Management of childhood infections in rural Ghana – Filling information gaps

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List of acronyms

AIDS	Acquired immunodeficiency syndrome
APH	Agogo Presbyterian Hospital
ART	Antiretroviral therapy
ARTI	Acute respiratory tract infections
CDC	Centre for Disease Control and Prevention
CHAG	Christian Health Association of Ghana
CHPS	Community-based Health Planning and Service
CI	Confidence interval
CRF	Conceptual research framework
DALY	Disability adjusted live year
DOTS	Directly Observed Treatment, Sort Course
EIEC	Enteroinvasive *Escherichia coli*
GHS	Ghana Health Service
HCW	Health care workers
HIV	Human immunodeficiency virus
IDSR	Integrated Disease Surveillance and Response
IPTI	Intermittent preventive treatment in infants
ITN	Insecticide-treated bed nets
MDG	Millennium Development Goal
MoH	Ministry of Health
MTCT	Mother-to-child transmission
NCD	Non-communicable diseases
NHIA	National Health Insurance Authority
NHIS	National Health Insurance Scheme
NMCP	National Malaria Control Programme
NTD	Neglected tropical disease
NTS	Nontyphoidal *salmonellae*
OPD	Outpatient department
OR	Odds ratio
SDG	Sustainable Development Goals
SES	Socioeconomic status
SMH	St. Michael Hospital
SSA	Sub-Saharan Africa

List of acronyms (continued)

SYSRA	Systematic Rapid Assessment Toolkit
TB	Tuberculosis
UN	United Nations
UNAIDS	Joint United Nations Programme on HIV and AIDS
USA	United States of America
VAT	Value-added tax
WHO	World Health Organization
YLD	Years Lived with Disability
YLL	Years of Life Lost

Publications of the cumulative dissertation

The following three first-authored and two second-authored articles were published within the frame of the cumulative dissertation:

Krumkamp, R., Kreuels, B., Sarpong, N., Boahen, K. G., Foli, G., Hogan, B., Jaeger, A., Reigl, L., Zeeb, H., Marks, F., Adu-Sarkodie, Y., & May, J. 2016. Association Between Malaria and Invasive Nontyphoidal Salmonella Infection in a Hospital Study: Accounting for Berkson's Bias. *Clin Infect Dis*, 62 Suppl 1: S83–9.

Krumkamp, R., Sarpong, N., Schwarz, N. G., Adelkofer, J., Loag, W., Eibach, D., Hagen, R. M., Adu-Sarkodie, Y., Tannich, E., & May, J. 2015. Gastrointestinal Infections and Diarrheal Disease in Ghanaian Infants and Children: An Outpatient Case-Control Study. *PLoS Negl Trop Dis*, 9(3): e0003568.

Krumkamp, R., Sarpong, N., Kreuels, B., Ehlkes, L., Loag, W., Schwarz, N. G., Zeeb, H., Adu-Sarkodie, Y., & May, J. 2013. Health care utilization and symptom severity in Ghanaian children - a cross-sectional study. *PLoS One*, 8(11): e80598.

Dekker, D., ***Krumkamp, R.***, Sarpong, N., Frickmann, H., Boahen, K., Frimpong, M., Asare, R., Larbi, R., Hagen, R., Poppert, S., Rabsch, W., Marks, F., Adu-Sarkodie, Y., & May, J. 2015. Drinking Water from Dug Wells in Rural Ghana - Salmonella Contamination, Environmental Factors, and Genotypes. *Int J Environ Res Public Health*, 12(4): 3535–3546.

Sothmann, P., ***Krumkamp, R.***, Kreuels, B., Sarpong, N., Frank, C., Ehlkes, L., Fobil, J., Gyau, K., Jaeger, A., Bosu, B., Marks, F., Owusu-Dabo, E., Salzberger, B., & May, J. 2015. Urbanicity and Paediatric Bacteraemia in Ghana - A Case-Control Study within a Rural-Urban Transition Zone. *PLoS One*, 10(9): e0139433.

The following publications, to which I have contributed, were published during the course of the dissertation and they contain relevant data on the thesis' topic:

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Frank, C., ***Krumkamp, R.***, Sarpong, N., Sothmann, P., Fobil, J. N., Foli, G., Jaeger, A., Ehlkes, L., Owusu-Dabo, E., Adu-Sarkodie, Y., Marks, F., Schumann, R. R., May, J., & Kreuels, B. 2016. Spatial heterogeneity of malaria in Ghana: a cross-sectional study on the association between urbanicity and the acquisition of immunity. *Malar J*, 15(1): 84.

Kang, H., Kreuels, B., Adjei, O., ***Krumkamp, R.***, May, J., & Small, D. S. 2013. The causal effect of malaria on stunting: a Mendelian randomization and matching approach. *Int J Epidemiol*, 42(5): 1390–8.

Nielsen, M. V., Sarpong, N., ***Krumkamp, R.***, Dekker, D., Loag, W., Amemasor, S., Agyekum, A., Marks, F., Huenger, F., Krefis, A. C., Hagen, R. M., Adu-Sarkodie, Y., May, J., & Schwarz, N. G. 2012. Incidence and characteristics of bacteremia among children in rural Ghana. *PLoS One*, 7(9): e44063.

Summary

Many low-income countries are facing high childhood morbidity and mortality rates. Especially in sub-Saharan Africa and Asia, infectious diseases are the predominant cause of disability-adjusted life years in children. Hence, several national and international health institutions prioritise childhood infectious disease control. However, the success of these control efforts differs between, as well as within nations, highlighting the relevance of local implementation challenges. The aim of this thesis was to provide missing information for childhood infectious disease management in Ghana, with a special focus on the Ashanti Region, and to present these results within the context of available public health data. A conceptual research framework (CRF) was established, which defines data needed to inform childhood infectious disease control. The CRF comprises the components (i) population, (ii) health system, (iii) health services, (iv) disease data, (v) diagnosis and treatment, and (vi) intervention and prevention. The data generated within the frame of the thesis, along with further published documents, were reviewed taking into consideration the objectives posed by the CRF. The thesis demonstrates that Ghana and the Ashanti Region have a well-planned childhood infectious disease management system. Priorities in childhood infectious disease control are identified and formulated into health policies. Various disease-specific interventions are in place to control disease transmission. Furthermore, the organisation of preventive and curative services emphasises childhood infectious disease control. However, implementation challenges constrain successful delivery of health services at all administrative levels. Shortage of medical equipment and health care personnel hamper health service provision, especially in rural areas. Generally, poor people living in remote areas are disadvantaged in several ways: they are more likely to be affected by resource constraints, they utilise health services less often, and they are at higher risk of contracting several diseases. Ghana has designed effective disease control programmes, which explicitly prioritise the poor and people living in remote areas. However, resource gaps as well as administrative barriers limit successful delivery of these measures. This thesis shows that no additional health programmes are needed to improve childhood infectious disease management, but the government needs to overcome obstacles identified at all administrative levels in order to successfully deliver established health services to the whole population.

1 Introduction

In recent decades the global mortality of children below five years of age has decreased notably. In 1990, 90 deaths per 1,000 livebirths were observed worldwide, which decreased to 43 by 2015. However, this rate still corresponds to 6 million childhood-deaths per year (United Nations, 2015a). Mortality is highest in young children and it is estimated that 40% of deaths in children below five years occur in neonates (i.e., children aged <29 days) (Liu et al., 2012). The regional distribution of childhood mortality differs tremendously. The majority of children die in sub-Saharan Africa (SSA) and in South Asia, namely 4.5 million and 3.0 million children in 2007, respectively. This corresponds to mortality rates of 147 and 78 per 1,000 livebirths, respectively. In comparison, 0.1 million children died in industrialised countries during the same year, accounting for a mortality rate of 6 per 1,000 livebirths (Loaiza et al., 2008). Furthermore, regional differences are observed in mortality reduction rates, and the decrease is among the slowest in SSA (Murray et al., 2007). The leading causes of death worldwide are pneumonia (18%), diarrhoea (15%) and malaria (8%) (Black et al., 2010). Generally, the role of non-communicable diseases (NCDs) is rising in developing countries and they are receiving more attention in current public health campaigns (Lim et al., 2012). However, in West Africa the ten leading causes contributing to years of life lost (YLL) are still dominated by communicable diseases and most deaths in children are due to infectious diseases. Estimates show that about 70% of deaths in children living in West Africa can be attributed to infectious diseases, while injuries are ranked second (Lozano et al., 2012). The aggregation of mortality and morbidity data may be misleading because the regional causes of death can differ significantly. While some SSA countries have little malaria, they are severely affected by human immunodeficiency virus (HIV) or vice versa. Using regional similarities in the causes of death to cluster countries allows most West African countries, including Ghana, to be grouped together. Here, 20–26% of deaths are attributed to diarrhoea, pneumonia, malaria and neonatal deaths (Black et al., 2003).

High childhood morbidity and mortality necessitates many low-income countries to define and implement prevention and treatment programmes. Reducing the spread of infectious diseases and improving care for affected children is high on the agenda of many nations and international organisations (Loaiza et al., 2008). For example, the German *Federal Ministry for Economic Cooperation and Development* (BMZ) prioritises health system strengthening, health care capacity building, infectious disease prevention and treatment, as well as women and child health in their global health strategy. To support partner countries, the BMZ facilitates direct and mutual cooperation with developing countries (BMZ, 2016). There is no

universal solution in how to successfully support nations to improve their population's health. Disease distribution varies widely between and within countries. Also the lethality of diseases differs regionally, because it heavily depends on available health service capacity and the population's health behaviour. Hence, the success of disease control is very context dependent and there is no generic approach for the control of infectious diseases. In order to tackle current health threats, an in-depth and holistic assessment of the local health situation is crucial to plan and implement disease control programmes (Bryce et al., 2013a).

The aim of the thesis was to generate and analyse public health-related data in order to inform the management of childhood infectious diseases in a rural Ghanaian area, located in the Ashanti Region, taking into consideration the local context. These studies were conducted within the *Research Group Infectious Disease Epidemiology* at the *Bernhard Nocht Institute for Tropical Medicine* in Hamburg, Germany. Five papers, three first-authored and two second-authored articles, form the publication skeleton of this cumulative dissertation thesis. These articles provide crucial information to guide childhood infectious disease management in a rural Ghanaian area. In order to assess newly generated as well as published data, a conceptual research framework (CRF) for childhood infectious disease management was established. The aim of this framework was to identify data needed to design disease management strategies, to systematically review and assess available data and to present this information in a structured manner to inform local disease control. Using the CRF the data generated within the thesis can be integrated into a greater public health context.

The thesis is structured as follows: (i) different control measures and programmes for childhood diseases are described, (ii) implementation challenges for disease interventions are outlined, (iii) the CRF is described and then (iv) the CRF is filled with available data and information generated within the dissertation. Finally, (v) the thesis is critically discussed and conclusions for local infectious disease management are provided. Within this document the five articles belonging to the cumulative thesis are cited using italic-bold font and within the text it is mentioned that these studies were conducted within the frame of the thesis.

2 Childhood infectious disease control

Several international, national as well as regional programmes are in place to save the lives of children and to improve their health. However, their achievements and success differ among geographical regions. Within this chapter important strategies for childhood infectious disease control implemented by *United Nations* (UN) institutions are summarised, their effectiveness is reviewed and obstacles for a successful implementation are discussed.

2.1 Strategies to reduce childhood morbidity and mortality

In September 2000 the UN's *Millennium Declaration* was signed by all member states and a series of targets was defined with the aim to reduce extreme poverty by 2015. These targets have become known as the *Millennium Development Goals* (MDGs) and they inspired and formed poverty reduction efforts as well as disease control efforts especially in the developing world. The MDGs include eight overarching aims. Reducing childhood mortality and morbidity is directly related to Goal 4 (i.e., reduce child mortality) and Goal 6 (i.e., combat HIV/acquired immune deficiency syndrome (AIDS), malaria and other diseases), although indirectly linked to all (United Nations, 2015b).

MDG 4 stipulates that the mortality rate of children under five years should be reduced by two-thirds by 2015, from levels present in 1990 (United Nations, 2013a). In this regard, the *World Health Organization* (WHO) highlights that effective and affordable interventions need to be scaled up and they promote the following four core strategies: (i) appropriate home care and timely treatment of complications for newborns; (ii) integrated management of childhood illness for all children under five years old; (iii) expanded programme on immunisation; and (iv) infant and young child feeding (WHO, 2015a). The *World Bank* underlines that nutrition, health care and infrastructure should be central to health campaigns and their child health strategy is focused on (i) strengthening national health systems; (ii) financing for children's health; and (iii) protecting the poor from ill health and unaffordable treatment (The World Bank, 2015a). Reducing child mortality is a core mission from the *United Nations International Children's Emergency Fund* (UNICEF). Their suggested strategies to reach MDG 4 are (i) providing high-impact health and nutrition interventions; (ii) improving family care practices; (iii) increasing access to improved water and sanitation; and (iv) providing a rapid response to emergencies (UNICEF, 2015).

MDG 6 addresses the management of globally widespread infectious diseases and is divided into three sub-aims, namely reducing HIV spread, reducing incidence of malaria and

other major diseases, and achieving universal access to treatment for HIV/AIDS by 2010 (United Nations, 2013b). The *Joint United Nations Programme on HIV and AIDS* (UNAIDS) was established in 1994 to coordinate the global action against HIV/AIDS and its own agenda is tailored to meet MDG 6. UNAIDS activities are manifold, however the most important strategies to control the HIV/AIDS epidemic are (i) to increase the number of people on HIV treatment, (ii) to support prevention programmes, (iii) to reduce mother-to-child transmission, (iv) to make legal resources work towards a HIV response and (v) to build a shared long-term investment to finance the HIV/AIDS response (UNAIDS, 2010). The WHO has identified six operational objectives to be supported in 2014 and 2015 to help countries meet their MDG 6 targets with respect to HIV/AIDS. In addition to UNAIDS' interventions, they call for the elimination of HIV in children, expanding access to paediatric treatment and implementing a stronger link between HIV control and related health services. With respect to malaria control, the second disease mentioned in MDG 6, WHO support the use of long-lasting insecticidal bed-nets and indoor residual spraying, increased use of diagnostics, treatment with anti-malarial medications, prevention therapies for infants, children as well as pregnant women, improved disease surveillance and measures to counter drug resistance (WHO, 2014a). Furthermore, MDG 6 is concerned with other major diseases. WHO emphasises the fight against tuberculosis (TB) infections. On that account they developed the *Directly Observed Treatment, Short Course* (DOTS) strategy, which focuses on political commitment, early case detection, standardised treatment, effective drug management and monitoring of TB programme performance (Volmink et al., 2000). Currently no vaccines are available against TB and HIV, however progress had been made in immunisation research (Rappuoli & Aderem, 2011). GlaxoSmithKline is currently applying for a licence for the recently tested RTS,S-malaria vaccine. The vaccine offers partial protection against plasmodium falciparum, the most deadly malaria parasite (RTS,S Clinical Trials Partnership, 2015). The malaria vaccine is not yet ready for large-scale roll-out, however its introduction to selected regions for further field evaluations is being considered. Due to the high death toll attributed to HIV, malaria and TB, these infections are also called "the big three". These infections receive much attention from influential funding organisations because successful control of these infections has a huge impact on morbidity and mortality reduction in both children and adults. For example, donors such as *The Bill and Melinda Gates Foundation* and *The Global Fund to Fight AIDS, TB and Malaria* primarily finance research and projects relating to the big three.

In MDG 6, the group "other diseases" is mentioned, however it is not specified which health conditions fall into this category. In recent years this group received much attention,

which led to the definition of neglected tropical diseases (NTDs), a group of communicable illnesses that mainly prevail in tropical countries and that often affect underprivileged people living in unhygienic conditions. In 2003 WHO intensified discussions on the control of NTDs and they listed 17 diseases to be prioritised: Buruli ulcer, Chagas disease, Dengue and Chikungunya, Dracunculiasis (guinea-worm disease), Echinococcosis, Endemic treponematoses (Yaws), Foodborne trematodiases, Human African trypanosomiasis (sleeping sickness), Leishmaniasis, Leprosy (Hansen disease), Lymphatic filariasis, Onchocerciasis (river blindness), Rabies, Schistosomiasis, soil-transmitted helminthiases, Taeniasis/ Cysticercosis and Trachoma (WHO, 2003). Producing robust estimates on the global distribution of NTDs is challenging due to the limited data available on these conditions. Hence, published prevalences and incidences for particular NTDs can vary greatly. Recent estimates from the *Global Burden of Disease Study* show that about 48 million disability adjusted life years (DALYs) are attributed to NTDs (Hotez et al., 2014). DALYs are a summary measure of YLL due to premature death and years lived with disability (YLD) (Murray et al., 2012a). For many NTDs the greatest part of the disease burden can be attributed to YLD because they progress chronically, impairing physical and mental development in children, and limiting work productivity in the adult population. The number of DALYs attributed to NTDs is comparable to the number attributed to TB (49 million) and it is more than half of the DALYs attributed to malaria (83 million) or HIV/AIDS (82 million) (Hotez et al., 2014). Particular NTDs have their distinct geographical distribution, but generally the highest NTD burden is observed in SSA (Hotez & Kamath, 2009; Hotez et al., 2014). Generally, children and adults are affected by NTDs but ascariasis (a soil transmitted helminth), trichuriasis, schistosomiasis and trachoma disproportionately affect children and infants (Barry et al., 2013). Currently, integrated control strategies are being promoted and applied that treat people with a combination of drugs, which target different NTDs. These so called “rapid-impact packages” are delivered as mass treatment in affected areas within which people are frequently infected with several NTDs simultaneously (Molyneux et al., 2005). However, treatment alone will not stop transmission. This will also depend heavily on the availability of improved water, sanitation, and hygiene as well as a change towards low-risk behaviour (Freeman et al., 2013).

The initiation and application of the MDGs were important steps to set the frame for poverty reduction. During the time this thesis was written the *Sustainable Development Goals* (SDGs) were adopted by the UN. They came into force in 2016 and this agenda is entitled “Transforming our world: the 2030 Agenda for Sustainable Development”. The SDGs

emphasise social and economic development, the careful use of natural resources and advocate the principle of equal opportunities and dignity for everyone. Seventeen goals were formulated and one of them (SDG 2: end hunger, achieve food security and improved nutrition and promote sustainable agriculture) has a direct link to childhood health. Many others, for example SDG 3 (ensure healthy lives and promote well-being for all at all ages) and SDG 6 (ensure access to water and sanitation for all) tackle heath related topics (United Nations, 2015c). The SDGs have been criticised for no longer containing health as a central component. Compared to the MDGs, the SDGs' health agenda has a much broader focus, with little specification on quantitative targets to be fulfilled. The future will show how the MDG-to-SDG shift will affect the development of local health policies in low income countries and the commitment of donors to invest in public health (Murray, 2015a).

2.2 Effectiveness of applied disease control

During MDG implementation and within the aftermath of the MDGs, health programmes around the globe have been evaluated to assess whether the postulated targets have been met. The 2015 UN MDG report presents detailed data on the improvements towards the formulated targets. This report shows that the global under-five mortality has nearly halved and dropped from 90 to 43 deaths per 1,000 live births between 1990 and 2015. However, this progress is below the MDG 4 target. Considering current progress, control measures would have to be applied for ten more years to achieve these targets (United Nations, 2015a). As outlined above, the two leading infectious causes of childhood morbidity and mortality are pneumonia and diarrhoea. Seventy-two per cent of pneumonia deaths and 81% of diarrhoea deaths occur in neonates and infants. The mortality from the two diseases has decreased remarkably during the last two decades, yet this success was greater for diarrhoea (Fischer Walker et al., 2013). Current intervention analyses suggest that, if global preventive and therapeutic control measures were scaled up by 80%, 95% of diarrhoea deaths and 67% of pneumonia deaths could be prevented by 2025 (Bhutta et al., 2013).

MDG 6 evaluation highlights that the defined targets were met in most countries. Globally, new HIV infections declined by about 40%, from 3.5 million to 2.1 million infections in 2000 and 2013, respectively. SSA is the most affected region, within which 1.5 million new HIV infections occurred in 2013. In SSA, HIV incidences vary significantly between countries and nearly half of the cases occur in Nigeria, South Africa and Uganda. Due to the increased use of antiretroviral therapies (ART), which are now more easily accessible in the developing world, people are able to live longer and hence HIV prevalence is rising. It is estimated that in

2013, 36% of HIV positive individuals in the developing world were on ART. However, in SSA only 22% of HIV infected individuals received ART, again highlighting the geographical differences in disease management success. For malaria, the MDG target has been achieved: the global incidence has fallen by 37% between 2010 and 2015, and the malaria mortality decreased by 58% during the same period. This success is mainly due to an increased use of anti-malarial therapies, which primarily reduced mortality in children aged below five years within the SSA region. Other successful control measures are improved access to prevention and treatment programmes, including the use of insecticide-treated bed nets (ITNs), indoor residual spraying, improved malaria diagnostics and the implementation of artemisinin-based therapies. The MDG targets are also likely to be met for TB. The global TB incidence declined to 9 million cases globally. However, the decrease is slow, on a rate of about 1.5% per year. Most success can be attributed to the above-mentioned DOTS strategy. To further reduce TB spread and disease lethality, access to treatment had to be improved in many affected regions. Further attention should be focused on HIV infected persons, who are at higher risk of contracting TB. They are often stigmatised and less able to utilise disease prevention and control programs (United Nations, 2015a).

As highlighted above, data on the global NTD situation is still scarce, which challenges the evaluation of disease control progress. However, the *Global Burden of Disease Study* presents estimates for some NTDs for 1990 and 2010, allowing an assessment of disease trends. The largest DALY decrease was recognised for ascariasis (5,877 to 1,314 DALYs), possibly due to intensified deworming activities and economic development in affected areas. Increased public health campaigns also reduced the burden of African trypanosomiasis (2,034 to 560 DALYs) and rabies (3,234 to 1,462 DALYs). In contrast, the burdens of trachoma (144 to 334 DALYs), schistosomiasis (2,125 to 3,309 DALYs), lymphatic filariasis (2,368 to 2,775 DALYs), and Dengue (712 to 825 DALYs) appeared to increase during the same period. These increases may be due to population growth, ecological factors, such as the construction of dams, and intensified surveillance leading to improved case detection (Hotez et al., 2014). Many NTDs can be controlled with a comparably low monetary effort, and cost effective interventions are available to reduce morbidity and mortality in developing countries. However, even though treatment and control strategies are available, many poor people are still affected by NTDs or are at risk of contracting these diseases (Molyneux, 2004).

2.3 Challenges to implement disease control

Even though childhood morbidity and mortality has been reduced significantly during recent decades, many children still die from preventable and treatable diseases. Hence, the question arises: why are well-studied diseases responsible for so many deaths, when substantial mortality reductions can be achieved with known intervention measures? Apparently, current implementation of disease interventions does not reach large parts of populations, including high-risk groups. Several research groups have analysed challenges to successfully implement disease interventions, which are summarised in Table 1.

In many low income countries the overall structure of the health system, which is required to plan, finance and deliver health services to the whole population, is weak. Interventions for different diseases are often stand-alone programmes, which are not integrated into the health system. Many diseases affect same population groups and coinfections are frequent. Hence, disease interventions could be applied jointly. NTD interventions, which are often underfunded, would especially benefit from an integration into national or regional disease control programmes (Hotez et al., 2006; Molyneux et al., 2009). Generally, disease programmes can be organised horizontally or vertically. Horizontal (or integrated) approaches nest disease programmes into the health system, so that established administrative and logistic structures can be used. In contrast, vertical programmes are stand-alone interventions, which are applied via an independent service pipeline (Mills, 2005). Integrated programmes benefit from existing infrastructures and are preferable if long-term interventions are implemented. In contrast vertical programmes run independently, which is costly yet provides flexibility, making them suitable for emergency responses or short-term programmes. A decision on which approach to use should be based on the country's health system structure, the health service capacity and the nature of the disease control programme (Laxminarayan et al., 2006).

In many developing countries a strong leadership for child survival is lacking. Disease programmes and their implementation are not adequately planned by governments as well as between ministries and international agencies (Gill et al., 2013). Health programmes are not ranked high enough on the political agenda and the national financial resources allocated to their control are inadequate. Also international cooperation between national health policy makers, donor institutions or academia may not be flexible enough to take country needs into account (Bryce et al., 2013c). Often clear health priorities are not identified and tailored health policies, focusing on defined health goals, are not in place. Health policy adaptation towards current disease problems, which are subject to change, is often too slow. Another obstacle is weak community participation, needed to make people responsible for their own

and for the public's health. Furthermore, lacking stakeholder involvement, an important knowledge base on local disease problems, is insufficiently incorporated (Kuruvilla et al., 2014). In cases where interventions are planned and procedures are formulated, they are often out-dated and not amended with up-to-date practice guidelines (Gill et al., 2013).

Table 1: Potential barriers and implementation challenges in childhood infectious disease control.

Barrier	Implementation challenge
Health system	• Health system structure to deliver health services is weak (Kuruvilla et al., 2014) • Interventions are disease specific and applied independently (Hotez & Molyneux, 2008; Hotez et al., 2006) • Vertical and horizontal approaches are not clearly balanced (Laxminarayan et al., 2006) • Costs of the heath system structures needed to deliver interventions not covered (Stenberg et al., 2007)
Leadership	• Child survival not high on the national agenda (Kuruvilla et al., 2014) • Funders and international academic institutions not flexible enough to work toward country needs (Gill et al., 2013) • No health policy strategies defined (Gill et al., 2013) • Inflexibility to deal with changes (Gill et al., 2013) • The public's participation is weak (Kuruvilla et al., 2014) • Low stakeholder participation in policy processes (Gill et al., 2013; Kuruvilla et al., 2014) • Poor coordination of planning and implementation actions (Bryce et al., 2013c) • Interventions are not up-to-date (Gill et al., 2013)
Financial resources	• Costs of interventions and of the delivery channel not covered (Laxminarayan et al., 2006; Stenberg et al., 2007) • Underfunded research on implementation challenges (Gill et al., 2013) • Illicit financial flows reduce resources (O'Hare et al., 2014; O'Hare & Makuta, 2015) • Resources may be blocked by bureaucratic processes (O'Hare & Makuta, 2015)
Data	• Local measurements on intervention coverage not available (Bryce et al., 2013c) • Data on intervention success not available (Bryce et al., 2013c) • Data collection is not harmonised, data is not analysed timely and results are not commutated to inform local health policy (AbouZahr et al., 2015; Gill et al., 2013) • Weak knowledge about evidence based interventions (Gill et al., 2013; Requejo et al., 2015)
Equity	• Rural areas are less connected to health facilities and health services (Kuruvilla et al., 2014; Laxminarayan et al., 2006) • The poor have lower access to health services (Barros et al., 2012; Gill et al., 2013; Requejo et al., 2015) • Gender and ethnic background can affect likelihood to use health services (Barros et al., 2012; Gill et al., 2013; Requejo et al., 2015) • Less educated people are often not aware about existing health services (Barros et al., 2012; Gill et al., 2013; Requejo et al., 2015)

Financial needs for health programmes are manifold, including the actual costs of an intervention (e.g., costs of vaccines or INTs) as well as the indirect costs for programme application (e.g., maintaining a cold-chain to stock drugs). Even though various low-cost interventions exist, which are proven to be highly effective, budget is needed to cover their application. Additionally, the whole delivery channel has to be financed, which includes a functional health system to provide interventions at household, community and facility level. For example, such costs include budgets for health care workers (HCW), training of staff, programme monitoring and evaluation, and the overall logistic infrastructure (Stenberg et al., 2007). Sometimes scarce financial resources further diminish through illicit financial flows, corruption or due to the servicing of debts (O'Hare & Makuta, 2015). Under some circumstances resources may be blocked due to ministerial overregulation or overburdened bureaucratic processes (Gill et al., 2013).

The lack of data affects planning of health programmes and their implementation. Timely data on disease occurrence should be available to plan health service provision and to recognise changes in disease occurrence (Requejo et al., 2015). Information on intervention coverage and access is often insufficient (Bryce et al., 2013c). This is needed to identify population groups which may benefit from disease programmes and those which are hard to reach with conventional measures. Furthermore, estimates on the effectiveness of interventions within the local context and on the success of different implementation strategies are needed (Bryce et al., 2013a). This includes analyses to develop evidence-based interventions for different diseases taking into account local conditions (Requejo et al., 2015). Often reliable national population data on the number of births, number of deaths as well as the causes of death are lacking. So called comprehensive civil registration and vital statistics systems would be an effective tool to deliver these data (AbouZahr et al., 2015). However, such systems are often substituted by surveys, studies or census, which do not provide continuous, timely and locally relevant data needed to inform public health policy making (Phillips et al., 2015). In cases where data is collected this is often not done in a harmonised manner and data is analysed too late, so that the current disease situation is not reflected. Finally, generated results may not be communicated to the health workforce, challenging disease responses at local level (Gill et al., 2013).

Within countries population groups benefit differently from health interventions. Within the same community under-five mortality in the poorest households is average twice as high as in children living in the wealthiest households. A similar effect is observed between rural and urban populations, where urban citizens experience lower mortality rates compared to

those living in rural areas (United Nations, 2015a). Risky health behaviour and insufficient knowledge about disease transmission are major risk factors for most infections. Again, people with lower socioeconomic status (SES) and those living in rural areas are at higher risk of contracting infections (United Nations, 2015a). Finally, the same inequality is observed in terms of health programme utilisation. However, lesser differences are observed if community-based interventions are applied, which are delivered by health workers based within villages (Barros et al., 2012; Requejo et al., 2015). In some areas gender and the individual ethnic background can further impede access to health services (Requejo et al., 2015). Education often dictates levels of health service utilisation. Thus, the provision of information, training and the application of tailored intervention tools enable people to access disease programmes, even if they are illiterate or lacking higher educational training (Laxminarayan et al., 2006).

3 Conceptual research framework

Infectious diseases are still a major cause of morbidity and mortality in many developing countries, like Ghana. Even tough disease interventions are implemented, various barriers challenge a successful delivery of these prevention and treatment measures to the population. Hence, information on the regional context is highly relevant to identify interventions needed, to plan appropriate prevention and treatment programmes and to facilitate their successful implementation. A tailored disease management approach is crucial to effectively control diseases locally (Bryce et al., 2013b).

The aim of this thesis was to fill existing information gaps in the management of childhood infectious diseases within a rural area in Ghana. The generated results as well as further available data had to be reviewed and structured according to a standard format required for disease management. For this purpose, a CRF was established to systematically identify areas within which data for childhood disease control is needed. So far no research framework for (childhood) infectious disease control in developing countries has been published. However, research frameworks exist from other public health disciplines and policy concepts for childhood infectious disease control have been published. Even though these frameworks and concepts may address other diseases, and are applied in different geographical areas and population groups, they may provide useful information to structure research on infectious disease control. Based on the identified health research frameworks, which are described briefly below, a CRF for the control of childhood infectious diseases was drafted. The CRF was used to systematically identify areas essential to successfully implement disease control interventions considering the local situation in the study area in Ghana. Finally, the health information generated within the dissertation along with other published data was used to fill the CRF.

3.1 Establishing the conceptual research framework

The health literature was reviewed to identify relevant CRFs and policy concepts for the control of infectious diseases. The search strategy applied included a review of websites from international health and development agencies, a search of electronic databases (i.e., Medline via PubMed and Google Scholar), scanning of reference lists from relevant published studies and direct contacts with technical and in-country experts. Preference was given to documents with a focus on low-income and middle-income countries. The selected documents were reviewed in order to identify research areas deemed relevant to manage childhood infectious

diseases or other health threats. These selected areas were assigned into generic research components, which finally frame the CRF on childhood infectious disease management. Below, the identified CRFs and policy concepts are described briefly and the generic research components are established and explained.

3.2 Review of frameworks and policy concepts

A well-established socio-behavioural and biomedical research framework was developed by Anderson in 1998, which he claimed to constitute a "unifying framework for all of the health sciences". Anderson highlights that the five levels - social/environment, behavioural/psychological, organ systems, cellular, and molecular level - should be integrated in interdisciplinary health research. Biomedical disciplines (e.g., immunology or microbiology) and social sciences (e.g., social epidemiology) are often seen as separate disciplines, yet research conducted in collaboration is able to address complex health issues to advance both clinical research and public health (Anderson, 1998). Rimer, amongst others, adopted Anderson's model to define research priorities in cancer control. He proposed a seven-component research strategy: (i) increasing fundamental knowledge, (ii) intervention and prevention, (iii) early detection, (iv) screening measures, (v) improve treatment decisions, (vi) proper dissemination strategies, and (vii) disease surveillance (Rimer, 2000).

A further framework to structure research on cancer is based on similar pillars and highlights the importance of operational components, like programme application and programme delivery, to guide policies and practice (Hiatt & Rimer, 1999). Furthermore, a review of cancer control frameworks from the United States of America (USA) and Canada pointed to the importance of populations, for which research is applied to and information for policymaking is needed (Best et al., 2003). The *Translational Framework for Public Health Research*, developed by Ogilvie et al., highlights four tasks to be conducted when framing translational research: elements and their link with the framework should be described, effective control measures should be identified, operational elements within the framework should be identified, and strategic areas where research should be concentrated needs to be revealed (Ogilvie et al., 2009). A review of childhood disease control programmes in developing countries underlines the importance of integrated approaches to understand disease occurrence and to manage health programmes effectively. Factors influencing disease progression (e.g., malnutrition, co-infections, or treatment effects) and disease control (e.g., health care delivery, service utilisation and health behaviour) have to be considered when designing disease control frameworks (Claeson & Waldman, 2000).

Baker et al. describe a mapping, monitoring and surveillance policy framework to eradicate particular NTDs. Identifying and describing disease distribution is important in defining the local context within which interventions have to function and to identify priority diseases and priority populations to target. Monitoring should be in place to measure the success of implemented interventions. This should be done via routine evaluation of defined indicators or, if continuous monitoring cannot be implemented, via periodic evaluations. Finally surveillance should be intensified after a disease has been successfully eliminated, in order to monitor a potential reintroduction into a population (Baker et al., 2010).

In 2011 the *Centre for Disease Control and Prevention* (CDC) in Atlanta, USA published a framework for infections disease prevention, which should guide USA policies on infectious disease control. This framework is tailored for an application in industrialised countries, however it provides aspects which can be translated to low- and middle-income countries as well. The CDC framework is divided into three elements: element one is concerned about strengthening public health fundamentals, defined as infectious disease surveillance, laboratory detection and epidemiologic investigation capacity. Element two focuses on public health interventions to reduce infectious diseases, and element three advocates policies to prevent, detect and control infectious diseases (CDC, 2011).

The *Child Health and Nutrition Research Initiative* propagates a research culture with the ultimate aim to reduce disease burden. Thus, academia should not only focus on generating new health knowledge, they also have to consider how their research results can be translated into successful and applicable public health interventions. They defined research criteria which should be considered in health research, namely (i) likelihood that an ethical answer can be produced, (ii) likelihood that research output will reduce disease burden, (iii) deliverability, affordability and sustainability of the resulting interventions, (iv) potential of an intervention to reduce disease burden, and (v) ability of interventions to improve equity within populations (Rudan et al., 2007).

The *Countdown to 2015 for Maternal, Newborn and Child Survival* (Countdown) initiative evaluated country's achievements towards the stipulated MDG 4 and MDG 5 goals. They identified areas important for successful implementation of health interventions. Countdown highlights that health targets need to be identified and proven health interventions have to be in place to reach these goals. The health system and corresponding health policies should build the basis to effectively deliver interventions to the whole population. Likewise, all population groups should have access to these interventions, regardless of wealth, gender, ethnic group and geographical location. Accurate up-to-date data should be available to make

informed decisions about priority interventions and to judge the effectiveness of measures in place (Requejo et al., 2015).

The *Systematic Rapid Assessment Toolkit* (SYSRA) provides a framework to assess a health system's structure and its capacity to successfully implement health programmes and disease interventions. It is designed to collect and analyse data on health system components, within which a health programme has to function. These components are defined as context (health system environment), the input (affected population and the infectious disease threats), the intervention (nature of health measures and services in place), mechanisms (ways the health programmes work within the population), the output (intermediate effect) and the outcome (overall effect on disease spread). SYSRA provides a framework to systematically collect health system component's information in order to analyse its capacity to deliver health interventions to the public (Hanvoravongchai et al., 2010; Krumkamp et al., 2010).

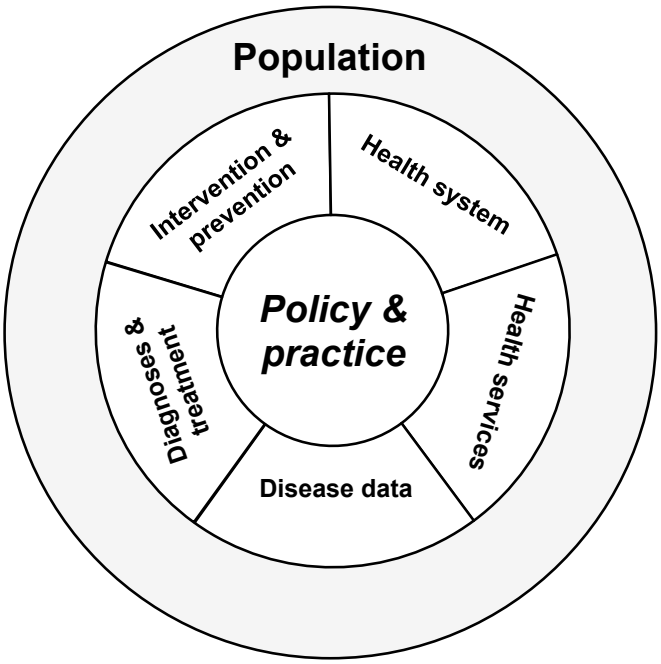

Figure 1: Graphical representation of the conceptual research framework for childhood infectious disease control

The aim of this review was to identify and evaluate published health research frameworks and health policy concepts, in order to establish a CRF on childhood infectious disease control in developing countries. Based on this review seven components were identified which form the CRF (Figure 1). These components, along with the basic research information needs, are:

1. **Population.** Information about the population to which the CRF is applied, data on the political system within which the health system is functioning.
2. **Health system.** Information on the national health system structure, on financing mechanisms and health budget generation, data on the available resources and on national early case detection and disease surveillance systems.
3. **Health services.** Overview of the health service structure, capacity to deliver prevention and treatment to individuals and health care utilisation constraints.
4. **Disease data.** Information on infectious disease threats, on high-risk populations, risk behaviour, and on geographic, demographic and socioeconomic associations.
5. **Diagnoses and treatment.** Current standard on how to diagnose and treat infectious diseases within the population.
6. **Intervention and prevention.** Measures to reduce disease occurrence and disease transmission within the population, intervention coverage and obstacles in intervention implementation.

In the literature review several research objectives were identified, which structure the CRF and which define information needs to manage childhood infectious diseases within the focus population. They are summarised in Table 2.

Table 2: Components of the conceptual research framework for childhood infectious disease management along with the corresponding information needs.

Component	Information needed
Population	• What is the target population to which the framework has to be applied (Best et al., 2003; Krumkamp et al., 2010; Rimer, 2000)? • What is the social and political situation within which the target population live (Best et al., 2003; Krumkamp et al., 2010; Rimer, 2000)?
Health system	• How is legislative power distributed in the health system (Krumkamp et al., 2010)? • How is the health system funded (Krumkamp et al., 2010)? • Are public health policy priorities defined (Claeson & Waldman, 2000; Ogilvie et al., 2009; Requejo et al., 2015)? • Are health policies ranked high on the political agenda (Ogilvie et al., 2009)? • Are reliable up-to-date population data available (Krumkamp et al., 2010; Rimer, 2000)? • Is comprehensive disease surveillance in place and is the generated data available (Baker et al., 2010; CDC, 2011; Hiatt & Rimer, 1999; Rimer, 2000)?

Table 2 (continued)

Health services	• Is the health service structure up-to-date and are resources sufficient to reach the population (Claeson & Waldman, 2000; Krumkamp et al., 2010)? • Are health services delivered to all people (Requejo et al., 2015; Rudan et al., 2007)? • Are health services utilised equally (Claeson & Waldman, 2000; Hiatt & Rimer, 1999; Requejo et al., 2015)?
Disease data	• What is the prevalence, incidence, disease lethality and mortality of relevant diseases in the population (Baker et al., 2010; Hiatt & Rimer, 1999; Krumkamp et al., 2010)? • What is the geographical distribution of diseases (Baker et al., 2010)? • What are high-risk populations (Anderson, 1998; Requejo et al., 2015)? • What are social, behavioural and environmental risk factors to contract diseases (Anderson, 1998; Hiatt & Rimer, 1999; Requejo et al., 2015; Rimer, 2000) ? • Are data on microbial drug resistances available (CDC, 2011)?
Diagnoses and treatment	• Are treatable diseases diagnosed early (Claeson & Waldman, 2000; Rimer, 2000)? • What is the laboratory capacity to diagnose relevant pathogens (CDC, 2011; Krumkamp et al., 2010; Rimer, 2000)? • Is treatment available to all sick people (Rimer, 2000)? • Are clinical guidelines published and applied (CDC, 2011)?
Intervention and prevention	• What are appropriate effective interventions and are they well evaluated (Baker et al., 2010; CDC, 2011; Hiatt & Rimer, 1999; Ogilvie et al., 2009; Requejo et al., 2015)? • Which interventions are in place (Requejo et al., 2015; Rudan et al., 2007)? • Are applied interventions efficient and cost-effective (Claeson & Waldman, 2000; Requejo et al., 2015; Rimer, 2000)? • Do ethnic background, gender, socioeconomic and educational aspects affect intervention coverage (Claeson & Waldman, 2000; Hiatt & Rimer, 1999; Requejo et al., 2015; Rimer, 2000; Rudan et al., 2007)? • Are applied interventions sustainable (Baker et al., 2010; Requejo et al., 2015; Rimer, 2000)? • What are functional channels to communicate and promote health behaviour (Anderson, 1998; CDC, 2011; Rimer, 2000)?

4 Management of childhood infectious diseases in rural Ghana

The CRF-guided review will provide necessary data to inform management of childhood infectious diseases, taking into consideration various public health aspects of the study population. The data available within the different CRF components may be of different quality, depending on current evidence available in the respective research fields. Below, available data and data generated within the thesis is allocated to the components of the established CRF.

4.1 Population

The studies conducted within the frame of the thesis were carried out in Ghana, a SSA-country situated in western Africa (Figure 2). The total population in 2015 was estimated at 26.328.000, with 38% of these inhabitants aged below 15 years and 14% below 5 years. The average live expectancy at birth in 2015 was 63 years for males and 68 for females (CIA, 2015). Ghana gained independence in 1957, the first SSA-country in colonial Africa to do so, and now has a constitutional democratic system (CIA, 2015). Ghana's judiciary has proven to be independent and is being generally trusted by the Ghanaian population. Ghana is classified as a lower-middle income country and reached the MDG 1A (i.e., halving the poverty rate). The proportion of people living in extreme poverty (i.e., below 1.25$ a day) was reduced from 54% in 1991 to 24% in 2013 (The World Bank, 2015b). Generally, Ghana's economy strengthened during the last decade, however it is currently challenged by national account deficits and a depreciating currency. The country still partially depends on international economic and technical support. The energy infrastructure did not grow at the same speed as the economy, which leads to frequent power cuts and which challenge further development (CIA, 2015). It is estimated that 67% of women and 71% of men of working-age are in employment, of which 80% work in the informal sector (Oduro, 2009). In 2014 Ghana's corruption perceptions index, calculated by *Transparency International*, was 48. The index ranges from 0 to 100, where 0 expresses a highly corrupt and 100 a corruption clean country. Ghana ranks worldwide 61 out of 175 countries, however it is the third least corrupted country within Africa (Transparency International, 2015). Ghana's Gini index, which measures income inequalities, was 42 in 2012/2013. The Gini index ranges from 0 to 100, where 0 expresses total income equality and 100 total inequality. Ghana ranked 52 out of 144 measured countries worldwide (CIA, 2015).

The area where the thesis' studies were conducted is located within the Ashanti Region, the largest region in Ghana, which had 4,780,280 inhabitants in 2010. The urban population in the region is steadily increasing and was estimated to be 61% of the total population in 2010. The capital of Ashanti Region is Kumasi, with 1,723,000 inhabitants. Ashanti Region is divided into 27 districts and municipalities (Ghanadistricts, 2015).

(source: www.un.org/Depts/Cartographic/english)

Figure 2: Map of Ghana (amended) showing the study hospitals located within the Ashanti Region.

The thesis' analyses were conducted at the Agogo Presbyterian Hospital (APH; www.agogopresbyhospital.org) and the St. Michael Hospital (SMH; no homepage available) in Pramso as well as within the corresponding hospital catchment areas. Agogo has a population of 37,000 inhabitants and is located 60 km east of Kumasi, and Pramso, with a population of 3,300 inhabitants, is located 20 km southeast of Kumasi (Figure 2). Both areas have a rural character with simple housing conditions and basic village infrastructures. The climate is tropical with two rainy seasons per year. The area is mainly covered by secondary rain forest and cultivated land. APH is the largest hospital in the Asante Akim North District, with capacity for 250 inpatients. It has specialised children's and adult's outpatient

departments (OPDs) and several specialised medical wards. SMH is the biggest health facility in the Bosomtwe District with a general OPD and about 90 patient beds.

Box 1: Population

- Ghana is a multi-party democratic low-middle income country within SSA.
- The studies of the thesis are conducted within the Ashanti Region, the largest region of Ghana.
- Study villages have a rural character, with simple housing conditions and limited urban infrastructures.

4.2 Health system

Several health policy reforms have taken place since Ghana gained independence in 1957. Because of worsening economic conditions the Ghanaian government initiated larger health sector reforms in 1980, which were guided by the *World Bank* and the *International Monetary Fund* (McIntyre et al., 2008). The current health system is primarily based on the three administrative pillars of policy-making, under *Ministry of Health* (MoH) control, health service delivery, under *Ghana Health Service* (GHS) control, and health finances, under the control of the *National Health Insurance Authority* (NHIA) (The World Bank, 2013). Health service provision is decentralised and preventive and curative measures are planned and managed at regional level. The *Community-based Health Planning and Services* authorities were established within the regions to administrate health service provision. However, the management of human resources and partly of health funds is still with the MoH and the GHS at national level. So far, decentralisation is not introduced in all administrative tasks within the health sector. The different political acts, which regulate health-policy among the legislative levels, are in some ways contradictory and a clear administrative framework, guiding health policy implementation, is not established yet. This hampers efficient health service provision, targeted health fund allocation and institutional legal empowerment (Kwamie et al., 2016; The World Bank, 2013).

In general, health policy is high on Ghana's political agenda. Ghana promotes different health initiatives, like universal health care programmes based on the *Ouagadougou Declaration for Primary Health Care and Health Systems* or the *Community Health Nurses Programme*, where HCW serve as an early contact point to provide care in remote areas. In cooperation with the WHO, a health agenda was developed which prioritises MDG-related interventions, interventions against communicable and NCDs, health system strengthening programmes, and actions on social determinates of health (WHO, 2014b). Furthermore, the

GHS has different national disease control programmes, like the *National Malaria Control Programme*, the *Guinea Worm Eradication Programme*, the *National Yaws Elimination Programme* and the *Non Communicable Disease Control Programme* (GHS, 2014).

Ghana's MoH launched the *National Health Insurance Scheme* (NHIS) in 2004, which pays for hospital visits, outpatient consultations, basic laboratory procedures and medications at accredited health facilities. Before the NHIS was established different heath-funding regulations were in place, ranging from free medical services to direct out-of-pocket payments. The drugs and the disease treatment covered by the NHIS are restricted. Basic treatment is covered by the insurance, while procedures like elective surgery, transplant medication and surgery, or haemodialysis are excluded (Drislane et al., 2014). People employed in the formal work sector provide their contribution via social security payroll and people working in the informal sector have to pay their direct mutual health-insurance fee of 5$ up to 35$ per year, depending on their individual SES. Reduced fees for children are available if one parent is already enrolled into the NHIS. Registration is mandatory for the formal sector, however voluntary for workers within the informal sector. Even though only NHIS members can benefit from free services, about 70% of the NHIS expenditures are funded from value-added tax (VAT) contribution. Generally, the establishment of the NHIS has strengthened the quality of the health service sector, however, it is estimated that only about 40% of the population were enrolled in the NHIS in 2009. Evaluations showed that people with higher SES benefit especially from the NHIS (Mills et al., 2012). People with a lower SES and families with several members can be heavily taxed by NHIS fees (Kusi et al., 2015). About 45% of health expenditures in Ghana are still covered by out-of-pocket payments (McIntyre et al., 2008).

In 1998 the *National Surveillance Unit* was established, which coordinates disease surveillance, based on WHO's *Integrated Disease Surveillance and Response* (IDSR) strategy (WHO, 2010a). IDSR aims to integrate existing disease surveillance systems and to merge collected data from different sources for public health action. Thereby, diseases of national and international concern are considered. Ghana's disease surveillance system is based on district health information units. Outpatient- and inpatient clinics, and hospitals are preparing surveillance reports. These reports are collected at district health directorates, who forward data to their regional officers. They finally submit reports to the central health information management located at national level (Adokiya et al., 2015a). Twenty-three priority diseases and health conditions have to be reported immediately, weekly or monthly, according to their defined reporting mode. The eight diseases, which have to be reported immediately are

cholera, meningitis, yellow fever, measles, viral haemorrhagic fever, poliomyelitis, dracunculiasis (guinea worm disease), and neonatal tetanus (MoH, 2002). Surveillance data can be submitted via paper form, electronically, by phone or text messages. Electronically entered data is immediately available to all users. However, so far this reporting procedure is only used by some regional hospitals. Evaluations of the national surveillance system identified obstacles at all administrative levels involved: within the health facilities training and supervision is low. Specimens are often not collected and stored correctly and participating laboratories are ill equipped, which hampers laboratory identification or confirmation. The different reporting formats are not properly linked, which leads to missing data and challenges data validation. In many health care facilities, surveillance is not a priority for HCW or facility administration, which limits cooperation with the surveillance units (Adokiya et al., 2015a; Adokiya et al., 2015b).

Box 2: Health system

- Ghana's health system is decentralised and health services are managed at regional levels
- In general health policy is high on the political agenda.
- A national health insurance system was established, which improved access to health services, however especially the poor are less frequently enrolled.
- A comprehensive surveillance system is established, faceing problems with data completeness.

4.3 Health services

Health services are provided by governmental clinics and hospitals (including university hospitals), missionary clinics and hospitals, mainly run by the *Christian Health Association of Ghana* (CHAG), as well as some private health facilities. Generally, clinics provide basic treatment and prescribe medications at defined opening hours, and at hospitals admitted patients are treated while physicians and other health care personnel are continuously available (Drislane et al., 2014). Ghana's official health service strategy stipulates that every region (in total 10) should have a regional hospital and every district (in total 216) should have a district hospital. Health clinics are important first level of care providers at sub-district level, which may refer patients to higher-level health facilities at district or regional levels. Regional hospitals are better equipped and able to provide specialised care. Also ambulance services are only available at some regional hospitals (The World Bank, 2013). Because people living in rural areas have limited access to formal health services, the *Community-based Health Planning and Service* (CHPS) system was established in 1990. This has enabled *Community Health Workers* to provide preventive and basic curative care within the

community, especially to pregnant women, young mothers, neonates, infants and children. They assist *Public Health Nurses* who are registered nurses with advanced qualifications. Both are in close contact with the rural population and they provide basic health services at people's homes (Sacks et al., 2015). Furthermore, pharmacies are a frequently used facility, where sick individuals can get medical treatment. Pharmacies must have a qualified pharmacist and they are not allowed to sell prescription drugs. Often traditional healers are the first point of contact before patients seek professional care at health care facilities. Local healers or village herbalists practice traditional medicine and they apply herbs or use spiritual healing methods. There is no regulation on the prescription of traditional drugs and this is a parallel health sector without any official quality control mechanisms (The World Bank, 2013).

Even though health service delivery has improved in recent decades, the resources available are still constrained. Ghana's medical system is heavily concentrated on the two largest cities Accra and Kumasi, whereas the rural areas are often underserved. Health centres and health clinics are important first level of care providers at sub-district level. However, the number of these centres is too few to meet demand. District hospitals are available in 42% of the districts, despite the national strategy recommending at least one such hospital per district. Their distribution is not coordinated, hence several districts lack appropriate medical care (The World Bank, 2013). Access to district or regional hospitals is further limited by low numbers of ambulances, lack of public transportation and long travel times, factors which particularly affect the poor living in rural areas (Atuoye et al., 2015). University hospitals are well equipped, however only available in the large cities. Private clinics often have advanced technical equipment, but only wealthier people can afford their services. There are relatively few specialised physicians in Ghana and specialised medical training is only provided abroad (Drislane et al., 2014). Medical universities are available in four cities, namely Accra, Kumasi, Cape Coast and Tamale. Ghana has about 1,400 physicians, but this number remains relatively stable because many trained medical doctors emigrate. It is difficult to motivate physicians to work in the rural areas, where the demand is greatest (Drislane et al., 2014). CHAG provides health services especially in rural areas, which are underserved by governmental health care providers (The World Bank, 2013). CHPS provides crucial health services to the rural population, but again evaluations show that employed health care personnel and available resources are constrained (Sacks et al., 2015).

Apart from health service delivery constraints, understanding health service utilisation behaviour is important to efficiently provide prevention and treatment measures to the

population. In the frame of the thesis we conducted a study within the APH catchment area to identify factors influencing health service utilisation in children with moderate or severe fever and moderate or severe diarrhoea. For all conditions, increased travel distance reduced the likelihood of attending the hospital while being enrolled in the NHIS increased the likelihood of attendance. SES had no additional influence on health care utilisation if modelled together with travel distance and NHIS enrolment (***Krumkamp et al., 2013***). These results are in line with other studies conducted in rural Ghana. They highlight that lower travel time and being health insured are the most important factors determining whether people seek formal health care. Non-insured individuals are more likely to use informal health services, like drug stores or unlicensed drug peddlers (Fenny et al., 2015).

Box 3: Health services

- Health services are provided at clinics and hospitals at regional and district level, and community health nurses deliver treatment within remote villages.
- Staff shortage and resource constrains are observed at many health facilities, particularly in rural areas.
- Travel distance and lacking health insurance are main barriers to seek professional health care.

4.4 Disease data

Within Ghana the major infectious causes for hospital admissions in children below five years are malaria (58%), diarrhoeal diseases (5%) and pneumonia (3%). The major causes of death within this group are malaria (20%), HIV/AIDS-related conditions (5%), pneumonia (5%), septicaemia (3%) and diarrhoeal disease (3%) (GHS, 2009). This data is in line with results from a reanalysis of verbal autopsy data (Liu et al., 2015).

Malaria is the most frequent infectious disease in Ghana and the whole country is classified as a high transmission area. The predominant species is *Plasmodium falciparum*. The estimated malaria incidence for all ages was 27,201 per 100,000 and the malaria death rate was 67 per 100,000 in 2012 (UNSTATS, 2015). We showed that *falciparum* malaria not only causes severe acute symptoms, it is also associated with long-term childhood growth deficits such as stunting (Kang et al., 2013). For the Ashanti Region the prevalence of Malaria in 2008 was 19,188 per 100,000 (GHS, 2009), which was below the national average of 21,376 per 100,000 (GHS, 2009). Furthermore, at our study hospitals, malaria is the predominant infection in febrile children. At APH (recruitment at the ward) 37% and at SMH (recruitment at the OPD) 38% of children below 15 years were infected with *P. falciparum* parasites (***Krumkamp et al., 2016***). Studies conducted within the study area show that the malaria

prevalence is very heterogeneous (Ehlkes et al., 2014). The risk of contracting malaria varies even between close geographical regions and between villages with different sizes. Higher population density and a more central location within a village are associated with reduced likelihood of malaria infection (Kreuels et al., 2008). Also individuals living in rural areas are at higher risk of contracting malaria compared to those in urban areas (Frank et al., 2016). Individual risk factors, similar to lower SES and young age, additionally increase the risk of contracting malaria (Sarpong et al., 2015).

Acute respiratory tract infections (ARTI), such as pneumonia, are the leading single cause of death in infants and children globally as well as on the African continent (Liu et al., 2012). In Ghana, ARTI is the second cause of death in children, after malaria (GHS, 2009). However, sufficient data on the distribution of ARTI and on the causes of respiratory tract infection is scarce. A meta-analysis estimated an incidence of 0.21–0.30 pneumonia episodes per child-year in Ghana in 2000. In comparison, for Africa the incidence is estimated to be 0.33 episodes per child-year. In Africa 7 to 13% of the pneumonia episodes required hospital treatment and the interquartile range of fatality rates was 1.3–2.6%. Treatment success is highly dependent on health seeking behaviour and on the quality of medical care provided. Common risk factors for ARTI are lack of exclusive breastfeeding, malnutrition, low birth weight and indoor and outdoor air pollution (Rudan et al., 2008). Another important respiratory tract infection is pulmonary TB. The incidence of TB in Ghana, based on the number of new and relapsed cases per year, decreased from 178 per 100,000 in 2011 to 165 per 100.000 in 2014 (UNSTATS, 2015). Figures on paediatric TB in Ghana are not published, but about 10% of the global TB morbidity and mortality can be attributed to children below the age of 15 years (WHO, 2015b). Within the study area 14.7% of TB strains are mono-drug resistant and 8.7% are multi-drug resistant, which threatens success of local treatment programmes (Owusu-Dabo et al., 2006).

Diarrhoea is the second leading cause of childhood mortality globally and it is estimated that 18% of the deaths in children below five years of age were attributed to diarrhoeal diseases in 2004 (Boschi-Pinto et al., 2008). Generally, regional data on the distribution of gastrointestinal infections are scarce and also for Ghana no regional frequency data on diarrhoeal disease were found. However, as outlined above diarrhoeal disease is one of the main reasons for hospitalisation and childhood death in Ghana. In the frame of the thesis we conducted a study at the OPD at APH to estimate the association of predominant gastrointestinal pathogens with diarrhoeal diseases and the corresponding attributable fractions. The highest risk for diarrhoeal disease was observed for rotavirus followed by

Cryptosporidium parvum/*hominis*, norovirus and *Salmonella enterica* and *Shigella* spp./Enteroinvasive Escherichia coli (EIEC) infection. The highest attributable fraction, which is the proportion of diarrheal disease within the study group, which can be attributed to a pathogen, was observed for rotavirus, *Shigella* spp./EIEC, norovirus and *Cryptosporidium parvum*/*hominis* infection. All associations were age dependent. For most infections, the risk for diarrhoeal disease as well as the risk of infection was highest in infants and young children (***Krumkamp et al., 2015***). Studies show that safe water, adequate sanitation facilities and hygienic behaviour are core factors in reducing the risk of diarrhoea (Cairncross et al., 2010). In the frame of the thesis, we assessed the quality of water taken from dug wells, a common drinking water source within the study area. In one village, sixteen wells were repeatedly sampled throughout one year. The study showed that nearly all wells (99%) were contaminated with faecal indicator bacteria, posing a potential health risk for the local population. *S. enterica* isolates were found in 7% of the water samples. However, only uncommon serovars were observed, which are rarely seen in humans, but which are more often found in reptiles or poultry (***Dekker et al., 2015***).

Another important cause of childhood mortality Ghana is septicaemia. Twenty per cent of the blood samples collected from febrile children between 2007 and 2009 at APH's children's ward were culture positive. The most frequently detected isolates were nontyphoidal *salmonellae* (NTS) (53.%), *Staphylococcus aureus* (13%), *Streptococcus pneumoniae* (9%) and *S.* Typhi (7%) (Nielsen et al., 2012). At SMH, 3% of the children who attended the OPD with fever were blood culture positive and, similar to APH, the main isolates detected were NTS (33%), *S.* Typhi (25%) and *Streptococcus pneumoniae* (21%) (***Sothmann et al., 2015***). These studies highlight the predominance of NTS and *S.* Typhi infection in that study region, which is in line with further studies conducted within Africa (Elizabeth et al., 2010). Reported case fatality rates for children hospitalised with NTS or with *S.* Typhi ranged between 20% and 27%. Generally, *S.* Typhi only causes systemic infections, while NTS causes both diarrhoeal and systemic disease. Information on the environmental reservoirs of *Salmonella* spp. are limited. Although human-to-human infection is the predominant transmission route, contaminated food and drinking water, and zoonotic transmission were also reported. Furthermore, malaria, malnutrition, SES and living conditions are reported risk factors for *Salmonella* spp. as well as for other bloodstream infections (Feasey, Dougan, Kingsley, Heyderman, & Gordon, 2012; Morpeth, Ramadhani, & John, 2009). Three studies conducted in the frame of the thesis investigated risk factors for bloodstream infections and septicaemia. The abovementioned study on drinking water from dug wells identified NTS isolates in water

samples, however, these may play only a minor role in human NTS infections (***Dekker et al., 2015***). Studies show that current malaria parasiteamia increases the risk of invasive NTS infection. Quantifying this association in hospital studies is prone to selection bias. Many health conditions are mutually exclusive in hospital attendees because the admission disease reduces the likelihood of an alternative disease (so called Berkson's Bias). We used a sampling approach, where only children with bacteraemia were selected, to account for that bias. Odds ratios (ORs) for the association between malaria and invasive NTS ranged between 2.4 and 3.2. These values were derived using simple bias adjustment, hence no confidence intervals (CIs) are available. We estimated that about 13% (5%–20%) of the NTS infections within the study group could be attributed to malaria (data not shown in the article) (***Krumkamp et al., 2016***). Another relevant risk factor for bloodstream infections is individual living conditions. We studied the effect of urbanicity and SES on contracting bloodstream infection. We established an urbanicity score, which quantified urban development of villages located within the SMH catchment area. The score was based on eight components, namely population size, economic activity, education, health services, transportation, services, sanitation, and housing. Also a SES-score was established via principal component analysis using individual assets data. For the study both scores were categorised into four groups. Multivariate analysis showed a preventive effect for both higher urbanicity levels (OR=0.8; 95%-CI: 0.7–1.0) and higher SES (OR=0.8; 95%-CI: 0.6–0.9). These effects were stronger if only NTS bacteraemia was considered (***Sothmann et al., 2015***).

Over recent decades HIV became an important cause of childhood mortality and morbidity, especially in SSA. Ghana has a comparably low disease burden and the number of new HIV infections has decreased during the last few years. In 2013 the national HIV prevalence among the adult population was 1.3%. The prevalence within the Ashanti Region was with 3.2% the second highest among the Ghanaian regions (Ghana AIDS Commission, 2013). The estimated number of newly HIV infected children below five years was 850 in 2012. Infections in children are mainly due to mother-to-child transmission (MTCT), which is transmission during pregnancy, delivery or breastfeeding (UNAIDS, 2015a). The national HIV prevalence in pregnant women attending antenatal care clinics was 1.9% in 2013 (Ghana AIDS Commission, 2013).

Data on NTDs in the study area is scarce. However, neglected tropical diseases affect all regions in Ghana. A list of prevalent NTDs, including a medical summary, is provided in Table 3. In the Ashanti Region Onchocerciasis, schistosomiasis, soil-transmitted helminths (especially hookworm infection) are endemic (GHS, 2014; WHO, 2010b). Furthermore,

Buruli ulcer causes a significant health problem, with an estimated prevalence of 30.8 per 100,000 in 2000. Generally, all Ghanaian regions report Buruli ulcer, but Ashanti Region had the second highest prevalence in Ghana. Buruli ulcer affects all age groups but is most frequently observed in children below 15 years (Amofah et al., 2002). The mode of transmission is still unclear but more infections are observed in areas near rivers (Raghunathan et al., 2005).

Table 3: List of neglected tropical diseases present in Ghana, the causative agents, the major clinical manifestations, treatment measures and control strategies. Summarised from WHO's web site (WHO, 2015c).

Disease / Pathogen	Medical summary
Buruli ulcer *Mycobacterium ulcerans (bacterium)*	**Transmission:** still unclear **Presentation:** *M. ulcerans* produces a toxin, which destroys tissue; mainly chronic debilitating ulcer, sometimes affecting bones **Treatment:** can be treated with antibiotics **Control:** lacking knowledge base to design interventions
Lymphatic filariasis *Wuchereria bancrofti, Brugia malayi* and *Brugia timori* (helminth)	**Transmission:** larvae transmitted via mosquito, nests into human lymphatic system and develop to worms **Presentation:** causes painful lymphoedema of the limbs; superinfection results in Elephantiasis, often leads to kidney damage **Treatment:** single dose of albendazole and diethylcarbamazine citrate **Control:** vector control
Onchocerciasis (river blindness) *Onchocerca volvulus* (parasite)	**Transmission:** via the bite of black flies **Presentation:** skin and eye disease, leads to disfiguring skin disease and/or visual loss **Treatment:** treatable with ivermectin **Control:** vector control
Schistosomiasis Schistosoma spp. (helminth)	**Transmission:** contact with fresh water infested with larvae **Presentation:** species cause urinary or intestinal schistosomiasis, anaemia, stunting in children, reduces ability to work, chronic complications can be fatal **Treatment:** treatable with praziquantel **Control:** mass treatment
Soil-transmitted helminthiases group of helmiths, e.g., roundworm or hookworms	**Transmission:** species dependent, can be faecal-oral or though skin contact **Presentation:** impaired nutrition status, physical and psychological development; concomitant infections likely **Treatment:** most helmiths infections within this group are treatable **Control:** periodic treatment and improved hygiene and sanitation
Trachoma *Chlamydia trachomatis* (bacteria)	**Transmission:** person-to-person contact or via flies **Presentation:** ocular infection, repeated episodes can damage eye's cornea and lead to blindness **Treatment:** antibiotic treatment **Control:** improved hygiene and sanitation

Box 4: Disease Data

- Malaria is the predominant childhood infections disease, with the highest disease mortality and morbidity in children.
- Diarrhoeal disease and acute respiratory tract infections are responsible for a large number of hospital admissions.
- NTDs are prevalent and Buruli ulcer causes a high NTD burden.

4.5 Diagnoses and treatment

Diagnoses and treatment of infectious diseases in Ghana are challenged by significant capacity constraints. Several health facilities lack the ability to provide standard services. In particular health facilities at district level are poorly equipped and they often lack basic medical devices, like filled oxygen cylinders, medical autoclaves as well as laboratories, pharmacies or operating rooms (The World Bank, 2013). The lack of laboratory facilities challenges the diagnoses of many bloodstream infections. However, these infections can often not be differentiated on the bases of their clinical presentation. For example, differentiation between bacteraemia and malaria without laboratory diagnostics is often not possible. An evaluation showed that only 10% of febrile children were tested for malaria at hospitals or clinics in the Ashanti Region. Rapid diagnostic tests for malaria, particularly useful in settings lacking laboratory facilities, are rarely available at health facilities (Webster et al., 2014). Hence, mistreatment and an overuse of antibiotics are observed, which reduces treatment success and which promotes drug resistance. For many infections, resistance to first line antibiotics has already been observed (Feasey et al., 2012; Morpeth et al., 2009). For example, within the study area multidrug resistance, defined as resistance against amoxicillin, chloramphenicol and cotrimoxazole, was observed 77% oft invasive NTS isolates (Nielsen et al., 2012). NTS resistance to ciprofloxacin, considered second line antibiotic in the region, is observed for some serotypes already, which could lead to serious treatment failures (Eibach et al., 2016). Fortunately, in Ghana resistances to artemisinin-based combination therapy, the first line therapy for uncomplicated malaria, is not reported so far (Quashie et al., 2007).

Generally, the number of hospital beds increased during the last decades. However, it did not meet population growth rates and the ratio of hospital beds per person actually decreased. Furthermore, due to successful health campaigns, health facility attendance increased in the last years, which additionally burdens available health service capacity. Staff shortages are reported at all levels, but again facilities at provincial level are heavily taxed by the lack of trained personnel. Since district hospitals are not appropriately equipped to deliver advanced

treatment, emergency-cases have to be referred to regional or teaching hospitals (The World Bank, 2013).

Treatment guidelines exist for many health conditions however, evaluations showed that they are not strictly followed and antibiotic misuse is often observed. Workload constraints are often reported as a reason for non-adherence to clinical guidelines and as the main reason why staff are not able to complete medical examinations (Febir et al., 2015). In comparison to regional hospitals, clinical guidelines are less strictly followed at district clinics and patients with a lower SES tend to receive less comprehensive medical care (The World Bank, 2013).

Box 5: Diagnoses and treatment

- Health service capacity gaps challenge diagnoses and treatment especially in rural areas and at district health facilities.
- Due to limited laboratory capacity, diagnosis in fever patients is often lacking, which leads to misuse of antibiotics. Antibiotic resistances are already observed against some isolates.
- Clinical guidelines exist but they are often not followed due to time constrains.

4.6 Intervention and prevention

Ghana runs several malaria campaigns, which are coordinated under the *National Malaria Control Programme* (NMCP). NMCP cooperates with different international health initiatives, such as the USA's *President's Malaria Initiative* (USAID, 2015) or UNICEF (UNICEF, 2007). Different proven preventive and therapeutic interventions, with a special focus on malaria in children and pregnant women, are in place. ITN are provided free of charge and each household is supposed to have one. The current ITN-strategy focuses on maintaining ownership through continuous distribution at schools and health facilities. In some areas, like in the Ashanti Region, indoor-residual spraying is offered to reduce mosquito presence within houses. Intermittent preventive treatment of malaria in pregnant women is provided to reduce the risk of malaria during pregnancy, which can cause serious complications in women, the unborn or the newborn child. Furthermore, intermittent preventive treatment in infants (IPTI) is applied to reduce malaria infection and anaemia in young children. Health behaviour programmes promote the use of ITN and motivate malaria related care seeking behaviour (GHS, 2015). The recently tested RTS,S malaria vaccine, which provides partial immunity in infants and children, could be an additional control strategy (Galactionova et al., 2015), however the official international vaccination strategy is still under discussion.

Water, sanitation and hygiene are key factors to reduce the risk of gastrointestinal infections and diarrhoeal disease. Rural areas, in particular, have a high dependency on unsafe water and lack access to improved sanitation facilities. Ghana's *Medium Term National Development Policy Framework*, which outlines current national development policies, summarises programmes to increase the proportion of the population with access to safe drinking water and sanitation. A national project also promotes the practice of key hygienic behaviours, including hand washing with soap, safe excreta disposal and safe storage of dinking water (NPC, 2014). The abovementioned dug-well study provides important data on how to improve drinking water in rural areas, where dug wells are a common water supply. The study highlights that the presence of a frame around the well reduces the likelihood of water contamination while, during the rainy season, well water has a higher chance of containing *Salmonellae* isolates. Such information is important to guide well construction and the maintenance of sufficient drinking water quality (***Dekker et al., 2015***). Ghana is committed to WHO's *Expanded Programme on Immunization* and several vaccine programmes are in place. In 2012 a vaccine against rotavirus (Gavi, 2012), the most important cause of diarrhoeal disease in the study area, was introduced (***Krumkamp et al., 2015***). Ghana was one of the first African countries that offered rotavirus vaccine. No official numbers on rotavirus vaccine coverage within the Ashanti Region are published. However, within a recent study, conducted with fever patients admitted to APH's paediatric ward, 44% of the children were vaccinated against rotavirus (data not published). A first evaluation study showed that after rotavirus vaccine introduction hospital admissions with severe diarrhoea could be reduced (Enweronu-Laryea et al., 2014).

HIV in children is mainly due to MTCT. In the absence of any intervention, transmission rates range from 15 to 45%. However, with effective control measures these rates can be reduced down to 5% (WHO, 2016). Ghana is one of 37 countries which are committed to the UNAIDS' *Global Plan* to eliminate new HIV infections in children and keep their mothers alive. Preventive MTCT measures include lifelong antiretroviral therapy for all pregnant and breastfeeding woman, initiation of preventive antiviral treatment for newborns as soon after birth as possible and early infant diagnosis via an HIV test at week 4 to 6 after birth. All pregnant women who register for antenatal services are provided with free HIV testing (UNAIDS, 2011). Within the Ashanti Region the proportion of women not tested for HIV, as part of a voluntary service, increased from 12% in 2011 to 19% in 2013. Those with a higher HIV risk are likely to be more hesitant to undergo testing. The proportion of HIV positive women decreased from 2.8% in 2011 to 1.4% in 2013 and there was a steep decline in women

not initiated on HIV treatment during the same period (68% to 19%, respectively) (Dako-Gyeke et al., 2016). Nationally, it is estimated that the rate of MTCT decreased from 32% in 2009 to 21% in 2013 (UNAIDS, 2015b).

Vaccines against the two leading causes of childhood pneumonia, *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* (pneumococcus), are recommended by the WHO (Madhi et al, 2008) and both are included in Ghana's national vaccination scheme. The national coverage for full Hib immunisation (three courses) is estimated to be 98% (WHO, 2014c). At our study site the full Hib vaccination coverage in children below 16 years was at 93% somewhat lower (data not published). Pneumococcus vaccine was introduced in 2012 along with the rotavirus vaccine (Gavi, 2012). No official figures on the immunisation coverage are published so far, however at our study site 51% of the children were vaccinated against pneumococcus (data not published). Generally, immunisation is an important pillar of Ghana's childhood infectious diseases management strategy. An evaluation showed high vaccination coverage among population groups. However, shortcomings were observed in the timeliness of childhood vaccination. Lower SES, lower education and rural residence are associated with delayed vaccine uptake (Gram et al., 2014)

The *Ghana NTD Control Programme* was launched in 2007 and it is managed by the GHS. The programme's aim is to harmonise established disease eradication programmes to efficiently control NTDs (GHS, 2014). Six diseases are managed within the programme using annual or bi-annual mass drug administration measures, education campaigns and enhanced disease surveillance. Targeted diseases are Buruli ulcer, Lymphatic filariasis, Onchocerciasis, Trachoma, Schistosomiasis and soil transmitted helminthiasis (GHS, 2014). Progress has been made in eradicating Lymphatic filariasis and trachoma due to mass treatment campaigns and it is currently believed that these programmes can be ended safely. Both infections are not observed within the Ashanti Region and hence not included into local disease control programmes. Strategies to control Buruli ulcer include early case detection, education as well as fostering research innovation, the transmission route having not been identified yet. Onchocerciasis programmes build on community mass-drug administration, vector control and education. All school-aged children should receive Schistosomiasis treatment annually and regular preventive treatment against prevalent soil transmitted helminths (GHS, 2013).

Box 6: Intervention and prevention

- Ghana has launched various programmes targeting high-burden infectious diseases.
- Data on the success of programmes are scarce, which challenge assessment of their efficiency.
- Infection risk is highest in the poor, but they are also less likely to utilise intervention programmes.

5 Discussion

The established CRF is a helpful tool to systematically identify and assess data needed for childhood infectious disease management in a defined study area. Applying the CRF allows available data from the study area to be reviewed, taking into consideration the broad spectrum of public health aspects involved at all administrative levels. This comprehensive public health data can be assessed and analysed following a systematic holistic approach. Generally, child health is high on Ghana's political agenda. Aims to improve children's health are formulated and several interventions and health programmes are in place to deliver prevention and treatment measures. However, the thesis identifies barriers which hamper successful provision and delivery of health services.

Ghana's health system underwent several reforms within recent decades. Planning and administrating health services is the role of regional administrative authorities. The health policy system is still in transition and the allocation of legislative power between the regional and national levels is not fully harmonised. Concentrating administrative power at regional level provides flexibility to tailor preventive and curative services according to the local needs. However, to do so a policy framework, defining responsibilities among all administrative levels involved, is urgently needed. Currently, contradictory regulations, especially in terms of health financing, challenge successful health service delivery within the regions. For example, in 2015 the NHIS was not reimbursing open health service claims for about seven months, which led to a withdrawal of local providers delivering care to subscribers (News-Ghana, 2015). Generally, the NHIS is a major achievement of the Ghanaian state, providing health services to the broader population, yet some population groups do not benefit from the insurance. It is estimated that about 60% of the population are not enrolled, of which the majority are people with lower SES (Mills et al., 2012). Ghanaians who work in the formal sector are more likely to take advantage of the health insurance, because their fees are paid via the social security payroll. People employed in the informal sector or those unemployed have to register voluntarily and the registration rates are the most frequently mentioned barrier to enrolling (Kusi et al., 2015). Ghana has an unemployment rate of about 30% and the proportion of employed people working in the informal sector is estimated to be 80% (Oduro, 2009). Hence the majority of the population have to pay NHIS fees out of their pocket, and are therefore less likely to enrol (Mills et al., 2012). Considering that a large part of the NHIS budget is VAT-funded, a more universal approach to covering heath service costs is warranted. Since people with lower SES present later and more infrequently to health services, an extension of the NHIS to these population groups could be

an opportunity to improve health for the whole population, especially those who are currently hard to reach.

Ghana has a comprehensive disease surveillance system in place, which collects data on major health conditions throughout all districts. However, harmonisation of data exchange between the different legislative levels is insufficient, leading to delayed data transfer and data incompleteness (Adokiya et al., 2015a; Adokiya et al., 2015b). Generally, the surveillance system follows the IDSR strategy and it has the capacity to generate the data needed to properly plan disease prevention and response measures. However, the established system could be more efficient if all players involved, from health facility level to the national authorities, would cooperate effectively. Gaps identified were concerned with the motivation of HCW to collect and report data, and the effectiveness of the established reporting chain. People involved in data collection at facility level could be encouraged to report fully if surveillance reports are communicated to the districts in a timely manner. Even though comprehensive surveillance is in place, the dissemination of health data is limited. While reviewing the data to complete the CRF it became clear that official health data is poorly accessible and data presented in health reports often lack sufficient analyses. However, such data is crucial for local HCW to properly plan disease responses and to have the required background information to diagnose and treat patients appropriately. Additionally, national and international health policy makers and researchers would benefit from fully accessible health data in order to plan and support prevention and treatment programmes.

Ghana's health services are designed to deliver prevention and care to the whole population, regardless of whether people are living in rural or urban areas. However, capacity gaps are reported from health care facilities at all regional levels, but in particular from providers at district and community level. Medical personnel are scarce in rural regions, and health centres and clinics are often poorly equipped. Many physicians are reluctant to work in rural areas and employed HCW are often overwhelmed by their daily work, so that clinical guidelines are followed insufficiently. Also the established CHPS, which aim to provide basic health care within rural communities, report resource constraints and lack of skilled personnel. Non-operational laboratories impede proper diagnoses of many infectious diseases and shortcomings in medical equipment hamper appropriate curative treatment. The lack of laboratory resources also challenges basic disease surveillance, since confirmed diagnoses are often not available (The World Bank, 2013). Health facilities in rural areas, which in particular serve people with lower SES, are heavily burdened by these constraints. Furthermore, people living in rural communities are at higher risk of contracting diseases,

including bloodstream infections (***Sothmann et al., 2015***) and malaria (Frank et al., 2016). CHPS has the potential to successfully support these deprived individuals, because it is designed to deliver prevention and treatment to people's home, but resource constraints hamper service delivery. Reallocation of resources should be considered to overcome the observed implementation constraints. Successful delivery of health services to people who are hard to reach with conventional disease programmes is an important step forward in reducing morbidity and mortality. This is particularly the case as this population group is burdened by high childhood infectious disease prevalences.

Ghana, including the Ashanti Region, is a high malaria transmission area, which poses a risk to infants and young children. They lack immune resistance, which increases the risk of severe disease complications (Kleinschmidt et al., 2009). Even though programmes have successfully reduced malaria incidence in many endemic areas (Murray et al., 2012b), this infection remains a priority for childhood disease control in Ghana. Ghana has several health programmes in place to control malaria in children, for example IPTI or the free distribution of ITNs to families. Preventing childhood malaria will also have a substantial effect on invasive NTS infections. We estimated that about 13% of the infections observed in our study group are associated with current malarial disease (***Krumkamp et al., 2016)***. Looking at further disease risks, it is striking that diarrhoea and pneumonia are less frequently observed in the study area compared to other SSA countries. Nevertheless, these conditions are still attributable for a high childhood disease burden in Ghana. The corresponding disease control measures are mainly based on vaccination strategies. However, access to improved water and sanitation are additional cost effective measures to control diarrhoea and ARTI (UNICEF, 2015), although they feature less prominently in national prevention strategies. These measures would also foster NTD control, some of which are prevalent within the Ashanti Region (Freeman et al., 2013). The prevalence of most NTDs is relatively low, which opens a window of opportunity to eradicate these infections. Buruli ulcer is one important exception, however it is difficult to prevent given that the mode of transmission is still unknown (Raghunathan et al., 2005). Thus, further research is warranted in order to improve the knowledge base for Buruli ulcer control.

For the thesis a CRF was constructed in order to systematically identify public health areas and aspects relevant for childhood infectious disease management. The framework provides the structure to review existing data from Ghana and the study area, and to integrate the data generated for the thesis, considering the larger public health context. In addition, the CRF incorporates boundaries around the research fields in order to focus attention on the most

relevant research areas where further data is needed. So far no research framework for (childhood) infectious disease control in developing countries has been published. However, research frameworks and policy guidelines exist for other public health disciplines, such as cancer control or health system assessment, and these have been used to establish the present CRF. The CRF is based on existing concepts and may not have considered all relevant aspects of childhood infectious disease control. New, unpublished opinions will not have been considered using the current review approach. A systematic literature review along with expert discussions would be an appropriate approach to establish a holistic and general CRF, which covers current research aspects in childhood disease control.

Scientific articles and published 'grey' literature, such as health reports, websites or doctoral theses, were considered when applying the established CRF. It is likely that further informative data are not officially published. Particularly, data from district and community levels, which reflect the local health context, are less likely to be publically available. As outlined above, infectious disease occurrence varies even over smaller geographical areas, thus extrapolating data from national summary reports could be highly misleading. However, national figures had to be used for some objectives, because district data were not available, particularly in the case of the CRF-component "Disease data". Also information on local disease management programmes and on locally observed barriers was not available in greater detail. Such data could be collected in expert interviews or with tailored evaluation studies, which are beyond the scope of the thesis. Another limitation is that the level of evidence of the cited data was not considered. Even though unreliable information sources were ignored in the review, the quality of the included data was not systematically assessed or graded, which can bias derived conclusions.

The CRF was established to allow a holistic assessment of the different public health aspects relevant to childhood infectious disease control. Some information required to complete the CRF needs comprehensive scientific data analyses. For example, the second component requests health system data, e.g., on the distribution of legislative power, on the structure of the health funding system or on the disease surveillance process. Commonly, the assessment of health system performance is based on in-depth evaluation of the present health policy structure, including legislative regulations, resource capacity as well as the social and political context of a geographical area (Hanvoravongchai et al., 2010; Krumkamp et al., 2010). The CRF component "Intervention and prevention" relies on information about the effectiveness of disease programmes and their sustainability. Such information should be based on evaluation studies, considering health programme integration into a political system

and their efficiency to reduce disease occurrence (Atun et al., 2010). Hence, to provide information on some CRF components, a research framework itself would be the appropriate method to gather and evaluate the needed data systematically. Such comprehensive assessments of the CRF's components would be beyond the scope of the thesis. However, it highlights that the CRF relies on conducted and published analyses. Even though comprehensive data may be lacking for some CRF components, the thesis is able to point to areas which risk preventing the successful management of childhood infectious diseases. To discuss how the established childhood infectious disease control system can be improved, an in-depth evaluation of the identified barriers would be warranted in order to base policy recommendation on timely and reliable information.

Studies conducted within the frame of the thesis are set at two local hospitals or their corresponding hospital catchment areas located in the Ashanti Region. For the thesis, these findings were extrapolated throughout the study area, which implies generalisability of the study results. The analyses conducted at the study hospitals relate to infectious causes of diarrhoea (***Krumkamp et al., 2015***), the association between malaria and invasive NTS infection (***Krumkamp et al., 2016***) and the association between urbanicity and bacteraemia (***Sothmann et al., 2015***). In all studies children attending local hospitals were recruited. The CRF-evaluation showed that HCU is influenced by distance to the health care facility and NHIS enrolment. Hence, children living in remote areas or in poor conditions are less likely to be included into our studies. This selection bias systematically reduces the number of study participants from rural environments with lower living standards. The results generated may still hold true for these individuals, however the overall disease burden in the studies could be underestimated since excluded individuals may have a higher chance of contracting infectious diseases. The HCU-study conducted for the thesis assesses factors influencing the likelihood to seek professional medical care (***Krumkamp et al., 2013***). This analysis is not based on observed health seeking behaviour; it rather evaluates how parents would utilise health services in the case of a child showing a potential set of disease symptoms. Hence, parents may tend to overestimate the use of professional care, as this is the expected health behaviour. It is likely that actual HCU is lower, as reported within the study. Finally, a study on the quality of drinking water collected from dug wells within Asankare village was conducted (***Dekker et al., 2015***). Asankare is a rural community with unique characteristics, which may not be representative of other communities in the study area. For example, the high proportion of water contamination could be associated with the specific sanitary or livestock husbandry situation. However, the study highlights the general threat posed by poorly maintained dug

wells. To reliably assess drinking water quality within the study area, a sampling approach covering a larger geographical area, including different communities, should be followed.

This thesis shows that many factors must be considered to successfully implement prevention and treatment measures. It also underlines that academic institutions should not only focus on research to develop new preventive or medical interventions. Attention should also be paid to how to deliver interventions, so that whole populations, including neglected and high-risk groups, receive the greatest benefit. Often the development and demonstrating the effectiveness of interventions is seen as a legitimate endpoint in health research. With such an approach however, we miss opportunities to reach affected people most in need in order to successfully reduce childhood morbidity and mortality (Rudan et al., 2007).

Within the frame of the thesis data was generated, reviewed and summarised to evaluate childhood infectious disease management in a systematic and comprehensive manner. The disease control barriers identified cannot be ordered according to their relevance in disease control, because the effect of the above limitations may be diverse. However, analyses show that no single factor is responsible for intervention success and there is no particular combination of constraints which must be addressed in order to accelerate programme success (Kuruvilla et al., 2014). To improve health programme performance, all disease management aspects should be considered and feasibility should guide system improvement. In order to become policy relevant, the generated results have to be discussed with local stakeholders as well as experts concerned with disease control. An important next step would be to involve local stakeholders and policy makers to discuss the CRF's results and to identify feasible improvements in childhood disease control. This may also identify areas where further data is needed to successfully overcome programme implementation barriers. Generally, communication between researchers and policymakers should be intensified, with the aim of making research more policy relevant and to foster research driven health policy (Lavis et al., 2010). Policy evaluation showed that research conducted within a local context or which was adjusted to the local context is more likely to be picked up by health experts than results extrapolated from superior studies (Guindon et al., 2010). Hence, the used CRF approach is a valuable method to systematically identify and assess comprehensive local public health data. Policy relevant information will be linked throughout the CRF's components to provide a holistic overview, which locally enables policy makers to locate barriers within a complex system and to tailor measures to improve disease management.

6 Conclusion

The thesis demonstrates that Ghana and the Ashanti Region have a well-planned system for childhood infectious disease management. Priorities in childhood infectious disease control are identified and formulated into health politics. The health service structure is tailored to provide care to infected children and various interventions are in place to control disease transmission. In comparison to other SSA countries, Ghana has achieved greater improvements in its population's health and reduced the burden of childhood infectious diseases notably. However, the results summarised within the CRF highlight the serious gaps that exist. Implementation challenges constrain successful delivery of health services at all administrative levels, which in particular affect the poor and people living in remote areas. These individuals are disadvantaged in several ways: they are at higher risk of contracting infectious diseases, they are more likely to be affected by health service resource constraints, and they are less likely to utilise prevention and treatment measures. Additionally, large parts of Ghana's population do not benefit from the economic growth. The Ghanaian government correctly identified lower SES groups and rural areas as an intervention priority, however the established prevention and treatment programmes are not effectively implemented to reach these people.

The thesis also supports an optimistic assessment of the quality of the health system structure in place in Ghana. Generally, additional childhood disease control programmes are not needed because various health programmes are already in place. The government should overcome existing barriers present at the different administrative levels, to successfully deliver health services to the whole population. Training and resource improvement focusing on the community and district level would bring health benefits to many of the more underserved population groups. Even though Ghana has a relatively strong economy, the current national financial crisis makes it unlikely that further resources will be available to strengthen the health system. Hence, to some extent the required changes will still rely on external donor support. If external contributions can be effectively channelled, the currently established childhood infectious disease management system will become more efficient and better reach neglected population groups.

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Annex I: Eidesstattliche Erklärung

Hiermit versichere ich (Ralf Krumkamp) an Eides statt, dass ich

- diese Arbeit selbständig und ohne unerlaubte fremde Hilfe angefertigt habe,
- keine anderen als die von mir angegebenen Quellen oder Hilfsmittel benutzt habe und
- die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Zudem erkläre ich, dass ich keine weiteren Promotionsversuche unternommen habe.

Hamburg, 22.03.2016

Annex II: Zusammenfassung

Viele Entwicklungsländer sind mit einer hohen Mortalität und Morbidität bei Kindern konfrontiert. Im subsaharischen Afrika und im asiatischen Raum sind Infektionskrankheiten für den größten Anteil der Krankheitslast verantwortlich. Internationale Organisationen und viele Staaten haben sich der Kontrolle von Infektionskrankheiten verpflichtet und haben zu diesem Zweck verschiedene Interventionen umgesetzt. Allerdings ist der Erfolg dieser Maßnahmen sowohl zwischen als auch innerhalb der Staaten unterschiedlich. Hierfür sind oft lokale Hindernisse verantwortlich, die eine Implementierung der Interventionen erschweren. Ziel dieser Dissertation war es, fehlende Informationen für die Kontrolle von Infektionskrankheiten bei Kindern in der Ashanti Region in Ghana zur generieren, um eine Verbesserung der Infektionskontrolle zu ermöglichen. Es wurde ein konzeptionelles Gerüst entworfen, worin relevante Komponenten des öffentlichen Gesundheitssystems zur Infektionskontrolle wie folgt definiert wurden: (i) Gesellschaft, (ii) Gesundheitssystem, (iii) Gesundheitsdienstleistungen, (iv) Krankheitsdaten (v) Diagnose und Behandlung und (vi) Intervention und Prävention. Innerhalb dieses Konzeptes wurden die Ergebnisse der Studien die im Rahmen der Dissertation entstanden sind, zusammen mit weiteren publizierten Daten, systematisch erfasst und beschrieben. Die Dissertation zeigt, dass Ghana und die Ashanti Region ein gut strukturiertes System zur Kontrolle von Infektionskrankheiten bei Kindern haben. Schwerpunkte in der Infektionskontrolle sind erfolgreich identifiziert und entsprechende gezielte Interventionen werden eingesetzt. Des Weiteren wurden kurative Maßnahmen, mit dem Fokus auf Infektionen bei Kindern, etabliert. Allerdings gibt es verschiedene Implementierungshindernisse, die eine erfolgreiche Umsetzung der Kontrollmaßnamen auf allen administrativen Ebenen erschweren. Besonders in ländlichen Gegenden mangelt es an medizinischer Ausstattung und Personal. Personen in ländlichen Gegenden sind zudem durch ein höheres Erkrankungsrisiko und durch eine geringere Inanspruchnahme von Gesundheitsdienstleistungen belastet. Die Dissertation zeigt, dass Ghana ein umfassendes System zur Krankheitskontrolle bei Kindern etabliert hat. Allerdings muss der Staat bestehende Hindernisse bewältigen, um Gesundheitsdienstleistungen und Krankheitsinterventionen für die gesamte Population zu gewährleisten.

Annex III: Publications

Association Between Malaria and Invasive Nontyphoidal *Salmonella* Infection in a Hospital Study: Accounting for Berkson's Bias.

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http://cid.oxfordjournals.org/content/62/suppl_1/S83.long

Abstract

Background: There is growing evidence for a positive association between malaria and invasive nontyphoidal Salmonella (iNTS) disease. However, case-control studies conducted within healthcare facilities also report inverse associations. This may be due to Berkson's bias, a selection bias that acts when both exposure and outcome are associated with hospital attendance and study participants are selected among attendees only. This study describes the effect of Berkson's bias on the malaria-iNTS association and provides a less biased effect estimate.

Methods: Data collected in 2 Ghanaian hospitals were analyzed using 2 case-control approaches. In both approaches, cases were defined as iNTS-positive children, and concomitant malaria infection was the exposure of interest. In the first conventional sampling approach, children without any febrile bloodstream infection served as controls. In the second control-disease approach, children with non-iNTS bacteremia were used as controls.

Results: Data from 6746 children were suitable for the analyses. One hundred sixty children with iNTS infection were study cases. In the conventional case-control approach 6301 children were controls, and in the control-disease approach 285 children were controls. In the conventional case-control study, malaria was estimated to protect against iNTS disease (odds ratio [OR], 0.4; 95% confidence interval [CI], .3–.7), whereas in the control-disease approach, malaria was identified to be a risk factor for iNTS disease (OR, 1.9; 95% CI, 1.1–3.3).

Conclusion: The study highlights how a selection bias may reverse results if an unsuitable control group is used and adds further evidence on the malaria-iNTS disease association.

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Gastrointestinal Infections and Diarrheal Disease in Ghanaian Infants and Children: An Outpatient Case-Control Study

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Abstract

Introduction

Diarrheal diseases are among the most frequent causes of morbidity and mortality in children worldwide, especially in resource-poor areas. This case-control study assessed the associations between gastrointestinal infections and diarrhea in children from rural Ghana.

Methods

Stool samples were collected from 548 children with diarrhea and from 686 without gastrointestinal symptoms visiting a hospital from 2007–2008. Samples were analyzed by microscopy and molecular methods.

Results

The organisms most frequently detected in symptomatic cases were *Giardia lamblia*, *Shigella* spp./ enteroinvasive *Escherichia coli* (EIEC), and *Campylobacter jejuni*. Infections with rotavirus (adjusted odds ratio [aOR] = 8.4; 95% confidence interval [CI]: 4.3–16.6), *C. parvum/hominis* (aOR = 2.7; 95% CI: 1.4–5.2) and norovirus (aOR = 2.0; 95%CI: 1.3–3.0) showed the strongest association with diarrhea. The highest attributable fractions (AF) for diarrhea were estimated for rotavirus (AF = 14.3%; 95% CI: 10.9–17.5%), *Shigella* spp./ EIEC (AF = 10.5%; 95% CI: 3.5–17.1%), and norovirus (AF = 8.2%; 95% CI 3.2–12.9%). Co-infections occurred frequently and most infections presented themselves independently of other infections. However, infections with *E. dispar*, *C. jejuni*, and norovirus were observed more often in the presence of *G. lamblia*.

Conclusions

Diarrheal diseases in children from a rural area in sub-Saharan Africa are mainly due to infections with rotavirus, *Shigella* spp./EIEC, and norovirus. These associations are strongly age-dependent, which should be considered when diagnosing causes of diarrhea. The presented results are informative for both clinicians treating gastrointestinal infections as well as public health experts designing control programs against diarrheal diseases.

Author Summary

Gastrointestinal infections are frequent in many low-income countries. However, their role in diarrheal diseases is still under discussion. Many epidemiological studies focus on individuals with diarrheal symptoms only, ignoring the fact that infections may progress asymptomatically as well. In order to identify infectious agents associated with diarrhea it is imperative to consider cases without symptoms as a control group. We conducted a case-control study, including 548 children with diarrhea and 651 children without gastrointestinal symptoms in order to untangle the role of gastrointestinal infections in diarrheal disease. As shown in other studies infections with rotavirus, *Shigella* spp./EIEC and norovirus are responsible for the main diarrhea burden. Co-infections are frequently observed in our study group and some organisms occur more frequently in the presence of a second one. Especially *Giardia lamblia*, which is not associated with diarrhea, is more often observed along with *Campylobacter jejuni* and norovirus, which are responsible for a high number of diarrheal episodes. This may be of particular interest since *G. lamblia* is, with a frequency of 40% within the study group, the most prevalent organism observed. Furthermore, the high number of co-infections challenged the identification of causative pathogens since diagnosing a particular isolate may not rule out the effect of another potentially infectious agent in diarrheal disease. We observed a strong effect of age on the course of an infection, which may guide clinicians when diagnosing causes of diarrhea.

Introduction

Diarrheal diseases are the second leading cause of childhood mortality worldwide. In 2010, diarrhea was responsible for 0.8 million deaths of children below the age of five years, accounting for 10.5% of all deaths within that age group [1]. Mortality and morbidity patterns differ across geographical regions, with 78% of all pediatric diarrhea-associated deaths occurring in the African and South-East Asian World Health Organization (WHO) Regions [2].

The etiology of diarrhea is often not completely understood, especially in developing countries, including those in sub-Saharan Africa. Knowledge of the distribution and impact of infectious agents in diarrheal diseases is crucial in guiding empirical medical treatment and in designing prevention programs. However, many studies on the epidemiology of gastrointestinal infections are restricted to only patients with diarrhea, ignoring the possibility that infections may progress asymptomatically or even influence one another. This may be of particular importance in areas in which certain infectious agents are endemic, which would results in a high probability of ongoing infections after the development of partial immunity and/or tolerance. Thus, an adequate control group is essential to determine the pathogenicity of infectious

agents, their fractions attributable to gastrointestinal symptoms (GIS), and the age-dependent association of infectious agents with GIS [3,4].

This hospital-based case-control study in a rural area of Ghana was designed to analyze gastrointestinal infections in children with and without diarrhea. The aims of this study were (i) to identify the causative pathogens linked to diarrhea, (ii) to describe their pathogenicity and contribution to the burden of diarrhea, and (iii) to analyze the frequency and interactive effects of co-infections.

Methods

Data were collected at the Agogo Presbyterian Hospital (APH), a district hospital with approximately 250 patient beds located in the Asante Akim North municipality in Ghana. Among other facilities, it has a children's Outpatient Department (OPD) and a pediatric ward. Asante Akim North municipal area has a population of approximately 142,400 inhabitants, spread over an area of 1,160 square kilometers. The region has a tropical climate and is mainly covered by secondary rain forest and cultivated land [5]. Falciparum malaria is highly endemic in this area [6] and HIV is, with a prevalence of 3.0% in pregnant women in 2009, at a stabile state [7].

This case-control study included children aged up to 13 years who visited APH between June 2007 and October 2008. Stool samples were collected from children with diarrhea attending the hospital's OPD. Diarrhea was defined as at least three episodes of loose stools within the previous 24 hours. Therefore, a stool container was handed to guardians of cases and controls to collect a sample. In case a child could not provide stool at the OPD, parents were asked to return a sample to the hospital within a day after collection. Assistance was provided if needed (3). The laboratory personnel was required to confirm loose stool consistency of the collected samples. Throughout the study period, each day stool samples were also collected from children who visited the hospital OPD without diarrhea and vomiting, again with the laboratory personnel's confirmation that stool samples were of solid consistency. Children with ongoing diarrheal disease, defined as repeated hospital visits because of diarrhea within a 6-week period, were excluded from the analysis. Data per individual were used to describe the study groups. Data per hospital visit were used for further analyses.

Ethics statement

The Committee on Human Research, Publications and Ethics, School of Medical Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, approved the study design and the informed consent procedure. All participants were informed of the study's purpose and procedures. Written informed consent was obtained from the parents or the legal guardian on behalf of the study children prior to study enrolment. Non-participation had no effect on the medical treatment provided.

Microbiological analyses

Stool samples were refrigerated (4°C) immediately after collection and transported within a day to the laboratories at the Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR). Sample transport took about 1.5 hours and a cool-box was used to maintain the cold chain. Upon arrival stool samples were aliquoted (3 x 0.2 mg) and frozen at -20°C while the reaming sample was processed for further analyses. Microscopy of blood and stool samples took place at the KCCR laboratory and DNA extractions, polymerase chain reaction (PCR) assays and ELISA-tests were conducted at the Bernhard Nocht Institute for Tropical Medicine (BNITM) in Germany. Therefore, frozen stool samples were transported on dry ice to the

BNITM maintaining a temperature of -20°C (3). All samples were handled, stored and transported according to good laboratory practice.

Sodium acetate-acetic acid-formalin (SAF) solution was added to the sample to preserve parasites. Following formalin-ether concentration of protozoan cysts and trophozoites, as well as helminth eggs, fractions of the concentrated material were stained with iodine or by the modified acid-fast method, the latter to improve detection of Coccidia species, such as *Cryptosporidium*, *Cyclospora*, and *Cystoisospora* [8,9]. The samples were then viewed under a light microscope.

For molecular detection, DNA was extracted from the frozen stool samples using QIAamp DNA Stool Kits (Qiagen, Hilden, Germany). Specific sequences were amplified by PCR to identify the following organisms: *Campylobacter jejuni* [10], *Cryptosporidium parvum/hominis* [11], *Cyclospora cayetanensis* [12,13], *Entamoeba dispar* [14,15], *Entamoeba histolytica* [14,15], *Giardia lamblia* [11], norovirus [16], *Salmonella enterica* [10], *Shigella* spp./enteroinvasive *Escherichia coli* (EIEC) [10], and *Yersinia enterocolitica* [10]. Diagnostic PCR assays give the Ct value as a semi-quantitative result and a cut-off of 35 cycles was applied to determine a positive test result. As *Shigella* spp. and EIEC both possess the *ipaH* gene, these organisms cannot be distinguished by PCR [10]. Further, the PCRs applied did not allow differentiation between typhoidal and non-typhoidal Salmonella or between norovirus genotypes. Rotavirus was identified by an enzyme-linked immunosorbent assay (ELISA; Ridascreen).

Blood samples were obtained from all participants by finger prick. Thick and thin smears were prepared from these blood samples, which were stained and examined by microscopy. Malaria was defined as any asexual parasitaemia with body temperature >38°C.

All PCR reactions, as well as malaria and stool microscopy, were regularly evaluated by external and internal quality assessments. The study personal was trained to adhere to the standard operational procedures for each laboratory method.

Statistical and epidemiological analyses

A sample size of about 500 children per group was estimated to identify isolates in 5% of the controls and 10% of the cases, considering an alpha-level of 5% and a power of 80%. Categorical variables are reported as frequencies and percentages, whereas continuous variables are reported as means ± standard deviations (SDs) or as medians with interquartile ranges (IQRs). Missing values were excluded from analyses, thus the denominators for some comparisons differ.

Because microscopy has decreased sensitivity and specificity in diagnosing diarrheal samples [8,17], all statistical comparisons were based on PCR- or ELISA-based diagnoses. The associations between diarrhea and gastrointestinal infections were determined by calculating odds ratios (OR) and 95% confidence intervals (CI). Subjects were stratified to show effects within categories of a third variable to assess and account for confounding or effect modification. Mantel-Haenszel adjusted ORs (aOR) were calculated from the stratified analyses. The attributable fractions (AF) on the diarrhea burden, defined as the proportion of diarrhea attributable to a certain pathogen, were calculated as described [18], from logistic regression estimates, including dummy variables, for age categories.

Heterogeneity in the occurrence of co-infections was assessed by comparing the probability of organism A in the presence of organism B over the probability of A in the absence of B. These associations were determined by calculating the risk ratio (RR), using the formula $RR = P(A|B = 1)/P(A|B = 0)$, in which a value of about one indicates independence and a value different from one indicating dependence. RRs were calculated for the total study group and for cases and controls separately. Organisms diagnosed by PCR or ELISA and detected in more

than 5% of stool samples in the respective study groups were included in this calculation to ensure a sufficient number of co-infections. Age-adjusted RRs (aRR) were calculated to account for the age-dependence of infections. Age was categorized into the groups 0–<1, 1–<2, 2–<5 and 5–15 years to analyze age specific infection dynamics.

All data analyses were performed with STATA 12 (StataCorp LP, College Station, USA).

Results

In total, 1,234 patient visits made by 1,168 children were included in the analysis. The majority of children visited the hospital once (n = 1,109; 94.9%), 52 (4.5%) visited twice and 7 (0.6%) three times. Fifty-seven (4.6%) patients were admitted to the children's ward; the other children were treated at the OPD. Girls were slightly under-represented (n = 536; 45.9%). The median age of the attendees was 33 months (IQR: 15–70 months). Stratification by age showed that, at the time of visits, 227 (18.4%) children were aged 0 to <1 year, 266 (21.6%) were aged 1 to <2 years, 375 (30.4%) were aged 2 to <5 years, and 366 (29.7%) were aged 5–13 years. Malaria was diagnosed during 236 (20.7%) visits. Differences shown in measles and yellow fever vaccination status were not observed in an age-stratified comparison.

Diarrhea was present in 548 (44.4%) cases (case visits), but absent in 686 (55.6%) instances (control visits). Fever was the most frequent disease symptom and more often observed in controls than in cases [n = 568 (82.8%) vs. n = 397 (72.5%)]. Acute malnourishment was observed in 57 (12.0%) case and 35 (9.4%) control visits., 218 (39.8%) cases suffered from vomiting. The proportion of children with diarrhea decreased gradually with age. The median ages of children with and without diarrhea were 18 months (IQR: 9–36 months) and 57 months (IQR: 26–93 months), respectively (Table 1).

Organisms detected in stool samples

Potentially pathogenic organisms as well as facultative and non-pathogenic parasites were detected 1,843 times in 915 (79.5%) stool samples. The most frequent infections were with *G. lamblia* (n = 470; 38.1%), *Shigella* spp./EIEC (n = 336; 27.2%), *C. jejuni* (n = 242; 19.6%), *Blastocystis hominis* (n = 144; 14.3%), and norovirus (n = 139; 11.3%). Sex-dependent differences were not observed. All parasites and protozoa, apart from *C. parvum/hominis*, tended to be less frequently observed in diarrheal compared to non-diarrheal samples. *Cyclospora cayetanensis*, *E. histolytica*, and *Yersinia* spp. were not detected in any of the stool samples tested (Table 2).

Fig 1 shows the age-stratified proportions and the median ages of case and control children infected with particular organisms. In children with diarrhea, rotavirus, norovirus, and *C. parvum/hominis* were most frequently observed in younger infants, with infected individuals having median ages of 12 months (IQR: 8–23 months), 13 months (IQR 8–22 months), and 14 months (IQR: 10–20 months), respectively. By contrast, control children with these organisms were older, with median ages of 30 months (IQR: 16–45 months), 40 months (IQR: 20–82 months), and 23 months (IQR: 18–28 months), respectively. For all other infections the median age in children with diarrhea ranged from 19 months (IQR: 11–36 months) to 68 months (IQR: 35–123 months). Infants below 6 months accounted for 95 (8.1%) hospital visits, with 16 stool samples (16.9%) diagnosed with rotavirus and 13 (13.7%) with norovirus. All virus-infected children, except one diagnosed with norovirus, had diarrhea. Within this age group other organisms were not detected in more than six stool samples (6.3%).

Age-dependence of gastrointestinal infections and diarrhea

Crude analyses showed that the strongest positive associations with diarrhea were for infections with rotavirus (OR = 11.9; 95% CI: 6.2–24.9), *C. parvum/hominis* (OR = 4.3; 95% CI: 2.3–8.6), and norovirus (OR = 2.6; 95% CI: 1.8–3.9). Inverse associations were found for

Table 1. Characteristics of the total populations and stratified groups of case and control children.

Characteristic	Total (N = 1,234)	Cases (N = 548)	Controls (N = 686)
Sex, female (%)[#]	536 (45.9)	241 (47.6)	296 (44.6)
Age, median months (IQR)[#]	33 (15–70)	18 (9–36)	56 (25–92)
Malaria (%)[§,a]	236 (20.7)	90 (17.7)	146 (23.0)
Referred to ward (%)[§]	57 (4.6)	42 (7.7)	15 (2.2)
Mother's age, mean years (SD)[#,b]	30.3 (7.0)	28.4 (6.2)	31.9 (7.2)
Immunisation (%)[#]			
DPT/HIB/Hep B1/Pol[c]	1,086 (93.5)	468 (93.2)	618 (93.2)
Measles[d]	925 (82.2)	370 (74.0)	583 (88.3)
Yellow fever[e]	922 (82.0)	368 (73.6)	582 (88.3)
Symptoms (%)[§]			
Fever	965 (78.2)	397 (72.5)	568 (82.8)
Acute malnourished	92 (10.8)	57 (12.0)	35 (9.4)
Vomiting	NA	218 (39.8)	NA

Abbreviations: IQR, interquartile range; SD, standard deviation; DPT/HIB/Hep B1/Pol: diphtheria, pertussis, and tetanus/Haemophilus influenzae type B/Hepatitis B/Polio; NA, not applicable.

[#]Per child (N = 1,168,)

[§]Per visit (N = 1,234)

Missing values (not considered in percentages):

[a]91,

[b]76

[c]6,

[d]8,

[e]9s,

[f]383.

Frequency (per cent) presented if not specified otherwise.

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G. lamblia and *E. dispar* suggesting that more infections were diagnosed in controls than in cases. However, this effect was attenuated after age stratification (Table 3). Age stratification revealed varying associations of most infections with diarrhea. For example, associations with *S. enterica* or *Shigella spp.*/EIEC increased with age, and associations with norovirus were highest in the youngest age group (OR = 4.7; 95% CI: 1.4–24.6) and lower in older children. Associations between rotavirus and diarrhea were strong amongst all age groups, although the frequency of infections decreased with age. Stratified estimates for *C. parvum/hominis* were lower than the crude OR, indicating that age confounded effect estimates. No associations between sex (being female) and diarrhea (OR = 0.9; 95% CI: 0.7–1.1) or rainy season and diarrhea (OR = 1.1; 95% CI: 0.9–1.4) were observed. Also, in logistic regression models these factors did not alter the association between infections and diarrheal symptoms.

Infections manifesting at younger ages tended to be more strongly associated with diarrhea. For example, the highest ORs were observed for rotavirus, *C. parvum/hominis* and norovirus, infectious agents most frequently diagnosed in the younger case age groups, with median ages of 12 months (IQR: 8–23 months), 14 months (IQR: 10–20 months), and 12 months (IQR: 8–23 months), respectively. By contrast, *E. dispar* and *G. lamblia* were not positively associated with diarrhea and were more frequently diagnosed in older cases, with median ages around 38 months (IQR: 35–57 months) and 28 months (IQR: 16–46 months), respectively.

Table 2. Frequencies (per cent) of gastrointestinal organisms identified in stool samples sorted by species group and frequency of occurrence.

Organism	Frequency (%)	
	Cases (N = 548)	Controls (N = 651)
Viruses		
Norovirus[$]	91 (16.6)	48 (7.0)
Rotavirus[§]	89 (16.2)	11 (1.6)
Bacteria		
Shigella spp./EIEC[$]	167 (30.5)	169 (24.6)
Campylobacter jejuni[$]	124 (22.6)	118 (17.2)
Salmonella enterica[$]	33 (6.0)	27 (3.9)
Yersinia spp.[$]	0 (0)	0 (0)
Protozoa		
Giardia lamblia[$]	176 (32.1)	279 (42.9)
Blastocystis hominis[a]	34 (7.6)	110 (19.6)
Entamoeba dispar[$]	30 (5.5)	62 (9.0)
Entamoeba coli[b]	9 (2.0)	68 (12.2)
Chilomastix mesnili[c]	11 (2.4)	37 (6.6)
Cryptosporidium parvum/hominis[$]	45 (8.2)	14 (2.0)
Iodamoeba buetschlii[c]	0 (0)	1 (0.2)
Cystoisospora[d]	1 (0.3)	0 (0)
Cyclospora cayetanensis[$]	0 (0)	0 (0)
Entamoeba histolytica[$]	0 (0)	0 (0)
Helminths		
Hymenolepis nana[e]	3 (0.7)	54 (9.5)
Strongyloides stercoralis[c]	4 (0.9)	6 (1.1)
Hook worm[f]	2 (0.4)	4 (0.7)
Ascaris lumbricoides[f]	0 (0)	1 (0.2)
Negative for all agents	101 (18.4)	152 (22.2)
Mono-infection	209 (38.1)	226 (32.9)
Mixed infection	238 (43.4)	308 (44.9)

Missing values (not considered in percentages):
[a]225;
[b]224,
[c]218,
[d]602,
[e]213,
[f]217
[$]identified via Polymerase Chain Reaction (PCR)
[§]identified via ELISA

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The highest AFs (proportion of diarrheal symptoms attributable to a certain organism) were observed for rotavirus (AF = 14.3%; 95% CI: 10.9–17.5%), *Shigella* spp./EIEC (AF = 10.5%; 95% CI: 3.5–17.1%), and norovirus (AF = 8.2%; 95% CI: 3.2–12.9%), whereas all other infections had AFs of about 5% and lower (Fig 2).

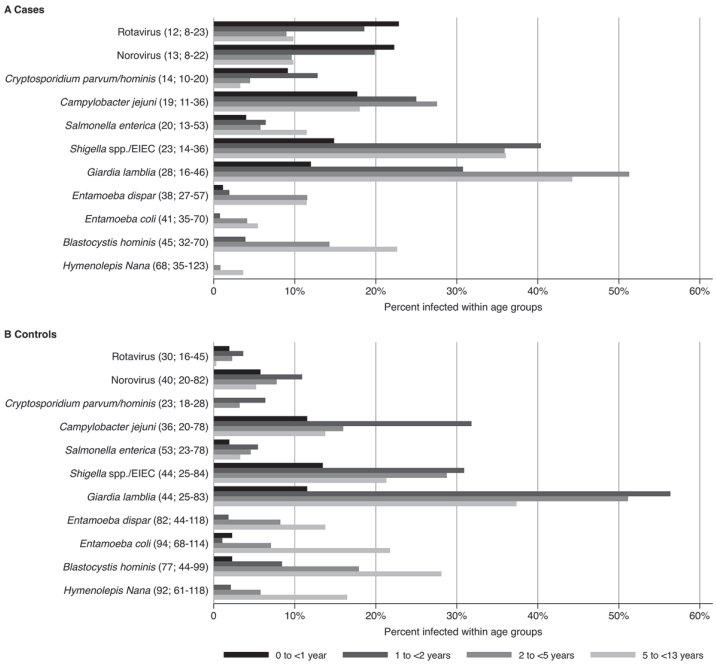

Fig 1. Proportions of case and control children by age group infected by various organisms. All organisms detected in more than 50 samples are shown. Median ages in months (IQR) at the time of infection are shown in brackets.

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Combined infections

Probabilities for the occurrence of distinct pairs of infectious agents were calculated for organisms detected by PCR or ELISA in more than 5% of the stool samples (Table 4). Considering the total study group, most of the infections occurred independently of other organisms. However, *E. dispar*, *C. jejuni*, and norovirus were observed more often in the presence of *G. lamblia*,

Table 3. Crude odds ratio (OR), age-adjusted OR (aOR), and age-stratified OR on associations between gastrointestinal infections and diarrhea, sorted by aOR.

			Age stratified OR (95%-CI)			
	Crude OR (95%-CI)	**aOR(95%-CI)**	**0 – <1 years**	**1 – <2 years**	**2 – <5 years**	**5–12 years**
Rotavirus	11.9 (6.2–24.9)	8.4 (4.3–16.6)	15.1 (2.4–623.3)	6.1 (2.0–24.3)	8.8 (3.3–27.0)[§]	
Cryptosporidium parvum/hominis	4.3 (2.2–8.8)	2.7 (1.4–5.2)	2.4 (1.0–6.4)[§]		3.5 (1.1–12.2)[§]	
Norovirus	2.6 (1.8–3.9)	2.0 (1.3–3.0)	4.7 (1.4–24.6)	2.0 (0.9–4.6)	1.3 (0.6–2.8)	2.0 (0.6–5.6)
Salmonella enterica	1.6 (0.9–2.7)	1.7 (1.0–3.0)	1.1 (0.3–97.6)	1.2 (0.4–4.1)	1.3 (0.4–3.6)	3.8 (1.2–11.6)
Shigella spp./ EIEC	1.3 (1.0–1.7)	1.5 (1.1–2.0)	1.1 (0.4–3.2)	1.5 (0.9–2.6)	1.4 (0.9–2.2)	2.1 (1.1–3.9)
Campylobacter jejuni	1.4 (1.1–1.9)	1.3 (1.0–1.8)	1.7 (0.6–5.1)	0.7 (0.4–1.3)	2.0 (1.2–3.4)	1.4 (0.6–3.0)
Entamoeba dispar	0.6 (0.4–0.9)	1.2 (0.7–1.9)	1.2 (0.2–31.0)[§]		1.5 (0.7–3.1)	0.8 (0.3–2.0)
Giardia lamblia	0.6 (0.5–0.8)	0.8 (0.6–1.0)	1.0 (0.4–3.3)	0.3 (0.2–0.6)	1.0 (0.6–1.5)	1.3 (0.7–2.4)

Abbreviations: OR, odds ratio; CI, confidence interval; aOR, age-adjusted odds ratio.

[§]Combined age groups to avoid empty cells in cross-tabulation.

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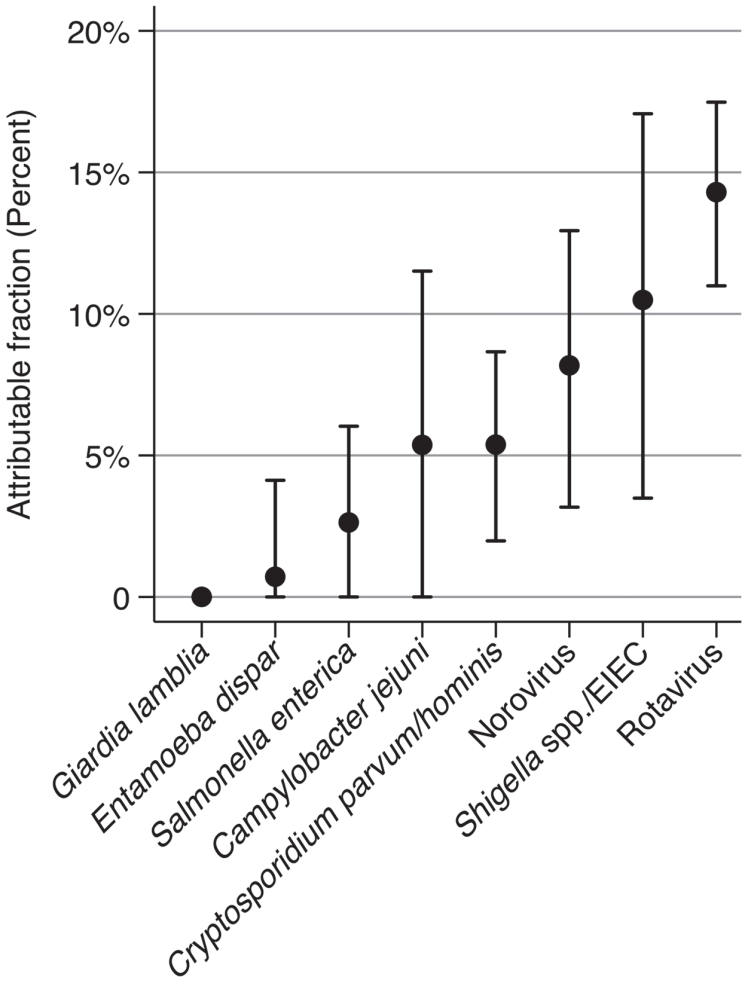

Fig 2. Age adjusted attributable fractions (AF) and their 95% confidence intervals of gastrointestinal infections on diarrhea.

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showing rate ratios of 1.6 (95% CI: 1.3–1.9), 1.3 (95% CI: 1.2–1.6), and 1.3 (95% CI: 1.1–1.6), respectively. These, estimates were comparable between cases and controls.

Discussion

The most important cause of diarrheal disease was rotavirus, with both the highest AF and the largest risk for diarrhea across all age groups. Frequency of rotavirus infections decreased with age, but its association with diarrhea was nearly constant throughout all age groups. Similarly, a multi-center study performed in seven resource-poor countries found that rotavirus was the leading cause of diarrhea in infants, with age-dependent AFs between 16% and 28% [3]. Furthermore, 96% of children in a Mexican birth cohort were infected with rotavirus at least once by the age of 2 years. Rotavirus infections conferred protection against re-infection, resulting in less frequent and less severe manifestations in older children [19].

The proportion of norovirus infections among cases and controls was, with 16.6% and 6.8%, respectively, comparable to figures from other high-mortality developing countries,

Table 4. Age-adjusted relative risk of the occurrences of selected gastrointestinal co-infections in all children and for cases and controls separately.

Combination of Organisms	aRR (95%-CI)		
	Total	Cases	Controls
G. lamblia & *Shigella* spp./EIEC	1.1 (0.9–1.2)	1.3 (1.0–1.6)	1.0 (0.8–1.2)
G. lamblia & *C. jejuni*	1.3 (1.2–1.6)	1.4 (1.1–1.8)	1.3 (1.0–1.6)
G. lamblia & Norovirus	1.3 (1.1–1.6)	1.4 (1.0–1.8)	1.3 (1.0–1.6)
G. lamblia & Rotavirus	0.8 (0.5–1.1)	0.8 (0.6–1.2)	NA
G. lamblia & *E. dispar*	1.6 (1.3–1.9)	1.5 (1.1–2.0)	1.6 (1.2–2.0)
Shigella spp./EIEC & *C. jejuni*	1.0 (0.8–1.2)	0.9 (0.7–1.3)	0.9 (0.7–1.3)
Shigella spp./EIEC & Norovirus	1.2 (0.9–1.6)	1.3 (0.9–1.7)	1.0 (0.6–1.6)
Shigella spp./EIEC & Rotavirus	0.8 (0.5–1.2)	0.8 (0.5–1.2)	NA
Shigella spp./EIEC & *E. dispar*	0.7 (0.4–1.1)	0.7 (0.4–1.4)	0.7 (0.4–1.2)
C. jejuni & *Norovirus*	0.8 (0.6–1.2)	0.7 (0.4–1.7)	1.0 (0.5–1.8)
C. jejuni & Rotavirus	0.6 (0.4–1.1)	0.6 (0.4–1.1)	NA
C. jejuni & *E. dispar*	1.3 (0.8–1.9)	1.4 (0.8–2.4)	1.1 (0.6–2.0)
Norovirus & Rotavirus	0.8 (0.4–1.4)	0.6 (0.3–1.1)	NA
Norovirus & *E. dispar*	1.0 (0.5–2.0)	1.2 (0.5–3.1)	0.8 (0.3–2.3)
Rotavirus & *E. dispar*	1.1 (0.4–2.9)	1.2 (0.5–3.3)	NA

Abbreviations: aRR, age-adjusted risk ratio; CI, confidence interval; NA: not applicable due to low frequency.

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where 14% (CI: 11–16) and 7% (CI: -2–16) are reported, respectively [20]. In children with diarrhea the frequency of norovirus infections was similar to that of rotavirus. However, compared to rotavirus the amount of norovirus infections was higher in asymptomatic (control) individuals, most probably due to increasing pathogen tolerance and limited sterilizing immunity. A transmission model showed that, in highly endemic settings, protection against severe norovirus gastroenteritis could be acquired early in life, resulting in frequent asymptomatic re-infections [21]. Likewise, our study found a significant association between norovirus infection and diarrhea in infants, whereas older children were more likely to be asymptomatic carriers.

In industrialized countries, cryptosporidiosis is primarily an opportunistic infection in HIV/AIDS patients and a major cause of water-born outbreaks reported from several countries [22]. In West Africa, however, cryptosporidiosis is the cause of diarrhea in 4.9% to 14.7% of immunocompetent children, depending on age and geographical location [3,23]. Similarly, our results showed that *C. parvum/hominis* infections were strongly associated with diarrhea throughout all age groups and was present in more than 10% of symptomatic children below the age of 2 years. Asymptomatic cryptosporidium carriers were not observed in this age group and rarely seen in older children. A review of cryptosporidiosis in sub-Saharan Africa reported the same age distribution, with a peak amongst children aged 6–12 months. Apparently, infection can occur throughout childhood, but symptoms become less severe with age [24].

Shigella spp./EIEC was, after *G. lamblia*, the second most frequent pathogen identified in children with diarrhea, increasingly occurring in older children. However, comparing our findings with other studies is challenging because our test was PCR based. Traditionally, diagnosing shigellosis relies on culturing techniques, which selectively isolates the pathogen, followed by a biochemical identification of one of the four Shigella species [25]. Introducing PCRs techniques has their benefits but the gene sequence used for the diagnosis is also carried by enteroinvasive

Escherichia coli (EIEC) [10]. Technically, our study cannot differentiate between Shigellosis and EIEC, which, in addition to the greater sensitivity of the PCR based test, might also be mirrored by the higher numbers of infections presented. Thus, despite its moderate association with diarrhea, the high prevalence of this pathogen group led to the second highest AF observed in our study.

Generally, the used PCR methods have a higher test sensitivity compared to conventional culture methods. This improves the ability to diagnose organisms in a stool sample. However, a drawback seems to be an increase in asymptomatic detections overall [26]. Since this affects diagnostics in both cases and controls the calculated ORs should not be affected. However, the estimated disease prevalence, and consequently the estimated AF, might be overestimated. Further studies using quantitative approaches [27] are needed to establish and improve diagnostic analyses for gastrointestinal diseases in low- and middle-income countries.

The burden of *G. lamblia* infections was quite high. Interestingly, the frequency of *G. lamblia* infections was lower in children with diarrhea than in asymptomatic carriers. A systematic review of the impact of *G. lamblia* on diarrhea highlighted that, although most studies show no or inverse effects, some studies report positive associations in children aged around 1 year, presumably as a response to initial *G. lamblia* infections [28]. However, the statistical power of the current study did not allow to disentangle such age-effects.

In both cases as well as controls high numbers of multiple infections were observed. Most co-infections were identified as statistically expected, although *G. lamblia* was more often found together with *E. dispar*, *C. jejuni*, and norovirus. *G. lamblia* has been reported to induce apoptosis of epithelial cells leading to increased epithelial permeability [29]. Further, *G. lamblia* was found to secrete proteins capable of impairing the innate immune response [30]. Alternatively, co-infections may be due to shared transmission routes. However, since most gastrointestinal organisms are transmitted via the fecal-oral route, it is unlikely that this alone explains the association among co-infections. Interestingly, a recent pooled case-control study from Ecuador also identified mechanistic interactions for diarrhea symptoms between rotavirus and *G. lamblia* as well as between rotavirus and *Escherichia coli* [31]. In vitro models have indicated that rotavirus may foster the adhesion, invasion, and multiplication of bacteria in enteric cells, mechanisms that may explain these synergistic effects [32–34]. Generally, the role of co-infections in diarrheal diseases deserves more attention in order to identify the associations between infections as well as interactions with GIS. Furthermore, strategies to identify causative pathogens in the presence of multiple infections are needed since diagnosing a particular isolate may not rule out other potential infectious causes in diarrheal disease.

The study presented here has several limitations, therefore the results should be interpreted with caution. Cases as well as controls were selected at a hospital OPD, thus the control group does not consist of healthy individuals. These are children seeking help for other health conditions that may increase the risk for gastro-intestinal infections. We have little background information on the total eligible study group, i.e., children that visited the OPD during the study period, from which we selected cases and controls. Thus, we cannot judge how well characteristics of cases and controls match. Table 1 highlights differences between cases and controls. For example, *falciparum* malaria is more frequently observed in controls, because controls need an alternative reason to attend the hospital, which is malaria in some attendees. Controls are also more likely to have a full vaccination schedule, which can be explained by age differences as well as due to possible differences in socio-economic status. In the analyses these factors cannot be controlled for, however, we believe that they do not act as a confounder. Even though some factors are associated with gastrointestinal infections, they are not associated with diarrheal symptoms, which would be needed to qualify as a confounder. Controls had to be diarrhea free at the point of study enrollment, yet GIS before enrollment were not assessed. Thus, controls

could be carriers of pathogens if infections occurred before study enrollment. For instance, norovirus can be found in stool for up to 60 days after infection [35]. In case some controls are pathogen carriers due to recent infections study results would underestimate the actual diarrheal association. Two preconditions need to be fulfilled to generalize AFs from case-control data: (i) the case-control selection must be representative of the source population and (ii) the OR must be a robust estimator of the RR. Cases in this study were recruited from children in the OPD, making this a select group of patients seeking professional care, thereby representing individuals with moderate to severe diarrheal disease. Considering our case-control sampling approach, the OR would approximate the RR only if the rare disease assumption is fulfilled. This, however, was applicable to all infections studied, especially not for *Shigella* spp./EIEC, *C. jejuni*, and norovirus. In these cases, the OR is likely to overestimate the true RR, resulting in a higher AF. Several possible infectious causes of diarrhea were not detected by these methods, including adenovirus [36] enteropathogenic *Escherichia coli* [37] and enterotoxigenic *Escherichia coli* [37,38].

The AFs express the proportion of diarrheal disease that would be reduced if an organism could be removed. This measure is highly relevant to public health concerns since it demonstrates the potential effects of disease prevention and control as well as empirical disease treatment measures. In particular, it highlights the potential roles of vaccinations against rotavirus and norovirus in sub-Saharan Africa, as well as of water purification, sanitation, and hygiene measures; effective options that can reduce the burden of diarrheal diseases [39].

Supporting Information

S1 Checklist. STROBE Checklist.
(DOCX)

S1 Dataset. Study data.
(CSV)

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Author Contributions

Analyzed the data: RK JA DE JM WL. Contributed reagents/materials/analysis tools: RMH ET JM. Wrote the paper: RK NS NGS DE RMH YAS ET JM. Designed the study: JA WL YAS ET JM. Conducted and supervised field work: NS JA WL.

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Health Care Utilization and Symptom Severity in Ghanaian Children – a Cross-Sectional Study

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Abstract

The aim of this study was to identify factors influencing health care utilization behavior for children with mild or severe disease symptoms in rural Ghana. Between March and September 2008 a cross-sectional health care utilization survey was conducted and 8,715 caregivers were interviewed regarding their intended behavior in case their children had mild or severe fever or diarrhea. To show associations between hospital attendance and further independent factors (e.g. travel distance or socio-economic status) prevalence ratios were calculated for the four disease symptoms. A Poisson regression model was used to control for potential confounding. Frequency of hospital attendance decreased constantly with increasing distance to the health facility. Being enrolled in the national health insurance scheme increased the intention to attend a hospital. The effect of the other factors diminished in the Poisson regression if modeled together with travel distance. The observed associations weakened with increasing severity of symptoms, which indicates that barriers to visit a hospital are less important if children experience a more serious illness. As shown in other studies, travel distance to a health care provider had the strongest effect on health care utilization. Studies to identify local barriers to access health care services are important to inform health policy making as they identify deprived populations with low access to health services and to early treatment.

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Introduction

The Millennium Development Goal (MDG) 4 stipulates that, between 1990 and 2015, the mortality rate of children aged younger than five years should be reduced by two thirds. Recent estimates suggest that childhood mortality is decreasing in the developing world but still not fast enough to reach MDG 4 [1]. Achievements to reduce childhood deaths differ strongly by geographical region and especially sub-Saharan-Africa is still burdened by high infant and child mortality rates [2,3]. Accessible professional health services make early treatment possible, thus reducing serious health consequences. Access to and provision of health services vary strongly between geographical regions, and even within countries [4,5]. Several studies in developing countries identified barriers to health care utilization (HCU) such as low socio-economic status, travel distance to service providers, poor knowledge about diseases or the perceived quality of the health care provider [6–9]. Disease severity in contrast, increased the likelihood of seeking professional medical treatment [7]. However, less is known about how HCU barriers differ between symptoms considering differences in symptom severity.

The aim of this study was to identify factors influencing health care utilization behavior for children with mild and severe fever or diarrhea in the Asante Akim North District, a typical rural area in Ghana.

Methods

Between March and September 2008 a cross-sectional health care utilization survey was conducted within the Asante Akim North District, Ghana.

The study and the informed consent procedure was approved by the Committee on Human Research Publications and Ethics of the College of Health Sciences, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Study area

The survey was conducted in two overlapping hospital catchment areas. The Agogo Presbyterian Hospital (APH) is a district hospital with about 250 patient beds. Among other facilities, it has a separate children's Outpatient Department (OPD) and a pediatric ward. The Konongo-Odumasi Hospital (KOH) is the government district hospital located in the district capital, with 42 patient beds of which eight belong to the children's ward. It has a general OPD for both children and adults. The study area has a rural character with an estimated population of 170,000 inhabitants spread over 1,160 square kilometers. The climate is tropical and the region is mainly covered by secondary rain forest and cultivated land.

Study population

Study households were randomly selected using a probability proportional to size cluster-sampling method. Sampling frame data was obtained from the Asante Akim North District Planning Office. Families had to live in the study area for at least six months in order to be selected. In a first step, local authorities (i.e. chiefs, assemblymen, opinion leaders) of the communities throughout the study area were approached to introduce the research team and to explain the purpose of the study carefully. When permission was given to conduct interviews residents were informed about the study highlighting that participation was voluntary. Appointments with the selected study households were made. Finally, field workers visited families at their home and interviewed caregivers of children aged up to twelve years after verbal informed consent was given. Consent was given in the presence of a witness and documented for each participant on a study form. Verbal consent was chosen because no samples or individual health data were collected. Study participants were free to terminate the interview at any time. One person refused to participate. Interviewees were asked questions regarding their health care seeking behavior in case their children showed a particular disease symptom, suggesting different severity stages of a febrile illness or a gastrointestinal infection. Symptom duration was used to differentiate between perceived disease severity. Symptoms considered for the study were (i) acute fever, (ii) fever for three days, (iii) acute diarrhea, and (iv) diarrhea for one week. In addition, demographic data, information about health behavior, and socio-economic data were collected, and the GPS (Global Positioning System) coordinates of the households were recorded.

The resulting dataset has previously been analyzed to identify the actual catchment population of APH in order to estimate incidences for childhood diseases [10–12], as well as to describe the association between socio-economic status and enrolment in the national health insurance program [13].

Study design

Participants could choose whether they would seek help at APH, KOH or another hospital, or whether they would visit a local healer, a pharmacy or treat their children at home. Interviewees who replied to visit APH or KOH if their children showed a particular disease symptom were classified as a hospital attendee. Non-attendees were defined as interviewees who would visit a local healer, a pharmacy, or treat their children at home. Due to the single choice character of the questioning the preferred hospital of non-attendees is unknown. To be included into the analyses, study participants had to belong to the respective hospital catchment groups of APH or KOH. Some study participants reported not to visit a hospital in case their child had acute fever or diarrhea, however they would visit a hospital other than APH or KOH if the respective more severe disease symptoms (i.e. fever for three days or diarrhea for one week) occurred. These individuals were removed from the analyses. Thus, different sets of study participants were selected, resulting in varying study sizes for different symptom groups.

Geographical data and distance measurements

The GPS coordinates of the households were mapped using ArcGIS 10 (Esri, Redlands, CA, USA). To calculate travel distances between the households and APH or KOH, a road map shapefile was acquired from DIVA-GIS (http://www.diva-gis.org/). In this shapefile, main streets and roads were recorded, however, smaller paths were not. Therefore, the air-line distance from each household to the nearest road, and the subsequent road distance to the hospitals were calculated with the Network Analyst extension (Esri, Redlands, CA, USA). The sum of path and road distance was used to estimate the individual travel distance to each hospital. Some study participants had an unreasonably long travel distance, especially when missing road information led to an unlikely combination of roads to travel. These study participants were identified via the distance ratio of travel distance over air-line distance. The travel distance of those study participants with a distance ratio above the 95th percentile were replaced using their air-line distance to the next hospital multiplied by the mean distance ratio in order to account for the longer road journey. For attendees the distance to their reported hospital was used in the analysis. For non-attendees the distance to their nearest hospital was used since no information about their preferred hospital was available. Consequently, only attendees that would attend their nearest hospital could be considered in the studies, as this restriction applied to non-attendees as well.

Data analysis

A relative socio-economic status (SES) score was constructed with a principal component analysis (PCA) [14]. Dichotomous asset and education variables were used to set up the score, namely living in a brick house, in-house toilet available, cooking inside, domestic tap-water available, electricity available, owning a fridge, difficulty to manage income, literacy of the mother, and literacy of the father. The constructed linear score was categorized to quartiles to generate the four SES-groups poor, low, moderate, and high.

To describe variable distributions the mean with the respective standard deviation (SD) was calculated for normally distributed continuous variables, or the median with the respective interquartile range (IQR) for the non-normally distributed continuous variables. Categorical variables were described showing the absolute frequency with the corresponding percent. Outcome measure of the study was the prevalence of hospital attendance, which is the number of attendees over the sum of attendees and non-attendees. To show associations between symptom-related HCU and travel distance, the prevalence of hospital attendance within a moving window along the sorted distance variable was calculated (moving prevalence). Each window contained 100 individuals and moved individual-wise through the travel distance. This method is adapted from the moving average approach to show smoothed changing proportions of a dichotomous variable along a sorted linear variable. Crude prevalence ratios (PR) and their corresponding 95%-confidence intervals (CI) were calculated via a cross-table to show associations between two dichotomous variables and effect differences along a second categorical variable were assessed by stratification. Finally, a Poisson regression analysis (with a robust error variance [15,16]) was performed to show interactions between independent variables and to control for potential confounding. The model was set up based on prior knowledge, including variables previously reported to be associated with HCU. To show how associations change between the different symptoms, the same set of independent variables was used to construct regression models for each symptom group. Observations with missing values in independent variables were ignored in the particular analyses. Thus, the denominator for some statistics may differ. Statistical analyses were performed using STATA 12 (StataCorp LP, College Station, USA).

Results

Study population

Throughout the study area 8,715 interviews were conducted in 138 villages. Village size varied from 18 to 15,383 inhabitants with a median size of 299 inhabitants (IQR 139-621). Almost all interviews were conducted with females (8,643; 99.2%) with a mean age of 36.2 years (SD 12.6). 3,321 interviewees (38.1%) reported to be enrolled in the national health insurance (NHI) scheme. Household size varied from two up to 32 people. The mean number of people per household was 6.0 (SD 2.8). The number of children per household varied between one and 25, with a mean number of 3.2 (SD 2.0) children. Living conditions differed strongly between families. Table 1 gives an overview of study participants' characteristics and the distribution of the variables used to model the SES-score.

According to the study participants, alternative health care facilities (HCF) were available in 27 of 138 villages, within which 4,631 (53.1%) of the interviewees lived. 2,618 (30.0%) reported to have some kind of medication at home. Of the children, 3,500 (40.2%) were born at home and 5,044 (57.9%) were born in a health facility. The place of birth for the remaining children was unknown. 519 (6.0%) of the interviewees reported to have never attended a hospital so far. Of the interviewees, 2,928 (33.6%) attended APH and 1,795 (20.6%) attended KOH at their last hospital visit. For 3,501 (40.2%) interviewees APH and for 5,214 (59.8%) KOH was the nearest hospital. Interviewees lived up to 72.9 km away from their nearest hospital, with a median travel distance of 12.9 km (IQR 3.1-23.8 km).

Table 1. Characteristics of the interviewees (N = 8,715), Asante Akim North District, Ghana, 2008.

Variable	frequency (%) or mean (SD[§])
Female interviewees	8,643 (99.2)
Age of interviewees, mean years	36.2 (SD 12.6)
Enrolled in National Health Insurance programme	3,321 (38.1)
People per household, mean number	6.0 (SD 2.8)
Children per household, mean number	3.2 (SD 2.0)
Illiteracy mother	6,101 (70.1)
Illiteracy father	3,152 (36.2)
Living in brick or stone houses	6,097 (70.0)
Domestic tab-water	6,797 (78.0)
In-house electricity	4,459 (53.5)
In-house cooking facilities	5,707 (65.5)
In-house toilets	2,846 (32.7)
Owning refrigerator	1,190 (13.7)
Difficulty to manage income	5,519 (63.3)

§. SD, Standard Deviation

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Hospital attendance

Different study groups comprising each around 50% of all observations were constructed for the symptom dependent HCU analyses. Table 2 shows the number of study participants who would attend a hospital for the different symptoms for all interviewees and the prevalence of hospital attendance within each symptom groups. A higher proportion of parents reported to visit a hospital in case their children had diarrhea compared to fever. For both fever for three days and diarrhea for one week, the prevalence of hospital attendance was more than doubled compared to the respective milder symptoms.

The binary associations between hospital attendance and potential influencing factors for all symptom groups are shown in Table 3. For all symptoms, the reported hospital attendance decreased with increasing travel distance, yet this trend was less marked for the more severe symptoms. The PR for distance suggests minor effects, however one has to consider that the prevalence change per 1 km unit was modeled. The PR of 0.98 (CI 0.98-0.99) as calculated for fever for three days, can be extrapolated to a respective PR of 0.85 (CI 0.83-0.86), 0.72 (CI 0.69-0.75), 0.61 (CI 0.57-0.65), and 0.51 (CI 0.47-0.56) for 10 km, 20 km, 30 km, and 40 km travel distance. Having a functional health care facility in the community did not influence the decision to attend a hospital. SES had the same effect for the milder symptoms, with the PR for clinic attendance increasing slightly towards the higher SES

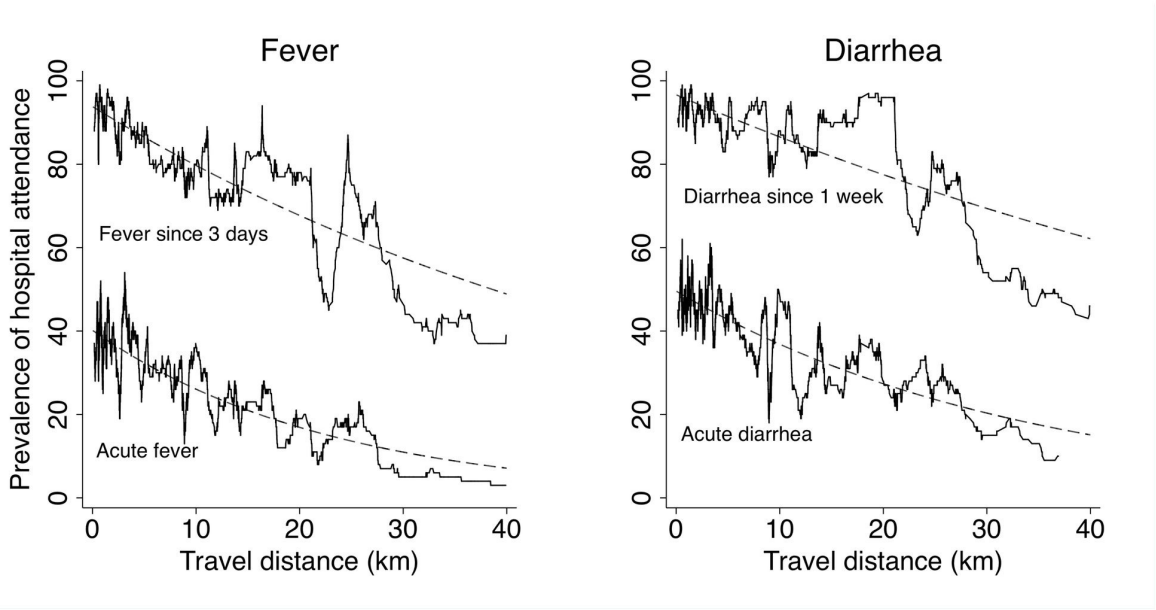

Figure 1. Observed prevalence (solid line) and predicted prevalence (dashed line) of intended hospital attendance by symptom along the travel distance to the nearest hospitals, Asante Akim North District, Ghana, 2008.
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Table 2. Disease symptoms and number of intended hospital visits within the whole group, the study size of the symptom-based study groups, and the intended hospital visits within the constructed symptom-based study groups, Asante Akim North District, Ghana, 2008.

Symptoms	Hospital attendance within the whole group[a]	Sample size of the symptom-based study group[b,c]	Hospital attendance within the symptom-based study group[b]
Acute fever	3,006 (34.5)	4,437 (48.3)	1,330 (30.0)
Fever for three days	7,944 (91.2)	4,320 (47.1)	3,570 (82.6)
Acute diarrhoea	3,952 (45.3)	4,285 (46.7)	1,738 (40.6)
Diarrhoea for one week	8,241 (94.5)	4,177 (45.5)	3,714 (88.9)

[a]. whole study group, comprising all interviewees (N = 8,715)

[b]. constructed study groups, comprising attendees and non-attendees for the respective disease symptoms

[c]. per cent of the whole study group

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quintiles. No association between SES and hospital attendance was observed for more severe symptoms. Similar associations were seen for enrolment in the NHI scheme.

The moving prevalence and the predicted prevalence (based on the Poisson regression analysis shown in Table 3) of hospital attendance along the travel distance is shown in Figure 1. The prevalence of hospital attendance continuously decreased with increasing distance between household and nearest hospital for all disease symptoms. However, the observed variability indicated that factors along the travel distance had an additional effect on the decision to visit a hospital. Some prevalence peaks occurred at distances where larger villages are located.

Multivariate Poisson regression model

Among the explanatory variables associations were observed between distance to hospital and SES group, as well as between being health insured and SES group. Individuals with a higher SES tended to live closer to a hospital, and individuals with a higher SES were more likely to be enrolled in the national health insurance scheme. To adjust for potential confounding and assess potential interaction between explanatory variables, multivariate regression models were built for each symptom. The regression models for all symptoms showed strong confounding and most variables lost their associations if modeled together with travel distance. In the models for mild fever and mild diarrhea, distance to hospital and health insurance were the only variables that showed a significant influence on hospital attendance (Table 4).

Discussion

The aim of the current study was to identify factors influencing a caregivers' decision to seek help in rural Ghana in case their children showed a particular disease symptom, taking varying disease severity into account. For the disease symptoms studied (i.e. acute fever, fever for three days, acute

Table 3. Bivariate associations (prevalence ratios with 95%-confidence interval) between hospital attendance and explanatory variables for the different disease symptoms, Asante Akim North District, Ghana, 2008.

Regressant	Distance (1 km unit)[a]	Alternative HCF[b$]	SES-groups (Q1 vs. Q2-Q4)[c$]	Health insurance[b]
Acute fever	0.96 (0.95-0.96)	1.0 (0.9-1.2)	Q1: ref.	1.4 (1.3-1.6)
			Q2: 1.4 (1.2-1.7)	
			Q3: 1.6 (1.4-1.9)	
			Q4: 1.7 (1.5-1.9)	
Fever for three days	0.98 (0.98-0.99)	1.0 (0.9-1.0)	Q1: ref.	1.1 (1.1-1.2)
			Q2: 1.1 (1.1-1.2)	
			Q3: 1.2 (1.1-1.3)	
			Q4: 1.2 (1.2-1.3)	
Acute diarrhoea	0.97 (0.96-0.98)	1.1 (1.1-1.3)	Q1: ref.	1.4 (1.3-1.5)
			Q2: 1.4 (1.2-1.5)	
			Q3: 1.5 (1.3-1.7)	
			Q4: 1.6 (1.4-1.8)	
Diarrhoea for one week	0.99 (0.99-0.99)	1.0 (1.0-1.0)	Q1: ref.	1.1 (1.0-1.1)
			Q2: 1.1 (1.1-1.2)	
			Q3: 1.1 (1.1-1.2)	
			Q4: 1.1 (1.1-1.2)	

[a]. calculated via Poisson regression, two decimal places shown to detail smaller effects

[b]. calculated via cross-tables

[c]. calculated via Poisson regression

[$]. HCF, health care facility; SES, socio-economic status; Q1-Q4, first quintile – fourth quintile

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Table 4. Poisson regression models of the relationship between hospital attendance and explanatory variables for the different disease symptoms, Asante Akim North District, Ghana, 2008.

Regressant	Distance (1 km unit)[a]	Alternative HCF[$]	SES-groups (Q1 vs. Q2-Q4)[$]	Health insured
Acute fever	0.96 (0.95-0.97)	1.0 (0.9-1.1)	Q1: ref.	1.3 (1.2-1.4)
			Q2: 1.1 (0.9-1.3)	
			Q3: 1.1 (0.9-1.3)	
			Q4: 1.1 (0.9-1.2)	
Fever for three days	0.98 (0.98-0.99)	0.9 (0.9-1.0)	Q1: ref.	1.1 (1.1-1.1)
			Q2: 1.0 (1.0-1.1)	
			Q3: 1.0 (1.0-1.1)	
			Q4: 1.0 (1.0-1.1)	
Acute diarrhoea	0.97 (0.97-0.98)	1.1 (1.0-1.2)	Q1: ref.	1.3 (1.2-1.4)
			Q2 1.1 (1.0-1.3)	
			Q3: 1.1 (1.0-1.3)	
			Q4: 1.1 (1.0-1.3)	
Diarrhoea for one week	0.99 (0.99-0.99)	1.0 (0.9-1.0)	Q1: ref.	1.1 (1.0-1.1)
			Q2: 1.0 (1.0-1.1)	
			Q3: 1.0 (1.0-1.1)	
			Q4: 1.0 (1.0-1.0)	

HCF, health care facility; SES, socio-economic status; Q1-Q4, first quintile-fourth quintile

a. two decimal places shown to detail smaller effects

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diarrhea, and diarrhea for one week) the intention to visit a hospital decreased constantly with increasing travel distance. Being enrolled in the NHI scheme increased the willingness to attend a hospital. The effect of SES and availability of a health facility in the home village diminished in the Poisson regression if modeled together with travel distance. The observed associations weakened with increasing symptom severity, which indicates that barriers to visit a clinic are less predominant if children experience a more serious illness.

Travel distance shows the strongest association with clinic attendance. Its effect has been shown in a number of previous studies and is often referred to as distance-decay. For instance, studies showed these decay effects for children [17–19], and adults [19] using actual health seeking behavior data, and for children [20,21] using reported health seeking behavior. Studies from Ethiopia [22] and Burkina Faso [23] demonstrated that child mortality increases with distance to health facilities. In the current study higher willingness to attend a hospital was observed at distances where larger villages are located suggesting that interviewees from larger villages have easier access to the hospitals, thus weakening the distance-decay. We used the estimated travel distance, which does not consider actual travel time or further travel related efforts. So far, little is known about the association of HCU and travel dependent factors. A study from Malawi showed higher likelihood of HCU for villages with access to public transport [24], and another study from Pakistan reported that the availability of public transport as well as the frequency of the provided services are important to access remote HCFs [25]. It appears highly plausible that availability of transport plays an important role, but issues such as affordability also need to be considered.

The current study does not show associations between SES and HCU after adjusting for travel distance. Associations shown in the bivariate analysis are confounded by travel distance, as interviewees living in remote rural areas tend to have a lower SES. Several studies have addressed the topic of SES and health seeking behavior and showed differing results across regions in the developing world. In Tanzania [26] and South Africa [27] higher SES groups had an increased likelihood of hospital attendance. Yet also in these studies, participants with a higher SES lived closer to the HCF, but the analyses were not controlled for potential confounders. In Uganda [28], Ghana [29], and Nigeria [30] individuals with lower SES utilize health services less often. Another study from Nigeria demonstrated that patients with a lower SES use professional malaria treatment less often but this association

could not be confirmed if controlled for the quality of the service provider [31]. The cited studies as well as the current study illustrate the methodological challenges to untangle confounded associations between SES and HCU.

In 2004 the National Health Insurance Scheme was implemented in Ghana. If insured, people receive free treatment for pre-defined health conditions. The insurance is social security and tax financed and had a coverage of 62% in 2009, with significant regional variations [32]. Evaluations showed that the NHI scheme improved the access to health care services but it is still fragmented [33] and less available for the poor [34]. In our study the enrolment in the NHI scheme increased the likelihood of HCU by approximately 30% (for the milder symptoms). This finding is supported by another study from Ghana, which analyzed the use of professional health care services for children with fever [35]. A universal health insurance, providing free access to professional treatment, could bridge the gap especially to poorer groups within the population.

Our data suggest that more efforts are mounted to overcome HCU barriers if the health conditions are perceived to be more serious. Disease severity has already been reported to be an important driver for treatment seeking in other studies [29,36]. Nevertheless, caregivers often underestimate the seriousness of diseases [37]. Easy access to professional medical treatment is crucial to prevent aggravation of diseases, and to reduce disease complications and mortality.

A limitation of the current study is that the analysis is based on self-predicted intended behavior of caregivers in case their children showed particular disease symptoms. This approach enables the analysis of health behavior with regard to different disease symptoms. However, we have to rely on reported behavior, which is prone to reporting bias. Interviewees are likely to over-report hospital attendance finally leading to an overall higher prevalence of HCU. An alternative approach would be the assessment of the actions taken by parents during the last illness of their child. This would also allow to differentiate effects of age and gender, which could not be assessed with the current methodology. Especially age differences are reported in the literature. For younger children caretakers tend to seek professional health earlier and more frequent compared to the elder ones [17,19,29].

Cross-sectional study designs allow the calculation of PRs, which yield comparable results among study groups even with different frequencies of the study outcome. This is not the case if the odds ratio (OR) is calculated from studies based on a cross-sectional sampling approach. Here the rare-disease-assumption has to be fulfilled to estimate the risk ratio; otherwise the OR is likely to overestimate the effect of a risk ratio [38]. Because the frequency of disease symptoms varies among the established symptom-based study groups, it was crucial to use a comparable effect estimator and thus PRs were calculated and the corresponding Poisson regression was applied.

Studies, like the current one, are informing public health policy makers to develop strategies to reach deprived population groups. Recent evaluation advocated this equity aspect in the provision of national health care services. Reaching most deprived population with less access to health services is a cost-effective approach to save lives in the developing world [39].

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Author Contributions

Analyzed the data: RK BK NGS JM. Contributed reagents/ materials/analysis tools: RK NS LE WL NGS YAS JM. Wrote the manuscript: RK NS BK LE NGS HZ YAS JM. Study design, supervision of interviews: NS YAS JM.

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Article

Drinking Water from Dug Wells in Rural Ghana — *Salmonella* Contamination, Environmental Factors, and Genotypes

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Abstract: Salmonellosis is an important but neglected disease in sub-Saharan Africa. Food or fecal-oral associated transmissions are the primary cause of infections, while the role of waterborne transmission is unclear. Samples were collected from different dug wells in a rural area of Ghana and analyzed for contamination with bacteria, and with *Salmonella* in particular. In addition, temporal dynamics and riks factors for contamination were investigated in 16 wells. For all *Salmonella* isolates antibiotic susceptibility testing was performed, serovars were determined and strains from the same well with the same serovar were genotyped. The frequency of well water contamination with Gram-negative rod-shaped bacteria was 99.2% (n = 395). Out of 398 samples, 26 (6.5%) tested positive for *Salmonella* spp. The serovar distribution was diverse including strains not commonly isolated from clinical samples. Resistance to locally applied antibiotics or resistance to fluoroquinolones was not seen in the *Salmonella* isolates. The risk of *Salmonella* contamination was lower in wells surrounded by a frame and higher during the rainy season. The study confirms the overall poor microbiological quality of well water in a resource-poor area of Ghana. Well contamination with *Salmonella* poses a potential threat of infection, thus highlighting the important role of drinking water safety in infectious disease control.

Keywords: *Salmonella*; disease transmission; drinking water; dug wells; risk factor

1. Introduction

Non-typhoid *Salmonella* (NTS) are distributed worldwide, in industrialized, as well as in resource-limited countries. The course of NTS infection in industrialized countries is usually a self-limiting diarrheal disease, and bloodstream infections are rare, occurring mainly in immunocompromised individuals [1]. In contrast, in sub-Saharan Africa, NTS are among the most common causes of bacteraemia [2,3]. In Ghana, studies demonstrated that *Salmonella enterica* is one of the most frequent blood culture isolates in febrile children [4–6].

Humans get infected with *Salmonella* through the consumption of contaminated food of animal origin, such as poultry, egg and milk products, and contaminated water, or via the fecal-oral route. In Africa, access to bottled drinking water especially in rural areas is rare and the role of drinking water in *Salmonella* infection is unclear. Main sources for drinking water are rivers, lakes and wells, which often are contaminated by soil, rubbish, dust, and animal droppings. Previous studies in Africa have emphasized the problem of drinking water contamination not only with fecal bacteria but also with *Salmonella*, indicating that such water may be unsafe for consumption [7,8]. In Ghana, little information is available on the prevalence of *Salmonella* serovars in water sources and whether these are associated with human infections. In addition, there is no data describing the association of well characteristics and other risk factors for well water contamination with *Salmonella*.

Supply of safe drinking water is high on the political agenda of international institutions. The Millennium Development Goal (MDG) 7.C, released in 2000 by the United Nations, aspires to halve the proportion of people without sustainable access to safe drinking water and basic sanitation by the end of 2015 [9]. The World Health Organization (WHO) has been regularly updating their guidelines for drinking water quality [10]. Even though much has been learned in recent years, data on risk factors for water and sanitation related infections and modes of water-borne pathogen transmission are scarce, but needed to guide local prevention programs. Hence, to assess drinking water quality, water sources have to be assessed for fecal contamination. To understand whether the same *Salmonella* strains present in water sources are associated with human infections, typing of *Salmonella* isolates found in drinking water sources is necessary. The question, how to protect a water source from fecal contamination seems to be simple, however, in order to protect the health of water users it is important to identify simple measures, such as putting a lid on the well, which may reduce contamination of water by enteric pathogens. The aim of this study was to generate information on (i) the frequency of water contamination, in particular with *Salmonella enterica*, in wells over time and (ii) to determine external factors associated with well water contamination in order to recommend on contamination preventing well construction and maintenance. The study was conducted in a rural village within the Asante Akyem District in Ghana, an area where many people use local dug wells as drinking water sources.

2. Methods

In 2009 and 2010, well water samples were collected in Asankare, a rural village situated in the Asante Akyem District in Ghana. Asankare is a typical rural village of Ghana, in which improved sanitation facilities are not available. Livestock is commonly found in the area of the wells and surface water from rivers and lakes is present. In the rainy seasons, flooding of the area is frequent.

Different sources for drinking water existed, however the main water sources were local dug wells (Figure 1). Sixteen wells with guaranteed access throughout the study period were randomly selected. These wells were sampled once every two weeks for a period of one year and temporal changes of contamination investigated. In total, wells were sampled 26-times throughout the study year.

For each sample, 200 mL water was taken from a well, 100 mL for culturing Gram-negative rod-shaped bacteria and 100 mL for culturing *Salmonella*. Samples were transported to the microbiology laboratory of the Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR) in a cool box containing ice packs within 4 hours after sample collection. At KCCR, each sub-sample was filtered using a 0.45 µm pore cellulose membrane filter (Millipore, Cork, Ireland). For the colony counts of Gram-negative rods, the filter was directly placed onto a MacConkey agar selective for Gram- negative bacteria (Oxoid Ltd., Basingstoke, Hampshire, England) and incubated at 35–37 °C for 18–24 h. Following incubation, the colony counts for total Gram-negatives were classified into three groups, *i.e.*, <10, 10–100 or >100 colony forming units (CFU) per 100 mL.

To culture *Salmonella*, the filter was placed in Selenite F broth (Oxoid) for enrichment, the broth streaked after 18–24 h incubation on a chromogenic selective *Salmonella* agar (Oxoid) and incubated at 35–37 °C for 18–24 h. From positive *Salmonella* cultures, one pure colony was picked and used for further testing. *Salmonella* were identified using a latex agglutination Test (Oxoid) and the API 20E

biochemical identification strip (bioMérieux, Marcy L'etoile, France). Susceptibility was tested for a set of antibiotics commonly used in the study area, *i.e.*, ampicillin, ampicillin/sulbactam, ceftriaxone, chloramphenicol, ciprofloxacin, cotrimoxazole, nalidixic acid (used as a screening test for ciprofloxacin) and tetracycline, following the Clinical and Laboratory Standards Institute (CLSI) guidelines. *Salmonella* isolates were stored at –80 °C until transport to Germany on dry ice. For all *Salmonella* isolates, the serovar was determined by slide agglutination following the Max von Gruber method [11] using *Salmonella* specific antisera (SIFIN GmbH, Berlin, Germany) and the White-Kauffmann-Le Minor Scheme [12] (carried out at the Bernhard Nocht Institute for Tropical Medicine).

Figure 1. Example of a covered well surrounded by a frame in the village of Asankare, Asante Akyem District, Ghana (copyright Denise Dekker).

To establish relationships amongst the isolated *Salmonella* from water samples, strains with the same serovar and from the same well were further typed by Pulsed-field gel electrophoresis (PFGE). After *XbaI* (Roche Diagnostics GmbH, Mannheim, Germany) restriction, PFGE settings were as follows: initial switch time 1 minute, final switch time 40 seconds, run time 22 hours, voltage 6 V/cm and included angle 120°. Gel Compar II software (Applied Maths, Sint-Martens-Latem, Belgium) was used to compare PFGE patterns, using DICE, UPGMA, 9.5% optimization, 1.0% position tolerance, with Lambda Ladder as a molecular marker (carried out at the Institute for Medical Microbiology, Virology and Hygiene, University Hospital Rostock).

For each well, contamination was assessed using the frequency with which CFU Gram-negative rod-shaped bacteria were found in water samples and the number of positive *Salmonella* cultures observed throughout the study year.

Furthermore, repeated water contamination in wells was analyzed. When a well was repeatedly contaminated with *Salmonella*, the serovar identified in each of these samples along with its clonal identity were assessed to determine *Salmonella* persistence.

The longitudinal data of the repeatedly sampled wells was analyzed to establish the link between *Salmonella* contamination, well characteristics, and seasonality. The dataset follows a hierarchical structure, where water samples are taken at different time points (level one-unit) from particular wells (level two-unit). Hence, a random-effect regression model for repeated binary responses was applied to analyze the data [13]. Sampling points at which identical *Salmonella* clones were identified in consecutive water samples were removed from the analysis, as persistent contamination was assumed. Missing data, e.g., when a well was locked or dried out, were excluded from the analysis.

The following covariates were considered to describe well characteristics: closure of well (wooden/ metal cover), presence of a frame (concrete frame, categorized into below or above 30 cm), presence of rubbish in well and seasonality (rainy *vs.* dry season). Odds Ratios (OR) and the corresponding 95% confidence intervals (CI) were calculated. Adjusted ORs are reported from multivariate regression models to account for confounding. Model selection was based on a likelihood-ratio test.

3. Results

Sixteen wells were sampled biweekly from 10 November 2009 to 17 November 2010, *i.e.*, at 26 sampling time-points. Eighteen samples could not be collected; hence 398 water samples were analyzed. The analysis is divided into the following parts: (i) well contamination with Gram-negative rod-shaped bacteria and *Salmonella*, (ii) *Salmonella* serovars and antibiotic susceptibility, (iii) PFGE genotypes of consecutively contaminated wells, and (iv) well characteristics and their associations with *Salmonella* contamination.

3.1. Contamination with Gram-Negative Rod-Shaped Bacteria

Out of the 398 water samples tested, 395 (99.2%) were contaminated with >100 CFU/100 mL Gram-negative rods. In the remaining three (0.8%) water samples between 10 and 100 CFU/100 mL were detected.

3.2. Salmonella Isolates and Serovars

Of the 398 tested well water samples, 26 (6.5%) tested positive for *Salmonella* (Figure 2). One isolate was lost during culture for serotyping and in one sample two serovars were detected. The following serovars were identified: *S.* Pramiso (n = 2), *S.* Stanleyville (n = 4), *S.* Rubislaw (n = 4), *S.* Duisburg (n = 3), *S.* Nima (n = 2), *S.* Saarbruecken (n = 3), *S.* Give (n = 1) and *S.* Colindale (n = 7). *Salmonella* were never isolated from six of the 16 study wells. The remaining ten wells tested positive between one and eight times each.

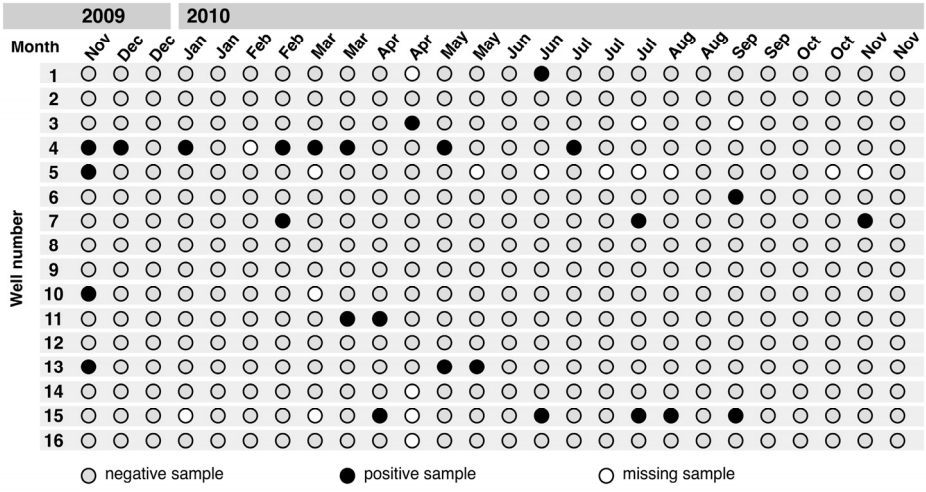

Figure 2. *Salmonella* contamination in 16 dug wells throughout the study period in Asankare, Asante Akyem District, November 2009–November 2010.

3.3. Clusters of Salmonella Isolates

Contamination of wells with *Salmonella* was highest in November 2009. *Salmonella* clusters were observed from February 2010 until May 2010. *Salmonella* colonization was less frequent from January 2010 until February 2010, and from September 2010 until November 2010. Seasonal associations and *Salmonella* clusters are further described below.

3.4. Antibiotic Susceptibility

Antibiotic susceptibility of *Salmonella* is shown in Table 1. Resistance was restricted to ampicillin and tetracycline in one of the isolates and to ampicillin and ampicillin/sulbactam in another isolate.

Table 1. Antibiotic susceptibility of *Salmonella* isolates (N = 26) found in Asankare wells, Asante Akyem District, November 2009–November 2010.

	Frequency (%)	
Drug (AC µg)	Susceptible	Resistant
Ampicillin (10)	24 (92.3)	2 (7.7)
Ampicillin/Sulbactam (20)	25 (96.2)	1 (3.8)
Ceftriaxone (30)	26 (100.0)	0 (0)
Chloramphenicol (30)	26 (100.0)	0 (0)
Ciprofloxacin (5)	26 (100.0)	0 (0)
Cotrimoxazole (25)	26 (100.0)	0 (0)
Nalidixic acid (30)	26 (100.0)	0 (0)
Tetracycline (30)	25 (96.2)	1 (3.8)

AC: antibiotic concentration.

3.5. PFGE Genotyping

Salmonella of the same serovar and isolated from the same well were eligible for PFGE to identify clonal clusters. Clonal identity was accepted at 95 % identity. In total, eight *Salmonella* isolates from three wells were discriminated to seven distinct clones by PFGE. Thus, individual wells were mostly colonized by different strains. From one well, a single clone persisted for about four weeks. PFGE results stratified by well are shown in Figure 3.

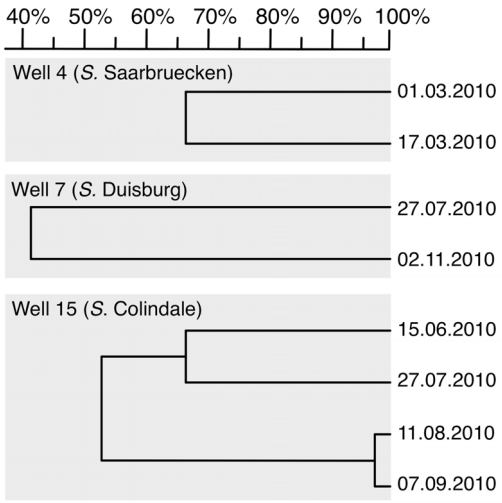

Figure 3. Dendrogram of pulsed-field gel electrophoresis (PFGE) profiles of three different serovars showing the genetic relationships for *Salmonella* isolates in Asankare wells, Asante Akyem District. The numbers on the right indicate sampling date. The numbers on the top (scale) indicate the genetic identity in percent.

3.6. Well Characteristics and Salmonella Contamination

Three hundred and ninety-seven well water samples were included in this analysis, of which 25 (6.3%) were *Salmonella* positive and 372 (93.7%) were negative. One observation was discarded because of persistent *Salmonella* contamination. Most samples were from covered wells (n = 269; 67.8%) and from wells with a frame (n = 294; 74.1%). The majority of samples were collected during the rainy seasons, from March to July, and September to November. Table 2 shows bivariate (Model A–D) and multivariate (Model E) random-effect models on well characteristics and their associations with *Salmonella* contamination. In the bivariate models, a frame with a minimum height of 30 cm indicated a possible protective association (OR: 0.3; 95% CI = 0.1–1.3) and rainy season a risk factor (OR: 2.6; 95% CI = 1.2–5.6) for *Salmonella* contamination. The multivariate model confirmed the protective effect of a well frame (OR: 0.3; 95% CI = 0.1–0.8) and the risk of rainy season (OR: 2.6; 95% CI = 1.2–5.5). In addition, an association between *Salmonella* contamination and the presence of rubbish was indicated (OR: 2.9; 95% CI = 0.9–9.6).

Table 2. Description of well characteristics and results from the random-effect models for well characteristics and *Salmonella* contamination, Asankare wells (397 sampling occasions from 16 wells), Asante Akyem District.

Independent Variables	Frequency (%)	Random-Effect Models OR (95% CI)				
		Model A	Model B	Model C	Model D	Model E
Presence of frame (>30 cm)	294 (74.1)	0.3 (0.1–1.3)	–	–	–	0.3 (0.1–0.8)
Well covered	269 (67.8)	–	2.0 (0.3–13.6)	–	–	–

Table 2. *Cont.*

Independent Variables	Frequency (%)	Random-Effect Models OR (95% CI)				
		Model A	Model B	Model C	Model D	Model E
Rubbish in well	75 (18.9)	–	–	2.6 (0.9–7.4)	–	2.9 (0.9–9.6)
Season (rainy *versus* dry)	272 (68.5)	–	–	–	2.6 (1.2–5.6)	2.6 (1.2–5.5)
Random Intercept Variance (SE)	–	0.89 (0.82)	2.96 (3.21)	0.96 (0.49)	1.68 (0.97)	0.76 (1.28)

OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval; SE: standard error.

4. Discussion

The main findings of this study are that (i) the majority of well water samples (99.2%) were contaminated with Gram-negative rod-shaped bacteria with >100 CFU/100 mL, (ii) 6.5% of water samples were contaminated with *Salmonella*, (iii) resistance to locally administered antibiotics was negligible in the *Salmonella* strains isolated from well water, (iv) the serovar distribution consisted of serovars not typically seen in clinical specimens, (v) wells were colonized by strains that persist for several weeks and (vi) certain well characteristics were associated with *Salmonella* contamination.

WHO standards for potable water demand the total absence of coliforms and fecal indicator bacteria [14]. In this study, nearly all samples were contaminated with Gram-negative rod-shaped bacteria above 100 CFU/100 mL, indicating a potential health hazard for the local population. The current study could not reveal the spectrum of organisms present in the water samples, nevertheless the colonial morphology pointed to fecal contamination. The number of infections actually transmitted via well water was not assessed since simultaneous gastrointestinal infections were not monitored during the study period.

At present, little information is available on the prevalence of *Salmonella* serovars in water sources in developing countries, yet studies have shown the presence of various human-pathogenic organisms in the aquatic environment [15–17]. Despite these reports, the *Salmonella* serovars identified in the present study are not those typically seen in clinical samples, but are rather known to be associated with reptiles or poultry. Serovars commonly found in reptiles are *S.* Pramiso [18], *S.* Rubislaw [19], and *S.* Nima [20]. *S.* Duisburg [21] and *S.* Saarbruecken [22] are found in poultry. Isolated serovars with pathogenic potential for humans are *S.* Stanleyville [23], *S.* Give [24] and *S.* Colindale [25]. Nevertheless, it has to be considered that all detected pathogens occur infrequently in clinical samples.

In the aquatic environment *Salmonella* are expected to be present in low concentrations. As only quite small amounts of water were sampled, the test sensitivity may be limited. In addition, the reported number of serovars per sample may be an underestimate as per positive sample only one colony was picked for further testing and, thus, for serotyping.

PFGE genotyping showed identical *Salmonella* clones at different sampling time points. This could be a result of repeated contaminations with the same genotype circulating in the environment but is more likely due to persistence of distinct clones for several weeks. Previous work has shown the capability of *Salmonella* to survive in water for several months [26].

Our study demonstrates well characteristics associated with *Salmonella* contamination. A frame around the well is likely to have a protective effect as it serves as a barrier against intrusion by animals, soil and dust. This effect was observed in a previous study on well water quality, where lined wells topped with a concrete parapet had lower concentrations of fecal indicator bacteria, especially at the beginning of the wet season [27]. A frame might prevent contamination of well water by surface run offs containing animal and human feces and soil, an assumption supported by the observed association between *Salmonella* contamination and rainy season. In rural areas, like in the Asankare village, animals roam freely. Studies in stray dogs and cats in the United States have shown carriages of *Salmonella* as high as 51.4% [28]. Reptiles and amphibians, that can easily fall or hide inside the wells, are also potential *Salmonella* carriers [29]. An additional risk factor for *Salmonella* contamination may be the presence of rubbish in well water. Rubbish might just indicate that the well is prone to contamination generally or it may contribute directly to *Salmonella* contamination.

Limitations of this research include the fact that the calculated odds ratios between environmental factors and *Salmonella* presence are of low precision. Furthermore, the assessed risk factors were not studied on a larger scale. For instance no data on the weekly precipitation was available and the frame height was analyzed without considering construction quality. Studying these associations with a larger sample size throughout a broader study region and including other potential risk factors would generate more valuable information to guide well construction and maintenance. In addition, the culture results of the Gram-negative rod-shaped bacteria were not reported as CFU per 100 ml but rather as categories, hence nearly all data fit a single category. As a consequence, it is not possible to identify measures of Gram-negative rods that might indicate relatively low probability of contamination.

It is estimated that 10% of the total burden of disease worldwide is related to water, sanitation and hygiene. Of this 62% are associated with drinking water and sanitation alone [30], mainly childhood diarrheal diseases. To evaluate progress towards the MDGs water targets, water sources are classified as improved drinking water sources (*i.e.*, piped water on premises, public taps, boreholes, or protected dug wells) or unimproved sources (*i.e.*, unprotected dug wells, unprotected springs or surface water) [31]. Globally, access to improved water sources has increased during recent years and the MDGs drinking water target has become one of the first Millennium Goals to be met [32]. However, significant disparities exist between and within regions, for example access to improved water sources is lowest in sub-Saharan Africa and in the poorer rural areas within countries [14]. In the current study, all wells were contaminated at nearly all sampling dates with fecal indicator organisms above the WHO threshold. Interestingly, the sampled wells belong to both categories, protected and unprotected dug wells. The current study suggests a more sophisticated classification may be necessary to differentiate between improved and unimproved sources.

5. Conclusions

The study results provide an overview of the level of contamination in wells with Gram-negative rod-shaped bacteria and on the presence of unusual *Salmonella* serovars seen infrequently in patients but more often in reptiles and poultry. Studying animal reservoirs would provide useful information to identify the source of such contaminations. External factors leading to contamination of water have been identified. Building a well with an impervious frame, for example, might be a first step protecting

water from contamination. In general, more insight into the risks of water contamination and its link to human infections are needed to promote measures effective in the reduction of water-borne disease. This is especially necessary as improved water sources are not necessarily safe water sources, given the high concentrations of Gram-negative rod-shaped bacteria and intermittent presence of *Salmonella*.

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Author Contributions

Denise Dekker planned the study, collected samples, led the microbiological analyses and drafted the manuscript; Ralf Krumkamp conducted the statistical analyses and drafted the manuscript; Nimako Sarpong planned and coordinated the fieldwork for the study; Hagen Frickmann planned the PFGE analysis and helped in the interpretation of all microbiological results and supported writing the manuscript; Kennedy Boahen, Michael Frimpong, Renate Asare and Richard Larbi collected samples and carried out the laboratory work for the water analyses; Ralf Hagen, Sven Poppert, Florian Marks and Yaw Adu-Sarkodie contributed to the conceptualization of this study and supported writing the manuscript; Wolfgang Rabsch supported the serotyping of *Salmonella* and helped writing the manuscript; Jürgen May planned the study and contributed to data analyses and manuscript drafting. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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RESEARCH ARTICLE

Urbanicity and Paediatric Bacteraemia in Ghana—A Case-Control Study within a Rural-Urban Transition Zone

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Abstract

Background

Systemic bacterial infections are a major cause of paediatric febrile illness in sub-Saharan Africa. Aim of this study was to assess the effects of social and geographical determinants on the risk of bacteraemia in a rural-urban transition zone in Ghana.

Methods

Children below 15 years of age with fever were recruited at an outpatient department in the suburban belt of Kumasi, Ghana's second largest city. Blood was taken for bacterial culture and malaria diagnostics. The socio-economic status of participants was calculated using Principle Component Analysis. A scale, based on key urban characteristics, was established to quantify urbanicity for all communities in the hospital catchment area. A case-control analysis was conducted, where children with and without bacteraemia were cases and controls, respectively.

Results

Bacteraemia was detected in 72 (3.1%) of 2,306 hospital visits. Non-typhoidal *Salmonella* (NTS; n = 24; 33.3%) and *Salmonella typhi* (n = 18; 25.0%) were the most common isolates. Logistic regression analysis showed that bacteraemia was negatively associated with urbanicity (odds ratio [OR] = 0.8; 95% confidence interval [CI]: 0.7–1.0) and socio-economic status (OR = 0.8; 95% CI: 0.6–0.9). Both associations were stronger if only NTS infections were used as cases (OR = 0.5; 95% CI: 0.3–0.8 and OR = 0.6; 95% CI: 0.4–1.0, respectively).

and analysis, decision to publish, or preparation of the manuscript.

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Conclusions

The results of this study highlight the importance of individual as well as community factors as independent risk factors for invasive bacterial infection (IBI) and especially NTS. Epidemiological data support physicians, public health experts and policy makers to identify disease prevention and treatment needs in order to secure public health in the transitional societies of developing countries.

Introduction

In 2012, three out of four deaths in African children under the age of five were due to communicable diseases and acute febrile illness was the most common cause of hospital admission on the continent [1,2]. Malaria is the predominant cause of systemic febrile illnesses in sub-Saharan Africa. Yet, additional causes of fever are increasingly the focus of research and public health campaigns [3,4]. Studies on paediatric febrile illness emphasize the role of invasive bacterial infections (IBI), which were found in up to 13% of febrile hospital admissions [1]. Moreover, little is known about the social and geographical determinants of IBI in Africa [5–7]. So far, only two studies from Kenya have described rural/urban differences in the incidence of *Salmonella* infections. Non-typhoidal *Salmonella* (NTS) was the leading rural pathogen with an incidence rate ten-fold higher compared to an urban site [6]. *S. typhi*, in contrast, had a fifteen-fold higher incidence rate amongst the urban population [7]. These findings suggest that factors related to the level of urbanicity may affect the distribution of IBIs. However, it is unclear whether individual or community factors are the major determinants of this discrepancy.

Developing countries are in the midst of massive social and economic transition. It is estimated that by 2050 Africa's urban population will have increased from 400 million to 1.2 billion. As a result, informal settlements with poor levels of infrastructure will extend and social differences will increase [8]. These profound changes in lifestyle and living conditions are likely to affect infectious disease epidemiology [9]. A better understanding of the epidemiological impact of social transition is crucial for future public health strategies in developing countries.

The aim of this study was to assess the effects of social and geographical determinants on the risk of bacteraemia in a rural-urban transition zone in Ghana. To account for the complexity of urbanization processes in developing countries [10], an urbanicity scale was developed, using rural/urban-key features representing the various aspects of urbanization in a developing region. In addition, the individual socio-economic status of study participants was estimated and both community-level and individual effects on the risk of childhood bacteraemia were assessed.

Methods

Study area

The study was conducted in the suburban belt of Kumasi, the capital of the Ashanti Region in Ghana. Kumasi is the region's economic and administrative centre and, with a total population of two million people, Ghana's second largest city. An annual population growth rate of 7.4% over the last decade has led to the expansion of Kumasi's periurban zone, particularly along the principal exit roads [11]. Study participants were recruited at St. Michael's Hospital (SMH) in Pramso, a town with 3,259 inhabitants located 20 km southeast of Kumasi along one of the major roads leaving the city. SMH is the biggest health care facility in the Bosomtwe District with a catchment area covering both Kumasi Metropolis and the Bosomtwe District. The

Bosomtwe District stretches from the suburbs of Kumasi to a largely rural countryside. Most of the primary vegetation has been cleared, giving way to predominantly bush and farmlands. Malaria is endemic with high transmission rates throughout the year [12]. Ghana's under-five mortality rate is 72 deaths per 1000 live births and the HIV prevalence is 1.4% amongst the adult population [13].

The Committee on Human Research, Publications and Ethics at the School of Medical Sciences, Kwame Nkrumah University of Science and Technology in Kumasi approved the study protocol and informed consent process.

Study population

Between January and December 2012, all children below 15 years presenting to the Outpatient Department (OPD) at SMH with fever (tympanic temperature of $\geq$38°C) were recruited if their caregiver gave written informed consent. Medical history and further socio-demographic data were obtained.

Laboratory diagnostics

A blood sample was taken from every child by venepuncture for malaria diagnosis and bacterial blood culture. One to three millilitres of blood were injected into vials for paediatric blood cultures (Becton Dickinson, NJ 07417, USA) and incubated in an automated BACTEC 9050 culturing instrument (Becton Dickinson). Broth from positive bottles was examined microscopically (Gram stain) and was further cultured on standard media (chocolate agar, MacConkey agar, and Columbia agar with 5% sheep blood). For malaria diagnosis Giemsa-stained thin and thick blood slides were prepared. Two independent readers examined the slides and a third reading was performed in case of discrepancies. In 39 (1.7%) patients malaria results were not available, and these patients were excluded from the respective analyses.

Urbanicity scale

A continuous numeric scale of urbanicity was constructed and validated according to the principles of scale development as described by DeVellis [14] and Netemeyer & Bearden [15]. To construct the scale, data on community characteristics were selected by literature review as well as extracted from the "2010 Population & Housing Census" conducted by the Ghana Statistical Service [16]. Additional information on available private services, road conditions and public transport within the communities was collected via systematic interviews in the communities. Variables were grouped into eight thematic scale components; namely population size, economic activity, education, health services, transportation, public and private services, sanitation, and housing. Each of these components accounted for ten points while variables within a component were weighted equally. Consequently, the final scale ranged from 0 to 80 points. To obtain a score for each community, points were accumulated according to the community's particular characteristics. Unidimensionality of the scale was tested by exploratory factor analysis (EFA), Kaiser-Meyer-Olkin (KMO) measure was used to assess sampling adequacy and internal consistency was validated using Cronbach's alpha [17]. For more details on scale construction and validation results please refer to the supplementary material (Supplement 1).

Socio-economic status

To estimate the socio-economic status (SES) at an individual level, a scale was established using Principal Component Analysis (PCA) [18]. It was conducted with variables containing individual data on mother's education, living conditions (i.e., type of cooking energy, source of

drinking water, type of sanitation) and ownership of assets (i.e., mobile phone, television, computer, fridge). The model's correlation matrix was constructed using tetrachoric correlation coefficients for dichotomous variables. Again, sampling adequacy and internal consistency were assessed by KMO measure and Cronbach's alpha, respectively.

Statistical analysis

Participants with a positive blood culture served as cases, while controls had a negative blood culture result. A second case-control set up was done on isolate level, where NTS infection, the most common blood isolate in the study, defined cases while the same control group was used. Patients who showed growth of probable or possible contaminants (e.g., *Bacillus spp*., *Micrococcus spp*. or coagulase-negative *Staphylococcus spp*.) were excluded from the study.

Associations with bacteraemia or NTS infection were calculated by logistic regression and displayed as odds ratios (ORs) with their corresponding 95% confidence intervals (CI). For this purpose, SES and urbanicity scores were categorized into four quartile-based groups (i.e., first quartile contains lowest SES/urbanicity score; fourth quartile contains highest SES/urbanicity score). Spearman's Rank Correlation Coefficient was calculated to assess associations between categorical variables. Finally, multivariate logistic regression models were established to calculate adjusted odds ratios (aOR) accounting for potential confounding. All analyses were carried out using Stata 12 (StataCorp LP, College Station, USA).

Results

A total of 2,306 hospital visits were included in the analysis. Patients were hospitalized for 375 (16.3%) of these visits, with an average hospital stay of 2.8 days [standard deviation (SD): ±1.3]. Girls were slightly underrepresented in the study group (n = 1,071; 46.4%). The median age of the study children was 34 months (Interquartile range [IQR]: 16–63 months). Characteristics of the study participants are summarised in Table 1.

Laboratory results

Bacteraemia was detected in 72 (3.1%) patients, who served as study cases. Median age of cases was 30 months (IQR: 18–65). Most frequent pathogens were NTS (n = 24; 33.3%), *Salmonella*

Table 1. Characteristics of cases and control living in the rural/urban study area.

Characteristics	Cases	Controls
Total, n (%)	72 (3.1)	2,234 (96.9)
Median age, months (IQR)	30 (18–65)	35 (16–62)
Female, n (%)	30 (41.7)	1,041 (46.6)
Malaria, n (%)	16 (22.2)	850 (38.8)
Median urbanicity, score (IQR)	46.5 (27.3–54.0)	53.5 (35.3–58.3)
Urbanicity quartile 1, n (%)	29 (40.3)	547 (24.5)
Urbanicity quartile 2, n (%)	13 (18.1)	440 (19.7)
Urbanicity quartile 3, n (%)	14 (19.4)	512 (22.9)
Urbanicity quartile 4, n (%)	16 (22.2)	735 (32.9)
Median SES, score (IQR)	1.4 (0.9–1.9)	1.7 (1.1–2.2)
SES quartile 1, n (%)	24 (33.3)	527 (23.6)
SES quartile 2, n (%)	24 (33.3)	573 (25.7)
SES quartile 3, n (%)	16 (22.2)	537 (24.0)
SES quartile 4, n (%)	8 (11.1)	597 (26.7)

Abbreviations: SES, socio-economic status; IQR, interquartile range.

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Typhi (n = 18; 25.0%) and *Streptococcus pneumoniae* (n = 15; 20.8%). Controls, which were blood culture negative, had a median age of 35 months (IQR: 16–62).

Malaria parasites were detected in 16 (22.2%) cases and 850 (38.8%) controls. In 1,345 (61.3%) patients neither bacteraemia nor parasitaemia were detected.

Study villages and urbanicity

The catchment area of SMH covered 73 communities spread over 375 km^2. Forty-nine (67.1%) communities were located in Bosomtwe District, 22 (30.1%) in Kumasi Metropolis and one each in Ejisu Juaben District and in Atwima Kwanwoma District. The population size per community ranged from 214 to 72,105 inhabitants, with a median of 1,608 (IQR: 738–8,150) inhabitants. The communities scored a minimum of 6.0 and a maximum of 78.5 points on the urbanicity scale, with a median of 31.3 (IQR: 20.5–65.3) points. A map with the study villages and their level of urbanicity is shown in Fig 1.

Validation of the scale showed good unidimensionality (eigenvalue of the first factor = 6.1; overall variance explained by the first factor: 96.6%), good sampling adequacy (KMO measure for the first factor = 0.93), and a high internal consistency (Cronbach's alpha = 0.96).

Socio-economic status

To quantify the individual SES of study participants, a PCA was conducted on eight socio-economic characteristics. The analysis yielded an eigenvalue of 4.1 for the first component, with eigenvalues of 1.0 and below for the remaining components. A SES score was calculated from the first component of the PCA, which accounted for an overall variance of >50%. The KMO measure of 0.86 indicated good sampling adequacy, KMO values for all used variables were greater than 0.78. Cronbach's alpha of 0.70 showed moderate internal consistency.

Urbanicity, SES and bacteraemia

Overall, the proportion of positive blood cultures decreased with increasing urbanicity. From the lowest to the highest urbanicity group bacteraemia was diagnosed in 29 (5.0%), 13 (2.9%), 14 (2.7%), and 16 (2.1%) patients, respectively (Fig 2). In the bivariate analysis, the proportion of patients with bacteraemia decreased with an OR of 0.7 (95% CI: 0.6–0.9) along the urbanicity groups.

Looking at NTS alone, the frequency and proportion of cases showed a marked linear trend along the urbanicity groups. NTS was found in 14 (2.5%), 6 (1.4%), 3 (0.6%), and 1 (0.1%) patients from the lowest to the highest category (Fig 1). The association between urbanicity and NTS was strong with an OR of 0.5 (95% CI: 0.3–0.7) per category step. When NTS cases were excluded from the group of bacteraemia cases no statistical association between bacteraemia and urbanicity could be shown (OR = 0.9; 95% CI: 0.7–1.2).

The proportion of patients with bacteraemia decreased towards higher SES groups as well. From the lowest to the highest SES group, bacteraemia was found in 24 (4.4%), 24 (4.0%), 16 (2.9%), and 8 (1.3%) patients, respectively (Fig 3). The proportion of patients with bacteraemia decreased with an OR of 0.7 per SES category (95% CI: 0.6–0.9). Among the SES groups, NTS infection was found in 10 (1.9%), 10 (1.7%), 3 (0.6%), and 1 (0.2%) patients (Fig 2). Similarly to urbanicity, the association between SES and NTS was strong (ORs = 0.6; 95% CI: 0.4–0.8), while SES showed reduced associations with bacteraemia when NTS cases were excluded from the analysis (OR = 0.8; 95% CI: 0.6–1.0).

There was evidence for a weak correlation between urbanicity and SES (Spearman's rho = 0.34; $p<0.001$), thus multivariate logistic regression models were established to account for potential confounding. In the adjusted models the association of both urbanicity and SES

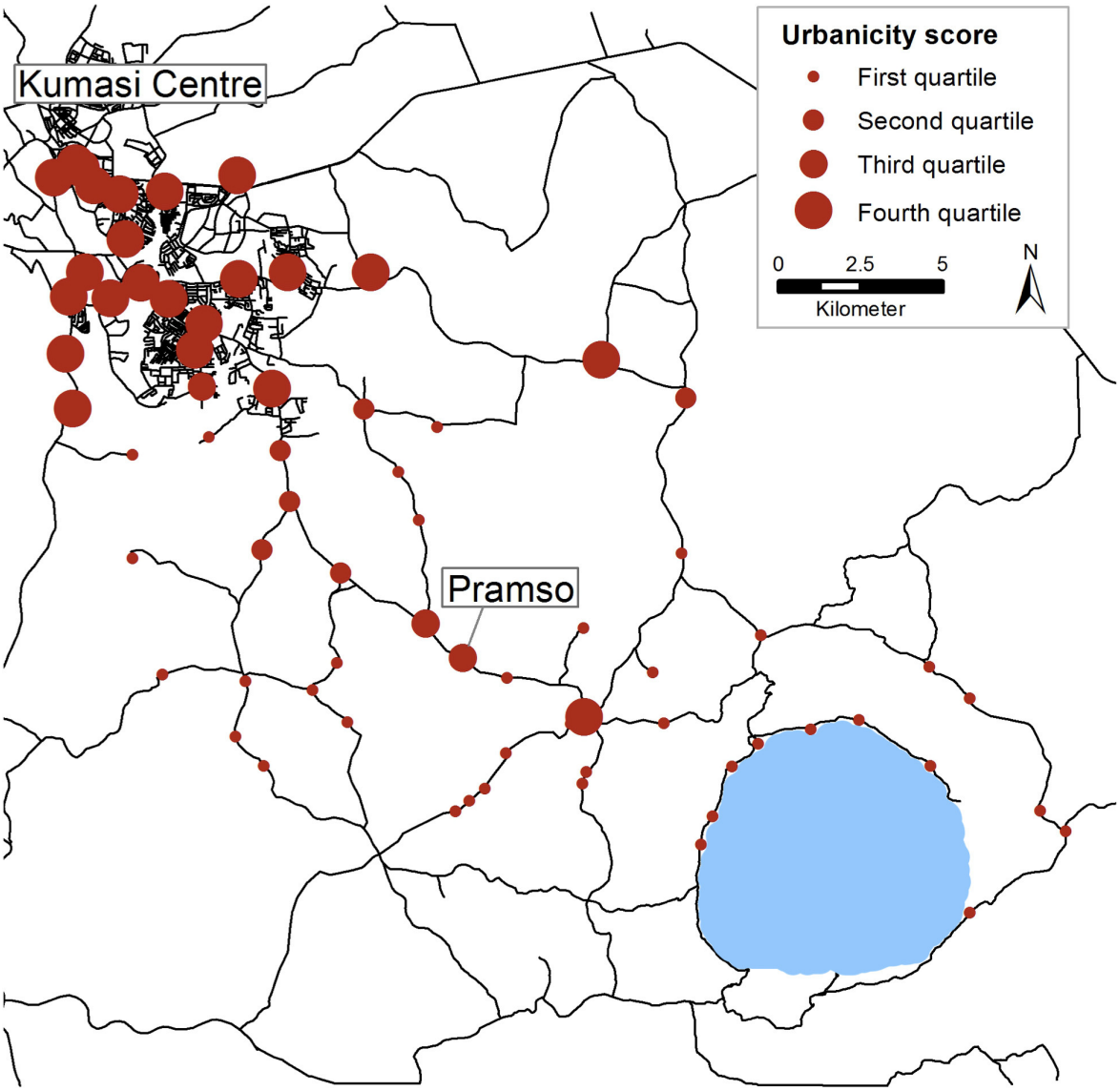

Fig 1. Map of the study area located within the Ashanti Region, Ghana. The level of urbanicity of a community is indicated by the size of the red dots.

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with bacteraemia remained comparable to the crude results (OR = 0.8; 95% CI: 0.7–1.0 and OR = 0.8; 95% CI: 0.6–0.9, respectively). Likewise, comparable effects were observed when modelling the correlation with NTS infection, with ORs of 0.5 (95% CI: 0.3–0.8) and 0.6 (95% CI: 0.4–1.0) for urbanicity and SES, respectively.

Discussion

Both higher urbanicity and SES were associated with lower odds of systemic bacterial infections in febrile children from a rural-urban transition zone in Ghana. These associations remained when urbanicity and SES were included in multivariate regression models highlighting that

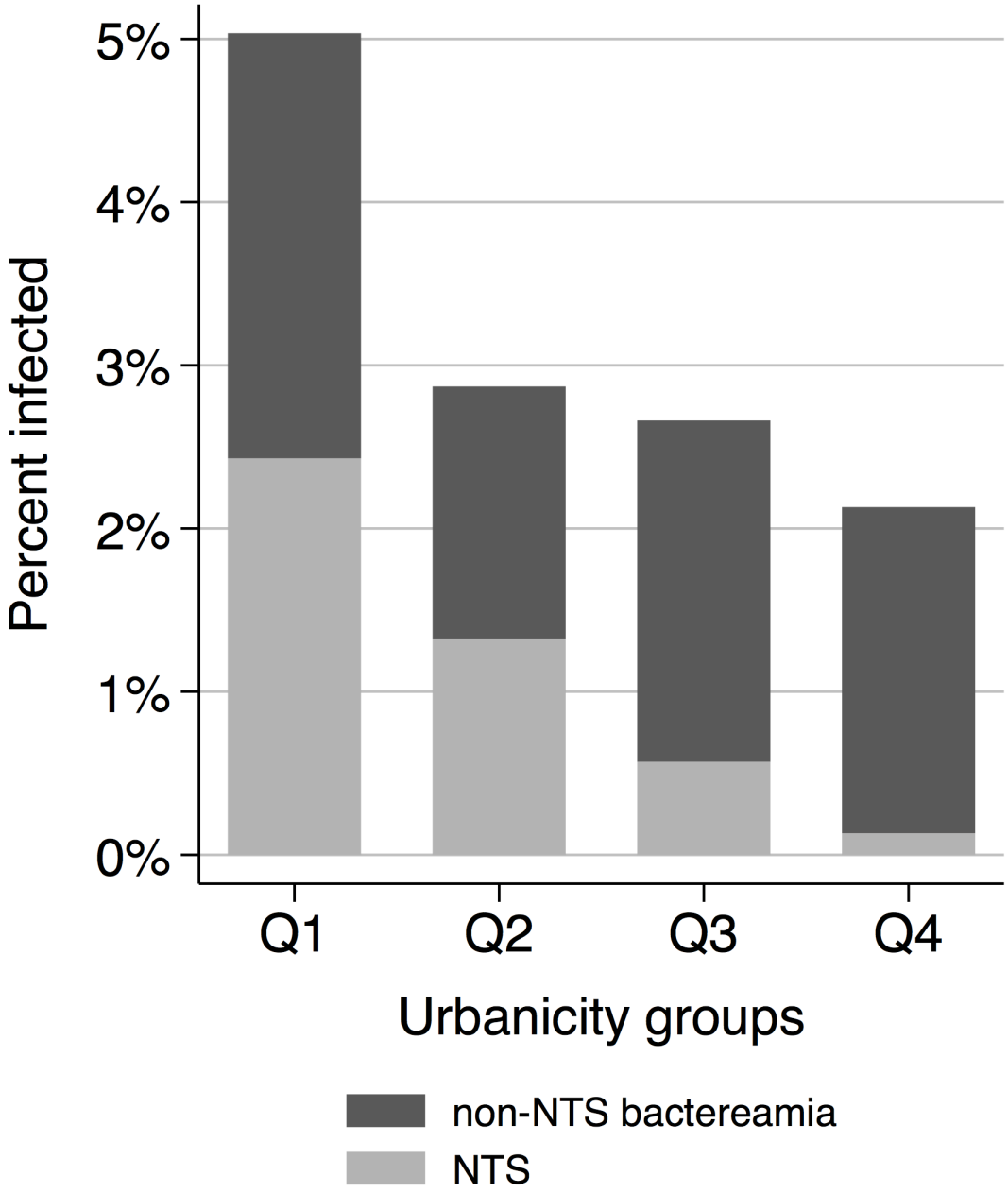

Fig 2. Frequency of non-typhoid *Salmonella* and other bloodstream infections among the four urbanicty groups (Q1 = low urbanicity and Q4 = high urbanicity).

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both are independent risk factors for IBI. As shown in a previous study [5], associations were mainly driven by NTS, the most frequent isolate in our study sample.

The proportion of bacteraemia decreased from the lowest to the highest urbanicity group. Low urbanicity status is generally associated with increased childhood mortality [19], but so far only a few studies have analysed the association between urbanicity and IBI in sub-Saharan Africa. Biggs et al. showed that the prevalence of bacteraemia was higher in a rural compared

to an urban site in Tanzania. These findings were largely driven by NTS, while other pathogens showed no urban and rural differences [5]. In accordance with that, our analyses yielded stronger effects of urbanicity on NTS than on unspecified bacteraemia and urbanicity showed no statistical association when cases of NTS were excluded. A comparable relationship was described from Kenya, where NTS accounted for 39% of rural and only 3% of urban blood culture isolates with an adjusted incidence rate ten times higher in the rural site [6].

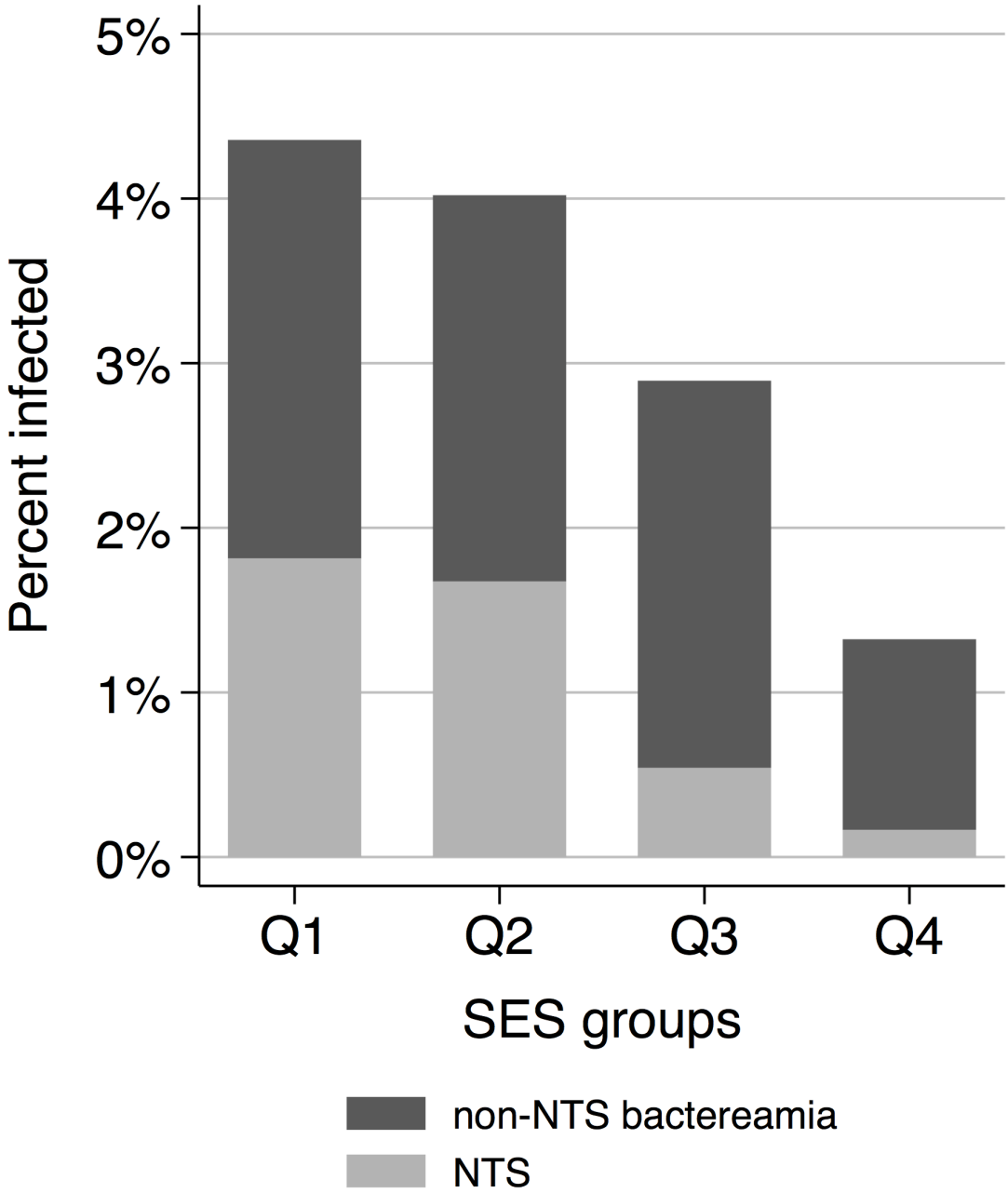

Fig 3. Frequency of non-typhoid *Salmonella* and other bloodstream infections among the four socio-economic status (SES) groups (Q1 = low SES and Q4 = high SES).

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We detected bacteraemia in 3.1% of febrile OPD children, a frequency similar to that in other OPD studies from sub-Saharan Africa. Brent et al. (2006) reported a rate of 2% in paediatric outpatients in Kenya [20] and Thriemer et al. (2012) reported a rate of 4% in outpatients of all ages in Zanzibar [21]. Yet, case numbers in our study were too small to assess effects at pathogen level apart from NTS. It remains unclear whether NTS was the main pathogen associated with urbanicity or if contrary effects in the heterogeneous group of pathogens obscured each other.

The observed association between NTS and urbanicity might be affected by associations between NTS and *Plasmodium falciparum* malaria. Several studies reported high NTS incidences in areas with high malaria prevalence and lower NTS numbers in areas with low malaria endemicity [22,23]. On a global scale, urbanization has been shown to coincide with decreased malaria transmission [24,25]. Even though it is unclear, whether these general findings can be applied to the complex epidemiological situation within a transition zone, they still indicate the role of *P. falciparum* malaria as a potential confounder for urbanicity. In our study malaria might consequently mediate the effect of urbanicity on NTS. Due to a selection bias (so called Berkson's bias) we were, however, not able to consider malaria in the regression analyses. In our hospital-based study, both cases and controls were recruited amongst symptomatic (febrile) patients. Hence, recruitment of patients without NTS was dependant on an alternative cause of fever, e.g. *P. falciparum* malaria. This biased the distribution of alternative pathogens among cases and controls and hampered further multivariate regression analyses [26,27].

In our study, the proportion of IBI decreased from the lowest to the highest SES group. Data on the relationship between SES and specific infectious diseases are scarce. In most studies, associations with non-specific acute febrile illnesses were reported [28] and these results are heterogeneous. While, for example, in one study conducted in four African countries, Ghana, Nigeria, Kenya and Sierra Leone, a higher likelihood of acute febrile illness in children with low SES was shown [29], studies from Tanzania and Ethiopia did not support this finding [28,30]. Furthermore, the analysis of demographic health surveys from 22 African countries yielded only a weak correlation between individual poverty and non-specific fever. However, there was evidence that the incidence of fever was influenced by the general level of wealth in the community [31]. To disentangle the effects of individual socio-economic and external community factors, we constructed multi-component measures of both determinants. In the multivariate model, effects of SES and urbanicity on bacteraemia were similar to those from the bivariate models. This provides evidence that both factors are considerable independent risk factors for childhood bacteraemia. Urbanicity and SES may influence the risk of bacteraemia via different mechanisms. Some key features of urbanicity are potential protective factors for bacteraemia, namely availability of tap water and sewage systems, access to education and health care, built environment and absence of agriculture [32]. However, low SES might prevent individuals from benefitting from them. Poor education and health related knowledge, low social status, and limited financial means might both increase disease risk, and also delay or prevent healthcare utilisation and the initiation of appropriate treatment measures [28,32,33].

Conventional rural/urban classifications based on surrogate markers, such as population size or density, are inadequate to capture the complex process of urbanization [10]. Especially in transitional societies, where urbanization leads to various forms of settlements, considering the population structure solely will lead to a biased assessment. For example, rapidly growing settlements without proper urban infrastructure would score highly on a population-based scale, but score considerably less on a multi-component scale. Including further urban features, such as public transport, water and sanitation, in this assessment enables the quantification of gradual differences between communities [34]. This allows analyses of health effects on a finer scale as required to differentiate epidemiological outcomes in heterogeneous transition zones.

Conclusions

The results of this study highlight the importance of individual as well as community factors as independent risk factors for IBI and especially NTS. The processes of urbanization and social transition in sub-Saharan Africa are complex and more data are needed to disentangle their effects on the epidemiology of infectious diseases. Such data will support physicians, public health experts and policy makers to identify disease prevention and treatment needs in the transitional societies of developing countries.

Supporting Information

S1 Dataset. Study data (CSF file).
(CSV)

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Author Contributions

Analyzed the data: PS RK BK LE. Contributed reagents/materials/analysis tools: FM. Wrote the paper: PS RK BK CF BS JM. Conducted laboratory analyses: KG BB. Coordinated hospital study: NS EO. Collected and managed data: JF AJ.

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