Populations in Sub-Saharan Africa are particularly vulnerable and 90 per cent of global deaths from both infections occur in the region (WHO, 2008).

Current literature suggests that HEU children are at increased risk of infectious disease morbidity. Signs of the increased susceptibility of HEU children emerged over two decades ago (Thea et al, 1993; Hersei et al, 1997).Thea and colleagues studied a cohort of infants born to HIV-positive and HIV-negative mothers in Zaire. Incidence of persistent diarrhoea amongst HEU infants was almost double that observed in HUU (4.9 episodes versus 2.7 episodes per 100 child-years). The study concluded that more public health interventions focussed upon HEU infants were needed. This study was amongst the first to show maternal HIV status was a key risk factor for diarrhoea morbidity regardless of an infants HIV status.

Increases in respiratory disease are linked to the increasing prevalence of HIV in South Africa (Gray and Zar, 2010). According to Spira et al (1999) HEU children are at increased risk of hospital admission and the development of severe pneumonia. This is supported by other studies that found that HEU children are at increased risk of pneumonia and mortality versus HUU. More recently a study in a tertiary hospital in Botswana found HEU children with pneumonia were at increased risk of mortality compared to HUU after adjusting for age and proximity to clinic (RR: 4.31, 95% CI: 1.44–12.87, p = 0.01) (Kelly et al, 2014). In Zimbabwe a sample of 14,000 infants born prior to the availability of HAART (1996 -2000) was used to investigate rates of hospitalisation in children with LRTI. The study found that during the neonatal period HEU infants were at greater risk of hospitalisation than HUU adjusting for age and maternal death (RR: 2.7, 95% CI: 1.6–4.7) (Koyangi et al, 2011). In Latin America, Mussi-Pinhata et al (2007) found 61 per cent of HEU infants experienced at least one infection with 17.5 per cent requiring hospitalisation. 40.7 per cent of respiratory infections led to hospitalisation. Mussi-Pinhata et al (2007) concluded that HEU infants were too often overlooked as a group at increased risk from severe forms of infectious disease, particularly LRTI. McNally et al (2007) found that HEU children were less responsive to treatment for pneumonia when compared to HUU (RR: 6.02, 95% CI: 1.55-23.38). McNally et al

concluded that pneumonia treatment was inadequate and more research was required to identify best practice. Studies by Yeung et al (2001), which used data from 70 cohort studies, and Kovacs et al (1999) reported significantly higher rates of infections via perinatal contagion in HEU children. Such findings indicate that there is an increased risk of the vertical and postnatal transmission of infections from HIV-positive mothers to infants (Mofenson et al, 2004)

A number of relevant studies have been conducted in South Africa. Morrow et al (2010), in their study at Red Cross Children's Hospital in Cape Town, reported that severe pneumonia and mortality occurred often in areas with large populations of HEU children. In a further Western Cape study Slogrove et al (2010) investigated several HEU children admitted with severe infections including pneumocystis, sepsis, and meningitis. They concluded that HEU children required better follow-up care. A recent study carried out by Slogrove et al (2012) in the Western Cape followed a cohort of 27 HEU and 28 HUU infants for 12 months to establish whether there was a significant difference in infectious disease morbidity. HEU infants were almost three times more likely to require hospitalisation than HUU (HR: 2.74, 95% CI: 0.85–8.78). Given the small sample size of this pilot study it failed to find a significant difference in the incidence of infectious disease between HEU and HUU infants. Such findings suggest that further investigation in the Western Cape is necessary (Slogrove et al, 2012). The literature relating to the role of HIV-exposure in infectious disease mortality is conflicting however. Findings by Zash et al (2014) detected no significant difference in antibiotic usage between HEU and HUU children. As such Zash et al ruled out the role of HIV-exposure in moderating susceptibility to infection or mortality.

Risk factors for mortality amongst HEU children

The apparent higher morbidity and mortality among HEU children could be the result of multiple risk factors encountered throughout the life course. As such, any attempts to reduce excess morbidity and mortality will rely upon identification of these factors. The need to account for the role of wider factors is essential in understanding the true association between HIV-exposure and mortality. For example Hong et al (2007) found the hazard ratio for mortality associated with HIV exposure increased from HR: 3.01 (95% CI: 1.64-5.50) to HR: 3.51 (95% CI: 1.87-6.61) after

controlling for factors relating to the child, mother, and environmental factors. A review of the literature reveals studies that have considered a range of risk factors. The studies we identified used different methods of study design, case finding, age groups, and locations. Despite this heterogeneity of method a set of common risk factors emerged. Risk factors included an impaired immune system, increased exposure to pathogens, low birth weight, nutrition, feeding practices, and maternal health. These risk factors shall be discussed in the remainder of this literature review.

Immune System

The tendency for HEU children to exhibit immunologic deficiencies has been known for some time. Two possible causes of this deficiency have been put forward by de Moraes-Pinto et al (1996) and Economides et al (1998). de Moraes-Pinto et al found inhibited transfer of antibodies from HIV positive mothers to their children. Economides et al noted greater levels of apoptosis of lymphocytes in HEU children due to excessive immune responses in HIV positive mothers. Clerici et al (2000) found that HEU children display impaired immune systems due to development within a microenvironment enriched with viral particles. Koyangi et al (2011) and Rombadi et al (2008) suggest the combination of exposure to increased concentrations of pathogens and reduced antibody transfer from HIV-positive mothers produces immuneologic abnormalities in HEU children. A study in South Africa found the innate immune response in HEU children was 'hyper-inflammatory' leaving them disproportionately vulnerable to infectious disease (Reikie et al, 2011). Despite these findings it is important to note that the routine provision of PMTCT, and ART in the Western Cape may mitigate the impact of immune deficiencies.

Low birth weight and nutrition

In Kenya Braddick et al (1990) observed that HIV exposed children were experiencing three times greater likelihood of having a low birth weight (<2500g) at term than their unexposed counterparts ($p = 0.007$). Following on from this a study from Rwanda by Lepage et al (1991) also found a propensity for HIV exposed children to have a low birth weight. Bulterys et al (1994) also identified that HIV-exposed children had significantly lower birth weights than unexposed children (2706g versus 2825g, $p = 0.002$). It should be noted however that Bulterys et al did not observe different mortality rates between HIV-exposed and unexposed children. Gangar (2009) identified that HIV-exposed newborns had significantly higher rates of foetal malnutrition than newborns of HIV-

negative mothers (36 per cent versus 10 percent, p = 0.0002). These results were corroborated by a randomised controlled trial comparing anthropometry of 125 HEU and 382 HUU Zambian infants by Filteu et al (2011). This highlighted that HEU infants had a lower birth weight, even when controlling for socio-economic status and feeding practice.

As we have demonstrated there is substantial evidence from across Africa of an association between HIV-exposure and low birth weight. Nutrition and low birth weight have also been found to be associated with mortality in HEU infants. Low birth weight was found to be independently associated with mortality in HEU infants mortality by Nathoo et al (2005) in Zimbabwe (HR: 2.80, 95% CI 1.52-5.13). Recently, Boerma et al (2014) showed that prematurity was an important independent risk factors for mortality in HEU infants on PMTCT in Cameroon with prematurity being one of them (HR: 6.4 (P < 0.001).

Maternal health

Studies show that the survival of HIV-exposed children is partially determined by the physical condition of parents and caregivers. Several studies were identified which support the above statement. In a multivariate analysis Nathoo et al (2005) found maternal death was independently associated with infant mortality (HR: 2.77, 95% CI: 1.09 -7.06). A pooled analysis of results from three longitudinal studies in Malawi, Tanazania, and Uganda by Zaba et al (2005) identified an increased risk of mortality associated with HIV-exposure (HR: 2.9, 95% CI: 2.3-3.6). The study also concluded that HIV influenced child survival indirectly through its influence on mother's survival. They found HIV-exposed children with a deceased mother were four times more likely to die (HR: 3.9, 95% CI: 2.8-5.5). This association increased upon controlling for the age of the child (HR: 5.2, 95% CI: 3.0–8.5).

Serious maternal illness also appears to have a bearing upon the survival of children. For example infant mortality was higher among infants whose mothers experienced serious maternal illness or death than among infants whose mothers experienced neither (HR: 5.8, 95% CI: 3.5, 9.4) (Chilongonzi et al, 2008). As expected, death and serious maternal illness were more common amongst HIV-positive mothers, and by the end of follow-up a greater proportion of HEU infants had died (7.2 per cent) than HUU (4.8 per cent). In their study of the well-being of children in rural Malawi, Crampin et al (2009) found maternal death was associated with mortality in HIV-exposed children but not unexposed. However, this study failed to separate exposed children according to

their HIV status. As such the association between mortality and HIV-exposure may actually be a consequence of a child being HIV-positive rather than exposure alone (Ryder et al, 1994; Crampin et al, 2009). Despite the above evidence, other Africa-wide studies have failed to find maternal health as a significant risk factor for mortality in exposed children (Newell et al, 2004; Kinney et al, 2010).

Feeding practice

Studies have shown that excess morbidity in HEU children was related to decreased levels of exclusive breastfeeding and increasing replacement feeding (WHO CST, 2000; Filteau, 2009). This is a particularly pertinent issue for the HIV-exposed child as many mothers tend to reduce breastfeeding either due to illness or to protect their child from HIV-infection. Initially HIV-infected mothers were discouraged from breastfeeding to reduce HIV transmission. When PMTCT was first initiated in South Africa it included a policy of providing free formula to all infected mothers (BIRTH, 2013). This led to low rates of breastfeeding and it was estimated that in 2012 only 8 per cent of infected mothers were breastfeeding in South Africa (Bloeman, 2012). Studies have shown that breastfeeding is essential for reducing infant mortality in resource poor and high HIV prevalence settings (Shapiro and Lockman, 2010).

Breastfeeding has also been implicated in determining the susceptibility of children to infectious diseases. One study sampled from HIV-positive mothers in Burkina Faso, Kenya, and South Africa, and compared a non-breastfed arm with an exclusively breastfed arm. Infants aged zero to 2.9 months who were not breastfed had a 6 fold increased risk of severe infections (95% CI: 2.2, 16.4; p = 0.001) compared to exclusively breastfed infants (Bork et al, 2014). Low levels of exclusive breastfeeding amongst HIV-positive women have also been linked to the increased mortality of HEU children. Kafulafula et al (2010) investigated the influence of feeding practice on gastroenteritis mortality in HEU infants in Malawi. The study found that mortality due to gastroenteritis was higher in HEU who experienced breastfeeding cessation by 6 months versus those on prolonged breast feeding (24 per 1000 versus 12 per 1000; p = 0.0002).

In Botswana Shapiro et al (2007) noted that discontinuation of breastfeeding was a major risk factor for the observed increases in morbidity amongst HIV-exposed children. Mugwaneza's (2011) cohort study at a PMTCT clinic in Rwanda identified feeding practice as one explanation for the poorer prognosis of HIV-exposed children over the 2 year follow up (aHR:3.5; 95% CI: 1.8-6.9). Kuhn et

al (2006) investigated the risk factors for mortality in a birth cohort of HEU infants from Zambia. Cessation of breastfeeding by 4 months resulted in increased mortality compared to those still breastfeeding (HR:14.92, 95% CI: 5.44–40.91). In an additional study in 2010 Kuhn et al identified the influence of early weaning upon the mortality of HEU infants. The trial, conducted in Zambia, found weaning by 5 months was associated with a 2 fold increased risk of mortality (95% CI:1.13–3.65) and weaning at 6–11 months was associated with a larger 3.5 fold increase (95% CI, 1.68–7.46) (Kuhn et al, 2010). Kuhn and colleagues findings are supported by another Zambian study which found mixed feeding was associated width a 5.6 fold increase in HEU infants mortality ($p = 0.025$) (Boerma et al, 2014). However it must be noted that Kuhn et al (2006) and Boerma et al (2014) failed to use a HUU control group so the value of these findings in relation to our research question is limited.

Of particular relevance to our study is a cohort study in Kwazulu-Natal conducted by Rollins et al (2013). This study found no difference in mortality between HIV-exposed and HIV-unexposed infants controlling for levels of breastfeeding practices. These findings suggest that exclusive breast feeding may moderate the impact of HIV-exposure. Rollins et al concluded that there is compelling evidence for the use of exclusive breastfeeding to reduce the mortality amongst HEU infants. Such findings clearly indicate that early weaning has a deleterious influence upon the survival of HEU infants.

Summary

Much research has been carried out into the impact of HIV-exposure on the survival of children in Africa. The literature highlights increased mortality and susceptibility to infectious disease amongst HIV-exposed children compared to their unexposed counterparts. The immune system, maternal health, birth weight, and feeding practice appear to mediate the association between exposure and mortality.

There is strong evidence of higher mortality in HEU compared to HUU children after controlling for confounding factors (Brahmbhatt et al, 2006; Hong et al, 2007; Chilongozi et al, 2008). HEU children also demonstrate an increased risk of hospitalisation and mortality with infectious diseases (McNally et al, 2007; Koyangi et al, 2011; Slogrove et al, 2012). Whilst conflicting findings do

exist (Zash et al, 2014), a systematic review by Afran et al (2014) concluded that the evidence for increased mortality risk in HEU children was compelling.

The literature also suggests that HEU children experience impaired immune function due to HIV-exposure Clerici et al, 2000; Rombadi et al, 2008; Koyangi et al, 2011). Reikie et al (2011) and Slogrove et al (2011) argue that investigation of this abnormality must be a priority if mortality in HEU children is to be reduced. Substantial evidence exists for an association between HIV-exposure and nutritional status (Filteu et al, 2011). Nutrition and low birth weight also show an association with mortality in HEU infants (Nathoo et al, 2005). Various studies, including a large pooled analysis (Zaba et al, 2005), suggest that maternal health is significantly associated with mortality in HIV-exposed children versus unexposed (Crampin et al, 2009). However, evidence to the contrary does exist (Newell et al, 2004; Kinney et al, 2010). Finally, the literature clearly indicates the need to consider feeding practice when investigating mortality in HIV-exposed children. Multiple studies found early cessation and formula feeding play a significant role in mortality (Kafulafula et al, 2010; Mugwaneza, 2011; Rollins et al, 2013; Boerma et al, 2014).

Despite the above evidence the size and importance of associations vary widely. There are also some common limitations in the literature. Our study is well placed to address some of these limitations. For example there is limited information available regarding mortality of HIV-exposed and unexposed children living in an urban areas, attending tertiary hospitals. There was also a preponderance of studies in locations without PMTCT coverage (Zijenah et al, 1998; Nathoo et al, 2005; Marinda et al, 2007). There is a clear need for more research into the influence of HIV-exposure on mortality in areas with high PMTCT coverage (Mugwaneza, 2011).There is also a shortage of studies looking at longer term survival of children beyond 24 months. Finally, many studies failed to separate HIV-exposed uninfected children from HIV-exposed infected children in their analysis. This makes it difficult to separate to influence of HIV infection from that of exposure alone (Ryder et al, 1994; Ota et al, 2002; Nakiyingi et al, 2003; Ng'weshemi et al, 2003; van der Loef et al, 2003; Crampin et al, 2009).

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PART C: MANUSCRIPT

In accordance with the journal:

Bulletin of the World Health Organisation (see Fig.6, Appendix D for guidelines)

Abstract

Objective

To establish whether there is an association between HIV exposure and infectious disease mortality in children admitted to hospital in the Western Cape. Also to identify additional risk factors associated with mortality and the prevalence of HIV exposure.

Methods

A case-control design was used to compare the HIV exposure status of 93 cases admitted with an infectious disease who died with 93 controls admitted with an infectious disease who did not die. Clinical and demographic data were collected via record review at three hospitals in the Western Cape. Factors associated with mortality were identified through regression analysis.

Findings

38.71% (36/93) of cases were HIV exposed versus 22.58% (21/93) of controls (p=0.017). 32.36% (30/93) of cases were HIV exposed uninfected (HEU) versus 18.28% (17/93) of controls (p=0.06).

Being HEU was a risk factor for mortality after adjusting for age, sex, feeding practice, and main infection. The odds of death in HEU children was 2.29 times greater than in HUU (aOR: 2.29, 95% CI: 1.06-5.0). Age, sex, and feeding practice confounded the association between HIV exposure-infection and mortality. LRTI, septicaemia, and meningitis were all significantly associated with mortality at the 5% level. Children admitted with septicaemia demonstrated a 13.44 times increased odds of death (aOR: 13.44, 95% CI: 3.35-53.99).

Conclusion

Children born to HIV positive mothers should be considered a vulnerable group even if vertical transmission has been prevented. Determination of exposure status could be a valuable tool for identifying children at increased risk of death. Septicaemia and meningitis also present a challenge in critical care in our sample.

Introduction

Over the past decade prevention of mother-to-child transmission (PMTCT) programmes have formed a key part of HIV control throughout Africa.[1] As a result transmission from mothers to infants has declined to 2% in the Western Cape.[2] One consequence of this success is an increase in the population of HIV exposed uninfected (HEU) children.

HEU children have a greater risk of mortality compared to HIV unexposed uninfected (HUU) children in studies from a range of contexts,[3,4,5,6,7] including South Africa.[8,9] The association between HIV exposure and mortality is mediated by multiple factors including maternal health,[10, 11] birth weight,[12] and feeding practice.[13, 14] There is also evidence that HEU children are more susceptible to infectious disease.[15, 16] However, risk factors identified in previous research differ widely and our knowledge on HIV-exposure in children in the Western Cape is limited.

HEU children have three times greater risk of mortality than HUU children[17] and an increased risk of severe infection and mortality from diseases such as pneumonia.[16] Despite this, HEU children are overlooked as a vulnerable group at increased risk from infectious diseases.[18] Identifying the causes of increased mortality amongst HEU children in South Africa should be a public health priority[19] and attempts to improve the health of HEU children must be informed by local investigations.[20]

In this study we determined the prevalence of HEU in children admitted to hospital for infectious diseases and investigated the association between HIV exposure and other risk factors and infectious disease mortality in the Western Cape.

Methods

A case control study was used to collect data from medical records at three hospitals in the Western Cape. Study sites included the three public hospitals that admit the majority of children in the Western Geographic Service Area (GSA) of the Cape Town Metro district.[21, 22] The sites comprised two tertiary hospitals of Red Cross Children's Hospital (RCCH) and Groote Schuur and the regional hospital New Somerset.

Permission to conduct the study was granted by the Provincial Research Sub-directorate, Western Cape Government, the relevant committees at each of the Hospitals, and the Human Research

Ethics Committee at the University of Cape Town Health Science provided research ethics approval (see Fig. 1-5, Appendix D).

Children were defined as cases if they were less than 13 years of age, diagnosed with an infectious disease upon admission and died due to that condition between January 2008 and December 2014. Controls were defined in the same manner, except that they survived the condition.

Data was collected between November 2014 and April 2015. Ninety-three cases were collected using digital records held on the Child PIP database. Ninety -three controls were collected from the three hospitals by accessing ward registers and medical records. At each of the sites cases and controls were numbered sequentially and a simple random sample was drawn for both groups.

The diagnosis on admission for all patients was confirmed through review of medical records. Children whose diagnosis on admission was infectious but changed to a non-infectious cause were considered ineligible. In accordance with past studies[23, 24] a missing value was assumed to indicate a worst case scenario regarding treatment received or patient outcome. The deletion of cases or controls with missing values was not a considered acceptable as it would have reduced sample size and potentially introduced bias.

The variables of interest are displayed in Table 1, Appendix A. Data were recorded on data collection sheets (see Fig 7, Appendix D), re-coded (see Table 1, Appendix D) and entered onto a data spreadsheet (Document Foundation. 2015. *LibreOffice:4.3.7*.Germany: Document Foundation). This was then exported to STATA 12 (StataCorp. 2011. *Stata Statistical Software: Release 12*.Texas: StataCorp) for statistical analysis. Data were double entered.

Data Analysis

Methods used during exploratory analysis included Pearson's Chi-square test, Fischer's exact test, and Wilcoxon Rank Sum test using a significance level of 5% to establish statistical significance. Exploratory analysis informed the analysis of variables in the preceding regression analysis and model selection.

Univariate logistic regression analysis was used to identify variables associated with mortality. Variables with a $p< 0.15$ were eligible for inclusion in model selection. Categorical variables were

treated as blocks. As such only one category within each variable was required to display a $p< 0.15$ to qualify a variable block for inclusion in model selection. Dummy variables were created to allow for the consideration of polychotomous categorical variables. The structure and coding of the dummy variables can been found in Table 2-6, Appendix A.

An a priori framework was used to identify confounders.[25] Age and sex were identified as a priori confounders as they consistently emerged as sources of confounding in the literature.[12, 6, 26] Additional confounders were identified through regression analysis. A variable was considered a confounder if its inclusion resulted in a >10% change in the estimate of association with the main exposures of interest. All confounders were forced into the base model.[27] The presence of collinearity was investigated prior to model selection and post model selection. Any variable with a VIF value >10 was considered for removal due to collinearity.[28] The final decision to remove a variable was informed by its clinical and analytical value.

Model selection

Multivariate logistic regression models, controlling for identified confounders, were built for the dependent variable (mortality) utilising independent variables identified via univariate analysis. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for each variable within a model. Firstly, all confounders were entered into the baseline model. Qualifying variables identified in univariate analysis (with $p< 0.15$) were added one at a time. At each step the Akaike information criterion (AIC) of each model was compared to that of the base model. The model with the lowest AIC was used as the base model for each successive step until all eligible variables had been added. In the penultimate step all previously discarded variables were readmitted one at a time to reassess their influence on the AIC. Finally, interaction terms were used to represent the interaction between HIV exposure-infection status and other variables. Interactions were retained if they produced a lower AIC than the model omitting them. Details of each step of model selection can be found in Table 1, Appendix C.

Results

Table 1: Child characteristics and treatments received (%) of cases and controls.

Parameter	Controls (n=93) (%)	Cases (n=93) (%)	Total (n=186) (%)	p-value
Sex				
Female	53 (56.99)	39 (41.94)	92 (49.46)	
Male	40 (43.01)	54 (58.06)	94 (50.54)	**0.040***
Median child age (months) (IQR)	10 (3-15)	7 (1-14)	8 (2-15)	0.128
Child HIV Status				
Negative	89 (95.70)	87 (93.55)	176 (94.62)	
Positive	4 (4.3)	6 (6.45)	10 (5.38)	0.747
Nutritional Status				
OWFA	0 (0.00)	0 (0.00)	0 (0.00)	
Normal	58 (62.37)	46 (49.46)	104 (55.91)	
UWFA	27 (29.03)	30 (32.26)	57 (30.65)	
Marasmus	4 (4.30)	6 (6.45)	10 (5.38)	
Kwashiorkor	4 (4.30)	11 (11.83)	15 (8.06)	0.156
Mother Health Status				
Dead/sick	9 (9.68)	12 (12.90)	21 (11.29)	
Alive/well	84 (90.32)	81 (87.10)	165 (88.71)	0.487
Primary Carer				
Other	13 (13.98)	12 (12.90)	25 (13.44)	
Mother	80 (86.02)	81 (87.10)	161 (86.56)	0.830
Feeding Practice				
Formula	28 (30.11)	38 (40.86)	66 (35.48)	
Exclusive Breast	49 (52.69)	40 (43.01)	89 (47.85)	
Mixed	16 (17.20)	15 (16.13)	31 (16.67)	0.293
Child ART				
Not Received	87 (93.55)	88 (94.62)	175 (94.09)	
Received	6 (6.45)	5 (5.38)	11 (5.91)	0.756
Mother ART				
Not Received	74 (79.57)	69 (74.19)	143 (76.88)	
Received	19 (20.43)	24 (25.81)	43 (23.12)	0.385
PMTCT				
Not Received	79 (84.95)	63 (67.74)	142 (76.34)	
Received	14 (15.05)	30 (32.26)	44 (23.66)	**0.007***

p-values derived from Rank Sum test for continuous variables and Chi-square test or Fischer's Exact test for categorical.

* = statistically significant (p<0.05).

Child characteristics and treatments received are shown in Table 1. Ninety-three cases and 93 controls were enrolled in the study. 58.06% (54/93) of cases were male compared to 43.01% (40/93) of controls (p=0.04). There was only one other significant difference between cases and controls. (30/93) of cases had received PMTCT compared to 15.05% (14/93) of controls (p=0.007). There was no significant difference between cases and controls in terms of age, primary carer, nutritional status, and feeding practices. There was also no significant difference in the presence of HIV infection between cases (6.45%; 6/93) and controls (4.3%; 4/93).

Child HIV exposure and infection status and infectious disease profiles

Table 2: HIV exposure and infection profiles and infectious disease profiles (%) of cases and controls.

Parameter	Controls (n=93) (%)	Cases (n=93) (%)	Total (n=186) (%)	p-value
HIV Exposure Status				
Unexposed	72 (77.42)	57 (61.29)	129 (69.35)	
Exposed	21 (22.58)	36 (38.71)	57 (30.65)	**0.017***
HIV exposure-infection status				
HUU	72 (77.42)	57 (61.29)	129 (69.35)	
HEU	17 (18.28)	30 (32.36)	47 (25.27)	
HEI	4 (4.30)	6 (6.45)	10 (5.38)	0.062
Main Cause				
Gastroenteritis	44 (47.31)	17 (18.28)	61 (32.80)	
TB	4 (4.30)	4 (4.30)	8 (4.30)	
LRTI	36 (38.71)	41 (44.09)	77 (41.40)	
Septicaemia	3 (3.23)	16 (17.20)	19 (10.22)	
Meningitis	6 (6.45)	15 (16.30)	21 (11.29)	**0.000***
Other Cause				
None	71 (76.34)	67 (72.04)	138 (74.19)	
Gastroenteritis	8 (8.60)	11 (11.83)	19 (10.22)	
TB	1 (1.08)	3 (3.23)	4 (2.15)	
LRTI	9 (9.68)	5 (5.38)	14 (7.53)	
Septicaemia	3 (3.23)	4 (4.30)	7 (3.76)	
Meningitis	1 (1.08)	3 (3.23)	4 (2.15)	0.528
Underlying Condition (e.g. congenital malformation				
No	83 (89.25)	79(84.95)	162(87.10)	

Parameter	Controls (n=93) (%)	Cases (n=93) (%)	Total (n=186) (%)	p-value
Yes	10 (10.75)	14 (15.05)	24 (12.90)	0.382

p-values derived from Chi-square test or Fischer's Exact test. * = statistically significant (p<0.05)

Table 2 shows the HIV and infectious disease profiles. 38.71% (36/93) of cases were HIV exposed compared to 22.58% (21/93) of controls (p=0.017). The prevalence amongst controls indicates that the estimated population prevalence of HIV exposure was 23%. There was no significant difference between cases and controls in terms of HIV exposure-infection status. 32.36% (30/93) of cases were HEU versus 18.28% (17/93) of controls (p=0.06). Cases and controls differed significantly according to their main infection (p<0.001). Gastroenteritis was more common amongst controls (47.31%; 44/93) than cases (18.28%; 17/93) whilst LRTI was more common amongst cases (44.09%; 41/93) than controls (38.71%; 36/93). Septicaemia and meningitis were also more common amongst cases.

Collinearity

HIV status of the child, HIV status of the mother, the health status of the mother and whether the mother received PMTCT were removed from consideration in the regression analysis as they displayed VIF values >10.

Logistic Regression Analysis

Table 3: Variable associated with death in the univariate logistic regression analysis.

Parameter	OR (95% CI)	p value
Sex	1.83 (1.03-3.28)	**0.041***
Age (months)	0.10 (0.98-1.01)	0.435
HIV Exposure	2.17 (1.14-4.11)	**0.018***
Primary Carer	1.10 (0.47-2.55)	0.830
Child ART	0.82	0.756

Parameter	OR (95% CI)	p value
	(0.24-2.80)	
Maternal ART	1.76 (0.90-3.44)	0.100
Underlying Cause	1.47 (0.62-3.50)	0.384
HIV exposure-infection status		
HUU	1.00	reference
HEU	2.23 (1.12-4.44)	**0.023***
HEI	1.89 (0.51-7.04)	0.340
Feeding Practice		
Formula	1.00	reference
Exclusive Breast	0.60 (0.32-1.14)	0.121*
Mixed	0.69 (0.29-1.63)	0.398
Nutritional Status		
Normal	1.00	reference
UWFA	1.40 (0.73-2.68)	0.308
Marasmus	1.89(0.50-7.10)	0.345
Kwashiorkor	3.47 (1.04-11.60)	0.228
Main infection		
Gastroenteritis	1.00	reference
TB	2.59 (0.58-11.54)	0.212
LRTI	2.95 (1.44-6.04)	**0.003***
Septicaemia	13.80 (3.56-53.47)	**0.000***
Meningitis	6.47 (2.15-19.44)	**0.001***
Other infection		
None	1.00	reference
Gastroenteritis	1.32	0.577

Parameter	OR (95% CI)	p value
	(0.49-3.56)	
TB	4.24 (0.46-38.90)	0.322
LRTI	0.59 (0.19-1.85)	0.364
Septicaemia	1.41 (0.30-6.55)	0.659
Meningitis	3.18 (0.32-31.32)	0.322

*= $p < 0.15$ (variable qualified for inclusion in model selection).

Table 3. presents the variables associated with death from the univariate logistic regression analysis. Boys were almost two times more likely to die than girls (OR: 1.83; 95% CI: 1.03-3.28). Children who were exclusively breastfed demonstrated a 40% reduction in the odds of death compared to children who were formula fed (OR: 0.6; 95% CI: 0.32-1.14).

Exposure to HIV (regardless of infection status) was associated with a greater than two-fold increased odds of death (OR: 2.17; 95% CI:1.14-4.11). The odds of death in HEU children was twice that of HUU children (OR: 2.23; 95% CI: 1.12-4.44). With gastroenteritis as the reference children with LRTI (OR: 2.95; 95% CI: 1.44-6.04), septicaemia (OR: 13.80; 95% CI: 3.56-53.47), and meningitis (OR: 6.47; 95% CI: 2.15-19.44) were more likely to die than children with gastroenteritis.

Confounders of the association between mortality and HIV exposure-infection status

In the regression analysis only feeding practice emerged as a confounder of the association between HIV exposure-infection status and mortality (see Table 1, Appendix B).

Model selection process and interpretation

Table 4: Variables associated with death in the multivariate regression model with the best compared to univariate regression model

	Model b1	Univariate analysis
Variable	Adjusted OR (95% CI)	Unadjusted OR (95% CI)
Age (months)	0.99	0.10

	Model b1	Univariate analysis
Variable	**Adjusted OR (95% CI)**	**Unadjusted OR (95% CI)**
	(0.98-1.01)	(0.98-1.01)
Sex	1.84 (0.96-3.53)	1.83 (1.03-3.28)
Feeding Practice		
exclusive formula	1.00	1.00
exclusive breast	0.76 (0.36-1.61)	0.60 (0.32-1.14)
mixed feeding	0.84 (0.31-2.28)	0.69 (0.29-1.63)
Main infection		
Gastroenteritis	1.00	1.00
TB	2.45 (0.52-11.45)	2.59 (0.58-11.54)
LRTI	**3.12 (1.50-6.61)**	2.95 (1.44-6.04)
Septicaemia	**13.44 (3.35-53.99)**	13.80 (3.56-53.47)
Meningitis	**8.59 (2.58-28.60)**	6.47 (2.15-19.44)
HIV exposure-infection status		
HUU	1.00	1.00
HEU	**2.29 (1.06-4.97)**	2.23 (1.12-4.44)
HEI	2.17 (0.51-9.22)	1.89 (0.51-7.04)

Model b1: Hosmer-Lemeshow chi2(8) = 7.83, Prob > chi2 = 0.4503, number of observations =186, number of groups = 10

Table 4 shows the association of variables with death in the multivariate regression model. The model selection process identified the model containing age, gender, feeding practice, main infection, and HIV exposure-infection status as the best model. As age, sex and feeding practice were identified as confounders they were retained in modelling despite their statistical non-significance at the 5% level. With regards to HIV exposure-infection status, the odds ratio for HEU is significant at a 5% level of significance (aOR: 2.29; 95% CI: 1.06-4.97). Of the main infections only TB was not significantly associated with mortality whilst LRTI, septicaemia, and meningitis were all significantly associated with mortality at the 5% level. From this model we can conclude

that age, gender, feeding practice, main infection type, and HIV exposure-infection status combine to have a significant influence over mortality.

Details of the final model output are shown in Table 3, Appendix C and the full model selection processes is displayed in Table 1, Appendix C**.** Multicollinearity was not present between any of the variables in the final model (see Table 2, Appendix C).

Role of infectious disease in mortality

After adjusting for age, sex, feeding practice, and exposure-infection status, a child admitted with a LRTI had a 3.12 increased odds of dying versus a child admitted with gastroenteritis (aOR: 3.12, 95% CI: 1.47; 6.61). Children admitted with meningitis had an 8.59 times increased odds of death (aOR: 8.59; 95% CI: 2.58-28.60). The largest association was found in children admitted with septicaemia who demonstrated a 13.44 times increased odds of death (aOR: 13.44; 95% CI: 3.35-53.99). TB was not significantly associated with morality.

Role of HIV exposure in mortality

After adjusting for age, sex, feeding practice and main infection being HEU remained a risk factor for mortality increasing from OR: 2.23 (95% CI: 1.12-4.44) to aOR: 2.29 (95% CI: 1.06-5.0). Compared to HUU children the odds of death for HEU children were 2.29 times greater. HEI children did not demonstrate a significant increased odds of death compared to HUU however (aOR 2.17, 95% CI 0.51; 9.22). No significant interaction effects were found in the association between HIV exposure-infection status and mortality (see Table 1, Appendix C).

Discussion

Based on findings from a range of studies[5, 17, 29, 30] we had reason to believe that HEU children are at increased risk of death when admitted to hospital with an infectious disease. Our findings support this hypothesis. After controlling for confounders and main infectious disease, HEU children demonstrated over twice the odds of death compared to HUU (aOR: 2.29; 95% CI: 1.06-4.97). Our model highlights an inherent risk associated with being HEU, independent of the type of infectious

disease. HIV prevalence in our controls was 5.38% (10/186), in line with estimates of early vertical transmission in the Western Cape.[31] The prevalence of HIV exposure of 38.71% (36/93) in our controls is larger than expected given a NDoH prevalence estimate of 17% in the Western Cape.[32]

Feeding practice was not found to be significantly associated with mortality. However, in agreement with prior studies[6, 13, 17] we found feeding practice confounded the association between HIV exposure-infection status and mortality. Our failure to identify significant interactions, particularly with age is surprising. Prior studies found the higher risk of death in HEU children declined with age.[30,33] This failure is likely due to a lack of power in our study.

In multivariate regression the association between each infection and mortality sharpened. Septicaemia remained most strongly associated with mortality (aOR: 13.44, 95% CI 3.34-53.99). Meningitis showed the largest increase in association in the final model with its unadjusted odds ratio rising from 6.47 (95% CI 2.15-19.44) to 8.59 (95% CI 2.58-28.60). Age and sex have consistently been identified as confounders of the association between infection and mortality[34, 35] which may explain this inflation in association in multivariate regression. Septicaemia presents a real challenge in our sample, and children with septicaemia had a 13 times increased odds of death (aOR: 13.44; 95% CI: 3.35-52.99). Inadequate access to diagnostic tools for septicaemia has been identified in the Western Cape[36] and this may offer some explanation for our finding. Studies have found that septicaemia survival is largely determined by prompt treatment[37] with every hour without resuscitation increasing the risk of mortality by 40%.[38]

A child's HIV status, mothers health status, nutritional status, and the presence of other infections and underlying conditions did not show significant association with mortality. This finding contrasts with other studies.[11, 29, 39] Possible explanations for our findings include the high standards of care,[40] better infrastructure, and higher levels of extended family childcare[41, 42] in the Western Cape relative to locations of previous studies.[43] These studies often sampled from rural clinics offering lower standards of clinical care compared to urban hospitals.[44]

Strengths

Mortality cannot precede HIV exposure, as such interpretation of our results is not complicated by concerns for temporality. The use of multivariate analysis allowed us to control for the confounders of age, sex, and feeding practice. This increases external and internal validity and increases the

likelihood that we are observing the true association between HEU children and mortality. Much of the previous research into HEU children has been conducted in countries and time periods when PMTCT and ART was not available. By researching Western Cape from 2008-2015 we have produced findings relevant to an increasing number of contexts where PMTCT is now part of routine care. Previous studies have failed to establish HIV infection status of HIV exposed children. As such their ability to identify the independent influence of HIV exposure on mortality was limited.[45, 46] As our study collected information on both HIV exposure and infection status we were able to investigate mortality in HIV exposed uninfected children and not simply HIV exposed children.

Limitations

Our sample size was sufficient to confirm the findings of larger studies regarding the association between being HEU and mortality.[5, 17, 29, 30] However, due to a relatively small sample our study lacked the power to detect associations of other variables with mortality. For example, there were just ten HEI children and we were unable to identify a significant association. This is despite larger studies identifying HEI children as being at increased risk of death.[3, 4] Sample size also diminished our capacity to identify confounders and interactions. Resource constraints meant some socio-economic variables could not be collected. Previous studies identified a range of socio-economic variables as confounders of the association between HIV exposure and mortality.[5, 30]

It is inevitable with a medical record review that the data used was not recorded with our research purpose in mind.[47, 48] Therefore the validity of our data is vulnerable to measurement error by health staff. Inaccuracies in recording, testing, and anthropometry measurements may have led to the misclassification of characteristics.

Our study sampled from three hospitals in the Western Cape and the results may not be generalisable to other regions. Data from the sites was also pooled which may have masked important differences in patient variables, standards of care, and diagnoses. However, as cases and controls were comprised of equal proportions from each hospital this should not influence the observed associations. Finally, older children in our study would have been born when PMTCT was not available and their experience of having a HIV positive mother would have differed compared to a child born after 2008. This limitation makes it difficult to apply our findings to children born from 2015 onwards.

It is possible that our study underestimates the odds of mortality in HEU children. The children in our sample may have easier access to hospital compared to children in rural Western Cape. We also only collected data from children attending hospital. Therefore one could argue that those attending hospital may be inherently healthier and more health aware than those not attending hospital. Some variables relied upon self-reporting and recall bias is a concern. For example, mothers of severely ill children may recall details more accurately than mothers of less severely ill children. Investigator bias is another concern as more severely ill children may have been investigated more thoroughly by clinicians. As a result the diagnosis of conditions may have differed between cases and controls. Missing values were another potential source of bias. We identified six medical records containing a missing values. These records were unequally distributed between cases and controls and may have biased our measures of association.

The cross-sectional nature of this study meant we were only able to establish patient characteristics at one point in time. We could not account for multiple transitions between variable categories over time. This limitation may have prevented us from identifying the true influence of feeding practice It is also possible that HIV-negative mothers may have seroconverted after delivery or most recent test. Therefore a child may have been misclassified as HIV unexposed. Added to this, some children may have been untested due to their age (18% were < 6 weeks) and others may have become infected after providing their last test. Therefore unidentified HIV infection in children may have been responsible for the association between being HEU and mortality.

Conclusion

HEU children demonstrate an odds of death that is over twice that of HUU children after controlling for confounders and type of infectious disease. This finding indicates an inherent risk associated with being HEU and is in line with studies across Africa.[5, 17, 29, 30] Children born to HIV positive mothers should be considered a vulnerable group even when MTCT has been prevented. The determination of exposure status could be valuable in identifying vulnerable children and reducing mortality. Children admitted to hospital with LRTI, septicaemia, and meningitis had an increased odds of dying. Septicaemia and meningitis present a challenge in critical care in our sample.

Future research utilising larger samples should consider how the association between HEU children and mortality is influenced by effect modifiers. Mixed-method studies should also consider the role

of socio-economic factors. Studies investigating underlying biological mechanisms should also be encouraged.

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PART D: APPENDICES

APPENDIX A

Table 1: Variables of interest that were collected.

Variable	Description
Age in months (Continuous)	Age of child in months. Recorded as: 0-155 months.
Child ART received (Binary)	Whether a child had ever received ART other than PMTCT before admission. Recorded as: Yes; No
Child HIV Status (Binary)	According to the results of ELISA or PCR as recorded on medical record or Child PIP record. Recorded as: HIV negative: HIV positive.
Feeding Practice (Categorical)	Feeding practice used by the mother during the first 6 months. Recorded as: Never breastfed; Exclusively breastfed; Mixed feeding
Sex (Binary)	Recorded as: Male; Female
HIV exposure status (Binary)	Whether a child was or was not HIV exposed through birth to a HIV positive mother. Recorded as: Exposed; Unexposed.
HIV exposure-infection status (Categorical)	Recorded as: HUU (HIV negative child born to a HIV negative mother); HEU: (HIV negative child born to HIV positive mother); HEI (HIV positive child born to HIV positive mother).
Main Infection (Categorical)	Main cause of admission or death as recorded on medical records. Recorded as: Gastroenteritis; TB; LRTI (bronchiolitis, bronchitis, pneumonia, croup); Septicaemia; Meningitis; Other.
Maternal ART received (Binary)	Whether the child had ever received ART other than PMTCT before admission. Recorded as: Yes/No
Maternal HIV Status (Binary)	Results of ELISA/PCR as recorded on medical record. Recorded as: Negative; Positive
Mother's health status (Binary)	Whether a mother was ‘Alive and well' and able to care for the child or ‘Dead/Sick ’and unable to care for child. Recorded as: Alive and well; Dead/Sick
Other infection (Categorical)	Whether an additional infection contributed to admission or death. Recorded as: None; Gastroenteritis; TB; LRTI (bronchiolitis, bronchitis, pneumonia, croup); Septicaemia; Meningitis; Other.
Primary Carer (Binary)	Whether the carer was the mother or 'other' (father, grandparent, foster parents, orphanage etc.). Recorded as: Mother; Other
Nutritional Status (Categorical)	Established by physician and use of WHO z-score weight-for-age charts (see Appendix). Recorded as: Overweight for Age (OWFA), Normal, Underweight for Age (UWFA), Marasmus, Kwashiorkor, Marasmus-Kwashiorkor (MK)
Underlying Condition (Binary)	Whether a child had an underlying condition such as congenital malformation, condition arising in perinatal period, disease of nervous system. Recorded as: Present; Not present

Table 2: HIV-exposure-infection status dummy coding

HIV-exposure-infection Status	Dummy variables	
	HEU	**HEI**
HUU	0	0
HEU	1	0
HEI	0	1

Table 3:Feeding practice dummy coding

Feeding ractice	Dummy variables	
	Exc. breast	Mixed
Formula	0	0
Exc. breast	1	0
Mixed	0	1

Table 4: Nutritional status dummy coding

Nutritional Status	Dummy variables		
	UWFA	Marasmus	Kwashiorkor
Normal	0	0	0
UWFA	1	0	0
Marasmus	0	1	0
Kwashiorkor	0	0	1

Table 5: Other infection dummy coding

Other Infection	Dummy Variables				
	Gastroenteritis	TB	LRTI	Septicaemia	Meningitis
None	0	0	0	0	0
Gastroenteritis	1	0	0	0	0
TB	0	1	0	0	0
LRTI	0	0	1	0	0
Septicaemia	0	0	0	1	0
Meningitis	0	0	0	0	1

Table 6: Main infection dummy coding

Main infection	Dummy Variables			
	TB	LRTI	Septicaemia	Meningitis
Gastroenteritis	0	0	0	0
TB	1	0	0	0
LRTI	0	1	0	0
Septicaemia	0	0	1	0

Meningitis	0	0	0	1

Appendix B

Table 1 Summary table of adjusted and unadjusted odds ratios to identify confounders

Adjusted for	Unadjusted OR	Adjusted OR	% Change
Unadjusted			
HEU	2.23	-	-
HEI	1.89	-	-
Primary carer			
HEU	2.23	2.25	-1.06%
HEI	1.89	1.92	-1.59%
Maternal ART			
HEU	2.23	2.2412	-0.54%
HEI	1.89	1.9029	-0.43%
Feeding practice			
HEU	2.23	2.0744	-6.94%
HEI	1.89	1.6904	-10.75%
Nutritional Status			
HEU	2.23	2.1626	2.98%
HEI	1.89	1.8545	2.12%
Underlying condition			
HEU	2.23	2.2259	0.14%
HEI	1.89	1.7820	5.95%

APPENDIX C

Table 1 Table showing a summary of the model selection process informed by AIC

Model	Independent variables in model	AIC
STEP 1 **Confounders**		
empty	none (constant only)	-
b	age + sex + [exc. breast + mixed feeding]	261.02
c	age + sex + [exc. breast + mixed feeding] + sex*age	263.02
d	age + sex + [exc. breast + mixed feeding] + sex*exc.breast + sex*mixed feeding	264.58
e	age + sex + [exc. breast + mixed feeding] + age*exc.breast + age*mixed feeding	262.11
STEP 2		
base b	***age + sex + [exc. breast + mixed feeding]***	***261.02***
a1	+ [TB + LRTI + septic.+ menin.]	242.80
a2	+ [HEU + HEI]	260.46
STEP 3		
base a1	***age + sex + [exc. breast + mixed feeding] + [TB + LRTI + septic.+ menin.]***	***242.80***
b1	+ [HEU + HEI]	241.79
STEP 4		
base b1	***age + sex + [exc. breast + mixed feeding] + [TB + LRTI + septic.+ menin.] + [HEU + HEI]***	***241.79***
c1	+ maternal ART	243.51
STEP 5		

Interactions with sex		
base ***b1***	***age + sex + [exc. breast + mixed feeding] + [TB + LRTI + septic.+ menin.] + [HEU + HEI]***	***241.79***
d1	+ [sex*TB + sex*LRTI + sex*septic + sex*menin.]	243.89
d2	+ [sex*HEU + sex*HEI]	243.35
STEP 6 **Interactions with age**		
base ***b1***	***age + sex + [exc. breast + mixed feeding] + [TB + LRTI + septic.+ menin.] + [HEU + HEI]***	***241.79***
d3	+ [age*TB + age*LRTI + age*septic. + age*menin.]	242.25
d4	+ [age*HEU + age*HEI]	251.35
STEP 7 **Interactions with feeding practice**		
base ***b1***	***age + sex + [exc. breast + mixed feeding] + [TB + LRTI + septic.+ menin.] + [HEU + HEI]***	***241.79***
d5	[exc. breast*HEU + mixed feeding*HEU + exc. breast*HEI + mixed feeding*HEI]	243.30
d6	[exc. breast*TB + mixed feeding*TB + exc. breast*LRTI mixed feeding*LRTI + exc. breast*septic.+ mixed feeding*septic + exc. breast*menin + mixed feeding*menin]	246.40
STEP 8 **Interactions with HEI/HEI**		
base	***age + sex + [exc. breast + mixed feeding] + [TB + LRTI + septic.+***	***241.79***

b1	***menin.] + [HEU + HEI]***	
d7	HEU*TB + HEI*TB + HEU*LRTI + HEI*LRTI + HEU*septic + HEI*septic + HEU*menin + HEI*menin	251.17
STEP 9 **Readmission**		
base ***b1***	***age + sex + [exc. breast + mixed feeding] + [TB + LRTI + septic.+ menin.] + [HEU + HEI]***	***241.79***
g1	mart2	243.51

As shown in Table 1 the model selection process identified mode b1 as the optimal model

Table 2. VIF results for final model

Variable	VIF
LRTI	1.83
Exclusive breast	1.70
sex	1.70
Age	1.37
Meningitis	1.32
HEU	1.26
Septicaemia	1.23
Mixed feeding	1.21
TB	1.12
HEI	1.07

As shown in Table 2. multicollinearity was not present between any of the variables in the final model according to the VIF threshold of >10.

Table 3. Logistic regression output for final model b1

LOGISTIC REGRESSION				**No. Obs**	186
				LR chi2(10)	38.06
				Prob > chi2	0.0000
LR	-109.89654			**Pseudo R2**	0.1476
death	**OR**	**SE**	**z**	**P>|z|**	**[95% Conf. Interval]**
age	.9928447	.0074438	-0.96	0.338	.9783617 1.007542
sex	1.840587	.6103002	1.84	0.066	.9609827 3.525307
exc. breast	.7613173	.2904315	-0.71	0.475	.3604497 1.608003
mixed feed	.8405495	.4274031	-0.34	0.733	.3102729 2.277103
TB	2.446383	1.926798	1.14	0.256	.5225272 11.45355
LRTI	3.116721	1.195579	2.96	0.003	1.469521 6.610283
septicaemia	13.44025	9.535949	3.66	0.000	3.345597 53.99341
meningitis	8.594364	5.272503	3.51	0.000	2.582352 28.60304
HEU	2.290338	.9044517	2.10	0.036	1.056237 4.966358
HEI	2.16548	1.600964	1.05	0.296	.5084534 9.222678
constant	.2744411	.1359788	-2.61	0.009	.1039202 .7247672

Model b1: Hosmer-Lemeshow chi2(8) = 7.83, Prob > chi2 = 0.4503, number of observations =186, number of groups = 10.

Table 3 shows the regression output of the final model. It is statistically significant ($p < 0.0001$) and we can conclude that age, gender, feeding practice, main infection type, and HIV exposure-infection status combine to have a significant influence over mortality. The model passed the Hosmer-Lemeshow test ($p = 0.4503$) confirming that model fit the data well.

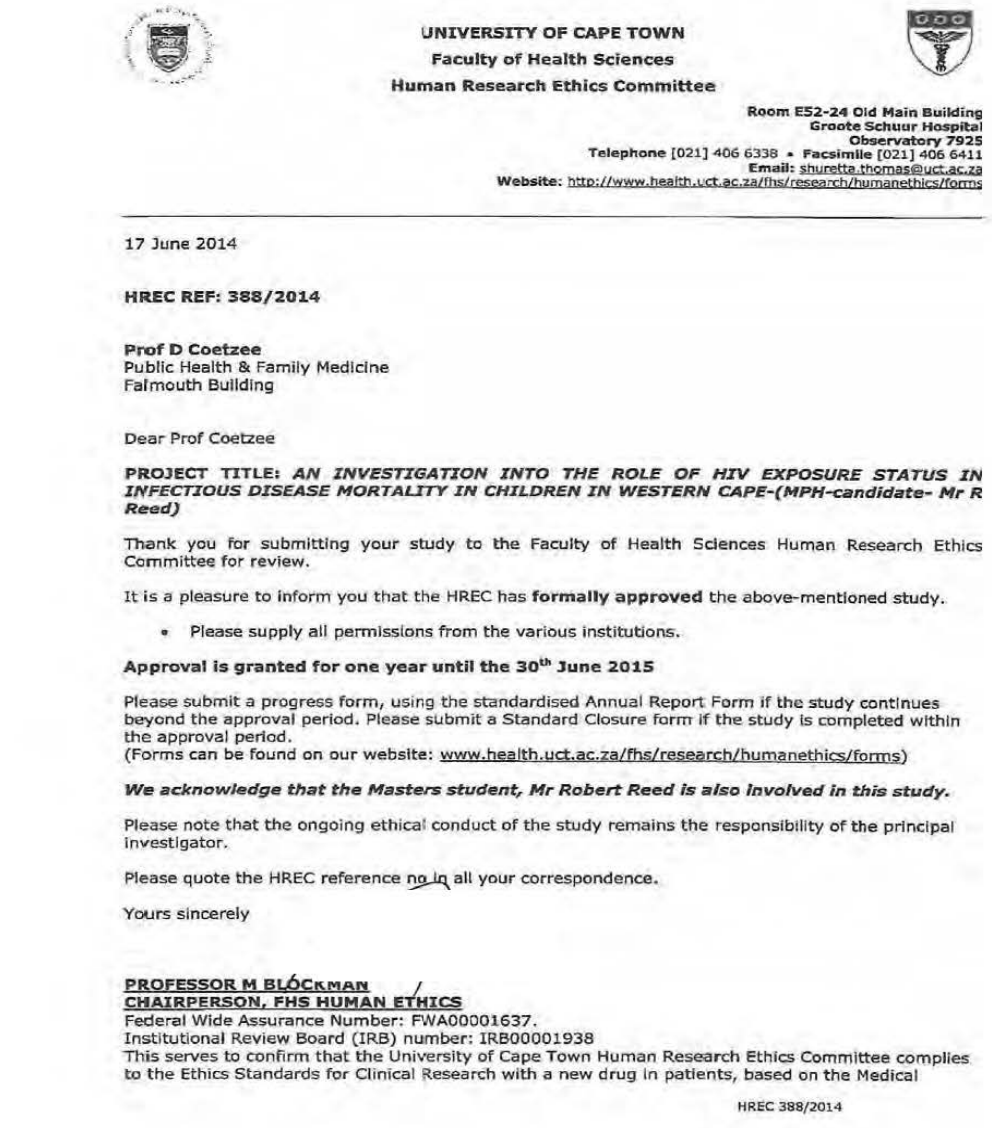
APPENDIX D

Table 1: Variable categories and codes for data collection

Variable	Measurement and Code
Death (dependent variable)	1 if Yes 0 if no
Sex	1 if make 0 if female
Age	Age in months
Weight Category	1 if Overweight for Age 2 if Normal 3 if Underweight for Age 4 if Marasmus 5 if Kwashiorkor 6 if Marasmus-Kwashiorkor
Mother health status	1 if Alive/well 0 if Dead
Primary care giver	1 if Mother 2 if Father 3 if Grandparent 4 if Other
Primary care giver	1 if Mother 0 if Other
HIV exposure status	1 if exposed 0 if unexposed
HIV exposure-infection status	0 if HIV unexposed uninfected (HUU) 1 if HIV exposed uninfected (HEU) 2 if HIV exposed infected (HEI)
Child HIV status	1 if HIV positive 0 if HIV negative
Mother HIV status	1 if HIV positive 0 if HIV negative
Feeding practice	0 if No breastfeeding

	1 if Exclusive breastfeeding 2 if Mixed feeding
Child ART	1 if ever received 0 if never received
Perinatal ART	1 if ever received 0 if never received
Maternal ART	1 if ever received 0 if never received
Main infection	1 if Gastroenteritis 2 if TB 3 if LRTI 4 if Septicaemia 5 if Meningitis 6 if Other
Underlying condition I	1 if present 0 if not present
Other infection	0 if None 1 if Gastroenteritis 2 if TB 3 if LRTI 4 if Septicaemia 5 if Meningitis 6 if Other

Fig.1. Health Research Ethics Committee Approval



UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
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Telephone [021] 406 6338 • Facsimile [021] 406 6411
Email: shuretta.thomas@uct.ac.za
Website: http://www.health.uct.ac.za/fhs/research/humanethics/forms

17 June 2014

HREC REF: 388/2014

Prof D Coetzee
Public Health & Family Medicine
Falmouth Building

Dear Prof Coetzee

PROJECT TITLE: ***AN INVESTIGATION INTO THE ROLE OF HIV EXPOSURE STATUS IN INFECTIOUS DISEASE MORTALITY IN CHILDREN IN WESTERN CAPE-(MPH-candidate- Mr R Reed)***

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

- Please supply all permissions from the various institutions.

Approval is granted for one year until the 30th June 2015

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the Masters student, Mr Robert Reed is also involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC reference no in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical

HREC 388/2014

Fig.2 Child PIP Executive Committee Approval

Application to use the Child PIP database outside of Child PIP structures

Surname: Reed	Name: Robert		Title: Mr
Address: Room 7045, Obz Square, Observatory, Cape Town, 7935			Email:rareed89@gmail.com
			Cell:
Current employer:	UCT MPH (postgraduate student)		
Current academic affiliation:	UCT MPH		
Research purpose:	**Degree: X**		Non-degree
	Publication		Conference presentation
Type of study:	Descriptive		Analytical: X
Data levels:	Province(s):	Western Cape	
	District(s):		
	Hospital(s):	Red Cross, Groote Schuur, New Somerset	
Data format requested:	Full use of the Child PIP software and database to generate tables		**Specifically prepared data tables: X**

Declaration of intent:

1. I declare that the data will be used solely for the purpose outlined above, and that absolute confidentiality will be maintained at all times. No patient identifiers will be sought nor used in my research.
2. I will acknowledge Child PIP as my data source, where Child PIP data are used.
3. I undertake to submit an electronic version of the publication(s) arising out of this study at completion to the Child PIP Exco through Dr Stephen

Name: Robert Reed	Signature: RAREED	Date:02/02/2015

Approval on behalf of the Child PIP Executive

Full use of the Child PIP software and database to generate tables		**Specifically prepared data tables X**
Name: Dr CR Stephen	Signature:	Date: 17.02.2015

Fig.3 Groote Schuur Hospital Approval

GROOTE SCHUUR HOSPITAL
Enquiries: Dr Bernadette Eick
E-mail : *Bernadette.Schmitz@westerncape.gov.za*

Professor D. Coetzee
Public Health & Family Medicine
MEDICAL SCHOOL

E-mail: rareed89@gmail.com

Dear Professor Coetzee

RESEARCH PROJECT: An Investigation into the Role of HIV Exposure Status in Infectious Disease Mortality in Children in Western Cape (MPH Candidate Mr. R. Reed)

Your recent letter to the hospital refers.

You are hereby granted permission to proceed with your research.

Please note the following:

a) Your research may not interfere with normal patient care.
b) Hospital staff may not be asked to assist with the research.
c) No hospital consumables and stationary may be used.
d) **No patient folders may be removed from the premises or be inaccessible.**
e) Please introduce yourself to the person in charge of an area before commencing.
f) Please discuss the study with Professor Heather Zar and Dr Paul Roux before commencing.
g) Please provide the research assistant/field worker with a copy of this letter as verification of approval.
h) Confidentiality must be maintained at all times.

I would like to wish you every success with the project.

Yours sincerely

Signed by

DR AGATA KRAJEWSKI
***(Acting)*CHIEF EXECUTIVE OFFICER**
Date: 9th January 2015

C.C. Mr. L. Naidoo
Professor H. Zar
Dr P. Roux
Dr R. Kirsten

G46 Management Suite, Old Main Building,
Observatory 7925

Private Bag X,
Observatory, 7935

Tel: +27 21 404 6288 fax: +27 21 404 6125

www.capegateway.go.v.za

Fig.4 New Somerset Hospital Approval

Western Cape Government
Health

STRATEGY & HEALTH SUPPORT
Health.Research@westerncape.gov.za
Tel: +27 21 483 6857; fax: +27 21 483 9895
5th Floor, Norton Rose House, 8 Riebeek Street, Cape Town, 8001
www.capegateway.gov.za

REFERENCE: RP116/2014
ENQUIRIES: Ms Charlene Roderick

Room 7045
Observatory
Cape Town
7935

For attention: **Robert Reed, Prof D Coetzee**

Re: An investigation into the Role of HIV Exposure in Infectious Disease Mortality in Children from the Western Cape.

Thank you for submitting your proposal to undertake the above-mentioned study. We are pleased to inform you that the department has granted you approval for your research.
Please contact the following people to assist you with any further enquiries in accessing the following sites:

New Somerset Hospital **D Stokes** **Contact No. 021 402 6992**

Kindly ensure that the following are adhered to:

1. Arrangements can be made with managers, providing that normal activities at requested facilities are not interrupted.
2. Researchers, in accessing provincial health facilities, are expressing consent to provide the department with an electronic copy of the final report within six months of completion of research. This can be submitted to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).
3. The reference number above should be quoted in all future correspondence.

Yours sincerely

DR J EVANS
ACTING DIRECTOR: HEALTH IMPACT ASSESSMENT
DATE: 15/09/2014
CC

Fig.5 Red Cross Children's Hospital Approval

Western Cape Government
Health

Dr R Mistry
Manager: Medical Services
Email: Roshni.Mistry@westerncape.gov.za
Tel: +27 21 658 5788 fax: +27 21 658 5166

Dr R Reed
University of Cape Town

Dear Dr R Reed

APPROVAL OF RESEARCH

PROJECT TITLE: AN INVESTIGATION INTO THE ROLE OF HIV EXPOSURE STATUS IN INFECTIOUS DISEASE MORTALITY IN CHILDREN IN WESTERNCAPE.

We have the pleasure of informing you that approval is hereby granted to conduct the above-mentioned study at Red Cross War Memorial Children's Hospital.

Kindly, note that no more than 10 folders per day are requested and returned to the Medical Records Department by the end of the working day.

Yours sincerely,

Dr Roshni Mistry
Manager: Medical Services
Date: 30 January 2015

www.westerncape.gov.za

Fig.6 Bulletin of World Health Organisation Submission guidelines

1. Scope and editorial policy

1.1 Content

The mission of the *Bulletin of the World Health Organization* is "to publish and disseminate scientifically rigorous public health information of international significance that enables policy-makers, researchers and practitioners to be more effective; it aims to improve health, particularly among disadvantaged populations".

The *Bulletin* welcomes a variety of unsolicited manuscripts (see below, 1.1.1.). These are initially screened in house for originality, relevance to an international public health audience and scientific rigour. If they pass the initial screening, they are sent to peer reviewers whose opinions are taken into account by the journal's editorial advisers when they decide whether to accept a manuscript for publication. Accepted papers are subject to editorial revision, which may involve substantive changes, shortening or restructuring the text and deleting superfluous tables and figures. The word limits given for each type of contribution do not include the abstract (where applicable), tables, boxes, figures and references or appendices, if any. The principal types of manuscripts are outlined below.

1.1.1. Unsolicited manuscripts

We welcome unsolicited submissions to the Research, Systematic reviews, Policy & practice, Lessons from the field and Perspectives sections of the *Bulletin*. All manuscripts destined for the first four of these sections must include two paragraphs indicating what they add to the literature. The paragraphs should briefly explain:

- what was already known about the topic concerned;
- what new knowledge the manuscript contributes.

Research

Research, methodologically rigorous, of relevance to international public health. Formal scientific presentations having not more than 3000 words and 50 references, plus a structured abstract (see below, 2.7); peer reviewed. As clear reporting is needed for readers and reviewers when judging the quality of research, studies should comply with the relevant reporting guidelines, available on the EQUATOR Network web site, at: http://www.equator-network.org/about-equator/equator-publications0/equator-network-publications-2010. Intervention trials as defined by WHO (i.e. "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes") require registration in a public trials registry acceptable to the International Committee of Medical Journal Editors (ICMJE) before submission, and the registration number must be provided at the end of the abstract. Acceptable registries are listed at: http://www.icmje.org/faq_clinical.html. Web publication constitutes prior publication. This includes institutional web sites that are open to the general public.

Systematic reviews

Exhaustive, critical assessments of published and unpublished studies (grey literature) on research questions concerning interventions, policies or practices in public health, with meta-analysis when feasible. Not more than 3000 words plus a structured abstract (see below, 2.7); the number of references in accordance with the scope of the review; peer reviewed. How studies were included and excluded should be illustrated in a flow diagram. Authors should strictly follow the reporting guidelines for systematic reviews and meta-analyses (PRISMA) available at: http://www.equator-network.org/reporting-guidelines/preferred-reporting-items-for-systematic-reviews-and-meta-analyses-the-prisma-statement.

Policy & practice

Analytical assessments, debates or hypothesis-generating papers; not more than 3000 words and 50 references, plus a non-structured abstract (see below, 2.7); peer reviewed.

Lessons from the field

Papers that capture experiences and practice gained in solving specific public health problems in developing countries. Convincing evidence of effect should be provided. Not more than 1500 words and 15 references, plus a structured abstract (see below, 2.7); not more than one table and one figure; must include one box listing three lessons learnt; peer reviewed (see: http://www.who.int/bulletin/volumes/84/1/3.pdf).

Perspectives

Views, hypotheses or discussions (with a clear message) surrounding an issue of public health interest; up to 1500 words, no more than six references; peer reviewed.

1.1.2. Commissioned manuscripts

The categories of articles shown below are normally commissioned by the editors. Authors wishing to submit an unsolicited manuscript for one of these categories should first contact the editorial office (see below, 2.1).

Editorials

Authoritative reviews, analyses or views of an important topic related to a theme or to one or more papers published in a given issue; not more than 800 words, maximum 12 references.

Commentaries

Explanatory or critical analyses of individual articles; not more than 800 words and 12 references.

Round tables

A base paper on a controversial current topic in public health (not more than 2000 words and an abstract) is the core of a debate by several discussants invited to contribute not more than 500 words each.

1.2 Ethical issues

The World Health Organization (WHO) publishes the results of research involving human subjects only if fully compliant with ethical principles, including the provisions of the World Medical Association Declaration of Helsinki (as amended by the 59th General Assembly, Seoul, the Republic of Korea, October 2008; available at: http://www.wma.net/en/30publications/10policies/b3/17c.pdf) and with the additional requirements, if any, of the country in

which the research was carried out. Any manuscript describing the results of such research must contain a clear statement to this effect and should specify that the free and informed consent of the subjects or their legal guardians was obtained and that the relevant institutional or national ethics review board approved the investigation. The *Bulletin* is a member of the Committee on Publication Ethics (COPE; see: http://publicationethics.org). Issues involving publication ethics may be referred to this committee by the editors. WHO Ethics Review Committee clearance is required for papers that report research supported by WHO or that are authored or co-authored by someone who was a WHO staff member while the research was conducted.

1.3 Competing interests

A competing interest arises when a professional judgement concerning a primary interest (such as patients' welfare or the validity of research) may be influenced by a secondary interest (such as financial gain or personal rivalry). We ask all authors to disclose at the time of submission any competing interests that they may have. Examples of competing interests may be found at: http://www.icmje.org. Further information on competing interests is available at: http://www.who.int/bulletin/volumes/83/9/645.pdf.

1.4 Funding

Authors should identify the sources that funded the work undertaken, affirm not having entered into an agreement with the funder that may have limited their ability to complete the research as planned, and indicate that they have had full control of all primary data.

1.5 Appeals process

Authors of rejected papers can appeal against the decision by following the procedures outlined in an editorial published in the *Bulletin* (see: http://www.who.int/bulletin/volumes/83/9/645.pdf).

2. Preparation and submission of manuscripts

2.1 Correspondence

Manuscripts should be submitted to the *Bulletin* via our submissions web site (http://submit.bwho.org), where full instructions are given. Queries about online submissions should be sent to: bulletin.submit.ask@who.int. Authors requiring assistance with online submission can contact the editorial office.

2.2 Uniform requirements

Manuscripts should be prepared in accordance with the *ICMJE recommendations for the conduct, reporting, editing and publication of scholarly work in medical journals*. The complete document, updated in August 2013, is available at: http://www.icmje.org/urm_main.html.

2.3 Languages

Manuscripts should be submitted in English and will be published in that language in the *Bulletin*; the abstracts are translated into Arabic, Chinese, French, Russian and Spanish.

2.4 Authorship

On the manuscript's title page authors should give their full names and the name, city and country of their institutions. The corresponding author must also provide a full postal address, which will be published with the e-mail address unless otherwise requested. Academic titles and the names of departments and subdepartments are unnecessary and are discouraged for reasons of space. If an author has several affiliations, only the most important one should be provided. The criteria for authorship described in the *ICMJE recommendations* (see above, 2.2) must be rigorously observed. Each author should have participated sufficiently in the work being reported to take public responsibility for the paper's content and should describe in detail on the online submission system (not within the manuscript itself) his or her particular contribution. The *Bulletin* encourages submissions from authors in developing countries, and in line with this policy at least one author should have a professional affiliation in the country where the study was conducted.

2.5 Licence for publication

If a manuscript is accepted for publication, the author(s) will be asked to sign a statement granting exclusive licence for publication (not copyright) to the WHO. A copy of the statement is available at: http://submit.bwho.org/journals/bullwho/forms/licence.pdf. Authors are responsible for obtaining permission to reproduce in their articles any material enjoying copyright protection. They should send the letter granting such permission to the editorial office when they submit their papers.

2.6 Figures, tables and boxes

These should be used only to enhance the understanding of the text, not to repeat what can be clearly communicated within the text. All tables, figures and boxes should be numbered consecutively (e.g. Fig. 1, Table 1 and Box 1).

2.7 Abstracts

Abstracts should highlight the text's most important points and should be provided for the following types of papers: Research, Systematic reviews, Policy & practice, base papers for Round tables and Lessons from the field. The abstract should not exceed 250 words. It appears in English at the beginning of the paper and in Arabic, Chinese, French, Russian and Spanish between the end of the text and the reference list. Structured abstracts are required for Research papers and Systematic reviews (Objective, Methods, Findings, Conclusion) and for Lessons from the field papers (Problem, Approach, Local setting, Relevant changes, Lessons learnt).

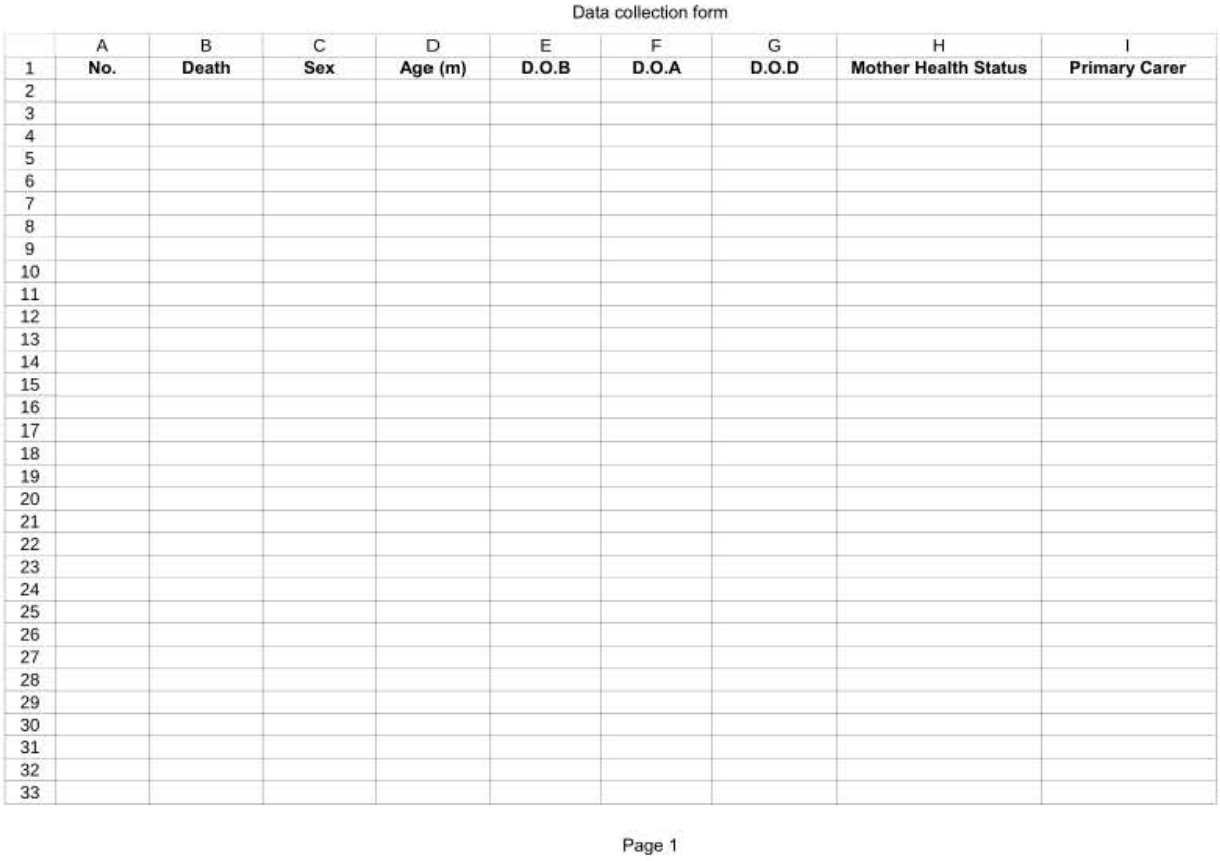
2.8 Bibliographic references

Reference citations should be numbered consecutively as they occur in the text and references should be listed in accordance with the *ICMJE recommendations* (http://www.icmje.org/manuscript_a.html). The accuracy of all references is the authors' responsibility and authors are also responsible for dating access to URLs, providing a record of when they were active.

2.9 Maps

Papers should contain no maps unless an important finding cannot be conveyed without them or unless they are needed to make an essential point. Maps that show international borders, partially or in full, must be created from one of the following sources, approved by the United Nations: http://www.un.org/Depts/Cartographic/english/htmain.htm, http://www.unsalb.org or http://apps.who.int/tools/geoserver and the vectorial EPS (Encapsulated PostScript) file must be submitted. ■

Fig 7 Data collection form



Data collection form

	A	B	C	D	E	F	G	H	I
1	**No.**	**Death**	**Sex**	**Age (m)**	**D.O.B**	**D.O.A**	**D.O.D**	**Mother Health Status**	**Primary Carer**
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